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Astrocyte Elevated Gene-1 (AEG-1) and the A(E)Ging HIV/AIDS-HAND¹

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Abstract

Recent attempts to analyze human immunodeficiency virus (HIV)-1-induced gene expression changes in astrocytes uncovered a multifunctional oncogene, astrocyte elevated gene-1 (AEG-1). Our previous studies revealed that AEG-1 regulates reactive astrocytes proliferation, migration and inflammation, all hallmarks of aging and CNS injury. Moreover, the involvement of AEG-1 in neurodegenerative disorders, such as Huntington's disease and migraine, and its induction in the aged brain suggest a plausible role in regulating overall CNS homeostasis and aging. Therefore, it is important to investigate AEG-1 specifically in aging-associated cognitive decline. In this study, we decipher the common mechanistic links in cancer, aging and HIV-1-associated neurocognitive disorders that likely contribute to AEG-1-based regulation of astrocyte responses and function. Despite AEG-1 incorporation into HIV-1 virions and its induction by HIV-1, tumor necrosis factor- α and interleukin-1 β , the specific role(s) of AEG-1 in astrocyte-driven HIV-1 neuropathogenesis are incompletely defined. We propose that AEG-1 plays a central role in a multitude of cellular stress responses involving mitochondria, endoplasmic reticulum and the nucleolus. It is thus important to further investigate AEG-1-based cellular and molecular regulation in order to successfully develop better therapeutic approaches that target AEG-1 to combat cancer, HIV-1 and aging.

Keywords

Astrocytes; Aging; HIV-associated neurocognitive disorders; Neuroinflammation; ER/Nucleolar/Oxidative stress; Excitotoxicity

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1. Introduction

With the introduction of antiretroviral therapy (ART), the perspectives on aging in human immunodeficiency virus (HIV)-1-infected people have been evolving. A new paradigm encompassing premature and/or accelerated aging has been recognized in the HIV+ population. The increased life expectancy of HIV patients allows a greater time span for comorbidities that include neurological outcomes, termed HIV-associated neurocognitive disorders (HAND). HAND is characterized by altered immunological and molecular changes in the brain such as pro-inflammatory signaling, oxidative stress pathways and cytotoxic neuroglial crosstalk. These conditions are exacerbated in the context of aging and may play an important role in influencing not just HIV-1 CNS disease pathogenesis, but also other comorbidities, thereby affecting patient quality of life and survival. Similar to the HIV-1-induced neurocognitive deficits, aging alone also leads to degenerative capacities in the brain. Therefore, understanding the mechanistic interplay among HIV-1 CNS infection, comorbidities and aging is highly warranted.

HIV-1 infection increases the risk of developing certain types of cancer, including Kaposi's sarcoma, non-Hodgkin's lymphoma, cervical and anal cancers, compared to the risk of the general population (de Sanjose *et al.*, 2008; Lederman *et al.*, 2013; Robbins *et al.*, 2015). However, the relationship between HIV-1 infection, oncogenic predisposition and aging has not been well studied. A widely applied tool for elucidating complex disease processes is initiating 'hypothesis generating studies'. To these ends, global gene expression changes were analyzed using HIV-1 patient samples in the quest to identify mechanistic targets to combat HIV-1 disease. Astrocyte elevated gene (AEG)-1 was one such protein associated with responses to HIV-1 infection, and later suggested as a prognostic marker in several cancers (Ke *et al.*, 2013; Li *et al.*, 2014a; Li *et al.*, 2008; Liu and Yang, 2013; Song *et al.*, 2010; Sun *et al.*, 2011; Tokunaga *et al.*, 2014; Xia *et al.*, 2014; Zhou *et al.*, 2012). Our new data presented herein suggest a novel link of human brain AEG-1 levels in context to age.

AEG-1 expression was detected at a basal level in naïve astrocytes and was significantly induced by HAND-relevant activation. Interleukin (IL)-1 β and tumor necrosis factor (TNF)- α are known astrocyte activators (John *et al.*, 2005), which were also proposed as inducers of AEG-1 in addition to HIV-1 (Kang *et al.*, 2005). These observations identify AEG-1 as an inflammatory responsive gene in astrocytes, as opposed to being solely an HIV-1-inducible transcript. Our prior studies established that AEG-1 binds to the key orchestrator of inflammation, nuclear factor (NF)- κ B, and facilitates NF- κ B nuclear localization (Vartak-Sharma *et al.*, 2014).

Detection of AEG-1 in the prefrontal cortex or frontal white matter regions provides physiological evidence for AEG-1 expression in the cognitive areas of the human brain, implying the need for functional studies to gauge the importance of AEG-1 in health and disease. In this review, we explore novel mechanistic links between the diverse AEG-1 roles in the context of aging and HAND. Further, we discuss several important pathways coupling AEG-1 to the aging HAND, including, but not limited to, inflammation, mitochondrial dysfunction, excitotoxicity and endoplasmic reticulum (ER), nucleolar and oxidative stress. As an astrocyte inflammatory gene relevant to HIV-1 CNS disease, we illustrate that AEG-1

is also associated with the aging brain. We present novel data regarding AEG-1 in aging human brains and its cellular redistribution in astrocytes under oxidative stress. Yet, despite the volume of information and perspectives we have summarized and discussed, we barely begin to answer some key questions: (1) What are the cellular functions of AEG-1 in human astrocytes at homeostasis? (2) How is AEG-1 expression induced in human astrocytes? (3) What are the implications of AEG-1 modulation in the aging brain? (4) Through what specific mechanism(s) does AEG-1 modulate astrocyte functions? (5) What is the disease relevance of AEG-1 in the CNS under multifactorial challenges? We propose that, AEG-1 regulation of multiple mechanisms related to neurodegeneration *via* astrocyte function is a key finding that can be utilized in developing therapeutic targets for CNS disorders including the aging HAND.

2. AEG-1: Historical Perspectives

AEG-1 was originally described in 2002 as an HIV-1 neuropathology-associated gene whose expression is significantly elevated upon HIV-1 or TNF- α exposure (Su *et al.*, 2002). Basic local alignment search tool analysis of AEG-1 gene indicated that AEG-1 has a unique gene structure, which does not resemble any other gene and is evolutionarily conserved among mammals and higher vertebrates. AEG-1 genomic environment is a known hot spot for genetic alterations in many cancers (Hu *et al.*, 2009a; Wan and Kang, 2013) and in other CNS pathologies such as migraine (Anttila *et al.*, 2010; Anttila *et al.*, 2011). AEG-1 is recognized as a pleiotropic protein localizing in various intracellular locations, including plasma membrane, cytoplasm, ER, nuclear envelope, nucleus and nucleolus. The physiological or cellular stimuli responsible for this differential localization remain unknown. At the same time, AEG-1 interaction with various protein/RNA partners and its wide-ranging intracellular localization are considered critical determinants of its function (Fig. 1) (Ash *et al.*, 2008; Sarkar *et al.*, 2008; Thirkettle *et al.*, 2009b). Despite detection of AEG-1 in the nucleus of many cancers, no DNA binding sites have been described in its protein structure. However, a putative RNA binding site and many protein-protein interaction sites on AEG-1 have been mapped, which suggest its role as a scaffolding protein or as a transcriptional co-activator/repressor. We elaborate on the specific role(s) attributed to these AEG-1 domains throughout the manuscript in relevant sections.

2.1. AEG-1 Discovery

AEG-1 research has evolved since its discovery and first report. While astrocytes are not the primary targets of HIV-1 CNS infection, in the current ART era inflammatory responses orchestrated by astrocytes continue to play a vital role in HIV-1 neuropathogenesis and hence remain a target of extensive research. In an attempt to understand the astrocyte-driven etiology of HAND, alterations in gene expression patterns in HIV-1-infected cultured primary human astrocytes were analyzed, and 15 “astrocyte elevated genes”, *i.e.* AEGs, were detected. Subsequently, mouse AEG-1, which was cloned using a phage screening approach, was found to mediate the metastasis of breast cancer cells to lung tissue (Qian *et al.*, 2011). Here, the AEG-1 protein was named metadherin (MTDH) to specify its role as a metastatic adhesion protein. Further cloning approaches categorized AEG-1 as a tight junction protein and was suitably named lysine-rich carcinoembryonic antigen-related cell

adhesion molecule 1 co-isolated (LYRIC)/3D3 (Sutherland *et al.*, 2004). AEG-1 was often induced as a late onset gene 3–7 days post-HIV-1 exposure and/or TNF- α treatment (Su *et al.*, 2002). In our studies in cultured human astrocytes, HIV-1 and TNF- α induced AEG-1 expression earlier, within 8 h for RNA and 24 h for protein (Vartak-Sharma *et al.*, 2014).

2.2. AEG-1 cloning, Biochemical interactions and Functional implications

As mentioned previously, gene discovery approaches have often been employed to delineate global gene changes in analyzing HIV-1 neuropathogenesis since HIV-1 primarily targets immune and resident brain cells including microglia and astrocytes, but rarely neurons. Following the original description of AEG-1 by Su *et al.* using rapid subtraction hybridization approach (Su *et al.*, 2002), four independent groups cloned the full-length cDNA and characterized AEG-1 by 2005 (Britt *et al.*, 2004; Brown and Ruoslahti, 2004; Kang *et al.*, 2005; Sutherland *et al.*, 2004). As mentioned earlier, AEG-1 is also known as MTDH and LYRIC/3D3, depending on the context in which it was characterized. The first description of AEG-1 as MTDH was when it was discovered as an ortholog in mice using *in vivo* phage screening, (Brown and Ruoslahti, 2004). This study also showed that AEG-1/MTDH, a cell surface protein, is overexpressed in breast cancer tissue facilitating cellular binding to lung vasculature through its extracellular, C-terminal lung homing domain (amino acids 378–440). This is the specific region that enhanced metastasis of breast cancer to the lung, earning its name “metastasis adhesion protein”. Finally, AEG-1 was cloned as LYRIC in polarized rat and human prostate epithelial cells, where it colocalized with tight junction proteins zonula occludens-1 and occludin (Britt *et al.*, 2004).

The *AEG-1* gene consists of 12 exons and 11 introns. The resultant mRNA encodes a single pass transmembrane protein consisting of 3611 nucleotides, excluding its poly A tail (Kang *et al.*, 2005). *AEG-1* promoter is regulated by the presence of negative transcriptional control elements located in the –2710/–459 base pair region. Deletion from –459 to –301 base pair resulted in 90% loss of basal Ha-ras-induced AEG-1 promoter activity, defining a transcription factor-binding element (Lee *et al.*, 2006). Further analysis uncovered two enhancer-box elements in the –356 to –302 region, that along with other transcription factor binding sites regulate AEG-1 promoter activity. Although AEG-1 is predominantly localized in ER and perinuclear region (Kang *et al.*, 2005), its membrane topology still remains incompletely defined.

AEG-1 is a lysine-rich, highly basic protein that contains 582 amino acids, with a predicted molecular weight of 64 kDa and isoelectric point of 9.3, which can be altered by post-translational modifications to regulate its intracellular localization (Fig. 1) (Kang *et al.*, 2005). It contains one transmembrane domain (TMD in Fig. 1) spanning amino acids 51–72. In addition, AEG-1 also contains N-terminal “LXXLL” domain that is used by co-activators to interact with transcriptional factors (Srivastava *et al.*, 2014). It has three putative nuclear localization signals (NLS) at amino acids 79–91, 432–451, and 561–580 (Fig. 1). Later on, targeting of AEG-1 to different sub-cellular compartments was attributed to the presence of intrinsic NLS and ubiquitination. Mutation and deletion studies of AEG-1 NLS indicated that extended sequences outside of canonical NLS (exNLS) were important AEG-1 localization determinants. Nuclear localization of AEG-1 is directed by extended NLS-3

(amino acids 546–582), whereas, extended NLS-1 (amino acids 78–130) mediates its nucleolar localization. The extended NLS-2 (amino acids 415–486) is modified by addition of ubiquitin that retains AEG-1 in the cytoplasm (summarized in Fig. 1).

AEG-1 exerts multiple functions with the aid of various binding domains that recruit diverse interacting proteins (Fig. 1 and Table 1). Consequently, interactions with several intracellular partners make AEG-1 a broad-spectrum effector protein (Fig. 1). AEG-1 is highly expressed in gliomas and recent studies suggest direct AEG-1 interactions with Akt2 in perinuclear regions of glioma cells. This interaction regulated cell proliferation and survival to augment glioma progression (Hu *et al.*, 2014). Breast cancer 2, early onset and cyclin-dependent kinase inhibitor 1A interacting protein (BCCIP) α , regulates cell cycle and homologous recombination (Lu *et al.*, 2005; Meng *et al.*, 2004). Yeast-two hybrid screening confirmed AEG-1 interaction with BCCIP isoforms, BCCIP α and BCCIP β (Ash *et al.*, 2008). While BCCIP α involvement in aging is not well studied, evidence supports that impaired function of breast cancer 2, a BCCIP α interacting partner, promotes p21-mediated senescence and premature aging (Cao *et al.*, 2003). It is likely that AEG-1 is indirectly involved in early aging processes as a BCCIP α interacting protein.

In colorectal carcinoma cell lines, AEG-1 overexpression increased β -catenin nuclear accumulation. AEG-1 activates Wnt/ β -catenin in other cancer types including hepatocellular carcinoma (HCC) (Srivastava *et al.*, 2015; Yoo *et al.*, 2009a), gastric cancer (Jian-bo *et al.*, 2011), diffuse large B-cell lymphoma (Ge *et al.*, 2012) and lung cancer (He *et al.*, 2015). Recent studies have suggested that Wnt/ β -catenin signaling plays a role in mammalian aging *via* reactive oxygen species (ROS) production (Naito *et al.*, 2010). Thus, AEG-1 likely contributes to aging *via* regulation of the Wnt/ β -catenin pathway.

In malignant glioma, chromatin immunoprecipitation studies revealed that AEG-1 was the bridge between NF- κ B and cAMP response element-binding protein-binding protein (CBP) on the CXCL8 promoter and augmented CXCL8 transcription (Sarkar *et al.*, 2008). Accordingly, in glioma cells AEG-1 may act as the NF- κ B co-activator and facilitate their invasion. AEG-1 also acts as a bridging molecule between yin yang protein 1 (YY1) and CBP on the excitatory amino acid transporter (EAAT) 2 promoter; thereby assisting YY1-mediated reduction of EAAT2 expression (Lee *et al.*, 2011). Further, high AEG-1 expression has been implicated in glioma-induced neurodegeneration (Lee *et al.*, 2011). Other studies indicated changes in NF- κ B transcriptional activity (Tilstra *et al.*, 2011) and CBP expression levels (Tomas Pereira *et al.*, 2013) in aging models. Our recent data shows that AEG-1 levels increase in human brains with a direct correlation to age (Fig. 2). We thus propose, that AEG-1 further exacerbates aging mechanisms through its versatility in binding a wide array of interacting proteins.

AEG-1 not only interacts with a variety of cellular proteins, in the setting of HIV infection, AEG-1 was recognized as an HIV-1 group-specific antigen (Gag) interacting protein using affinity purification experiments. Expression of Gag binding domain of AEG-1 enhanced Gag levels and viral infectivity (Engeland *et al.*, 2011). Consequently, AEG-1 interaction with the HIV-1 Gag polyprotein is considered to influence inflammation since the binding domain for HIV-1 Gag overlaps with the NF- κ B and BCCIP α binding domains.

Promyelocytic leukemia zinc finger protein (PLZF) is a transcriptional repressor that facilitates cell growth suppression and apoptosis (Bernardo *et al.*, 2007). Using yeast two-hybrid approach, AEG-1 interacts with PLZF and may block PLZF-facilitated function(s) to evade apoptosis and promote tumorigenesis (Thirkettle *et al.*, 2009b). Staphylococcal nuclease and tudor domain containing 1 (SND1) is a multifunctional protein that mediates transcriptional activation, spliceosome regulation and formation of RNA-induced silencing complex (Caudy *et al.*, 2003; Yang *et al.*, 2002; Yang *et al.*, 2007). Two independent studies have corroborated that AEG-1 physically interacts with SND1 in different cancer types (Blanco *et al.*, 2011; Yoo *et al.*, 2011). Consequently, SND1 interaction with AEG-1 suggests their role in tumor progression. Furthermore, SND1 mediates AEG-1 function by promoting chemoresistance and metastasis in breast cancer (Blanco *et al.*, 2011).

In a Huntington disease (HD) knock-in mouse model, AEG-1 interacts with regulator of ribosome synthesis (Rrs) 1 to determine ER stress responses in neuronal cells (Carnemolla *et al.*, 2009). Another recent study recognized AEG-1 as a binding partner of ubinuclein-1 (Ubn-1) in epithelial cells through co-immunoprecipitation and confocal microscopy (Lupo *et al.*, 2012). While the binding sites for β -catenin, Ubn-1, Rrs1 and some other proteins have not yet been clearly mapped, the functional outcomes from these interactions have been indirectly verified. Overall, these data strongly suggest that AEG-1 interactions with various proteins significantly broaden its functional repertoire and place it at the center-stage of cellular signaling networks.

2.3. AEG-1, aka MTDH, and its Role in Cancer and other CNS pathologies

While AEG-1 was first described in the setting of HIV-1 CNS infection, no discussion about AEG-1 and its role in any context will be complete without due justice to its role in cancer. As described earlier, AEG-1 has been widely investigated as the aliases MTDH, LYRIC/3D3 and metastasis adhesion protein. AEG-1 mRNA is ubiquitously expressed in all normal tissues, with elevated expression in the tissues of the skeletal muscle, heart and endocrine glands (Kang *et al.*, 2005). A number of clinical studies in cancers identified a positive correlation between AEG-1 expression and clinicopathological parameters (Table 2). Therefore, further in-depth research will promote AEG-1 as a vital prognostic tool in cancer diagnosis to enhance therapy efficacy and patient survival. AEG-1 is not only significantly overexpressed in most, if not all cancers, it is also implicated in oncogenic progression, with a considerable effect on disease prognosis (Hu *et al.*, 2009b). Numerous studies also highlight the role of AEG-1 in regulating cell growth, proliferation, anchorage-independent growth, migration, invasion, chemoresistance, angiogenesis, *in vivo* tumorigenesis and metastasis of several cancers (Hu *et al.*, 2009b). Table 3 provides a summary of the diverse role(s) AEG-1 plays in a variety of cancers. Taken together, AEG-1 is emerging as a novel target for the development of therapies to tackle multiple carcinogenic processes, through a single, centralized protein.

AEG-1 is also a principle regulator of tumor progression and metastasis in brain malignancy (summarized in Table 4). Many studies documented higher AEG-1 expression levels in CNS malignancies including glioblastoma multiforme (Emdad *et al.*, 2010; Liu *et al.*, 2010), neuroblastoma (Lee *et al.*, 2009), oligodendroglioma (Xia *et al.*, 2010), and astrocytoma (He

et al., 2014). Significant elevations in AEG-1 levels correlated with high-malignant astrocytoma tissues and glioma cell lines as compared to low-malignant astrocytoma, normal brain tissues and glial cells, which did not exhibit robust AEG-1 expression changes (He *et al.*, 2014). AEG-1 impacts CNS tumorigenesis by enhancing proliferation, migration and invasion, angiogenesis, cellular metabolism and chemoresistance (Table 4). Dysregulation of cell cycle or cell growth is a fundamental characteristic of any cancer. AEG-1 activates Akt signaling to promote tumorigenesis (Lee *et al.*, 2006; Lee *et al.*, 2008); and Akt regulates multiple biological processes including cell survival, proliferation and glycogen metabolism (Lawlor and Alessi, 2001; Song *et al.*, 2005). However, recent studies documented AEG-1 direct interaction with Akt isoform-2 in glioblastoma cells, stabilizing Akt2 phosphorylation at S474 to enhance glioma cell proliferation and survival (Hu *et al.*, 2014). Another study emphasized the role of AEG-1 in neuroblastoma pathogenesis by elevating cancer cell proliferation *via* activation of the phosphoinositide 3-kinase/Akt pathway (Lee *et al.*, 2009). Furthermore, in HCC, AEG-1 regulated Wnt/ β -catenin signaling and increased cell cycle regulatory proteins including c-Myc, cyclin D1 (Yoo *et al.*, 2009a), known upregulated markers in glioma (Cenci *et al.*, 2012; Wang *et al.*, 2012). Therefore, a better understanding of AEG-1 mediated Wnt/ β -catenin signaling in glioma cells is warranted in order to develop a strategy to control tumor cell proliferation.

AEG-1 also contributes to glioma cell migration and metastasis. Accordingly, AEG-1 expression was high at the glioma boundary and was associated with markers including matrix metalloproteinase-2 and -9, and the angiogenic marker, cluster of differentiation (CD)31 (Emdad *et al.*, 2010). Consistent with this notion, higher AEG-1 and matrix metalloproteinase-9 expression in intracranial xenografts of glioma cells have been well documented. AEG-1 induces matrix metalloproteinase-9 to promote tumor cell invasiveness (Liu *et al.*, 2010). Besides upregulating the expression of specific proteins, AEG-1 directed malignant glioma cell tumorigenic activities *via* NF- κ B regulation (Sarkar *et al.*, 2008). AEG-1 likely acts as a transcriptional co-activator for NF- κ B and CBP to alter genes expression in glioma cells. Recent studies documented cytoplasmic polyadenylation-element-binding protein, a transcriptional regulator, mediating AEG-1 expression and migration in glioma (Kochanek and Wells, 2013). Therefore, future strategies for targeting and disrupting cytoplasmic polyadenylation-element-binding protein 1 function will present novel therapeutic options for many cancers.

In addition to its tumorigenic role, AEG-1 mediates protective autophagy to promote chemoresistance and cell survival in glioma (Bhutia *et al.*, 2010). Further AEG-1 expression levels correlate to CD133, glioma stem cell marker and protein phosphatase 6c, radioresistance marker expression in human glioblastoma tissues (Guo *et al.*, 2014). AEG-1 knockdown studies also showed chemosensitivity of neuroblastoma cells to cisplatin or doxorubicin (Liu *et al.*, 2009). AEG-1 contributes to glutamate-induced neurodegeneration, by negatively regulating EAAT2 levels in glioma (Lee *et al.*, 2011). In summary, further investigation into the role of AEG-1 and its expression patterns in normal and glioma cells may aid in developing effective AEG-1 based therapies for CNS tumors.

In addition to the significant AEG-1 expression and its role in cancers, it is also implicated in other CNS pathologies. For example, in a genome-wide association study, a potential role

for AEG-1 was denoted in migraine (Anttila *et al.*, 2011). Immunohistochemical analysis of HD brains demonstrated AEG-1 upregulation by 1.7-fold over control brains, suggesting its involvement in the pathogenesis of HD (Carnemolla *et al.*, 2009). Recent studies on the pathogenesis of dengue hemorrhagic fever and dengue shock syndrome suggest that AEG-1 promoted illness by targeting endothelial cell dysfunction, thereby increasing vascular permeability (Liu *et al.*, 2011). We recently described AEG-1 expression in HIV-1 and HIV-1 encephalitic (HIVE) human brain tissues. HIVE is a pathological manifestation of the clinical stage IV of HIV-1 CNS infection. Our immunohistochemical and immunoblotting analysis of 12 HIV-1 without encephalitis and 11 HIVE brain tissue specimens revealed significant AEG-1 upregulation with HIV-1 CNS disease progression, suggesting its involvement in HIV-1-associated neurological complications (Vartak-Sharma *et al.*, 2014).

AEG-1 regulates astrocyte glutamate clearance by controlling the expression of the key glutamate transporter, EAAT2 (Lee *et al.*, 2011). In gliomas, AEG-1 downregulated EAAT2 promoter activity and thus decrease glutamate uptake. Consequently, AEG-1 was implicated in glutamate-mediated neurotoxicity, highlighting its plausible role in other neurodegenerative disorders including amyotrophic lateral sclerosis, Alzheimer's disease (AD), epilepsy, ischemia/stroke, HIVE, traumatic brain injury and hepatic encephalopathy. Besides, neurodegeneration, AEG-1 was suggested to play a key role in neuroinflammation, as studies in cancer have recognized AEG-1 as a downstream effector and interacting partner of the key inflammatory response pathway, NF- κ B (Sarkar *et al.*, 2008). Despite the fact that AEG-1 is evolutionarily conserved in vertebrates and is expressed ubiquitously in all cell types, AEG-1 expression has not been well explored in CNS cells including neurons and astrocytes. Further studies to explore AEG-1 in neurons and its physiological outcomes are therefore warranted.

3. AEG-1 in HAND

3.1. Introduction to HAND

The advent of combined ART has prolonged the life of HIV-1-infected individuals and reduced the progression to acquired immune deficiency syndrome (AIDS). With the advent of ART, there is tremendous success in keeping HIV patients alive, yet, the commonly termed 'successful failure' is that ART has also increased the incidence of HAND (Heaton *et al.*, 2010), a spectrum of neurological complications occurring in HIV-1-infected individuals. Currently, HAND is prevalent in 18–50% of those diagnosed with HIV and is considered as a major factor decreasing their quality of life (Cysique and Brew, 2011; Heaton *et al.*, 2010). In the early 1990s, prior to highly effective ART, HIV-associated dementia (HAD) affected 15–20% of AIDS patients (Power and Johnson, 1995). In 2007, a more precise and sensitive "Frascati" classification system was introduced, which broadly classified HAND into asymptomatic neurocognitive impairment, mild neurocognitive impairment and HAD, the most severe form of neurocognitive deficit (Antinori *et al.*, 2007). Successful therapeutic advancements in the post-ART era decreased HAD prevalence to 5% (Heaton *et al.*, 2010; Simioni *et al.*, 2010); whereas the incidence of milder cognitive deficits, remain high.

In the pre-ART era, HAD in AIDS patients was histopathologically characterized by white matter changes, demyelination, microglial nodules, multinucleated giant cells and perivascular infiltrates corresponding to mild to severe encephalitis (Gray *et al.*, 2003). In contrast, HAND is characterized by milder forms of brain parenchymal inflammation that can be detected by magnetic resonance spectroscopy and measurable markers of inflammation in cerebrospinal fluid (Valcour *et al.*, 2012). CD4+ T lymphocytes, dendritic cells and monocyte-derived macrophages are the major cells types that can support productive replication of HIV-1. Despite its immune privileged status, the CNS is now recognized as an important target of HIV-1 productive infection and an early viral reservoir following primary infection (Valcour *et al.*, 2012). While many theories have been postulated to explain HIV-1 neuroinvasion ranging from free viral entry to cell-mediated entry, the most widely accepted is the “Trojan horse” hypothesis, which suggests that HIV-1 crosses the blood brain barrier (BBB) *via* infected leukocyte infiltration from peripheral blood (Liu *et al.*, 2000). CNS tissue penetration leads to HIV-1-infected monocyte maturation into perivascular macrophages that allow productive replication and release viral particles. These progeny virions infect resident brain microglia and initiate a neuroinflammatory cascade that promotes development and progression of HAND. Other neurological comorbidities manifest as well. These include acute aseptic meningitis, distal sensory polyneuropathy and vacuolar myelopathy, other opportunistic viral or non-viral neurological infections, cytomegalovirus encephalitis, toxoplasmosis encephalitis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy, primary CNS lymphoma, cerebral vascular disease, and nucleoside neuropathy (Heaton *et al.*, 2011; Xia *et al.*, 2011). Amongst the multitude of neurological complications associated with HIV-1 infections, HAND is the most prevalent problem in the current ART era (Heaton *et al.*, 2011).

3.2. Role of Astrocytes in HAND

Inflammatory responses orchestrated through activated glia have emerged as important mechanisms in neurodegeneration associated with multiple diseases including HAND pathogenesis. Initially considered as merely passive bystanders, astrocytes are now recognized as important regulators of neuronal function by integrating neuronal inputs and modulating synaptic activity at the neuroglial tripartite synapse (Araque *et al.*, 1999). As astrocytes have both a ‘neural’ and an ‘immune’ repertoire, through which they are able to exert both neuroprotective and neurotoxic outcomes, an imbalance in any facet of astrocyte function likely has lasting repercussions, perpetuating neuropathogenesis.

In the case of HIV-1 CNS infection; however, the major targets for productive infection are not astrocytes, but rather, mononuclear phagocytes are primary sources of progeny virions in the CNS. Productive HIV-1 infection of perivascular macrophages and microglial cells can also activate surrounding microglia, which express higher levels of cell surface antigens, such as CD14, CD16, CD68 and major histocompatibility complex class II, leading to further leukocyte trafficking into the CNS, strongly implicated in HIV-1 neuropathogenesis (Lescure *et al.*, 2013). Current research established significant clinical correlations for cerebrospinal biomarkers of monocyte activation and chemotaxis, including soluble CD14, CCL2 and others, to magnetic resonance spectroscopy metabolites during chronic HIV

disease (Anderson *et al.*, 2015). HIV-infected patients with ongoing drug abuse also exhibit CD14+CD16+ monocyte migration and accumulation in the brain induced *via* elevated extracellular dopamine in the CNS (Coley *et al.*, 2015). Dendritic cells, macrophages or microglia, the primary targets of productive HIV-1 infection in the brain, along with astrocytes release several pro-inflammatory cytokines, such as IL-1 β , TNF- α , and interferon- γ , in addition to HIV-1 viral proteins, *e.g.* trans-activator of transcription (Tat), glycoprotein (gp)120 and negative regulatory factor. These cells also release platelet activation factors and other neurotoxic compounds like nitric oxide, glutamate, and inducible nitric oxide synthase, along with chemokines, such as CCL2, IL-6 and CXCL8. Recently, several excellent reviews have highlighted the role of astrocytes in HAND. (Hellmuth *et al.*, 2015, Churchill, 2013 #17073, Nath, 2015 #17265). Seminal work in this regard from Churchill *et al.*, highlighted astrocytes as a significant source of HIV-1-infected cells in the post-ART era. They showed that the percentage of infected astrocytes may be as high as 20%, and taking into account the relative abundance in the brain, astrocytes presented as significant HIV-1 reservoirs in the CNS (Churchill *et al.*, 2009). In HIVE and HAD cases, a strong relationship between the magnitude of astrocyte infection and proximity to perivascular macrophages in the brain tissue regions (Churchill *et al.*, 2009), confirms the notion of the Trojan Horse hypothesis for CNS infection. Considering HIV-1 entry into CNS via infiltrating HIV-1-infected monocytes and CD4+ T lymphocytes, another study demonstrated that robust infection of astrocytes can occur through a novel route *i.e.*, cell-cell contact between astrocytes and HIV-1 infected CD4+ T lymphocytes, albeit in a restricted manner. The existence of intercellular nanotube formation between CD4+ T cells and astrocytes also supports a cell-cell contact-mediated CNS infection (Luo and He, 2015). Yet another study indicated pH-mediated-endocytosis and subsequent viral destruction in the endocytic vesicles leads to non-productive infection of astrocytes (Chauhan and Khandkar, 2015). All together, astrocyte infection in HAND is now well established.

Interactions of infected and/or activated mononuclear phagocytes and astrocytes propagate disease processes. Resultant persistent CNS inflammation has been recognized as a hallmark of HIV-1 infection and is accompanied by cerebrospinal fluid pleocytosis and immune activation (Ho *et al.*, 2013). HIV-1 proteins, particularly Tat, potentiate inducible nitric oxide synthase in astrocytes through the activation of NF- κ B and CCAAT/enhancer binding protein β (Liu *et al.*, 2002). In addition, IL-1 β and TNF- α also trigger nitric oxide synthase production in astrocytes (Hu *et al.*, 1995; Lee *et al.*, 1993). Astrocyte exposure to HIV-1 proteins, Tat and gp120 were reported to induce IL-6 and TNF- α , which in turn, induce the release of excitatory amino acids, for example glutamate and potassium, that further trigger toxic calcium accumulation in astrocytes and neurons (Holden *et al.*, 1999). HIV-1 gp120 animal models documented that this protein disrupts the astrocyte glutamate-glutamine homeostasis by modulating the glutamate transporter expression on astrocytes, leading to memory impairment (Fernandes *et al.*, 2007). Besides the direct influence of viral proteins, oxidative stress induced by pro-inflammatory cytokines is also implicated in CNS disease progression. Reactive astrocytes exhibit impaired glutamate clearance leading to excitotoxicity, which is a source of ROS in addition to inflammatory stimuli (Rego and Oliveira, 2003). Astrocytes also extend foot processes surrounding the endothelial cell tight junctions and remain critical regulators of BBB integrity. Both brain microvascular

endothelial cells and astrocytes express molecular drug pumps and transporters, such as permeability glycoprotein (P-gp) and organic ion transporters, that dictate drug elimination (Pardridge *et al.*, 1997; Sanchez-Covarrubias *et al.*, 2014). Taken together, inflammation, excitotoxicity and oxidative stress result in neurotoxicity, leading to dysfunction or, ultimately, neuronal death.

Although release of toxic levels of pro-inflammatory cytokines, inability to clear extracellular glutamate and BBB damage endorse astrocytes as the central offenders in HAND pathogenesis, astrocytes are innately neuroprotective. Thus, it is imperative to take into consideration the neuroprotective repertoire of astrocytes and its dysregulation in disease. Astrocytes exposed to HIV-1 proteins secrete neuroprotective tissue inhibitor of metalloproteinases-1 (Ashutosh *et al.*, 2012) and also induce nuclear factor erythroid-2 related factor-2 (Nrf-2), a universal regulator of antioxidant cellular mechanisms, thereby triggering oxidative stress combating enzymes (Reddy *et al.*, 2012). Astrocytic sirtuin elicited neuroprotective effects by attenuating oxidative stress *via* increased production of super oxide dismutase 2 and catalase (Cheng *et al.*, 2014). Similarly, astrocytic tumor growth factor β -mediated neuroprotection was demonstrated in a mouse model for subacute neuroinflammation (Cekanaviciute *et al.*, 2014). Although deleterious effects of intracellular accumulation of amyloid beta (A β) is well known in AD, a recent study using an amyloid precursor protein/presenilin-1 transgenic mouse model revealed that HIV-1 Tat can contribute to HAND by modifying amyloid precursor protein processing to increase A β production (Kim *et al.*, 2013).

In balance, astrocytes represent an intriguing therapeutic target for re-establishing CNS homeostasis or for promoting neuroprotective behaviors in the context of HAND. Thus, further investigations discovering HIV-1-induced gene expression changes in astrocytes and how they influence astrocyte function during HAND are timely.

3.3. Astrocyte AEG-1 in HAND

Despite being a significantly upregulated gene in astrocytes following HIV-1 infection and/or TNF- α treatment (Su *et al.*, 2002), AEG-1 has not been investigated thoroughly in the context of HAND. In addition to high levels of AEG-1 in HIV+ patient brains (Vartak-Sharma *et al.*, 2014), AEG-1 contribution towards HAND pathogenesis is evident by its physical interaction with HIV-1 Gag protein, the main structural protein driving virion assembly and release from infected cells, and by the incorporation of AEG-1 into the viral particles and cleavage by viral proteases (Engeland *et al.*, 2011). Although there is no direct evidence to unequivocally confirm that AEG-1 facilitates HIV-1 viral entry into the CNS, recent inferences for AEG-1 in dengue virus-associated endothelial cell malfunction suggest that AEG-1 may regulate BBB permeability (Liu *et al.*, 2011). In this study, AEG-1 expression on human endothelial cells mimicked dengue virus non-structural protein 1, thereby facilitating the targeting of endothelial cells for destruction by dengue virus auto-antibodies and increasing vascular permeability (Liu *et al.*, 2011). Following entry of the virus *via* BBB, HIV-1 replicates in the primary sites of productive viral replication *i.e.*, CD4+, chemokine receptor CXCR4 and CCR5 expressing brain resident microglia, peripheral macrophages and infiltrating monocytes (Bissel and Wiley, 2004). In the current

ART era, the hippocampus, along with the surrounding entorhinal and temporal cortices, are the main sites for HIV-associated neuroinflammation (Anthony and Bell, 2008). A parallel analysis of AEG-1 expression in these distinct brain regions following viral BBB cross over are thus warranted to investigate whether increased astrocyte AEG-1 expression is a consequence of direct HIV-1 infection and/or HIV-associated neuroinflammation or an indirect downstream outcome. AEG-1 is a late onset gene in human astrocytes, with induction at days 3–7 post-HIV-1 exposure (Su *et al.*, 2002), indicating AEG-1 could be largely regulated by downstream signaling events and early onset genes induced by HIV-1. In attempt to address this issue, our *in vitro* short-term studies confirmed that astrocyte AEG-1 induction is mainly driven by neuroinflammation, but can be exacerbated by the presence of HIV-1 (Vartak-Sharma *et al.*, 2014).

3.3.1. AEG-1 and Reactive Gliosis—Our *in vitro* studies on reactive astrocytes revealed a fundamental role for AEG-1 in regulating proliferation and migration of astrocytes, which is important for post-injury/inflammation recovery to homeostasis (Vartak-Sharma and Ghorpade, 2012). As discussed above, in the context of cancer, elevated AEG-1 levels enable successful proliferation, migration and metastasis of cancer cells in a variety of tissue environments. These properties, in the context of HAND, translate into the phenomenon of reactive gliosis. Although AEG-1 expression, *per se*, was not significantly altered in injury-induced reactive astrocytes, intracellular localization changed dramatically. AEG-1 was localized to the nucleolus in a wound healing scratch model and hydrogen peroxide (H₂O₂) exposure. Differential trends of AEG-1 intracellular distribution were identified in HIV-1 or TNF- α treated astrocytes. AEG-1 remained in the cytoplasm in HIV-1-exposed astrocytes, whereas AEG-1 translocated to the nucleus in response to TNF- α treatment (Vartak-Sharma *et al.*, 2014). AEG-1 intracellular localization is a critical determinant of its cellular function. While AEG-1 nuclear localization is symbolic of its transcriptional role in response to inflammation, its retention in the cytoplasmic is indicative of its role as a scaffolding protein, regulating signaling events in the cell. These observations validate differential regulation of astrocyte AEG-1 in response to various stimuli and are testimonial to its diverse roles in the context of inflammation, oxidative stress and/or physical injury.

3.3.2. AEG-1 in HAND-associated Neuroinflammation—AEG-1, with its pleotropic functions and influences on inflammation, likely plays a central role in regulating HAND-associated neuroinflammation. One study demonstrated an early indication for AEG-1 involvement in regulating inflammatory cellular responses in U937 human promonocytic cells, wherein lipopolysaccharide induced AEG-1 and prostaglandin E2 production (Khuda *et al.*, 2009). Endogenous AEG-1 stable interaction with HIV-1 Gag protein, its incorporation into HIV-1 viral particles and cleavage by viral protease (Engeland *et al.*, 2011) is also an indicator of AEG-1 manipulation as a host factor to enhancing viral infectivity. This is supported by the fact that the AEG-1 and Gag interaction is also conserved for other retroviruses, such as murine leukemia virus and equine infectious anemia virus, and inhibition of this interaction leads to decreased viral infectivity (Engeland *et al.*, 2011). Therefore, analysis of AEG-1 expression and function in primary cellular targets of HIV-1 in the brain, including dendritic cells, microglia, macrophages and leukocytes, in addition to astrocytes, is necessary. In parallel with its direct interaction with

HIV-1 viral proteins, our recent works illustrate that AEG-1 also regulates signaling events in astrocytes post-HIV-1 exposure (Vartak-Sharma *et al.*, 2014). We suggest that this is mainly orchestrated by AEG-1 functioning as a co-activator to facilitate protein-protein interactions based on its spatiotemporal availability at distinct intracellular locations. AEG-1 interaction with the HIV-1 Gag protein has been speculated to influence astrocyte inflammatory responses as the binding domain for HIV-1 Gag overlaps with binding domains for NF- κ B and BCCIP α . In such, HIV-1 Gag creates a competitive binding environment, which ultimately influences cellular proliferation and cytokine/chemokine production through AEG-1. Astrocytes, the most abundant cells in the brain, respond to the inflammatory challenge and undergo reactive gliosis, a process that involves a graded continuum of progressive alterations in molecular expression, hypertrophy, proliferation and, in severe cases, scar formation. Reactive astrocytes secrete a wide variety of neurotropic factors, pro- and anti-inflammatory molecules that can potentially mediate neuroprotective or neurotoxic effects, depending upon the severity of the insult. IL-1 β and TNF- α are major pro-inflammatory cytokines in the brain and both stimulate astrocyte AEG-1 changes and subsequent inflammatory responses. Increased AEG-1 levels in reactive astrocytes, as noted in HAND brain tissues, signifies a positive correlation to increasing degree of neuroinflammation (Vartak-Sharma *et al.*, 2014). Moreover, AEG-1 modulation of astrocyte inflammatory responses was demonstrated *via* regulation of NF- κ B signaling, a master regulator of inflammation. AEG-1 interacts with the p65 subunit of NF- κ B and alters its nuclear availability in response to inflammatory stimuli. Further, AEG-1 transcriptionally induces the expression of the upstream activator of NF- κ B, I κ B kinase (IKK) β (Vartak-Sharma *et al.*, 2014). As NF- κ B is a significant regulator of numerous cytokines and chemokines, AEG-1 modulation of NF- κ B and IKK β in the context of HIV-1-induced inflammation is an important finding with far-reaching implications. Astrocytes can also facilitate immune cell extravasation into the CNS by secreting chemokines and by regulating the neurovasculature (Bajetto *et al.*, 2001). Astrocytes enhance T cell activation by modulating innate immune cells *i.e.*, microglial activity (Barcia *et al.*, 2008; Trapp and Nave, 2008). Moreover, in malignant glioma, astrocyte AEG-1 induction in response to neuroinflammation demonstrated transcriptional regulation of CXCL8, a neurotrophil chemoattractant (Emdad *et al.*, 2006) that also mediates productive HIV-1 infection in monocyte-derived macrophages and microglia (Mamik and Ghorpade, 2014). In response to injury or neuroinflammation, hypertrophic reactive astrocytes upregulate cytoskeletal proteins, such as glial fibrillary acidic protein (GFAP), vimentin and nestin, which are important for wound healing and homeostasis (Rolls *et al.*, 2009). AEG-1 may also modulate astrocyte cytoskeletal protein expression, as AEG-1 regulates the epithelial-mesenchymal transition proteins: vimentin, snail and slug, in cervical cancer, non-small cell lung cancer and HCC (He *et al.*, 2015; Liu *et al.*, 2015; Shi *et al.*, 2015; Zhang *et al.*, 2015). All together, AEG-1 plays a central role in orchestrating HAND-associated neuroinflammation.

3.3.3. AEG-1 at Astrocyte Intracellular Crossroads—Astrocyte intracellular signaling molecules, in addition to cytoskeletal proteins, are also heavily implicated in the molecular mechanisms of gliosis during neuroinflammation; *viz.* increased expression of NF- κ B, cAMP, oligodendrocyte transcription factor 2, mammalian target of rapamycin,

SOX9, *etc.* (Liberto *et al.*, 2004; Silver and Miller, 2004; Sofroniew, 2009). Besides NF- κ B, AEG-1 also physically interacts with a number of transcription factors and to regulate downstream pathways (Table 1). AEG-1 interacts with β -catenin to influence Wnt/ β -catenin signaling in colorectal carcinoma (Zhang *et al.*, 2013), which is critical for cytoskeletal changes that recruit astrocytes to the injury site (Yang *et al.*, 2012). Consequent to injury/neuroinflammation, reactive astrocytes can induce rapid activation of microglial cells and release of inflammatory mediators like IL-1 β and TNF- α , triggering a cascade of autocrine signaling events to amplify the neuroinflammatory barrage (Ransohoff and Tani, 1998; Tichauer *et al.*, 2007). Identification of AEG-1 as a downstream target of IL-1 β and TNF- α , along with its regulation of NF- κ B signaling, implies that AEG-1 is a key component of autocrine inflammatory signaling in astrocytes. Astrocyte production of IL-17A, IL-6, IL-12 and CXCL10 induce significant neuroglial activation (Pagenstecher *et al.*, 2000; Zimmermann *et al.*, 2013). Disrupting astrocyte-derived inflammatory signaling and cytokine production in a rat model of neuroinflammation, resulted in attenuated neuro/glial activation (Soliman *et al.*, 2013). Similarly, AEG-1 knockdown in astrocytes exposed to IL-1 β and TNF- α reduced NF- κ B activation and signaling, suggesting that AEG-1 can be utilized as a target associated with astrocyte-mediated neuroinflammation (Vartak-Sharma *et al.*, 2014). This observation is further supported by a recent phosphoproteomics study conducted in breast cancer cells, wherein it was demonstrated that AEG-1 is a direct target of IKK β at serine 298 and that AEG-1 phosphorylation is essential for I κ B α degradation, as well as, NF- κ B-dependent gene expression (Krishnan *et al.*, 2015).

In addition to NF- κ B, signal transducer and activator of transcription 3 (STAT3) is an important transcriptional regulator in reactive astrocytes that alters chemokine expression. Further, due to its inflammation regulatory potential, STAT3 is considered as a target to control neuroinflammatory pain (Liu *et al.*, 2013; Okada *et al.*, 2006). Moreover, HIV-1 Tat transactivates GFAP expression in astrocytes *via* STAT3 activation to mediate direct and indirect neurotoxicity (Fan *et al.*, 2015). Conditional knockout of STAT3 from reactive astrocytes markedly attenuated several aspects of gliosis and scar formation, including astrocyte hypertrophy and GFAP synthesis; resulting in greater spread of inflammation, increased lesion size, demyelination and impaired function (Herrmann *et al.*, 2008; Okada *et al.*, 2006). Although AEG-1 regulation of STAT3 signaling in astrocytes has not been investigated, recent insights from HCC studies demonstrated AEG-1 regulation of STAT3 activation and downstream processing (Deng *et al.*, 2014; Robertson *et al.*, 2014); a compelling argument for AEG-1 / STAT3 studies in astrocytes.

3.3.4. AEG-1 in HAND-associated Excitotoxicity—The functional outcome of neuroinflammatory cascades, consequent to reactive astrogliosis and traumatic CNS injuries or infections, is neurodegeneration. Neurodegeneration is characterized by rapid or gradual progressive decline in neuronal structure or functions, ultimately leading to neuronal loss, and manifests as cognitive, behavioral or motor deficits (Bredesen *et al.*, 2006). As the more resilient counterpart to neurons in the brain, the principle role of astrocytes is to protect and nourish neurons. In such, neurodegenerative disorders are consequences of astrocyte functional failures. Astrocytes affect neurodegeneration over a graded continuum comprising

of initial, immediate effects on neuronal survival post-injury; followed by delayed, effects, which ultimately influence neuronal recovery and function long-term.

Efficient removal of the neurotransmitter glutamate from the synapse is a critical function of astrocytes, which is often dysregulated during neuroinflammation and leads to excitotoxic neuronal damage. Prime examples are HIV-1-associated neuropathies, which develop gradually over time, due to excitotoxic damage to neurons, owing to persistent chronic low-level neuroinflammation (Lindl *et al.*, 2010). With an increase in the number of reactive astrocytes at various spatiotemporal locations of the brain, in response to neuroinflammation, there is significant clustering of glutamate transporters, which are responsible for efficient clearance of extracellular glutamate. Failure to clear glutamate in the astroglial-neuronal tripartite synapse results in seizures and neurodegeneration implicated in HAND (Petr *et al.*, 2015; Rothstein *et al.*, 1996; Watase *et al.*, 1998). The principle regulator of extracellular glutamate in the brain is the transmembrane sodium and potassium coupled transporter, EAAT2, which is expressed mainly on astrocytes (Yi and Hazell, 2006). Thus, EAAT2 expression and activity are important determinants of excitotoxicity in the neuronal microenvironment. In studies on glioblastoma, which express high levels of AEG-1, implicated AEG-1 in downregulating EAAT2 promoter activity, and thus, in mediating glioblastoma-associated necrosis and neurodegeneration (Lee *et al.*, 2011). We have demonstrated AEG-1-mediated EAAT2 downregulation and reduced glutamate clearance in primary human astrocytes treated with IL-1 β , a prominent cytokine regulating astrocytes during HAND (Vartak-Sharma *et al.*, 2014). AEG-1 is, thus, also implicated in HAND-associated neurodegeneration. Further, in glioblastoma, it was revealed that AEG-1 interacted with transcriptional repressor YY1 to downregulate EAAT2 (Lee *et al.*, 2011). Our studies on cultured human astrocytes overexpressing AEG-1, displayed induction of YY1, thereby implying that AEG-1 interaction with YY1 may be able to regulate EAAT2 levels. Additionally, studies described EAAT2 expression as highly regulated at the translational level (Tian *et al.*, 2007), indicating that, despite changes in EAAT2 transcription, the resultant EAAT2 protein levels may or may not resemble mRNA transcription. In this regard, AEG-1 regulation of protein translation has been demonstrated in certain cancer types; *viz.* AEG-1-facilitated association of multi-drug resistance gene 1 (MDR1) mRNA to polysomes to induce MDR1 protein translation (Yoo *et al.*, 2010), and microRNA processing as a component of RNA-induced silencing complex in HCC (Yoo *et al.*, 2011). However, AEG-1-mediated regulation of EAAT2 translation needs to be investigated. Yet another unidentified important indicator for AEG-1 regulation of extracellular glutamate is its genomic location on chromosome 8q22, flanking the migraine susceptibility gene and in the vicinity of the glutamate regulatory element, plasma glutamate carboxypeptidase, which increases extracellular glutamate concentrations by cleaving N-acetyl-L-aspartyl-L-glutamate into N-acetyl aspartate and glutamate (Anttila *et al.*, 2010; Anttila *et al.*, 2011).

Impaired glutamate transport and dysregulation of the glutamate-glutamine cycle are critical elements of most neurodegenerative disorders. Enhancing glutamate uptake by astrocytes would thus be an effective strategy to overcome resultant neuronal malfunction. Upregulation of astrocyte glutamine synthetase is linked to protection in ischemic stroke with reduced infarct size and plays a role in ischemic intolerance induced by inhibition of

succinate dehydrogenase (Gorovits *et al.*, 1997; Hoshi *et al.*, 2006). Taking into account AEG-1 regulation of astrocyte glutamate uptake, analysis of the effect of AEG-1 on glutamine synthetase activity in astrocytes is warranted. Similarly, ablation of astrocyte glutamate transporter EAAT2 significantly influenced neural stem cell population, neuronal regeneration and synaptic recovery post-injury (Rao *et al.*, 2001), suggesting that astrocyte glutamate clearance capacities can dictate neurogenesis.

Accumulation of extracellular glutamate, an important excitatory neurotransmitter in the brain (Meldrum, 2000), was observed in several neurodegenerative disorders like dementia, multiple sclerosis, AD, amyotrophic lateral sclerosis, Parkinson's disease, alcoholism, epilepsy and HD (Mehta *et al.*, 2013). Excessive glutamate in the neuronal microenvironment allows high levels of calcium influx into the neurons, thereby activating phospholipases, endonucleases and proteases like calpain that damage the neuronal cytoskeleton, membrane and DNA (Arundine and Tymianski, 2003; Atlante *et al.*, 2001). Since, EAAT2 expression and function in astrocytes is an important determinant of neurotoxicity, identification of AEG-1 as a negative regulator of astrocytic glutamate clearance *via* EAAT2 downregulation is an important finding that implies AEG-1 controls the level of neurodegeneration associated with neuroinflammation in HAND.

4. Astrocytes and AEG-1 in Aging

4.1. Astrocytes and Aging

Aging is a major risk factor of neurodegenerative diseases and is associated with gradual deterioration in brain functioning by neuronal loss or impairment. Whereas, the fundamental functions of astroglia help maintain CNS homeostasis; aging induces astroglial activation, resulting in the failure of CNS homeostasis (Amenta *et al.*, 1998; Jiang and Cadenas, 2014). Reactive astrogliosis is implicated in several chronic neurodegenerative diseases including AD, Parkinson's disease, HD and amyotrophic lateral sclerosis (Phatnani and Maniatis, 2015). Astrocytes, in conjunction with microglia, become reactive to CNS insults and are characterized by morphological and physiological changes including hypertrophy, upregulated GFAP expression, and proliferation. Age-dependent, increased GFAP expression and astrocyte proliferation was observed in several regions of brain, including the hippocampus (Amenta *et al.*, 1998). During aging astrocytes display elevated expression of S100 β and GFAP, a probable outcome of inflammatory and oxidative stress episodes (Barreto *et al.*, 2009). One characteristic observation in aging brains is that the number of astrocytes increases by approximately 20% (Pilegaard and Ladefoged, 1996; Salminen *et al.*, 2011). The ubiquitous presence of astrocytes in aged brain tissue is often considered as harmful, producing cytokines and chemokines that provoke inflammation (Jiang and Cadenas, 2014; Salminen *et al.*, 2011). Reactive astrocytes release several factors including cytokines, in particular, IL-1 β and TNF- α , which perpetuate inflammatory responses (Jiang and Cadenas, 2014). Several age-related neurodegenerative diseases are accompanied by neuroinflammation, indicating that brain vulnerability is due to the factors triggered during inflammation. In our immunohistochemistry studies using a brain injury mouse model for reactive astrogliosis, significant AEG-1 colocalization was observed with reactive astrocytes

(Vartak-Sharma and Ghorpade, 2012), thereby proposing AEG-1 role in modulating astrocyte function in the context of inflammation.

Due to onset and increased presence of aging-associated neuropathies, there is increased burden on astrocytes for their protective roles, resulting in exacerbation of the disease manifestations by skewed astrocytic functions. Gene expression analyses of aging mouse brains have provided evidence for aging-associated astrocyte inflammatory and oxidative stress response pathways, particularly in the neocortex, hippocampus and cerebellum, similar to that of neurodegenerative disorders (Lee *et al.*, 2000; Zeier *et al.*, 2011). A hallmark of molecular aging is a dramatic increase in the levels of damaging free radicals, including nitric oxide, superoxides and peroxynitrites (Shibata and Kobayashi, 2008). Astrocyte-derived antioxidants are remarkable neuroprotective molecules against the barrage of oxidative stress during aging (Dringen and Hirrlinger, 2003). However, as mentioned earlier, astrocytic physiological functions are impaired during aging, exacerbating their deleterious effects. Although astrocytes play a role in clearing A β peptides during AD, (Li *et al.*, 2011) A β activates astrocytes to decrease glutamate uptake, increase inflammatory mediators and oxidative stress, hence, mediating neuronal loss (Agostinho *et al.*, 2010). One study showed that neither pro-oxidants nor A β alone were significantly toxic to astrocytes; however, in combination, they resulted in oxidative stress and astrocyte apoptosis *in vitro*, and enhanced inflammation and neurodegeneration *in vivo*. Therefore, reduced oxidative stress coping mechanisms in astrocytes may contribute towards neuronal death and development of AD (Garcia-Matas *et al.*, 2010).

Cognitive impairment is a major functional outcome associated with aging and is characterized by synaptic deficiency, particularly in the hippocampal and neocortical circuits, owing to loss of dendritic and axonal arborization [reviewed by (Yankner *et al.*, 2008)]. In humans, significant age-dependent GFAP increase is mainly reported in the hippocampus, indicating the potential role of astrocytes in modulating the age-associated cognitive deficits (David *et al.*, 1997). A study utilizing GFAP knockout mice revealed increased cellular proliferation and neurogenesis in the granular layer of the dentate gyrus in aged animals (Larsson *et al.*, 2004). Similarly, GFAP null astrocytes demonstrated a better capacity for supporting neuronal survival and neurite outgrowth (Menet *et al.*, 2000). This is most likely attributed to the temporal discordance between neurogenesis and GFAP expression during development, with neurogenesis occurring much earlier than astrocyte maturation and GFAP expression. However, these studies provide profound insight that regulating 'reactive' responses of astrocytes may provide effective novel approaches to ameliorate neuropathogenesis associated with aging and other neurodegenerative diseases.

4.2. Accelerated Aging in HIV-infected Individuals

The commonly accepted definition of physiological aging is "the process of progressive deterioration of physiological functioning as a consequence of tissue damage" (Hayflick, 1998). In the setting of HAND and ART; however, there is an increasing consensus that HIV infection or treatment affects and alters physiological aging, typically observed as accelerated, premature or early aging in infected patients. Thus, accelerated aging in HIV infection refers to the observation that HIV+ individuals may encounter advanced or faster

aging than the normal, uninfected, age-matched population. As a result, HIV-infected people are designated to be “elderly” at age 50 years, compared with the general population at 65 years (Capeau, 2011). In the absence of proper care or treatment, HIV-infected patients also have a shorter lifespan because of immune system failure.

With the advent of ART, life expectancy of the HIV+ patients has dramatically improved. Life duration of HIV-infected people is dependent upon the age they started treatment and expected to be 10–30 years less compared to control population (Lohse *et al.*, 2007). Analysis of 14 HIV+ patient cohorts identified that combination therapy significantly extended life expectancy and declined mortality rates of HIV-infected people; however, a dramatic gap compared to the uninfected population remains (Antiretroviral Therapy Cohort, 2008). As a result, the numbers of HIV+ patients aged 50+ years have significantly increased. Worldwide, approximately 3.6 million people over 50 years old are living with HIV (UNAIDS, 2013). In 2013, HIV-infected people aged 50 and older accounted for about one-quarter of estimated total HIV diagnoses in United States. In addition, 37% of the 6,995 AIDS-related deaths in 2013 were among people aged over 55 years (CDC, 2015).

The real concern; however, is not that HIV-infected people are getting older, but the risk of age-related complications associated with HIV infection at any given age, compared to age-matched controls. Consequently, early HIV-associated aging exhibits several clinical similarities with biological aging, including but not limited to, malignancy, cardiovascular disease, low muscle mass (sarcopenia), osteoporosis, hypertension, atherosclerosis, renal failures, neurocognitive decline and frailty, collectively termed polypathology (Deeks and Phillips, 2009; Desquilbet *et al.*, 2007; Desquilbet *et al.*, 2009; Ikezu, 2009; Kaplan *et al.*, 2007; Pawelec *et al.*, 1999; Richert *et al.*, 2011). Polypathology is commonly seen in individuals aged over 55 years, but occurs, on average, 15 years earlier in HIV-infected people aged only 40 years. Cardiovascular pathologies and coronary heart disease are prominently increased in HIV-infected people versus uninfected age-matched controls (Triant, 2013). Other findings indicate that functional impairment in middle-aged adults with HIV-1 infection is associated with sarcopenia and loss of mineral density (osteopenia) (Erlandson *et al.*, 2013). Liver volume, hepatocyte numbers and drug metabolism gradually decrease with age. In the post-ART era, higher mortality rates associated with liver cirrhosis were observed in HIV-infected patients (Weber *et al.*, 2006). Both aging and HIV-1 infection are also associated with neurological complications. Neuroimaging studies in HIV+ and seronegative individuals over 50 years and older showed significant atrophy in gray and white matter (Becker *et al.*, 2012). Furthermore, Tau protein deposition, a marker for AD and HD, was also elevated in the brains of HIV-infected patients (Anthony *et al.*, 2006). Metabolic complications are also observed in HIV+ patients including dyslipidemia, body composition changes, insulin resistance and diabetes (Feeney and Mallon, 2011; Lo, 2011; Stanley and Grinspoon, 2012). HIV infection and therapy also contribute to immunosenescence, or declining immune function, which is also characteristic of physiological aging. One study confirmed HIV-infected patients (median age 56 years) with reduced viral load had immune changes *i.e.*, T-cell characteristics comparable to older HIV-negative people (median age 88 years) (Desai and Landay, 2010). Taken together, HIV-1 infection and ART influence mortality and morbidity rates through inflammation and treatment-related toxicities, including renal dysfunction, liver cirrhosis and abnormal fat

deposition; along with chronic viral diseases and comorbidities often associated with physiological aging. Drug interactions between existing ART medications and those used to treat these polypathologies and/or comorbidities is also poorly studied.

Despite effective ART regimens and well-managed HIV infections, overall health issues in HIV+ patients are comparable to HIV-negative population approximately 15 years older. In 2013, adults >50 years accounted for 21% of new HIV diagnoses in the United States (2009; CDC, 2015). This, together with the continued aging of HIV+ patients on ART, the average age of patients living with HIV-1 in the USA will continue to rise. Better understanding of age-associated effects in HIV+ people, and management of HIV infection in older patients, is thus warranted. Further exploration of accelerated aging in HIV infection with the circumstances associated with age-associated polypathologies and other comorbidities will provide novel interventions to benefit both HIV-infected patients and senior adults.

4.3. AEG-1 in the Aging Human Brain

As introduced earlier, AEG-1, a multifunctional oncogene, is an HIV-1 and TNF- α -inducible transcript in astrocytes (Su *et al.*, 2002). Several important oncogenic roles have been attributed to AEG-1, specifically, tumor initiation, proliferation, metastasis, angiogenesis, chemoresistance and protective autophagy. The contributions of AEG-1 towards the hallmarks of aging, including reactive astrogliosis (Vartak-Sharma and Ghorpade, 2012), inflammation (Vartak-Sharma *et al.*, 2014), oxidative stress and ER/nucleolar stress (Carnemolla *et al.*, 2009), indirectly suggest a role for AEG-1 in regulating the aging process. We evaluated the age-related changes in AEG-1 expression in cognitive centers of the human brain. In such, we analyzed AEG-1 protein expression in the prefrontal cortex and frontal white matter brain regions of 22 HIV-1 seronegative individuals between the ages of 21–90 years old (Fig. 2). Materials and methods employed in our experimental studies are described in detail in the supplementary information provided with the manuscript. Real-time-PCR, densitometry and correlation analyses demonstrated a significant increase in AEG-1 protein that exhibited positive correlation with age ($P=0.0031$, Fig. 2A,B). Consistent with these findings, AEG-1 mRNA levels in a human brain tissues from 25 patients >50 years were significantly higher than those from 37 patients <50 years (Fig. 2C). Together, our results confirm increased AEG-1 levels in the aging human brain. Hallmarks of aging include increased excitotoxicity in the cerebral parenchyma and in the vicinity of the microvasculature due to reduced glutamate clearance, elevated pro-inflammatory processes, loss of neural circuits and plasticity owing to calcium dysregulation (Kumar *et al.*, 2009; Michaud *et al.*, 2013; Vatassery *et al.*, 1998). Furthermore, accumulation of extracellular glutamate in the synaptic cleft could upregulate production of ROS, ultimately resulting in neuronal death (Reynolds and Hastings, 1995). An inverse relationship between levels of AEG-1 and EAAT2 expression, an astrocyte glutamate transporter, is well known (Lee *et al.*, 2011). In addition, we determined AEG-1-mediated EAAT2 downregulation and reduced glutamate clearance coincided with increased interaction between AEG-1 and YY1 in primary human astrocytes treated with IL-1 β (Vartak-Sharma *et al.*, 2014), thus implicating AEG-1 in glutamate-mediated excitotoxicity of neurons. Recently, a study conducted in aged mice revealed differential regulation of

EAAT2 gene expression by NF- κ B and N-Myc during post-natal development (Gupta and Prasad, 2014). As AEG-1 levels are elevated in the brains of aged individuals and since AEG-1 is a target of both NF- κ B (Emdad *et al.*, 2006) and c-Myc (Lee *et al.*, 2006), analysis of AEG-1 regulation of EAAT2 in the context of accelerated aging of HIV+ patients is important.

4.4. Common Factors Contributing to Normal aging and Accelerated aging in HIV+ patients

There are several common factors that influence both physiological aging as well as premature aging of HIV-infected individuals; including life style associated risk factors *i.e.*, smoking, alcohol consumption, drinking, and drug abuse. Fig. 3 summarizes several mechanistic features associated with aging including inflammation/immunosenescence, increased oxidative stress, owing to long-term effects on mitochondrial DNA and mitochondrial damage, ER stress, and the direct toxicities of antiretroviral drugs and other HIV-associated comorbidities. Each of these pathways is discussed below, in the context of increased AEG-1 expression in aged human brain, expected age-related outcomes, and plausible mechanisms through which AEG-1 may contribute to these aging processes.

4.4.1. Inflammation—Inflammation is a complex and tightly regulated process that recruits immune cells to and clears pathogen from the site of infection. Any sort of tissue damage generally induces acute inflammation, a beneficial process characterized by swelling, redness, pain and warmth. This process involves a complex array of soluble pro-inflammatory mediators including cytokines, chemokines and acute phase proteins. While acute inflammation is an immediate or short-term response that helps in healing process, chronic inflammation, as is developed with sustained viral infection, has been implicated in age-related diseases and loss of young functions (Chung *et al.*, 2009; Jones, 2001; Pearson *et al.*, 2003). Inflammation is associated with many age-related pathologies, such as cardiovascular diseases, diabetes, AD, osteoporosis and atherosclerosis (Chung *et al.*, 2006; Franceschi and Campisi, 2014; Jones, 2001; Libby and Simon, 2001; Pearson *et al.*, 2003).

Aging is a gradual degenerative process underlined by low-grade, chronic inflammation, collectively referred as “Inflammaging” (Guaraldi *et al.*, 2014). Chronic inflammation commonly leads to tissue degeneration and is considered as a high risk factor for morbidity and mortality in aged people (Franceschi and Campisi, 2014). The aging process triggers the pro-inflammatory NF- κ B signaling pathway and upregulates inflammatory mediators including TNF- α , IL-6, IL-1 β , inducible nitric oxide synthase and C-reactive protein (Chung *et al.*, 2009; Ferrucci *et al.*, 2005; Franceschi and Campisi, 2014). Oxidative stress and DNA damage are classic features of the aging process and age-related diseases and also activate the NF- κ B pathway (Janssens and Tschoop, 2006; Kratsochnik *et al.*, 2005; Morgan and Liu, 2011), thus solidifying NF- κ B as a critical mediator of aging process. As we have discussed earlier, many studies describe the involvement of AEG-1 in NF- κ B-dependent gene expression enhancing tumor progression, migration and invasion (Emdad *et al.*, 2006); yet its specific contribution(s) to inflammatory responses is not well known. We recently identified AEG-1 as a novel regulator of HIV-1-induced inflammation *via* augmented NF- κ B nuclear translocation and CCL2 production (Vartak-Sharma *et al.*, 2014). In another study, AEG-1 was implicated in lipopolysaccharide-induced NF- κ B activation (Khuda *et al.*,

2009) and AEG-1 inhibition abrogated TNF- α and prostaglandin E2 production, two distinguished upregulated markers of aging (Paolisso *et al.*, 1998; Wu and Meydani, 2004; Wu *et al.*, 1998). In our previous studies, we established AEG-1 as an inflammatory responsive gene and showed that IL-1 β and TNF- α induced AEG-1 expression in astrocytes *via* the NF- κ B pathway (Vartak-Sharma *et al.*, 2014). Since these inflammatory mediators are also upregulated during aging, they may be driving AEG-1 expression during the aging process. Consequently, the fundamental participation of AEG-1 in inflammatory responses through the regulation of NF- κ B pathway (Emdad *et al.*, 2006; Li *et al.*, 2014b; Sarkar *et al.*, 2008) designates AEG-1 as a critical immune modulator in aging and age-related diseases (Fig. 3).

Several serum pro-inflammatory markers are correlated to cognitive decline clinically presented as dementia, commonly associated with aging. Elevated IL-6 and C-reactive protein blood levels are designated as risk factors of mortality. In one aging study, elevated C-reactive protein levels were observed in vascular dementia and AD (Schmidt *et al.*, 2002). Though the rationale for elevated inflammatory markers was not well understood, one possible mechanism could be mitochondrial dysfunction and oxidative damage associated with age and related diseases (Cui *et al.*, 2012; Tschopp, 2011). In HepG2, a human HCC cell line, AEG-1 knockdown inhibited cell proliferation and induced apoptosis through the suppression of IL-6 secretion (Deng *et al.*, 2014), suggesting that AEG-1 may upregulate IL-6 levels during aging.

Studies showed increased expression of similar markers, including TNF- α and IL-6 in both young HIV+ and normal aged individuals, proposing that the nature of inflammation in aging correlates to the HIV-associated premature aging (Ferrucci *et al.*, 1999; Keating *et al.*, 2011). Various pathogens, including HIV-1 and hepatitis virus B and C, remain in the body for a long time; therefore, the immune system is chronically activated, resulting in immune dysregulation and age-related diseases. HIV-1 viral proteins, such as Tat, negative regulatory factor and gp120, trigger inflammation by regulating cytokine signaling (Holguin *et al.*, 2004; Olivetta *et al.*, 2003; Pu *et al.*, 2003). AEG-1, which is also induced by HIV-1, could exacerbate the infection by interacting with the HIV-1 Gag pro-protein to increase viral load (Engeland *et al.*, 2011).

ART causes metabolic disorders and plays an indirect role in inflammation. Though ART reduces systemic HIV-1-induced inflammation, the low-grade, ongoing, chronic inflammation through the disease span remains a significant contributing factor for accelerated aging. For example, non-nucleoside reverse transcriptase inhibitors, such as efavirenz, could inhibit adipocyte differentiation, leading to lipid accumulation and atherosclerosis (Diaz-Delfin *et al.*, 2011). Efavirenz also promotes IL-6 accumulation, a phenomenon also observed in elderly people (Diaz-Delfin *et al.*, 2011). In addition, the *Strategies for Management of ART* study showed patients on abacavir have elevated pro-inflammatory and coagulation biomarkers (Highleyman, 2010). All together, ART has significant effects on age-associated parameters. AEG-1 was identified as a chemoresistance molecule in cancers including HCC and neuroblastoma (Hu *et al.*, 2009a; Liu *et al.*, 2009; Yoo *et al.*, 2009b). AEG-1 facilitated association of the MDR1 mRNA to polysomes, resulting in increased translation and drugs efflux from cancer cells. Analysis of AEG-1

regulation of MDR1 expression or P-gp pump expression in endothelial cells of HAND patients, will therefore help to evaluate the BBB penetration capacities of ART, which remains a continued therapeutic challenge (Fig. 3). It is conceivable that increased AEG-1 expression during HIV-1 infection likely offers chemotherapeutic resistance to the HIV-1-infected cells against ART, thus playing a significant role in aggressive progression of HIV CNS infection.

4.4.2. Immunosenescence—Immunosenescence is the gradual impairment of the immune system brought on by aging, which reduces both the individuals ability to respond to infection and their long-term immune memory. This phenomenon is characterized in elderly people by elevated levels of terminally differentiated CD8+ cells, reduced T-cells proliferation, altered CD4+ and CD8+ T-cell homeostasis and heightened inflammatory markers (Czesnikiewicz-Guzik *et al.*, 2008). Moreover, alterations in T-cell characteristics, exhaustion of replicative capacity of CD8+ cells, aging of lymphocytes and dysregulated hematopoiesis were also reported in HIV-infected population (Appay *et al.*, 2002; Moses *et al.*, 1998). One study showed HIV-infected people with median age of 53 years had similar T-cell characteristics and higher immune activation as compared uninfected people with a median age of 88 years (Desai and Landay, 2010). Therefore, early immunosenescence in HIV+ individuals due to infection-mediated depletion and prolonged immune activation could lead to accelerated aging. Chronic cytomegalovirus co-infection associated with HIV-infected individuals could also contribute to immunosenescence and accelerated aging (Alonso Arias *et al.*, 2013; Hadrup *et al.*, 2006).

Immunosenescence not only affects adaptive but also influences innate immune responses (Solana *et al.*, 2012). As significant activators of innate immunity, toll-like receptors (TLR), recognize molecular patterns on microbes and induce antimicrobial host responses by triggering pro-inflammatory cytokines production (Kawai and Akira, 2007; Takeda and Akira, 2005). One study identified age-associated deficits in TLR1 and TLR3 on the surface of monocytes and macrophages (Kong *et al.*, 2008; van Duin *et al.*, 2007). Similarly, reduced TLR levels have been noted in macrophages and plasmacytoid dendritic cells of aged mice, indicating age-dependent alterations in TLR expression (Murciano *et al.*, 2008; Renshaw *et al.*, 2002; Stout-Delgado *et al.*, 2008). However paradoxical to other studies, another study demonstrated age-associated increases in CD56+ monocyte subpopulations, which are high producers of TNF- α via TLR2 and TLR4 (Nyugen *et al.*, 2010). As significant receptor molecules that link innate and adaptive immunity, it is important to study TLR expression and function in aging models. Recently, AEG-1 was suggested to be a lipopolysaccharide-TLR4-responsive gene triggering inflammatory responses (Khuda *et al.*, 2009). Consequently, future studies investigating the role of AEG-1 in innate immunity and TLR signaling will help elucidate its function in immunosenescence and aging (Fig. 3).

4.4.3. Oxidative Stress—Oxidative stress reflects an imbalance between free radicals levels, or ROS, and the availability of detoxifying antioxidants. The resultant oxidative damage to biomolecules, including lipids, proteins and DNA, impacts chronic ailments such as cancer, atherosclerosis, stroke, septic shock, aging and other degenerative diseases. Denham Harman's "free radical theory of aging" states that aging is caused by deleterious

effects resultant from the accumulation of free radicals (Harman, 1956). A hallmark of aging brain is increased oxidative stress. Moreover, it is interesting that gene expression profiling by oligonucleotide array showed altered inflammatory responsive and oxidative stress associated genes in the brains of aged mice (Lee *et al.*, 2000). Oxidative stress is often associated with HIV-1 infection. For example, HIV-1 proteins induce oxidative stress; viral Tat protein released from HIV-infected cells increase mitochondrial ROS production (Kruman *et al.*, 1998). Moreover, lower intracellular glutathione levels are observed in Tat transgenic mouse liver and erythrocytes through modulation synthesis of glutathione, an important antioxidant pathway (Choi *et al.*, 2000). HIV-1 viral protein R also dampens the astrocyte glycolytic pathway by impairing glyceraldehyde 3-phosphate dehydrogenase activity, leading to reduced intracellular ATP and glutathione concentrations, along with increased ROS and neurotoxicity (Ferrucci *et al.*, 2013). Likewise in Jurkat T cells, reactive oxygen intermediates, by activating NF- κ B, may indirectly activate the HIV-1 long terminal repeat promoter NF- κ B regulatory elements to enhance HIV-1 infection (Schreck *et al.*, 1991). Other findings suggest that NF- κ B acts as a sensor of oxidative stress and further contributes to mitochondrial ROS production (Mariappan *et al.*, 2010). Subsequently, AEG-1 interactions with NF- κ B could enhance its nuclear translocation and activate HIV promoter to enhance HIV infection and oxidative stress.

In addition to the direct consequences of HIV-1 inducing a cytotoxic extracellular environment, ART drugs may also exacerbate oxidative stress and cause neuronal damage (Akay *et al.*, 2014). In HIV-1 patients undergoing therapy, serum oxidant levels increased while antioxidant status decreased when compared to untreated and control groups (Mandas *et al.*, 2009). Moreover in HIV-1-infected adults, higher plasma F2-isoprotane concentrations, a reliable index of oxidative stress, were observed. Further, plasma F2-isoprotane concentrations were more likely to be elevated in patients on ART compared to untreated HIV+ subjects (Hulgan *et al.*, 2003). Nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors cause premature immunosenescence by accelerating oxidative stress (Smith *et al.*, 2012). Overall, oxidative damage caused by ROS impairs physiological function and can impact life span of HIV+ patients. Taken together, it can be inferred that oxidative stress resulting from HIV infection and ART toxicity possibly serves as a driving factor for premature aging in HIV-infected individuals.

The antioxidant responsive element (ARE) is an important transcriptional regulator during cellular adaptation to oxidative stress (Jaiswal, 2004). Genes regulated by ARE are involved in antioxidant enzymes production, glutathione synthesis and protein turnover (Ishii *et al.*, 2000; Kobayashi and Yamamoto, 2005; Kwak *et al.*, 2003; Prestera *et al.*, 1995). A master regulator of antioxidant cellular responses is the transcription factor Nrf-2, which is known to regulate the expression of numerous antioxidant enzymes. Under unstressed conditions, Nrf-2 is degraded by ubiquitination. Oxidative or electrophilic stress disrupts critical cysteine residues in the adaptor protein that facilitates Nrf-2 binding to its ubiquitinating partner, cullin 3, thereby preventing Nrf-2 degradation. Nrf-2, thus stabilized, translocates to the nucleus and regulates ARE promoter activity. AEG-1 interacts with various other proteins, including NF- κ B p65 subunit, CBP, Rrs1, β -catenin and ubinuclein-1, to elicit diverse functions (Table 1) (Carnemolla *et al.*, 2009; Lupo *et al.*, 2012; Sarkar *et al.*, 2008; Zhang *et al.*, 2013). Numerous *in vitro* studies in cancer cell lines have demonstrated AEG-1

induction in response to oxidative stress, hypoxia and glucose deprivation, and have identified important roles in modulating survival pathways (Baumann and Dorsey, 2011; Bhutia *et al.*, 2010; Noch *et al.*, 2011). We thus evaluated whether astrocyte AEG-1 levels, RNA and/or protein, changed in response to oxidative stress (Fig. 4). Much to our surprise, in cultured human astrocytes exposed to a concentration range of 0–400 μM H_2O_2 , we did not observe any significant changes in AEG-1 mRNA (Fig. 4A). However, we did observe greater amounts of AEG-1 in the nucleus while cytoplasmic levels of AEG-1 remained relatively stable (Fig. 4B). Most notably, our data revealed novel binding of astrocyte-AEG-1 to Nrf-2 in the nucleus, regardless of whether the cells were exposed to H_2O_2 , but not the cytoplasm, (Fig. 4C). While it remains to be seen whether AEG-1 participates in Nrf-2-related events, the fact that AEG-1 interacts with Nrf-2 suggests a plausible role in regulating oxidative stress-induced responses in astrocytes.

Heme oxygenase-1 (HO-1) is another candidate among the genes upregulated by AEG-1/MTDH in cancer cells (Hu *et al.*, 2009a) and is also regulated by Nrf-2, an AEG-1 interacting partner during antioxidant responses, as shown by our studies. Age-linked inflammatory markers, including TNF- α , IL-1 β , and A β , upregulated HO-1 expression (Serviddio *et al.*, 2011), which is essential for free iron and carbon monoxide degradation (Espada *et al.*, 2010). Pathological iron deposition and mitochondrial damage, which are well-known characteristics of age-related neurodegenerative diseases, were augmented by HO-1 (Serviddio *et al.*, 2011). AEG-1 is thus strongly implicated in oxidative stress and age-associated diseases. In addition, as a scaffold protein, AEG-1 binding to Nrf-2 perhaps influences further Nrf-2 post-translational modifications and activation, which is required for ARE-related adaptive responses (Kobayashi and Yamamoto, 2005). Further studies investigating the potential molecular mechanisms of post-translational modifications to AEG-1 that allow nucleolar translocation are therefore warranted.

In most cell types, AEG-1 is primarily detected in the cytoplasm or in the perinuclear regions *i.e.*, the nuclear envelope and/or ER (Thirkettle *et al.*, 2009a). Although, three nuclear localization sequences have been recognized in the AEG-1 protein (Thirkettle *et al.*, 2009a), AEG-1 nucleolar localization is reported in very few cell types (Sutherland *et al.*, 2004). AEG-1 translocation to the astrocyte nuclear pockets and colocalization with nucleolar protein fibrillarin, upon oxidative or physical stress such as injury is an important finding highlighting the yet unknown nucleolar function of AEG-1. The main function of the nucleolus is the transcription of ribosomal RNA and synthesis of small and large ribosome subunits (Cisterna and Biggiogera, 2010; Hernandez-Verdun *et al.*, 2010), a tightly regulated process to achieve proper cellular proliferation and cell growth. AEG-1 colocalization with fibrillarin, a dense fibrillar component of the nucleolus participating in ribosome synthesis (Ochs *et al.*, 1985), indicates that AEG-1 may play a role in pre-ribosomal RNA processing in response to oxidative stress, a hallmark of all neuroinflammatory and neurodegenerative disorders including biological aging. Disrupted nucleolar integrity and impaired ribosomal RNA synthesis were observed in aging and neurodegenerative diseases (Parlato and Kreiner, 2013). We recently investigated whether oxidative stress affected AEG-1 sub-cellular localization in human astrocytes. In Fig. 2, we showed AEG-1 nuclear translocation and binding to Nrf-2 in the nuclear compartment at 200 μM H_2O_2 exposure. Upon comparable H_2O_2 treatments, AEG-1 translocated to the astrocyte nucleus and further coalesced into

dense nuclear pockets (Fig. 5A–D). To distinguish these nuclear compartments, confocal analyses were performed using the nucleolar marker fibrillarin. AEG-1 and fibrillarin colocalization within the astrocyte nucleus following treatment with H₂O₂ confirms AEG-1 localization to the astrocyte nucleolus during oxidative stress (Fig. 5E–H). The nucleolus senses stress and is a central hub for coordinating stress responses, leading to changes in its organization and composition (Parlato and Kreiner, 2013). Hence, the present finding of oxidative stress-induced AEG-1 colocalization with fibrillarin, within human astrocyte nuclei, is vital for further comprehending cellular stress response pathways.

4.4.4. Mitochondrial Dysfunction—Oxidative damage affects mitochondrial DNA transcription and disrupts mitochondrial function that in turn enhances ROS production (Cui *et al.*, 2012). Higher rates of oxidative stress-induced DNA damage are seen in mitochondrial DNA as compared to nuclear DNA, suggesting that mitochondrial DNA is more susceptible to oxidative stress (Richter, 1992; Santos *et al.*, 2002). Mitochondria play a significant role in apoptosis and mitochondrial redox imbalance could enhance apoptotic cell death and aging. During oxidative stress, cellular p53 translocates into mitochondria and triggers apoptosis (Marchenko *et al.*, 2000); however, upon mild oxidative stress, p53 mitochondrial transport stabilizes DNA (Achanta *et al.*, 2005). In such, the outcomes of p53 regulation depend on the intensity and duration of oxidative stress. Interestingly, apart from its conventional pro-apoptotic function, p53 loss in cancer cells amplifies genetic instability and increases ROS (Achanta *et al.*, 2005), which are critical elements of aging process. AEG-1 overexpression suppressed apoptosis by regulating phosphorylation of Akt substrates glycogen synthase kinase 3 β , c-Myc, p53, and B-cell lymphoma-2 associated death promoter (Lee *et al.*, 2008). AEG-1 was also induced after DNA damage and implicated in p53 independent apoptotic pathway by activating NF- κ B (Gnosa *et al.*, 2012). Additionally, opening of mitochondrial transition pores, an indicator of cell death, is enhanced in tissues of aged mice (Mather and Rottenberg, 2000). In contrast, downregulation of AEG-1 increased caspase 3/7 activities and reduced mitochondrial potential disruption and cell survival (Nikpour *et al.*, 2014). More investigations are clearly needed to better understand the role of AEG-1 in regulating ROS-mediated mitochondrial dysfunctions that could supplement potential impact of AEG-1 in aging and related diseases.

Mitochondrial DNA damage and replication defects are induced by NRTIs (Kohler *et al.*, 2008; Kohler and Lewis, 2007). The principal tenet for this observation is that NRTIs affect DNA polymerase γ function, the only known mitochondrial DNA polymerase. Both, the HIV reverse transcriptase and the mitochondrial DNA polymerase γ are distinctively sensitive to NRTIs-guided aberrant DNA replication. A competition between the NRTI triphosphates with the physiological nucleoside triphosphates leads to aberrant mitochondrial DNA replication, loss of mitochondrial DNA, and introduces mutations, ultimately leading to, reduced cellular numbers of mitochondria. This culminates in overall cellular energy deprivation. In addition, NRTIs also compromise mitochondrial membrane integrity resulting in electron leakage, thus increasing the ROS load on the cell (Kohler *et al.*, 2008; Kohler and Lewis, 2007). Mitochondrial DNA mutations increase with age and aberrant DNA replication causes premature aging *via* multiple mechanisms including DNA polymerase γ malfunction, improper synthesis of key proteins required for oxidative

phosphorylation, energy deprivation and electron leakage across mitochondrial membrane that all together manifest as oxidative stress (Bratic and Larsson, 2013; Cui *et al.*, 2012). Overall, increased mitochondrial oxidative stress is an important contributing factor for aging, particularly in the setting of ART, as despite the long-term implications, NRTIs continue to be mainstream treatments for HIV patients in the absence of effective alternatives. ART therapy weakens mitochondrial integrity, induces oxidative stress and inflammation, which are directly related to aging. Delineation of AEG-1 contributions to mitochondrial damage in context to HAND and ART could further expand the role of mitochondria and oxidative stress in normal or premature aging in HIV+ individuals.

4.4.5. ER Stress—Aging and age-related neurodegenerative diseases are commonly accompanied by accumulation of disease-specific misfolded proteins in the CNS, supporting a relationship between age and protein misfolding. Classical examples include A β peptides and tau proteins in AD (Glenner and Wong, 1984; Kosik *et al.*, 1986), α -synuclein in Parkinson's disease (Spillantini *et al.*, 1998), huntingtin in HD (Bates, 2003) and superoxide dismutase in amyotrophic lateral sclerosis (Chia *et al.*, 2010). The link between aging and protein misfolding is well-accepted; for instance, quality control of protein synthesis wanes with age. Under stress, the ER initiates adaptive mechanisms termed the “unfolded protein response (UPR)” to limit accumulation of misfolded proteins. However, prolonged or sustained ER stress leads to apoptosis and inflammatory response.

Aging causes impairment of UPR-related components and enzymes *i.e.*, binding immunoglobulin protein (BiP), protein disulfide isomerase, calnexin and glucose-regulated protein 94. BiP, a chaperon in the ER lumen serves as a sensor for protein quality control challenges and thus changes in BiP levels are significant for activation of UPR pathway and quality control of proteins. However, several studies suggest that BiP expression levels are reduced with age. Both BiP mRNA and protein levels are reduced in hippocampus of old (23–26 month old) compared to young (4–6 month old) Wistar rats (Paz Gavilan *et al.*, 2006). BiP protein levels are also reduced in cerebral cortex of aged C57/B6 mice (Naidoo *et al.*, 2008). In addition to BiP, other components of the UPR, including protein kinase RNA-like ER kinase, protein disulfide isomerase, and calnexin are significantly reduced in aged rat hippocampus (Paz Gavilan *et al.*, 2006). During aging, there is a shift from UPR protective response *i.e.*, proper protein folding to pro-apoptotic signaling. Prolonged ER stress induces the expression of pro-apoptotic factor CCAAT/enhancer binding protein homologous protein (CHOP). Studies show elevated CHOP levels in aged mouse cortex and in rat hippocampus and cortex (Hussain and Ramaiah, 2007; Naidoo *et al.*, 2008; Paz Gavilan *et al.*, 2006). Furthermore, aging causes oxidation of ER chaperones and reduces protein quality control. Many studies suggest age-associated oxidation or oxidative damage of ER resident proteins, including BiP, protein disulfide isomerase, calnexin and calreticulin, impairing their function (Naidoo, 2009; Nuss *et al.*, 2008; van der Vlies *et al.*, 2002). In response to stress from the accumulation of unfolded proteins, the ER transduces signals to downregulate protein translation. We thus propose that AEG-1 may orchestrate ER functions locally in the ER and at the site of ribosome biosynthesis, in the nucleolus (Fig. 3).

HIV-1 infection and antiretroviral drugs also induce ER stress response. During infection, increase in viral load and proteins activate UPR. For instance, HIV-1 Tat upregulates X-box

binding protein that could further activate UPR pathway (Norman *et al.*, 2008). However, certain downstream mechanisms are not beneficial for viral infection *e.g.*, ER-associated degradation. Quality control mechanism *via* the ER-associated degradation pathway induces proteosomal degradation of gp160, an essential precursor for productive viral replication (Casini *et al.*, 2015). Depending on its localization, AEG-1 can serve as an ER and nucleolar stress sensor. AEG-1 also colocalized with Rrs1, a protein involved in ribosome maturation, nuclear export and assembly. Colocalization was observed in both nucleolus and ER of immortalized striatal cells in a model of HD disease (Carnemolla *et al.*, 2009). Surprisingly, both Rrs1 and AEG-1 have ER localization sequences and are targets of ER stress (Carnemolla *et al.*, 2009; Sutherland *et al.*, 2004). Recent studies identified induction of ER stress markers, including BiP, CHOP, and homocysteine-inducible, ER stress-inducible, ubiquitin-like domain member 1, along with Rrs1 and AEG-1, in human HD postmortem brains. HD is marked by the elevation of misfolded proteins and ER stress. During UPR, ER transmits signals to the nucleolus to inhibit protein synthesis, so based on its localization sequence and close proximity to ER and nucleolus, AEG-1 may assist as an ER stress sensor. Moreover, aging and other age-related neurodegenerative diseases are also associated with ER stress (Brown and Naidoo, 2012), implicating that AEG-1 may serve as a key molecule in aging process (Fig. 2).

The ER stress response can also induce autophagy and increase cell survival (Ogata *et al.*, 2006; Yorimitsu *et al.*, 2006). Macroautophagy, the most common pathway, generally termed autophagy, is a process wherein cellular cytoplasmic components are encapsulated in double membrane vesicles called autophagosomes and then are delivered to, and degraded in, the lysosomes. While bulk autophagy is primarily induced upon starvation, physiological baseline levels of autophagy are important for cellular health. Unlike the ubiquitin-proteasome pathway, which is responsible for specific degradation of proteins, autophagy is the less selective counterpart of the cellular clearing house. Since autophagy helps in the elimination of aggregated proteins and defends cells from further damage, it serves a protective function, in addition to the main purpose of autophagy, to deliver amino acids and other nutrients to the cell under starvation stress. *In vivo* studies in HD transgenic mice demonstrated that sequestration of mutant huntingtin into inclusions induces autophagy and clears mutant huntingtin fragments (Ravikumar *et al.*, 2004). Perturbations in autophagy process *via* suppression of autophagy-related gene 5 lead to neurodegeneration in mice even in the absence of any mutant proteins or gain-of-function neurotoxicity (Hara *et al.*, 2006; Komatsu *et al.*, 2006), thus confirming an important role for homeostatic baseline autophagy as a prerequisite for neural health. Recent evidence in *Drosophila* indicates age-related decrease in autophagy-related gene protein expression and suggests autophagy declines with aging (Simonsen *et al.*, 2008). Moreover, aged human subjects show reduced levels of Beclin 1, a protein essential for autophagy in HD (Shibata *et al.*, 2006). Beclin 1 regulates initiation and formation of the autophagosome by providing a molecular interactome, *i.e.* a molecular scaffold to facilitate interactions among suppressing and activating partners (Salminen *et al.*, 2013). Beclin 1 levels are also reduced in AD brains. Beclin 1 is a multifunctional interactome critical to autophagy and also known to participate in phagocytosis, endocytosis and apoptosis (Salminen *et al.*, 2013). Interestingly, AEG-1 was recognized as a potential regulator of autophagy, by protecting cells from serum-starvation-

induced cell death (Bhutia *et al.*, 2010). All together, the significance of autophagy in aging and neurodegeneration is increasingly apparent (Fig. 3), and elucidation of AEG-1-regulated autophagy mechanisms will assist in targeting AEG-1 to alleviate chronic neurological disorders.

4.4.6. Comorbidities—Increasing the life expectancy of HIV+ individuals has resulted in an augmentation of comorbidities compared to the uninfected age-matched population. Oxidative stress, chronic inflammation, severe mitochondrial toxicity serve as critical mechanisms for the development of comorbidities and premature aging. Increasingly, deaths in HIV+ patients receiving ART are due to non-infectious comorbidities (Phillips *et al.*, 2008). These include several age-related diseases such as cardiovascular diseases, diabetes mellitus, stroke, hypertension and renal failure. One study found that risk of death during the non-AIDS condition is higher compared to that during AIDS conditions of HIV infected persons with CD4 count above 200/mm³ (Lau *et al.*, 2007). However, data from another large case-control study confirmed that non-infectious comorbidities are more frequently observed in HIV-infected people than general population (Guaraldi *et al.*, 2011).

5. AEG-1 as a therapeutic target

Identification of the cellular functions of AEG-1 in human astrocytes, and implications for its role(s) in CNS diseases including injury, inflammation, stroke and cancer, have warranted further studies on AEG-1 as a therapeutic target. In astrocytes, AEG-1 regulates migration, proliferation, chemokine production and glutamate clearance following its induction *via* the NF- κ B pathway in response to neuroinflammation and in the setting of aging. AEG-1, in turn modulates astrocyte inflammatory responses by regulating NF- κ B signal transduction, and neurotoxic responses by regulating EAAT2 expression. Taken together, AEG-1 plays a central role in regulating astrocyte function in neural health and perturbations in CNS injuries, neuroAIDS, neurodegeneration, aging and cancer. At a molecular level, transcriptional co-activator/scaffolding function of AEG-1 proposes a novel stress-responsive function in astrocytes, thereby recommending AEG-1 as a potential candidate for therapeutic targeting for numerous CNS diseases.

Astrocytes are heavily directed towards the progression of neuroinflammatory diseases, particularly A β toxicity in AD, α -synuclein immunoreactivity in Parkinson's disease and amyotrophic lateral sclerosis (Guillamon-Vivancos *et al.*, 2015). The extensive capabilities of astrocytes to drive neuroinflammation have singled glia out as therapeutic targets for neuroinflammatory disorders. For example, genetic modification of astrocytes to express brain derived neurotrophic factor revealed enhanced neuroprotective effects in mouse model of HD (Giralt *et al.*, 2010). Similarly, pharmacological targeting of inflammatory processes in astrocytes using MW01-5-188WH and ONO-2506, selective inhibitors of glial inflammation, exhibited promising neuroprotective outcomes in experimental models of AD and Parkinson's disease (Himeda *et al.*, 2006; Mori *et al.*, 2006; Ralay Ranaivo *et al.*, 2006). Subsequent to the molecular dissection of reactive astrogliosis several novel therapeutic strategies are under development. Parawixin 1, a molecule capable of augmenting the function of astrocyte glutamate transporter EAAT2, protected retinal neurons from ischemic degeneration (Fontana *et al.*, 2007; Fontana *et al.*, 2003). Beta-lactam antibiotics boosted

glutamate uptake by astrocytes, enhancing neuroprotection in models of stroke and amyotrophic lateral sclerosis (Leung *et al.*, 2012). Owing to the fact that AEG-1 intracellular localization is an important determinant of its functional outcome, future studies should be directed towards understanding the nucleolar functions of AEG-1, especially in the context of oxidative stress and aging. It is vital to identify AEG-1 binding partners in the nucleolus and to understand whether AEG-1 participates in the complex processes of ribosome assembly, transfer and ribosomal RNA synthesis. Taking into account the key roles attributed to AEG-1 in cancers, it would also be worthwhile to investigate how AEG-1 regulates astrocyte migration. As a downstream effector of neuroinflammation and as an important regulator of NF- κ B signaling in astrocytes, AEG-1 participation in non-canonical NF- κ B signaling pathways and death receptor activation for cellular apoptosis remains to be investigated. Our studies documented AEG-1 as a major regulator of EAAT2 in astrocytes and investigations by other groups implicate AEG-1 in glioma-associated necrosis; therefore, it would be beneficial to understand AEG-1's role in neurodegeneration associated with aging, a central morbidity factor in the context of HAND.

6.0. Perspectives and Concluding Remarks

In this review, we have brought together multifactorial neurodegenerative processes involved in aging and HAND, and evaluated common mechanistic link(s), centered on the multifaceted protein AEG-1. This review not only brings together diverse pieces of data from the literature, it encompasses our novel findings about AEG-1 regulation in the setting of oxidative stress, a common link in multiple CNS pathologies. Fig. 6 provides an overview of the processes through which AEG-1 likely regulates neurodegeneration. On-going, low-level inflammation coupled with HAND-relevant stimuli and aging induce AEG-1 expression in human astrocytes. In addition, IL-1 β and TNF- α , and ROS promote AEG-1 nuclear translocation. **(A)** Upon induction, AEG-1 upregulates the expression of YY1, an EAAT2 repressor in astrocytes. In glioma cells, increased AEG-1 interaction with YY1 leads to EAAT2 transcriptional repression. Thus, AEG-1-mediated EAAT2 downregulation would in turn lead to reduced glutamate uptake by astrocytes. Subsequent high levels of glutamate in the synaptic cleft will ultimately enhance neuronal excitotoxicity. Both HIV-1 infection and aging are associated with increased extracellular glutamate levels; thus, it is significant to focus on the role of AEG-1 in glutamate-associated neurodegeneration. **(B)** Our recent investigations implicated AEG-1 interactions with Nrf-2, a master regulator of antioxidant gene expression in astrocytes. Although Nrf-2 mainly regulates cellular antioxidant responses, one study also demonstrated augmented HO-1, a downstream target of Nrf-2, in aging astrocytes that contributed to pathological iron deposition and mitochondrial damage. Accordingly, further studies are warranted to understand AEG-1-mediated Nrf-2 regulation in aging and neurodegeneration. **(C)** The NF- κ B signaling pathway is a key pathological mediator in HAND and aging. In astrocytes, AEG-1 induces NF- κ B pathway activation by elevating the expression of upstream regulatory kinase IKK β and NF- κ B p65 subunit nuclear translocation. AEG-1 induction with inflammatory stimuli and activation of NF- κ B pathway may exacerbate both inflammation and ROS production, resulting in neurodegeneration. **(D, E)** We have shown that oxidative stress and injury induced AEG-1 nucleolar translocation in astrocytes. Due to various localization sequences, increased

AEG-1 may act as sensor, regulating UPR signals between the ER and nucleolus. Aging and neurodegenerative diseases are significantly marked by ER/nucleolar stress. In an HD model, AEG-1 colocalized with Rrs1 in the ER and nucleolus, suggesting a role in regulating ribosome synthesis. In addition to AEG-1 upregulation in HD, other ER stress markers such as BiP were also augmented, indicating AEG-1 role in ER stress responses in aging and HAND. **(F)** Furthermore, AEG-1 was found to be upregulated in vascular endothelial cells of neuroblastoma patients, implicating AEG-1 in regulation of BBB permeability. Investigating AEG-1 expression and regulation of drug import/export across BBB endothelial cells of HIV-1 patients could enhance ART penetration into the brain and ultimately, better control of HIV replication in the CNS.

In this review, we not only provide in-depth knowledge regarding the role of AEG-1 in a diverse spectrum of diseases, from cancer to HIV-1 and aging; but more importantly, we highlight mechanistic processes that synergize with known AEG-1 functions. Our work promotes the notion that AEG-1 occupies a unique niche in inflammation, mitochondrial, ER and nucleolar stress, culminating in excitotoxicity and other neurodegenerative outcomes. Furthermore, we have highlighted key unanswered questions and identified several new lines of investigation that will provide the necessary clues to solve them. Considering the detrimental effects of astrocyte AEG-1 in the context of aging and neurodegenerative diseases including HAND, we propose that this gene could serve as a valuable broad-spectrum therapeutic target to protect neurons from inflammation, toxicity and death.

Supplementary Material

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Abbreviations

Aβ	amyloid beta
AEG-1	astrocyte elevated gene-1
AD	Alzheimer's disease
AIDS	acquired immune deficiency syndrome
ARE	antioxidant responsive element
ART	antiretroviral therapy
BBB	blood brain barrier

BCCIP	breast cancer 2, early onset and cyclin-dependent kinase inhibitor 1A interacting protein
BiP	binding immunoglobulin protein
CBP	cAMP response element-binding protein-binding protein
CD	cluster of differentiation
CHOP	CCAAT/enhancer binding protein homologous protein
EAAT	excitatory amino acid transporter
ER	endoplasmic reticulum
exNLS	extended nuclear localization signal
Gag	group-specific antigen
GFAP	glial fibrillary acidic protein
gp	glycoprotein
H₂O₂	hydrogen peroxide
HCC	hepatocellular carcinoma
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorders
HIV-1	human immunodeficiency virus-1
HIVE	HIV encephalitis
HD	Huntington's disease
HO-1	heme oxygenase-1
IKKβ	I κ B kinase β
IL	interleukin
LYRIC	lysine-rich carcinoembryonic antigen-related cell adhesion molecule 1 co-isolated
MDR1	multi-drug resistance gene 1
MTDH	metadherin
NF-κB	nuclear factor- κ B
NLS	nuclear localization signal
Nrf-2	nuclear factor erythroid-2 related factor-2
NRTI	nucleoside reverse transcriptase inhibitors

PLZF	promyelocytic leukemia zinc finger protein
ROS	reactive oxygen species
Rrs1	regulator of ribosome synthesis 1
SND1	staphylococcal nuclease and tudor domain containing 1
STAT3	signal transducer and activator of transcription 3
Tat	trans-activator of transcription
TLR	toll-like receptors
TMD	transmembrane domain
TNF	tumor necrosis factor
Ubn-1	ubiquitin-1
UPR	unfolded protein response
YY1	yin yang protein 1

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Highlights

- Elevated AEG-1 levels are associated with aging and HAND in human brain.
- Astrocytes elevate AEG-1 in response to neuroinflammation and are critical players in HAND and the aging brain.
- H₂O₂ induces nucleolar translocation of AEG-1 in astrocytes and AEG-1 binds to Nrf-2, a master regulator of oxidative stress.
- AEG-1 may regulate neuroinflammation, oxidative stress, reactive astrogliosis, mitochondrial damage and ER/nucleolar stress.
- Future therapeutic approaches may pursue AEG-1 as a novel target for the treatment of HAND and age-related disorders.

Figure 1. AEG-1 sub-cellular localization and protein interaction domains

AEG-1, together with other proteins, exerts diverse functional outcomes. The open reading frame for AEG-1 spans from 220 to 1968 base pairs, and encodes for a 582 amino acid protein with a predicted molecular weight of 64 kDa. This diagram highlights select AEG-1 binding domains and their respective functions. An extended list is presented in Table 1. In addition to the known interactions, specific elements that are responsible for sub-cellular localization of AEG-1 are also shown. AEG-1 has multiple nuclear localization sequences (NLS) with embedded extended segments (exNLS). However, AEG-1 regions that bind β-catenin, ubinuclein-1 (Ubn-1), and regulator of ribosome synthesis (Rrs)1 are not yet identified. Nuclear factor erythroid-2 related factor-2 (Nrf-2) binding site on AEG-1 is also unknown. In this study, we present novel data to illustrate AEG-1 interacts with Nrf-2, a transcription factor regulating antioxidant gene expression. Future investigations for mapping specific AEG-1 binding regions for Nrf-2, other proteins, and related functional changes will facilitate better understanding of its pleiotropic role in health and disease.

Figure 2. Brain AEG-1 levels correlate with age in humans

AEG-1 protein levels in the prefrontal cortex/frontal white matter regions of the brain tissue specimens obtained from 22 (21–90 years old) HIV-1 seronegative individuals were analyzed by immunoblotting (**A**). Total AEG-1 protein levels significantly correlated with age (**B**, $R^2=0.36$, $P=0.0031$). A larger cohort of HIV-1 seronegative samples from individuals aged <50 ($n=37$) compared to those aged >50 years ($n=25$), also demonstrated significant upregulation in AEG-1 mRNA with increased age (**C**, $***P<0.001$). Statistical analyses were performed using Prism 6.0 (GraphPad Software, La Jolla, CA) with Pearson's correlation coefficient analysis and student's t-test. Significance was set at $p<0.05$ and data represent means $\pm$ standard error of the mean (SEM).

Figure 3. AEG-1 links physiological aging mechanisms to premature aging in HIV patients
 Several factors (tan boxes) contribute to the healthy aging process and can induce premature aging during HIV-1 CNS infection (clear arrows). Antiretroviral therapy (patterned arrow), also cause accelerated aging in HIV+ population. We propose that AEG-1 mediates many aging-related processes through its diverse regulatory mechanisms (hatched, purple arrows) both in physiological aging and HIV-associated premature aging.

Figure 4. AEG-1 translocates to the nucleus following H₂O₂ exposure and interacts with transcription factor Nrf-2 in human astrocytes

Astrocytes were treated with increasing concentrations (50–400 μM) of H₂O₂ for 8 h and AEG-1 mRNA levels were analyzed by real-time RT-PCR. AEG-1 mRNA levels did not change significantly upon H₂O₂ treatment (A). Astrocytes were treated with different doses of H₂O₂ and cytoplasmic and nuclear extracts were isolated at 24 h. H₂O₂ induced AEG-1 nuclear translocation even at 50 μM; the lowest H₂O₂ concentration tested (B). Cytoplasmic GAPDH and nuclear lamin A/C were used as subcellular fraction normalizing controls. Co-

immunoprecipitation analyses were performed using anti-AEG-1 and mouse IgG antibodies, followed by immunoblotting for AEG-1 and Nrf-2 (C). These novel results clearly establish Nrf-2 as an AEG-1 binding partner in human astrocytes. Future investigations may reveal, if and how, AEG-1 contributes to the management of oxidative stress, a key pathway implicated in aging.

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Figure 5. Oxidative stress induces AEG-1 nucleolar translocation

Astrocytes treated with 0 μM (A), 100 μM (B), and 200 μM H₂O₂ (C–D), were fixed and immunostained for GFAP (astrocyte marker, red) and AEG-1 (green). AEG-1 localized mainly to the cytoplasm under control conditions (A) whereas upon H₂O₂ treatment, AEG-1 translocated to the nucleus and localized to nuclear pockets at both 100 μM (B) and 200 μM H₂O₂ (C). DAPI was used to visualize nuclei and immunostaining without and with DAPI is shown (C–D, respectively). Nucleolar localization was confirmed using astrocytes exposed to 200 μM H₂O₂ (E–H). Confocal micrographs depict fibrillarin (E, nucleolar marker; red), AEG-1 (F, green), DAPI (G, blue) and merged images (H, fibrillarin, AEG-1 and DAPI), confirming that AEG-1-positive nuclear pockets (B–D) are indeed nucleoli. Representative confocal images from two individual donors are shown. Original magnification 200×

Figure 6. Collective perspectives on AEG-1 regulated pathways in HAND and aging

Increased AEG-1 levels and/or its nuclear-nucleolar translocation occur in astrocytes during inflammation, oxidative stress and injury, which contribute towards neurodegeneration in HAND and aging. AEG-1 exerts a multifactorial impact on these outcomes, through mechanisms including but not limited to: EAAT2 dysregulation leading to glutamate-induced excitotoxicity (A), Nrf-2 mediated oxidative stress responses (B), enhanced neuroinflammation through NF- κ B (C), ER/nucleolar stress following altered sub-cellular localization (D–E, respectively), and increased antiretroviral drugs efflux at the BBB (F). AEG-1 thus bears the prospects of being an attractive therapeutic target for the not so distant future.

Table 1**AEG-1 binding partners in cancer**

AEG-1 Interacting Protein/s	AEG-1 Role	Implications	Cell/Cancer type	References
Akt2	Cell survival and proliferation	Glioma progression	U87 cells	(Ash <i>et al.</i> , 2008)
BCCIP ¹	Negative regulator of BCCIP α	Promote proteosomal degradation of BCCIP α , cell cycle regulator	293 T cells	(Zhang <i>et al.</i> , 2013b)
CBP ² & p65	Induce CXCL8 transcription & NF- κ B dependent gene expression	Invasion and migration of glioma cells	Malignant glioma	(Sarkar <i>et al.</i> , 2008)
CBP & yin yang (YY)1	EAAT2 ³ transcriptional repression	Reduced glutamate uptake by glial cells or glioma-induced neurodegeneration	Glioma	(Lee <i>et al.</i> , 2011)
HIV-1 group specific-antigen (Gag)	Increased Gag expression levels & AEG-1/LYRIC incorporation into HIV-1 virions	Regulation of HIV-1 viral infection	293 T cells	(Engeland <i>et al.</i> , 2011)
Nuclear factor (NF)- κ B p65 subunit	NF- κ B nuclear translocation & gene expression	Anchorage independent growth and invasion (cancer progression)	HeLa cells	(Emdad <i>et al.</i> , 2006)
		HAND ⁴ -induced inflammatory responses	Primary human astrocytes	(Vartak-Sharma <i>et al.</i> , 2014)
PLZF ⁵	Suppression of PLZF binding to c-Myc promoter	Supports tumorigenesis	HeLa cells	(Thirkettle <i>et al.</i> , 2009b)
Rrs1 ⁶	ER stress response marker	Regulation of ribosome biosynthesis and ER stress	Neurons	Carnemolla <i>et al.</i> , 2009)
SND1 ⁷	Facilitate RISC ⁸ activity (component of RISC)	Hepatocellular carcinogenesis	HCC cells, HepG3 cells	(Yoo <i>et al.</i> , 2011)
Ubinuclein (Ubn)-1	Scaffold epithelial cell junction protein	Potential role in tumorigenesis	Epithelial cells (HT29 cells)	(Yoo <i>et al.</i> , 2011)

¹ breast cancer 2, early onset and cyclin-dependent kinase inhibitor 1A interacting protein,

² cAMP response element-binding protein-binding protein,

³ excitatory amino acid transporter 2,

⁴ HIV-associated neurocognitive disorders,

⁵ promyelocytic leukemia zinc finger protein,

⁶ regulator of ribosome synthesis 1,

⁷ staphylococcal nuclease and tudor domain containing 1,

⁸ RNA-induced silencing complex.

Table 2

Seminal roles of AEG-1 in diverse cancers

AEG-1-regulated phenomena	Cancer Type	References
Angiogenesis	Hepatocellular carcinoma	(Srivastava <i>et al.</i> , 2012; Yoo <i>et al.</i> , 2009a)
	Triple negative breast cancer	(Li <i>et al.</i> , 2011a)
	Cervical carcinoma	(Z <i>et al.</i> , 2013a)
	Aggressive tumors in nude mice	(Emdad <i>et al.</i> , 2009)
Autophagy	Cervical cancer	(Zhang <i>et al.</i> , 2013c)
	Immortalized human astrocytes	(Bhutia <i>et al.</i> , 2010)
Chemoresistance	Breast cancer	(Hu <i>et al.</i> , 2009a; Qian <i>et al.</i> , 2011)
	Neuroblastoma	(Liu <i>et al.</i> , 2009)
	Osteosarcoma	(Liu <i>et al.</i> , 2013a)
Enhanced proliferation	Acute myeloid lymphoma	(Long <i>et al.</i> , 2013b)
	Breast cancer	(Li <i>et al.</i> , 2009; Zhang <i>et al.</i> , 2011)
	Hepatocellular carcinoma	(Yoo <i>et al.</i> , 2009a)
	Esophageal squamous cell carcinoma	(Yu <i>et al.</i> , 2009)
	Neuroblastoma	(Lee <i>et al.</i> , 2009; Liu <i>et al.</i> , 2009)
	Prostate cancer	(Kikuno <i>et al.</i> , 2007)
	Gastric carcinoma	(Zhang <i>et al.</i> , 2013a)
Epithelial-mesenchymal transition	Renal tubulointerstitial fibrosis	(Wei <i>et al.</i> , 2013)
	Hepatocellular carcinoma	(Zhu <i>et al.</i> , 2011)
	Non-small cell lung cancer	(Liu <i>et al.</i> , 2013b)
	Osteosarcoma	(Tang <i>et al.</i> , 2014)
Inhibition of apoptosis	Non-small cell lung cancer	(Ke <i>et al.</i> , 2013b)
	Prostate cancer	(Kikuno <i>et al.</i> , 2007)
	Gastric cancer	(Jian-bo <i>et al.</i> , 2011)
Metastasis	Breast cancer	(Brown and Ruoslahti, 2004; Hu <i>et al.</i> , 2009a; Zhang <i>et al.</i> , 2014)
	Colorectal cancer	(Jiang <i>et al.</i> , 2012)
	Epithelial ovarian cancer	(Li <i>et al.</i> , 2011b)
Migration and invasion	Breast cancer	(Zhao <i>et al.</i> , 2011)
	Colorectal carcinoma	(Zhang <i>et al.</i> , 2013b)
	Glioma	(Sarkar <i>et al.</i> , 2008)
Orientation chemotaxis	Hepatocellular carcinoma	(Zhou <i>et al.</i> , 2012b)
Radioresistance	Cervical cancer	(Zhao <i>et al.</i> , 2012)

Table 3

Clinical observations of elevated AEG-1 levels in cancers

Cancer Type	References
Breast cancer	(Hu <i>et al.</i> , 2009a; Li <i>et al.</i> , 2011a; Li <i>et al.</i> , 2008; Qian <i>et al.</i> , 2011)
Cardiac myxoma	(Fang <i>et al.</i> , 2015)
Cervical carcinoma	(Long <i>et al.</i> , 2013a)
Colorectal carcinoma	(Gnosa <i>et al.</i> , 2012)
Diffuse large B-cell lymphoma	(Ge <i>et al.</i> , 2012)
Esophagus squamous cell carcinoma	(Song <i>et al.</i> , 2009; Yu <i>et al.</i> , 2009)
Gall bladder cancer	(Sun <i>et al.</i> , 2011)
Gastric cancer	(Jian-bo <i>et al.</i> , 2011)
Glioblastoma multiforme	(Liu <i>et al.</i> , 2010)
Hepatocellular carcinoma	(Ahn <i>et al.</i> , 2013; Sarkar, 2013; Srivastava <i>et al.</i> , 2015a; Yoo <i>et al.</i> , 2009a)
Laryngeal squamous cell carcinoma	(Liu <i>et al.</i> , 2013d)
Neuroblastoma	(Lee <i>et al.</i> , 2009; Liu <i>et al.</i> , 2012)
Non-small cell lung cancer	(Ke <i>et al.</i> , 2013b)
Osteosarcoma	(Wang <i>et al.</i> , 2011)
Oligodendroglioma	(Xia <i>et al.</i> , 2010)
Ovarian cancer	(Meng <i>et al.</i> , 2011; Yuan <i>et al.</i> , 2012)
Prostate cancer	(Erdem <i>et al.</i> , 2013b; Thirkettle <i>et al.</i> , 2009a; Zhang <i>et al.</i> , 2015a)
Renal cell carcinoma	(Chen <i>et al.</i> , 2010; Erdem <i>et al.</i> , 2013a)
Salivary gland carcinoma	(Liao <i>et al.</i> , 2011)
Tongue carcinoma	(Ke <i>et al.</i> , 2013a)
Breast, colorectal, lung, liver and gastric cancers ¹	(Chen <i>et al.</i> , 2012)

¹ Elevated serum AEG-1 antibody levels in multiple cancers

Table 4

AEG-1 serves multiple roles in CNS cancers and other disorders

CNS Cancer Type	AEG-1 Role	References
Malignant glioma	Tumor cell proliferation and survival	(Hu <i>et al.</i> , 2014)
	Invasion of glioma cells	(Emdad <i>et al.</i> , 2010; Liu <i>et al.</i> , 2010)
	Migration and invasion	(Sarkar <i>et al.</i> , 2008)
	Angiogenesis	(Emdad <i>et al.</i> , 2010)
	Glutamate-induced neurodegeneration	(Lee <i>et al.</i> , 2011)
	Protective autophagy	(Bhutia <i>et al.</i> , 2010)
	Chemoresistance	(Bhutia <i>et al.</i> , 2010)
	Radioresistance	(Guo <i>et al.</i> , 2014)
Neuroblastoma	Chemoresistance to cisplatin	(Liu <i>et al.</i> , 2009)
	Cancer cell proliferation	(Lee <i>et al.</i> , 2009)
Oligodendroglioma	Unknown	(Xia <i>et al.</i> , 2010)
Astrocytoma	Unknown	(He <i>et al.</i> , 2014)
Other CNS related disorders		
Migraine	Possible glutamate signaling dysregulation	(Anttila <i>et al.</i> , 2011)
Huntington's disease	ER stress sensor	(Carnemolla <i>et al.</i> , 2009)
HAND ¹	Neuroinflammation	(Vartak-Sharma <i>et al.</i> , 2014)
	EAAT2 ² downregulation	(Vartak-Sharma <i>et al.</i> , 2014)

¹ HIV-associated neurocognitive disorders,² excitatory amino acid transporter 2