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Protease resistant protein cellular isoform (PrP^c) as a biomarker: Clues into the pathogenesis of HAND

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Abstract

HIV infection and HIV neurocognitive impairment are major global health problems. The prevalence of HIV associated neurocognitive disorders (HAND) is increasing as people with HIV are living longer due to the success of antiretroviral therapies. Our laboratory identified PrP^c, the cellular non-pathogenic isoform of the prion protein, as a biomarker of HAND. In this review we discuss the published data addressing PrP^c biology in normal conditions and pathologies, as well as the mechanisms of PrP^c shedding and secretion. Lastly, we discuss our studies that demonstrated that PrP^c is a biomarker of neurocognitive impairment in the HIV infected population.

Introduction

Human immunodeficiency virus-1, HIV, affects approximately 34 million people worldwide and continues to be a global health crisis (UNAIDS: Report on the Global AIDS Epidemic 2011, Geneva, World Health Organization). Despite successful antiretroviral therapy (ART), it is estimated that greater than 50 % of HIV infected people have HIV associated neurocognitive disorders (HAND) (Antinori et al., 2007; Heaton et al., 2010; Heaton et al., 2011). HAND is becoming more prevalent, with milder types of impairments being most frequent, as HIV infected people are living longer with the use of antiretrovirals (Nath et al., 2008; Cysique et al., 2009).

HIV enters the central nervous system (CNS) early during primary infection, predominantly transmitted by HIV-infected monocytes that constitutively cross the blood-brain barrier (BBB) (Koenig et al., 1986; Valcour et al., 2011). The transmigration of HIV-infected $CD14^+$ $CD16^+$ monocytes introduces virus into the CNS and causes the release of neuroinflammatory mediators including the chemokine CCL2/monocyte chemoattractant protein-1. CCL2, which is elevated in the CNS and cerebrospinal fluid (CSF) of individuals with HAND (Conant et al., 1998) and is an important mediator of HIV-associated CNS inflammation and neuropathology, induces transmigration of additional monocytes across the BBB (Williams et al., 2012b). HIV infected monocytes that cross the BBB differentiate

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into perivascular macrophages and become viral reservoirs, infecting resident macrophages, microglia, and to lesser extent astrocytes (Eugenin and Berman, 2003; Buckner et al., 2006; Eugenin et al., 2006a; Williams et al., 2012a). Infected and activated phagocytes and glia produce additional inflammatory and cytotoxic factors that cause BBB dysfunction and neuronal injury (Letendre, 2011).

HIV-associated neurocognitive impairment impacts the functional capacity and quality of life of an individual (Albert et al., 1995; Heaton et al., 2010; Scott et al., 2011). Predicting which individuals will develop HAND and identifying those with subtle impairment is difficult and presents a therapeutic challenge. Neurocognitive testing may not accurately reflect the dynamic nature of the disorder, as individuals with HAND frequently have fluctuating neurocognitive deficits. Furthermore, education, cultural background, local norms, and other environmental factors may affect the results of neurocognitive testing (Ances and Clifford, 2008). Thus, identification of a biomarker that is specific for HIV-associated CNS disease will facilitate the identification of individuals at risk of developing neurocognitive impairment. Biomarkers may also guide therapy and provide direction for the initiation of ART.

Our laboratory demonstrated that the soluble form of the cellular prion protein ($sPrP^C$) is a potential biomarker of HAND. PrP^C is the nonpathogenic cellular isoform of the human prion protein and is constitutively expressed in the CNS by neurons, astrocytes, brain microvascular endothelial cells (BMVEC), monocytes, macrophages, and microglia (Fournier et al., 2000; Brown, 2004; Viegas et al., 2006; Roberts et al., 2010b). We found that PrP^C is increased in brain tissue of macaques with SIV (simian immunodeficiency virus) encephalitis and in humans with HAND, but not in HIV-infected individuals without neurocognitive impairment. Soluble PrP^C levels in the CSF of those with HAND were also significantly elevated when compared with $sPrP^C$ levels in the CSF of HIV-infected people without cognitive impairment (Roberts et al., 2010b). These results suggest that CSF $sPrP^C$ may predict HIV-mediated CNS dysfunction and HAND.

PrP can be found in two isoforms. One is the normal non-pathogenic cellular isoform discussed above and that is the subject of this review, and the other is the scrapie isoform (PrP^{SC}). PrP^{SC} has the same amino acid sequence as PrP^C, but a higher content of β-sheet structures that makes it resistant to proteases. This infectious form of prion protein mediates the pathogenesis of neurodegenerative diseases termed transmissible spongiform encephalopathies (TSEs) (Prusiner, 1998). In our studies, it is important to note that we are not examining PrP^{SC}.

Structure of Cellular Prion protein

Cellular prion protein is a GPI-anchored glycoprotein located on the plasma membrane in specialized lipid raft microdomains (Brügger et al., 2004). While most PrP^C is found at the plasma membrane, the protein can also localize to the cytoplasm after clathrin-mediated endocytosis (Shyng et al., 1993; Shyng et al., 1994; Sarnataro et al., 2009) and can be secreted into the extracellular environment (Roberts et al., 2010b). This 27–30 kDa protein is encoded by the PRPN gene on human chromosome 20 (Liao et al., 1986) and contains two exons with an open reading frame found in the second exon (Puckett et al., 1991).

The three dimensional structure of PrP^C (Figure 1a) consists of a flexible N-terminal region containing a copper-binding octapeptide repeat (residues 51–90) (Stöckel et al., 1998), a neurotoxic region, and a hydrophobic region (residues 112–130) (Figure 1b). The C-terminal region of the protein (Figure 1b) is a globular structure composed of three α-helices (amino acids 144–153, 172–94 and 200–224), two β strands (amino acids 128–131 and 161–164), and a GPI anchor that attaches the protein to the plasma membrane (Riek et al., 1997; Zahn

et al., 2000). During the biosynthesis of PrP^c in the endoplasmic reticulum, the N-terminal signal sequence (residue 1–22) is removed, the GPI anchor is attached to the C-terminus, and N-linked oligosaccharides are added (Stahl et al., 1987; Borchelt et al., 1993). Human PrP^c is found unglycosylated, monoglycosylated, or diglycosylated at amino acids Asn 181 and Asn 197, and may have 60 different sugars attached to it, enabling binding to a broad range of partners (Haraguchi et al., 1989). PrP^c can also homodimerize, which facilitates the trafficking of PrP^c to the plasma membrane and confers protective properties to PrP^c during stress (Béland et al., 2012).

Physiological Functions of PrP^c

In the CNS, PrP^c has diverse biological functions that include cellular adhesion, cytoprotection, and neuroinflammation. However, most studies of PrP^c are in animal models and there are fewer reports examining the role of PrP^c in human cells or within the human CNS. Studies in zebra fish embryonic cell cultures showed that PrP^c mediates homophilic cell adhesion and that down regulation of PrP^c results in decreased morphogenetic movement which eventually leads to developmental arrest. It was also shown that PrP^c modulates cell adhesion by recruiting E-cadherin to the plasma membrane (Málaga-Trillo et al., 2009). The homophilic interactions of PrP^c on monocytes and on endothelial cells were shown to be essential for the migration of monocytes across an endothelial monolayer (Viegas et al., 2006). PrP^c recruits neural cell adhesion molecule (NCAM) into lipid rafts and interacts with it, promoting cell adhesion and neurite outgrowth by activating Fyn kinase (Schmitt-Ulms et al., 2001; Santuccione et al., 2005).

Glutamate induced calcium flux, which is the result of aberrant activation of the NMDA receptor, causes neuronal damage and death by excitotoxicity (Gillessen et al., 2000). PrP^c interacts directly with NMDA receptors to reduce glutamate mediated excitotoxicity. This was demonstrated in neurons from PrP^c null mice that had a greater cytotoxic response to NMDA receptor stimulation as compared to wild type neurons (Khosravani et al., 2008). In another study, spontaneous current activity and excitotoxicity caused by a mutated form of prion protein with the central region (amino acids 105–125) deleted was suppressed by over expression of WT PrP^c (Biasini et al., 2013). In astrocytes, PrP^c increased glutamate uptake that diminished excitotoxic neuronal death (Brown and Mohn, 1999; Brown, 2004). Another study demonstrated that PrP^c increased uptake of synaptic zinc in neurons through AMPA receptors. Reduced zinc uptake by neurons has been implicated in neurodegenerative diseases and the involvement of PrP^c in this process demonstrates the neuroprotective function of the protein (Watt et al., 2012). In addition, overexpression of PrP^c protected from apoptotic stimuli such as serum deprivation (Kim et al., 2004), or Bax dependent apoptotic death (Bounhar et al., 2001; Roucou et al., 2003). A recent study also showed that stress inducible protein-1(STI1) and Laminin-γ1 induced increases in axonogenesis in wild type neurons, while PrP^c null neurons showed no change, demonstrating that PrP^c is involved in axonal differentiation and growth (Santos et al., 2013).

The shed form of PrP^c, soluble PrP^c ($sPrP^c$), also has many biological functions including increasing the survival of wild type and PrP^c null neurons (Lima et al., 2007). Through its interaction with the secreted cytoplasmic co-chaperone molecule, STI1 and neural cell adhesion molecule (NCAM), $sPrP^c$ propagates survival signals increasing axonal differentiation, neurite outgrowth and synaptic development (Kanaani et al., 2005; Santuccione et al., 2005).

Although PrP^c is neuroprotective, it is also involved in neuroinflammatory processes. A study using blocking PrP^c antibodies showed that it is essential for monocyte migration across an endothelial monolayer (Viegas et al., 2006). PrP^c is also involved in macrophage

phagocytosis, cellular taxis (de Almeida et al., 2005) and microglia activation (Brown et al., 1998). These processes are dysregulated during HIV infection and microglia activation, taxis and aggregation are important features of HIV infection of the CNS (Visentin et al., 2001). In addition, our laboratory showed that sPrP^{C} induces production of the inflammatory mediators CCL2 and IL-6 from human astrocytes (Roberts et al., 2010b).

We previously demonstrated that CCL2 is neuroprotective against NMDA or HIV-tat-induced neuronal apoptosis (Eugenin et al., 2003; Eugenin et al., 2007). Thus, molecules considered inflammatory and/or pathogenic depending upon their expression pattern can also be neuroprotective (Eugenin et al., 2003; Eugenin et al., 2007). We propose that PrP^{C} has a similar dual role in the pathogenesis of HIV infection, one in neuronal/glial survival and another that results in CNS inflammation and compromise.

The duality of the neuron damaging and neuroprotective property of PrP^{C} is further demonstrated in CNS amyloid disease. Studies showed that PrP^{C} binds with high affinity to Aβ oligomers which are cleavage products of amyloid precursor protein (APP) involved in Alzheimer disease, suggesting that PrP^{C} may be a receptor mediating impairment of synaptic plasticity by the oligomers (Laurén et al., 2009). In contrast, sPrP^{C} was shown to prevent the aggregation of amyloid β fibrils and to inhibit the formation of spherical oligomers and their toxic effect on neurons (Nieznanski et al., 2012) therefore suggesting a protective role. However, others reported that PrP^{C} did not have a role in Aβ oligomers synaptoxocity and that the absence of PrP^{C} did not prevent the blocking of long term potentiation (Balducci et al., 2010; Calella et al., 2010; Kessels et al., 2010), and learning and memory deficits (Cissé et al., 2011) caused by Aβ oligomers. Therefore, the function of PrP^{C} in Alzheimer's disease is still not well understood.

Mechanisms of PrP^{C} release from the cell membrane

PrP^{C} is released from cells through endoproteolytic processing, exosomal release, and cleavage from the GPI anchor. The endoprotelytic processing of prion protein occurs through α-cleavage, β-cleavage, and ectodomain shedding. Alpha cleavage, which takes place between Lys110/His111 or His111/Met112, gives rise to an 11 kDa N1-fragment and an 18 kDa C1-fragment, destroying the neurotoxic region which is associated with fibril formation (Vincent et al., 2000; Vincent et al., 2001; Mangé et al., 2004). Several proteases including members of the A disintegrin and metalloproteinase family, ADAM 10 and ADAM 17, and TNF-α converting enzyme, TACE were thought to mediate α-cleavage of PrP^{C} (Vincent et al., 2000; Vincent et al., 2001). However, recent studies have argued against the involvement of these proteases in α-cleavage of PrP^{C}. ADAM10 knock-out mice did not have a significant decrease in α-cleavage (Altmeppen et al., 2011) and transgenic mice with neurons that over expressed ADAM 10 did not show increased PrP^{C} cleavage products (Endres et al., 2009). βeta cleavage of PrP^{C} occurs in response to oxidative stress and occurs near the octapeptide region (McMahon et al., 2001).

PrP^{C} can also be cleaved near the GPI anchor, releasing an almost full-length protein lacking any GPI modification, through protease-mediated shedding (Borchelt et al., 1993). The cleavage occurs between Gly228 and Arg229, three amino acids away from the GPI anchor (Zhao et al., 2006). Cell culture experiments identified ADAM 10 as the sheddase of PrP^{C} (Taylor et al., 2009) and demonstrated that ADAM 9 indirectly regulates the sheddase activity of ADAM 10 (Tousseyn et al., 2009; Moss et al., 2011).

PrP^{C} is transported from the plasma membrane to endosomes and is recycled back to the cell surface (Shyng et al., 1993; Shyng et al., 1994) or stored within intraluminal vesicles in multivesicular bodies (Laine et al., 2001; Peters et al., 2003). PrP^{C} can be transferred from late endosomes, to lysosomes for degradation (Peters et al., 2003) or released into the

extracellular space on 50–90 nm lipid vesicles known as exosomes, with the GPI anchor still attached to the membrane surface (Vella et al., 2007; Vella et al., 2008). Multiple CNS cells including neurons, macrophages, endothelial cells, and leukocytes release exosomes, and exosomes have been detected in human plasma and CSF (Caby et al., 2005; Vella et al., 2008). This suggests that exosomal microparticles are an important mechanism for PrP^c shedding and likely promote $sPrP^c$-mediated signaling during HIV infection of CNS.

PrP^c as a biomarker for HAND

HIV infection of the CNS leads to cytotoxic and protective responses that mediate the dynamic processes of HIV CNS pathogenesis. Therefore, the severity of an individual's neurocognitive impairment is likely to change during the course of the disease (Antinori et al., 2007). For this reason, indicators of neurologic dysfunction prior to the onset of clinical symptoms are important for identifying individuals who are likely to develop cognitive impairment with the goal of enabling long term monitoring of their CNS functions.

PrP^c is an adhesion and signaling molecule involved in several processes including transmigration of leukocytes across the endothelium (Roberts et al., 2010a). The process of transmigration is disrupted during HIV infection of the CNS (Eugenin et al., 2006c; Eugenin et al., 2006b; Roberts et al., 2010a; Roberts et al., 2010b; Williams et al., 2012b). Thus, we proposed that dysregulation of adhesion molecules, including PrP^c can result in increased monocyte influx into the CNS and damage to the BBB. We found that CSF $sPrP^c$ was elevated in HIV infected people with neurocognitive disorders as compared to uninfected people and HIV infected individuals with no cognitive impairment (Roberts et al., 2010a; Roberts et al., 2010b). The significant increase of $sPrP^c$ in individuals with HAND was independent of several factors including viral load or $CD4^+$T cells but correlated with increased CSF CCL2. These data indicate that increased $sPrP^c$ was not a consequence of immune suppression but rather was mediated by HIV infection and CCL2, demonstrating the importance of this chemokine in PrP^c release. Elevated CCL2 level in the CSF of humans is an indicator of neurocognitive impairment (Conant et al., 1998), while in pigtail macaques it is predictive of severity of encephalitis (Zink et al., 2001). Our *in vitro* studies examining the effect of CCL2 on BMVEC, neurons and astrocytes also showed that this chemokine induced increased PrP^c release between 30 min to 24 hrs (Roberts et al., 2010b). Therefore we propose that high CCL2 levels together with HIV infection induce shedding of PrP^c in the CSF as one of the pathogenic processes leading to HAND.

Our findings suggest that astrocytes, BMVEC and neurons are the main source of $sPrP^c$ in HIV infected individuals with HAND. In addition, HIV infected peripheral blood monocytes showed increased release of PrP^c followed by a sudden decrease 4 days post infection which was maintained for up to 7 days, the last time point assayed (Roberts et al., 2010b). We suggest that this shed PrP^c may facilitate monocyte entry into the CNS by dysregulating the normal homotypic PrP^c - PrP^c interactions that mediate baseline transmigration for surveillance.

To study the release of PrP^c during the course of HIV CNS pathogenesis, the pigtail macaque model of NeuroAIDS, in which 90% of animals develop SIV encephalitis (SIVE) within 3 months of infection (Clements et al., 2008), was used. CSF samples from different stages of SIV infection showed that animals that developed severe encephalitis had elevated $sPrP^c$ levels during early and late stages of infection as compared to uninfected animals and animals with mild SIVE. As these stages are characterized by elevated CCL2 in the CNS, thus, supporting our proposal that CCL2 and/HIV infection regulate $sPrP^c$ release.

Our previously published immunostaining and confocal microscopy studies showed that PrP^C was selectively increased in the brain tissue of HIV infected people with HAND as compared to uninfected people and HIV infected people with no cognitive impairment. Neuronal and astrocytic PrP^C was increased in individuals with both minor motor cognitive disorders (MCMD) and HIV encephalitis (HIVE) (Roberts et al., 2010a; Roberts et al., 2010b). Astrocytes in individuals with MCMD had morphologic changes including hypertrophy, proliferation and extensive process formation along with elevated PrP^C expression (Roberts et al., 2010a; Roberts et al., 2010b). An example of this increased PrP^C in the brain tissue of individuals with HAND is shown in Figure 2. Astrocytes from HIV (−) people had minimal PrP^C staining, while tissue from individuals with MCMD or HAD had highly increased PrP^C.

Our studies identified CSF $sPrP^C$ as a potential biomarker for HAND. We are expanding upon these finding using CSF samples from a large cohort of HIV infected people with and without cognitive impairment. If validated with this larger cohort, we propose that $sPrP^C$ will provide a new tool to diagnose and manage CNS disease in HIV infected individuals. CSF biomarkers are useful because the CSF reflects events occurring in the CNS. The correlation of HAND and elevated PrP^C was not observed in sera samples where $sPrP^C$ levels were significantly decreased relative to uninfected individuals. In addition, in the sera samples there was no significant difference between $sPrP^C$ levels in HIV infected people with or without cognitive impairment (Roberts et al., 2010b).

Is PrP^C a biomarker for other neurocognitive disorders?

Although altered PrP^C levels have been described in several neurocognitive disorders, they do not appear to be a biomarker of cognitive impairment. Individuals with Alzheimer's disease have upregulated PrP^C in several regions of the brain (Voigtländer et al., 2001; Rezaie et al., 2005). However, levels of $sPrP^C$ in the CSF of people with Alzheimer's disease were lower and did not correlate with brain PrP^C expression or with cognitive impairment. In people with Lewy body dementia (Rezaie et al., 2005) and in those with Creutzfeldt-Jakob disease (CJD) (Torres et al., 2012), CSF PrP^C was lower than in individuals with no neurocognitive disorders. CSF $sPrP^C$ was also decreased in people with other neurocognitive dysfunctions and neuroinflammatory diseases such as Parkinson's disease and multiple sclerosis (Meyne et al., 2009). Although CCL2 plays a role in the pathogenesis of multiple sclerosis (Mahad and Ransohoff, 2003), there was no increase in shed PrP^C in this disorder, underscoring the importance of HIV infection in concert with elevated CCL2 in mediating the shedding of PrP^C. These findings indicate that increased CNS PrP^C is not a general indicator of dementia and that elevation of both CNS PrP^C and CSF $sPrP^C$ occurs specifically during CNS HIV infection in the presence of CCL2. A study demonstrating that PrP^C expression in the brain is increased after acute stroke but not acute ischemia also supports the fact that PrP^C is not correlative with general neuroinflammation and neuronal injury.

Conclusion

Despite the success of ART, the prevalence of HIV associated neurocognitive disorders continues to increase as HIV infected individuals live longer. Although the mechanisms that mediate HAND are not fully characterized, HIV infected $CD14^+$ $CD16^+$ monocytes that transmigrate across the BBB initiate CNS infection and the subsequent neuroinflammation that mediates HAND. HIV infected monocytes cross the BBB through homophilic interactions between the junctional proteins on the surface of EC and on monocytes. PrP^C is one adhesion and signaling molecule that is essential for monocyte transmigration. This protein is involved not only in neuroinflammatory but also in cytoprotective processes such as neurite outgrowth, regulation of ionic current and protection against apoptosis. Studies

showed that PrP^C is involved in several neurocognitive disorders including Alzheimer's disease. However, the role of PrP^C in HAND had not been previously examined. Our data showed that PrP^C is increased in the brain of individuals with HAND as compared to uninfected individuals or HIV infected people with no cognitive impairments. In addition, we demonstrated that in HIV infected individuals, $sPrP^C$ in the CSF is a potential biomarker. Therefore we propose that HIV infection of the CNS and its associated inflammation mediated, at least in part, by increased CCL2 altering PrP^C expression and release, resulting in ongoing neuroinflammation and subsequent cognitive impairment. PrP^C as a biomarker for HAND would (1) identify individuals who are at risk of developing cognitive impairment, (2) inform management decisions regarding initiation of therapy and (3) monitor the efficacy of treatment of individuals with HAND.

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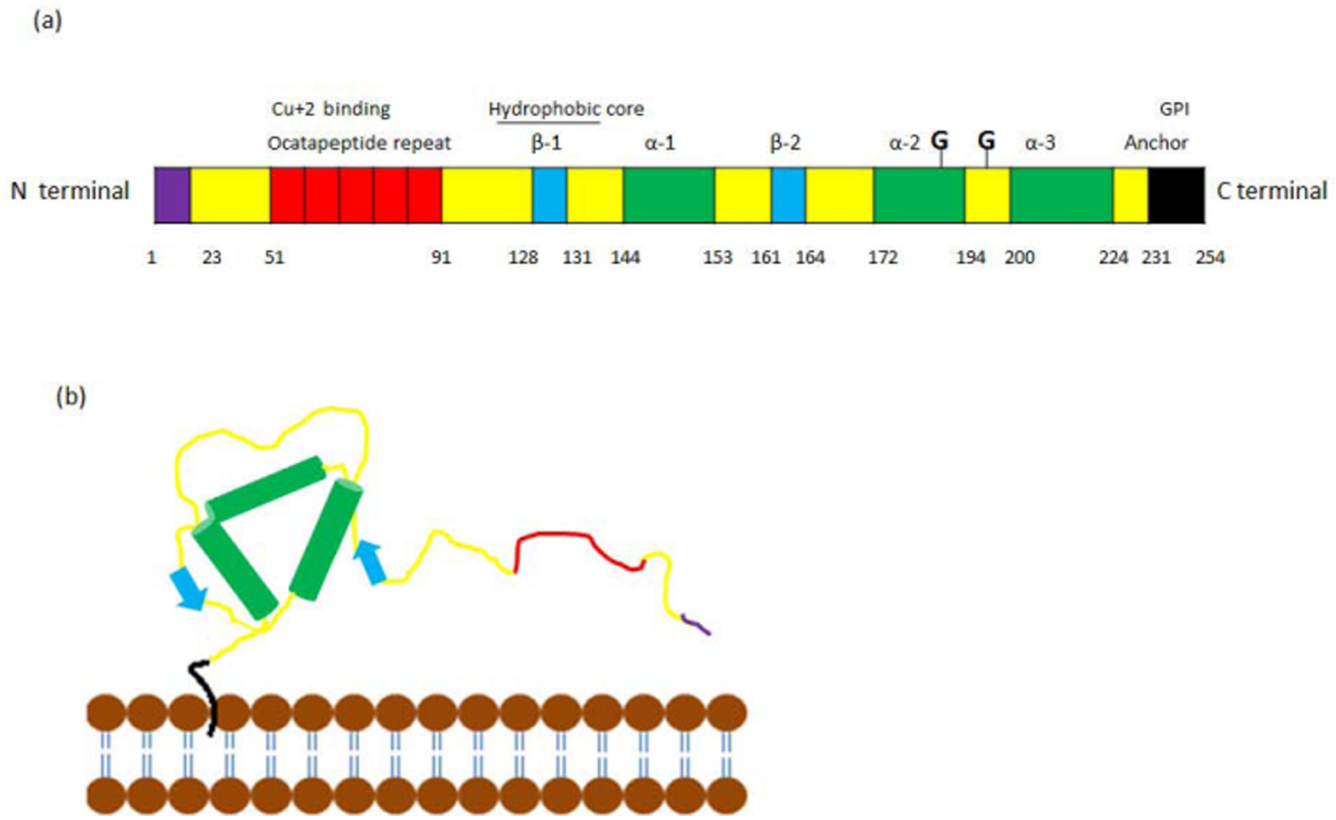

Figure 1.

Schematic representation of the structure of PrP^C a) linear representation of the primary sequence of prion protein. b) PrP^C is attached in the plasma membrane through the GPI anchor.

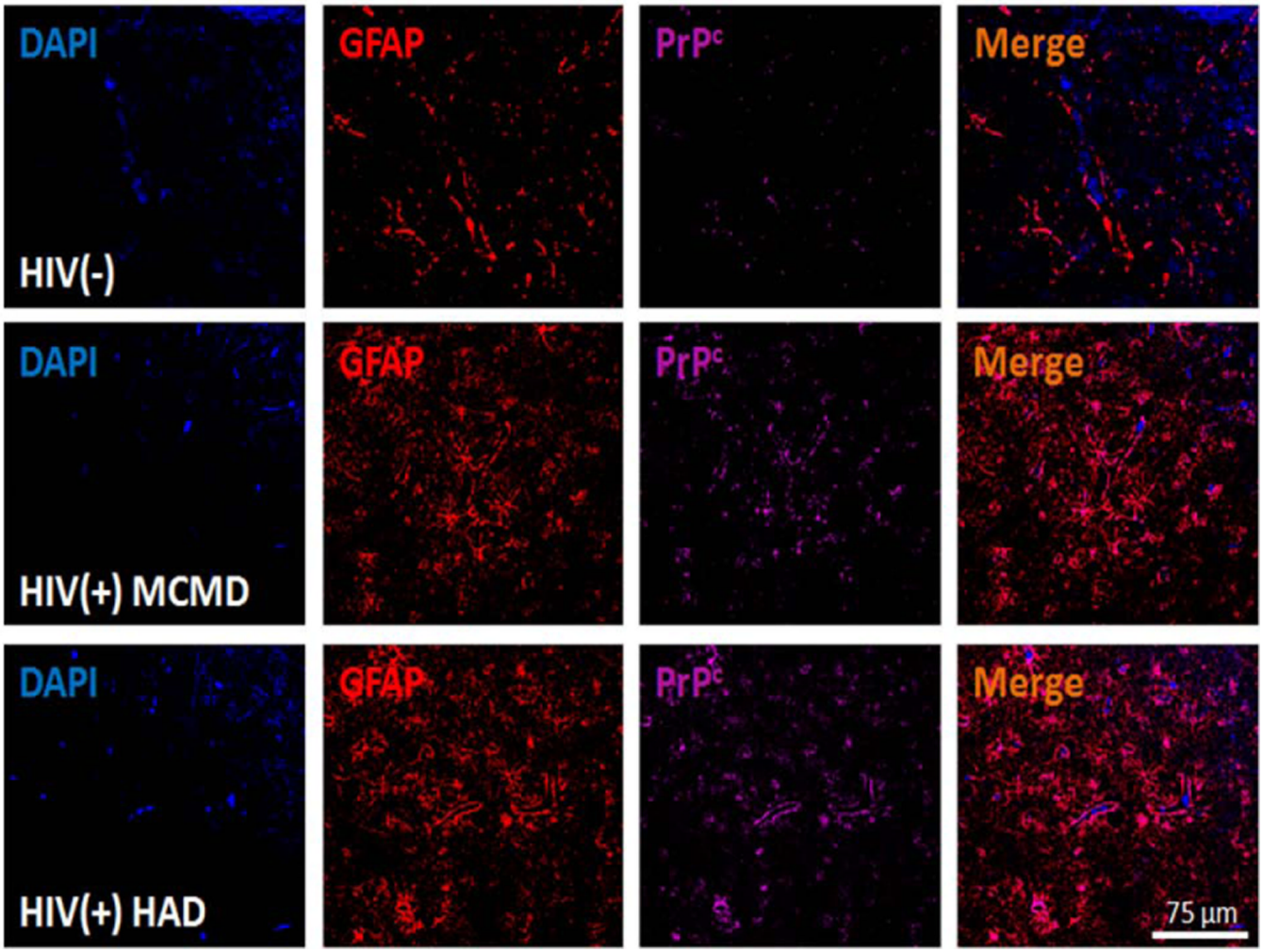

Figure 2.

PrP^c is increased significantly in the brain of individuals with HIV-1-associated neurocognitive impairment. Brain tissue sections from uninfected (HIV (−)), HIV infected with minor cognitive mild disorders (HIV(+) MCMD) and HIV infected with dementia (HIV (+) HAD) individuals were evaluated by immunohistochemistry using confocal microscopy. Astrocyte specific expression of PrP^C is demonstrated by GFAP (Cy3-red; astrocytic marker), and PrP^C (FITC-Cy5, cyan) co-localization indicates the presence of this protein in areas close to the BBB. PrP^C expression was elevated in MCMD and HAD individuals with HIV infection as compared to uninfected individuals.