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REVIEW

14-3-3s are potential biomarkers for HIV-related neurodegeneration

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Abstract Over the last decade, it has become evident that 14-3-3 proteins are essential for primary cell functions. These proteins are abundant throughout the body, including the central nervous system and interact with other proteins in both cell cycle and apoptotic pathways. Examination of cerebral spinal fluid in humans suggests that 14-3-3s including 14-3-3ε (YWHAE) are up-regulated in several neurological diseases, and loss or duplication of the YWHAE gene leads to Miller–Dieker syndrome. The goal of this review is to examine the utility of 14-3-3s as a marker of human immune deficiency virus (HIV)-dependent neurodegeneration and also as a tool to track disease progression. To that end, we describe mechanisms implicating 14-3-3s in neurological diseases and summarize evidence of its interactions with HIV accessory and co-receptor proteins.

Keywords 14-3-3 · Hepatitis C virus · Neurocognition · HIV accessory proteins · gp120 · Vpr · Vpu · GPR15 · Nef

Abbreviations

ACD	AIDS dementia complex
AD	Alzheimer's disease
ADHD	Attention deficient hyperactivity disorder
AIDS	Acquired immunodeficiency virus
BAD	B-cell lymphoma 2 antagonist of cell death
Bax	Bcl-2-associated X
BBB	Blood–brain barrier
Bcl-XL	B-cell lymphoma-extra large
C. elegans	*Caenorhabditis elegans*
Cdc25	Cell division cycle phosphatase 25
CDKs	Cyclin-dependent protein kinases
CJD	Creutzfeldt–Jakob disease
CME	Cytomegalovirus encephalitis
CNS	Central nervous system
CRK	Viral oncogene causes increased tyrosine-phosphorylated proteins
CSF	Cerebral spinal fluid
CXCR4	CXC chemokine receptor 4
DCAF-1	DNA binding protein 1 and Cullin 4a-associated factor
FoxO	Forkhead transcription factor
Gp120	Glycoprotein 120
GPR15	G protein receptor 15
GPRs	G protein cell receptors
HAD	HIV-associated dementia
HADC	HIV-associated dementia complex
HAND	HIV-associated neurocognitive disorders
HBMECs	Human brain microvascular endothelial cells
HCV	Hepatitis C virus
HEK293	Human embryonic kidney
Hela	Human cervical carcinoma
HepG2	Human hepatoma
HIV	Human immune deficiency virus
HIVE	HIV encephalitis
HMC	Human mesangial growth cells
HUVEC	Human umbilical vein endothelial cells
IL	Interleukin
ILK	Isolated lissencephaly

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K2P	Potassium channel
LB	Lewy bodies
LIS1	Encodes subunit of platelet-activating factor acetylhydrolase 1B (PAFAH1B1)
MDS	Miller–Dieker syndrome
MS	Multiple sclerosis
MYO1C	Myosin-1C
Nef	Negative factor
PKA	Protein kinase A
PKC	Protein kinase C
Raf	Proto-oncogene serine/threonine-protein kinase
RNAi	RNA interference
S. pombe	*Schizosaccharomyces pombe*
siRNA	Single stranded RNA\
SIV	Simian immunodeficiency virus
TAU	Tubulin-associated unit
TUSC5	Tumor suppressor candidate 5
Vpr	Viral protein R
Vpu	Viral protein U
Ywhae$^{-/-}$	*Ywhae*/*14-3-3ε*-deficient mice
YWHEA	14-3-3ε (human gene)

Dynamics of 14-3-3s

14-3-3s are proteins that regulate many cellular processes relevant to multiple pivotal points in the life cycle of a cell, such as apoptosis, mutagenic signaling, and cell-cycle checkpoints (Aitken 2006; Aitken et al. 2002; Berg et al. 2003b; Fu et al. 2000; Obsil and Obsilova 2011; Steinacker et al. 2011; Takahashi 2003; van Heusden 2005; Wang and Shakes 1996; Yaffe 2002). 14-3-3s were first described in 1967 from bovine brains as proteins with an acidic pI and molecular masses between 29–32 kD in an attempt to identify proteins unique to the nervous system (Moore and Perez 1967). These were later resolved to comprise nine proteins (α, β, γ, δ, ε, ζ, η, θ/τ, and σ) encoded by seven distinct genes, with the α and δ isoforms being the phosphorylated forms of β and ζ genes, respectively (Aitken et al. 1995). In addition, 14-3-3s contain a number of known common modification domains, including regions for divalent cation interaction, phosphorylation, acetylation, and proteolytic cleavage, among others (Aitken 2006; Obsil and Obsilova 2011; Rittinger et al. 1999; Xiao et al. 1995). The 14-3-3 family is ubiquitous, with members identified in all eukaryotic species examined, including mammals, insects, nematodes, frogs, plants, and yeast (Aitken et al. 1992a; Aitken et al. 1992b; Berg et al. 2003a; Fu et al 2000; Muslin and Xing 2000; Takahashi 2003; Wang and Shakes 1996). Whether particular modifications are present in orthologs or homologs from different species and their potential functional significance is a question under active investigation.

In vertebrates, 14-3-3s are highly enriched in the cerebellum, certain cerebral areas (including the hippocampus), and motor neurons in the brainstem and spinal cord (Boston et al. 1982; VanGuilder et al. 2011; Watanabe et al. 1991). Their abundance in the brain and recent evidence of up-regulation in various neurological disorders imply that 14-3-3s may play a significant role in neuronal functions (VanGuilder et al. 2011).

14-3-3s as diagnostic tools in the CSF

14-3-3 proteins have also been detected in the cerebrospinal fluid of patients with various diseases that lead to neurodegeneration, including those with Creutzfeldt–Jakob disease (CJD), Alzheimer's disease (AD), multiple sclerosis (MS), and HIV and these proteins also are aggregated in Lewy bodies (LB) in those with Parkinson's disease (PD) (Berg et al. 2003a; Ellis et al. 2007; Steinacker et al. 2011; Wakabayashi et al. 2001; Zerr and Poser 2002). Despite this apparent correlation, the question remains as to whether 14-3-3s are truly biomarkers that can be used to track neurodegeneration. Are different isoforms specific to a particular disease? Should specific isoforms be examined for different diseases? Additionally, it is important to understand whether 14-3-3s actually interact with the pathogens to regulate or affect in any way the progression of the disease because that would likely lead to potential therapeutic interventions.

Creutzfeldt–Jakob disease

It has been suggested that changes in the distribution of 14-3-3s in the CNS may be linked to spongiform encephalitis (Berg et al. 2003a). Transmissible spongiform encephalopathy, or prion disease, was first described by Gerhard Creutzfeldt and Alfons Jakob in the 1920s (Creutzfeld 1920), but recent clinical diagnostics indicate two forms: sporadic Creutzfeldt–Jakob disease (CJD) and variant CJD (Zerr and Poser 2002). Sporadic CJD occurs in patients in their 70s and is characterized by rapid dementia progressing to mortality within 6–14 months. In contrast, variant CJD occurs in patients from 14 to 74 years of age and typically presents slower progression (Zerr and Poser 2002).

To look for disease-specific biomarkers, clinical investigations have focused on potential changes in the levels of various proteins in the cerebrospinal fluid (CSF) and described increases in different 14-3-3s in CJD patients (Table 1). Monitoring 14-3-3 levels in the CSF by Western blot has revealed that using the anti-14-3-3β antibody, also called the pan-14-3-3 antibody, appears to be both sensitive and specific for sporadic CJD (Table 1) (Bahl et al. 2008; Baxter et al. 2002a; Bertrand et al. 2009; Brandel et al. 2000; Castellani et al. 2004; Chohan et al. 2010; Collins et al. 2010; Huang et al. 2003; Irani and Kerr. 2000; Otto et al.

Table 1 14-3-3 protein expression in the CSF of individuals with other neurodegenerative diseases

Disease	Isoforms	Effect	References
sCJD	γ	Present	Green et al. 2001
sCJD	ε, β, γ, η	Examined 16 different antibodies; found only four isoforms present, all others not detected	Wiltfang et al. 1999
sCJD	Pan	Present, variant subtype should be considered when using 14-3-3 as a biomarker	Bahl et al. 2008; Baxter et al. 2002a; Bertrand et al. 2009; Brandel et al. 2000; Castellani et al. 2004; Chohan et al. 2010; Collins et al. 2010; Huang et al. 2003; Irani and Kerr 2000; Otto et al. 2002; Peoc'h et al. 2006; Poser et al. 1999; Sanchez-Valle et al. 2002; Zerr et al. 2000a
sCJD	ε, γ	Increased using mouse antibodies more specific then polyclonal	Takahashi et al. 1999
CJD with PNDs	Pan	Double bands present only in patients with PNDs	Saiz et al. 1999
AD	η	Present and in those with herpes simplex encephalitis	Wiltfang et al. 1999
AD	ζ, pan	Binds to tau and co-purifies with microtubules. The ε or γ isoforms are not associated with tau	Hashiguchi et al. 2000
AD	Pan	Not present	Hsich et al. 1996; Tschampa et al. 2001
MS	ζ, pan	Present, dimeric and trimeric	Fiorini et al. 2007
MS	Pan	No change or not present	Bartosik-Psujek and Archelos 2004; de Seze et al. 2002; Hsich et al. 1996
HIV/AIDS	ε, γ, ζ	Increased only in patients with AIDS dementia complex or CMVE	Wakabayashi et al. 2001
HIV	γ	Increased in those with CNS lymphoma	Miller et al. 2000

sCJD Sporadic Creutzfeldt–Jakob disease; *CMVE* Cytomegalovirus encephalitis; *AD* Alzheimer's disease; *MS* Multiple Sclerosis; *PNDs* Paraneoplastic neurological disorders; pan – antibody against β cross-reacts to ε, ζ, γ, and η

2002; Peoc'h et al. 2006; Poser et al. 1999; Sanchez-Valle et al. 2002; Zerr et al. 2000a; Zerr and Poser 2002). Furthermore, using isotype-specific antibodies, increases in the levels of γ, ζ, and ε proteins have been reported in the CSF of CJD patients compared to non-CJD subjects (Table 1) (Green et al. 2001; Takahashi et al. 1999; Wiltfang et al. 1999). Distinct levels of the different 14-3-3s in the CSF appear to correlate with damage in particular areas of the brain and the rate of neurodegenerative changes (Huang et al. 2003; Zerr and Poser 2002). Hence, increased 14-3-3 levels in the CSF may result from their release upon cell death and reflect their abundance in the particular neurons affected as well their relative stability.

In fact, the appearance in the CSF is hypothesized to be due to release and local loss of 14-3-3β, γ, η, and ζ in areas of severe degeneration, particularly in the hippocampus and thalamus shown in scrapie-infected mice (Baxter et al. 2002b; Berg et al. 2003a). However, 14-3-3 levels in the CSF may not remain elevated if the damage is not sustained. For example, in cases of herpetic encephalitis, 14-3-3s are only present in the CSF initially and decline later on. Consequently, examining the level of 14-3-3 proteins in the CSF may not suffice for safely diagnosing such diseases, but rather provides independent support for diagnosis and characterization, in conjunction with clinical data (Table 1) (Zerr and Poser 2002; Zerr et al. 2000b). There is evidence of misdiagnosis using the pan-14-3-3 antibody potentially because of cross-reactivity with several other isoforms, including 14-3-3ε, ζ, γ, and η). In these cases, samples tested positive for CJD, while patients were actually affected by AD or dementia with LBs (Table 1). Thus, we suggest that the isoform-specific antibodies are likely more appropriate for diagnostic applications (Chitravas et al. 2011; Tschampa et al. 2001). It should be pointed out, however, that most studies aim to use the presence or levels of 14-3-3s as a potential acute diagnostic tool, assessing their profile longitudinally as a tool to track disease progression remains largely unexplored.

Alzheimer's disease

Increased levels of 14-3-3ζ, γ, and ε have also been reported in the CSF of AD patients (Table 1) (Hashiguchi et al. 2000; Tschampa et al. 2001; Wang et al. 1995). Interestingly, the 14-3-3ζ isoform has been suggested to affect the stability of the microtubule-associated protein Tau (tubulin-associated unit) (Table 1) (Hashiguchi et al. 2000). Furthermore, association of Tau and 14-3-3ζ appears to lead to its abnormal phosphorylation via PKA, and Tau hyper-phosphorylation is thought to be one of the key events in the development of AD pathology (Hashiguchi et al. 2000; Wang et al. 1995). In support of these data, 14-3-3ζ, but not 14-3-3ε and γ, was found to co-purify (Hashiguchi et al. 2000). However, it should be pointed out that other studies did not find changes in the 14-3-3 levels of AD

patients (Table 1) (Hsich et al. 1996; Tschampa et al. 2001), unless these patients were also infected with herpes simplex encephalitis virus (Table 1) (Wiltfang et al. 1999). These results suggest that 14-3-3s may not be appropriate as a biomarker for AD.

Multiple sclerosis

Studies have demonstrated that elevated signal is observed with the pan-14-3-3 and 14-3-3ζ-specific antibodies in the CSF of MS patients who present severe inflammation-induced extensive damage of the central nervous system (Table 1) (Fiorini et al. 2007; Sanchez-Valle et al. 2002). However, other studies reported absence, or at least no elevation of 14-3-3s in the CSF of MS patients (Table 1) (Bartosik-Psujek and Archelos 2004; de Seze et al. 2002; Hsich et al. 1996). Again, additional broad and specific antibodies should be tested to unequivocally establish whether 14-3-3 elevation in the CSF is also characteristic of MS patients.

Human immunodeficiency virus/acquired immune deficiency syndrome

Acquired immunodeficiency virus (AIDS) patients may develop AIDS dementia complex (ADC), also known as HIV dementia, HIV-associated dementia (HAD), and HADC. The CSF of such patients as those with CME was reported to contain 14-3-3ε, 14-3-3γ, and 14-3-3ζ (Wakabayashi et al. 2001). However, these 14-3-3 isoforms were not present in AIDS patients who did not have neurological symptoms (Table 1). Wakabayashi et al. (2001) also found that the isoforms present in AIDS patients were different from those reported in CJD and herpes simplex encephalitis, suggesting that isotype patterns in the CSF may facilitate differential diagnosis. High levels of 14-3-3ε, ζ, and γ were observed in the CSF of seriously ill AIDS patients, particularly those with low CD4 levels (Table 1). They suggest that 14-3-3 proteins may have been released from destroyed neurons and making them a marker of cellular destruction (Wakabayashi et al. 2001). Both 14-3-3ζ and ε levels were found twofold elevated in brain sections from HIV encephalitis (HIVE) and HIV-associated neurocognitive disorders (HAND) compared to those of non-HIV controls (Gelman and Nguyen 2010). The 14-3-3ε levels also correlated with the viral load of HIV-1 in the brain and CSF. Finally, another study examined HIV patients with lymphoma and found the 14-3-3γ isotype in their CSF in the 3 months preceding death (Table 1) (Miller et al. 2000). Collectively, these results strongly suggest that 14-3-3 proteins are involved in changes associated with HIV infection particularly in the CNS (Gelman and Nguyen 2010).

In *Macaques* infected with simian immunodeficiency virus (SIV), the continued presence of 14-3-3 proteins in the CSF was tightly linked with the amount of viral replication in the CNS (Helke et al. 2005). Animals with 14-3-3 protein in the CSF harbored the highest viral loads after acute infection and the highest levels of both viral RNA and protein in the brain. Hence, it was proposed that 14-3-3 protein levels may serve as a biomarker for early neuronal damage correlating to viral replication in the CNS and disease progression in individuals with HIV (Helke et al. 2005). A reasonable question which arises from all these results is: Are 14-3-3s regulating disease progression?

14-3-3s and HIV/SIV accessory and co-receptor proteins

The rate of disease progression with which HIV-1 infection leads to AIDS varies among individuals. Reasons for this variance include host susceptibility, genetics, immune function, and co-infections, and the regulation and modulation of the HIV gene products (including accessory proteins). Within the brain, HIV-1 infection is associated with the degeneration due to apoptosis (Jones and Power 2006; Shi et al. 1996) of the frontal cortex, substantia nigra, cerebellum, and striatum (Everall et al. 1993). This leads to development of HAD or HIVE (McArthur et al. 2003). Most studies of the pathogenic mechanism, thus far, agree that the modulation of HIV accessory and co-receptor proteins leads to neurodegeneration (Ellis et al. 2007; Iskander et al. 2004; Jones and Power 2006; Jones et al. 2007; Kogan and Rappaport 2011; Malim and Emerman 2008; McArthur et al. 2003; Strazza et al. 2011; Toggas et al. 1994). These are summarized below.

Glycoprotein 120

Both HIV-free and virus-infected monocyte/macrophages traverse the blood–brain barrier (BBB), infecting neighboring resident microglia, astrocytes, and other cell types (Valcour et al. 2011). The HIV-1-encoded glycoprotein 120 (gp120) envelope protein mediates and stimulates the entry of the virus into the host cell and induces neurotoxicity via multiple pathways, including the B-cell lymphoma-extra large (Bcl-XL)/B-cell lymphoma 2 antagonist of cell death (BAD) apoptosis pathway (Fig. 1) (Bazan et al. 1998; Ellis et al. 2007; Gallo et al. 2003; Iskander et al. 2004; Lipton 1992a; Lipton 1992b; Ushijima et al. 1995). Understanding the players involved in this pathway may help to block the effects of gp120.

14-3-3 proteins appear to play a role in gp120-mediated cytotoxicity in human umbilical vein endothelial cells (HUVEC) (Table 4) (Yano et al. 2007), which, like neuronal cells, have alpha- or beta-chemokine receptors but no CD4 receptor to induce their apoptosis (Ullrich et al. 2000). The 14-3-3τ protein protects against cell death when it is associated with BAD, preventing its interaction with Bcl-XL. Gp120 associates with BAD, preventing the 14-3-3 protein from binding, thereby allowing the BAD/Bcl-XL interaction. Suppression of BAD activity or expression seems to be

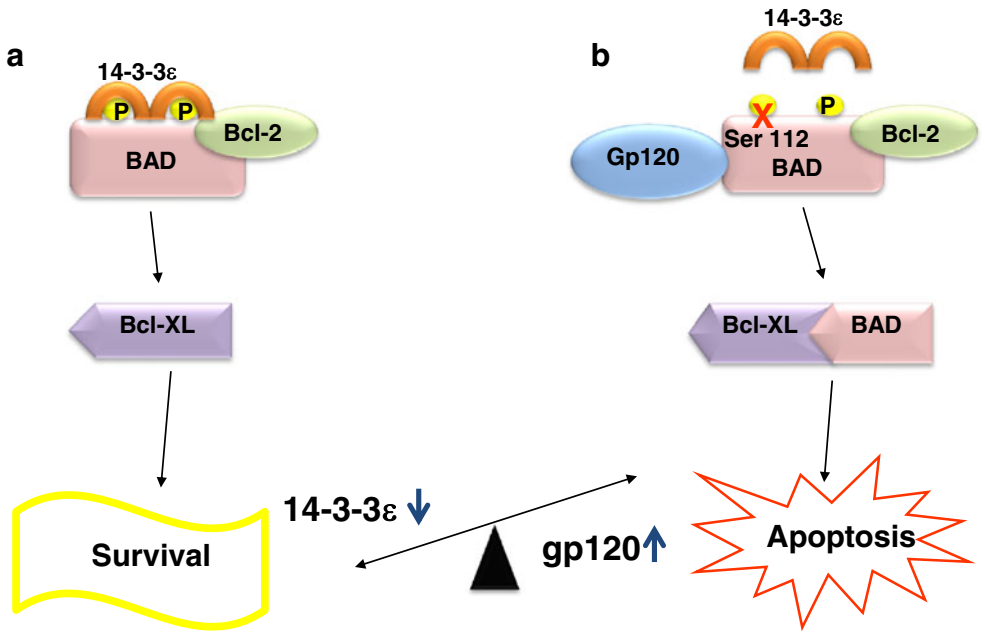

Fig. 1 Proposed relation of 14-3-3ε- and gp120-mediated apoptosis. **a** Binding of 14-3-3ε suppresses apoptosis in cells via phosphorylation of the pro-apoptotic Bcl-2 family protein BAD. The phosphorylation results in reduced association of BAD with Bcl-XL, thereby suppressing apoptosis. **b** Gp120-dependent dephosphorylation of BAD at serine-112, preventing 14-3-3ε binding, and its association with the Bcl-XL in mitochondria, promoting gp120-mediated apoptosis (Yano et al. 2007)

the reason cells are rescued from gp120-triggered apoptosis (Fig. 1) (Yano et al. 2007). In fact, 14-3-3τ is specifically up-regulated after a 24-h treatment with recombinant gp120 protein, while its down-regulation by RNAi accelerated gp120-dependent dephosphorylation of BAD at serine-112 and its association with the Bcl-XL in mitochondria, promoting apoptosis (Yano et al. 2007). Furthermore, in human brain microvascular endothelial cells (HBMECs), an association between gp120 and 14-3-3τ protein levels appears to regulate alpha or beta-chemokine receptors, but no CD4 receptors to induce apoptosis (Ullrich et al. 2000). In addition, an association between gp120 and 14-3-3τ protein levels appears to regulate the BBB breakdown by interfering with tight junctions between endothelial cells (Table 4) (Nakamuta et al. 2008). Furthermore, 14-3-3ε levels were inversely associated with gp120 amounts, with the lowest levels of 14-3-3ε at their highest concentrations of gp120 (Table 4 and Fig. 1) (Kapasi et al. 2001). These results suggest that 14-3-3 levels in the CSF may reflect either the level of HIV infection and/or neurodegeneration.

Negative factor

Negative factor (Nef) is a protein with a role in HIV-1 replication and pathogenesis (Foster et al. 2011; Kestler et al. 1991). Nef contributes to immune modulation of T cells upon HIV-1 infection through its association with PKCθ (Meller et al. 1998; Smith et al. 1996). 14-3-3τ interacts directly with PKCθ resulting in inhibition of interleukin 2 (IL-2) by preventing its translocation to the membrane in Jurkat T cells (Table 2) (Meller et al. 1998; Meller et al. 1996). This suggests that 14-3-3τ interaction with PKCθ is necessary for normal immune function via T-cell activation (Meller et al. 1996). These results are in agreement with the notion that 14-3-3s can modulate HIV disease progression by interacting with proteins whose functions are affected by the presence of HIV accessory proteins.

Viral protein U

The viral protein U (Vpu) accessory protein mediates proteasomal degradation of newly synthesized CD4 receptors, leading to their down-regulation (Cohen et al. 1988; Dube et al. 2010; Goff 2007). In addition, Vpu enhances the release of newly synthesized virions by regulating Tetherin, an interferon host restriction factor responsible for linking virons on the host cell surface (Dube et al. 2010). The two-pore domain potassium channel (K2P) K2P3 has been shown to interact with Vpu, leading to the dissociation of the channel (Hsu et al. 2004). K2P3 also binds to 14-3-3s suppressing beta-coatomer protein (β-COP) binding and aids in the trafficking of the channel (Table 2) (Mathie et al. 2010; Plant et al. 2005). Although, no one has examined whether there is direct relationship between Vpu and 14-3-3s, the fact that these proteins both bind and regulate the same receptor suggests that 14-3-3s would have a regulatory role in Vpu function.

Viral protein R

Viral protein R (Vpr) is a multifunctional accessory protein that plays a role in CD4+ T-cell and macrophage viral infection (Cohen et al. 1990b; Kino and Pavlakis 2004; Kogan and Rappaport 2011; Zhao et al. 1994a; Zhao et al.

Table 2 14-3-3 protein interactions with HIV accessory and co-receptor proteins

14-3-3 Isoform	Cell type	Related proteins	Relationship to 14-3-3 proteins	References
ε	HMC	gp120	Low-level stimulate cell proliferation and high-level inhibit of cell proliferation	Kapasi et al. 2001
τ/θ	HBMEC	gp120	Increase expression of gp120 and blood–brain barrier permeability	Nakamuta et al. 2008
τ/θ	HUVEC	gp120	Binding to BAD protects it from dephosphorylation regulating gp120/CXCR4-mediated cell death	Yano et al. 2007
τ/θ	T cells	Nef	Binding and suppression of PKCθ–dependent IL-2 promoter activity may relate to T-cell impairments by PKCθ/Nef	Meller et al. 1998; Meller et al. 1996
β	T cells	Vpu1	Binding effects translocation of K2P3 which interacts with Vpu1 releasing progeny virions from infected cells	Plant et al. 2005
Pan	Hela, HepG2	Vpr	Vpr leads to loss of 14-3-3/FoxO3a binding contributing to tissue-selective insulin resistance	Kino et al. 2005a
ε/rad24	*S. pombe*	Vpr	Binding with Vpr potentiates G2 cell-cycle arrest	Matsuda et al. 2006
η, σ	HepG2, σ knockout, Hela, *S. pombe*	Vpr	Triple complex with Cdc25 promotes G2/M cell-cycle arrest	Kino et al. 2005b; Kino and Pavlakis 2004
τ/θ	T cells	Vpr	Dissociation of 14-3-3θ to centrosomal proteins correlates to G2 cell-cycle arrest	Bolton et al. 2008
Pan	HEK293	GPR15	Binding with GPR15 increased its stability and trafficking	Chung et al. 2009; Okamoto and Shikano 2011
ε	*S. pombe,* HEK293	GPR15	Binding motif SWTY in ε interacts with GPR15	Shikano et al. 2005

Vpu-1 HIV-1 membrane protein, *K2P3* (*TASK1*) potassium channel, *HBMEC* human brain microvascular endothelial cells, *GPR15* G protein-coupled receptor 15, *Vpr* viral protein R, *HCV* hepatitis C virus, *Cdc25* cell division cycle phosphatase 25, *FoxO3a* forkhead in human rhabdomyosarcoma, *S. pombe Schizosaccharomyces pombe*, *HepG2* human hepatoma, *Hela* human cervical carcinoma, *SWTY* RGRSWTY, *HEK293* human embryonic kidney, *PKC*θ protein kinase C (Ca^{2+}-independent), *gp120* glycoprotein 120, *HMC* human mesangial growth cells, *HUVEC* human umbilical vein endothelial cells, *CXCR4* CXC chemokine receptor 4, *Nef* negative factor, *Pan* antibody against β cross-reacts to ε, ζ, γ, and η

1994b) and the role of HIV-1 Vpr in the inhibition of normal cell growth is well known. It is suggested that the interruption of cell division by Vpr increases virus replication and induces programmed cell death. Vpr mediates cell-cycle arrest at the G2/M transition in various mammalian cells. G2 arrest provides a replication advantage for the virus because the proviral transcription level is known to be elevated during the G2 phase of the cell cycle (Belzile et al. 2007; Elder et al. 2001; Goh et al. 1998; Tyson et al. 2002). In the virions, Vpr transports the virus for integration into the host genome (Cohen et al. 1990a; Vodicka et al. 1998).

The eukaryotic cell cycle is controlled by a complex network of proteins and genes including cyclin division cycle (cdc) proteins. Cyclin-dependent protein kinases (CDKs) initiate the essential events of the cell cycle by phosphorylating specific target proteins. The phosphorylation activity of CDKs is dependent on binding to cyclins. The CDK/cyclin complexes can be down-regulated either by inhibiting the phosphorylation of the CDK subunit or by binding to inhibitory molecules (designated cyclin-dependent kinase inhibitors) (Tyson et al. 2002). G2 arrest is distinguished by low levels of cyclin B1/p34Cdc2 activity and the inhibitory phosphorylation of p34Cdc2. It has been shown that Vpr directly inhibits the in vitro activity of a phosphatase, Cdc25C, which normally activates cyclin B1-p34Cdc2 (Fig. 2). Although the Vpr does not seem to bind on the catalytic site of Cdc25C, it nevertheless inactivates the phosphatase. In the absence of the Cdc25C, phosphatase activity cyclin B1-p34Cdc2 remains in its inactive phosphorylated form (Goh et al. 1998; He et al. 1995).

14-3-3 proteins normally regulate cell-cycle progression by modulating the activities of cyclins, including Cdc25C (He et al. 1995). DNA damage results in Cdc25C phosphorylation, which provides an active binding site for 14-3-3. Studies have shown that the C-terminal region of Vpr interacts with the C-terminal region of 14-3-3, leading to the association of 14-3-3 with Cdc25C (Fig. 1) (Kino et al. 2005a, b). The complex is not able to activate cyclin B1-p34Cdc2; therefore, the cell cycle is arrested (Fig. 2) (Kino et al. 2005a, b).

Inactivating Cdc25C is only one of the pathways utilized by Vpr to arrest the cell cycle. In addition, Vpr also plays a role in cell-cycle arrest because it binds directly onto DCAF-1, which in turn results in T-cell disruption (Kogan and Rappaport 2011; Stewart et al. 1997; Stewart et al. 2000). Results from a *Schizosaccharomyces pombe* model indicate that 14-3-3ε protein increases the levels of Wee1, a serine/threonine kinase (Wang et al. 2000), which

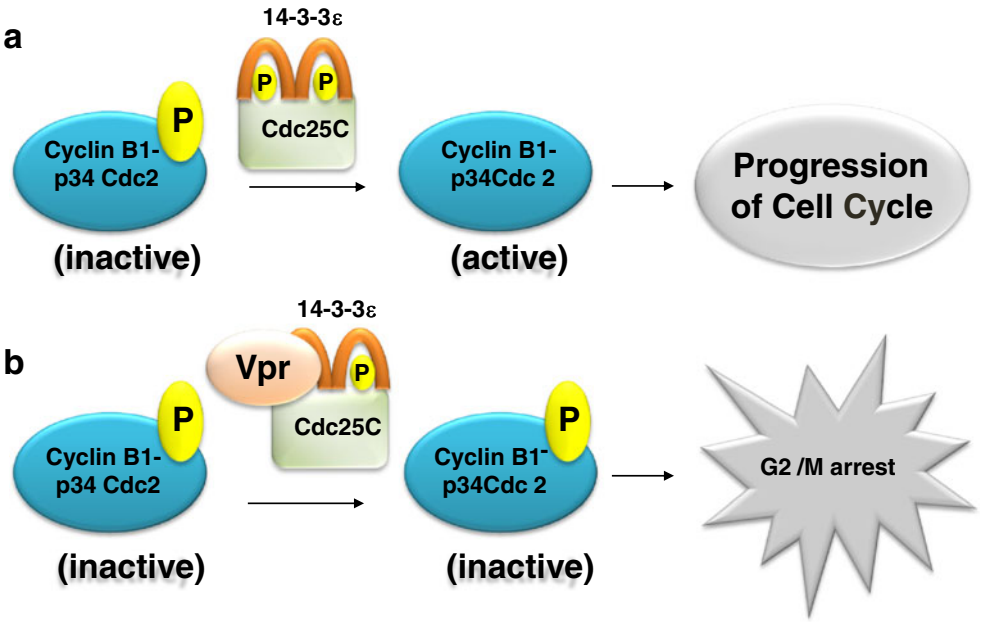

Fig. 2 Vpr functions and molecular interactions with 14-3-3e and Cdc25C to induce cell-cycle arrest. **a** 14-3-3ε proteins bind to Cdc25C, resulting in a complex that promotes phosphatase activity. The complex removes the phosphate molecule from the inactive form of cyclin B1-p 34 Cdc2, altering it to the active form that drives the progression of the cell cycle. **b** Vpr binds to the 14-3-3ε protein and Cdc25C and inactivates this complex. In the absence of the phosphatase activity of Cdc25C, cyclin B1-p34Cdc2 remains inactive, resulting in G2 arrest

contributes to Vpr-dependent G2/M cell-cycle arrest (Table 4) (Bolton et al. 2008; Matsuda et al. 2006). The 14-3-3η and σ isotypes have also been shown to bind directly to Vpr in a complex with Cdc25, which also promotes cell-cycle arrest (Table 4) (Kino et al. 2005b; Kino and Pavlakis 2004). In addition, Vpr disrupts 14-3-3η and σ, binding to a member of the Forkhead transcription factor (FoxO), FoxO3a, resulting in tissue-selective insulin resistance, a condition often presented by HIV-1-infected individuals (Table 4) (Kino et al. 2005a). Collectively, this evidence suggests a strong relationship between the Vpr accessory protein and 14-3-3 proteins mediating cell-cycle arrest that ultimately leads to neurodegeneration.

G protein receptor 15

G protein cell receptors (GPCRs) (Bernier et al. 2004) are cell-surface receptors, whose role in the pathophysiology of human diseases is dependent on their density (Dunham and Hall 2009). One GPCR that is expressed in the T cells of both HIV-1- and SIV-infected subjects, GPR15/BOB, serves as a co-receptor for the virus (Farzan et al. 1997; Unutmaz et al. 1998). 14-3-3 proteins play a role in the trafficking of GPR15/BOB, hence controlling its cell-surface density in response to phosphorylation signals (Table 2) (Chung et al. 2009; Okamoto and Shikano. 2011). Furthermore, 14-3-3ε binding substantially increases the stability of GPR15 (Table 2) (Shikano et al. 2005).

In summary, these data suggest that there is a strong relationship between HIV accessory and 14-3-3 proteins and that the latter, in addition to providing potential biomarkers for infection and disease progression, might be also utilized in the development of therapeutic interventions.

14-3-3s and the hepatitis C virus core protein

Best estimates are that 20–30 % of HIV-infected individuals and as high as 90 % within the infected intravenous drug users are also co-infected with the HCV. HCV infection results in liver diseases and accelerates death in those with HIV infection (Bica et al. 2001; Hernandez and Sherman 2011). In co-infected individuals, there appears to be a link with neurocognitive impairments (Anand et al. 2010; Letendre et al. 2007) and the development of HAD (Nath et al. 2008; Valcour et al. 2011). Expression of HCV core proteins leads to the translocation of Bcl-2–associated X (Bax) protein from the cytosol to the mitochondria, where it leads to apoptosis (Aoki et al. 2000). 14-3-3ε binds to the HCV core protein and blocks Bax binding leading to caspase-dependent and independent apoptotic pathways (Lee et al. 2007). In addition, binding with HCV core protein activates Raf-1 kinase, which in turn affects hepatocyte growth regulation (Aoki et al. 2000; Nakamura et al. 2011). Interaction between 14-3-3s and FoxO1 is also important in translocation from the nucleus to the cytoplasm, which is blocked in HCV core-expressing cells (Banerjee et al. 2010). The direct interaction between 14-3-3s and HCV core proteins emphasize the importance of understanding 14-3-3s in HIV disease progression and underlines their potential as therapeutic targets in co-infected individuals.

What are the consequences of genetic alterations to YWHAE/14-3-3ε?

YWHAE/14-3-3ε is expressed in cultured astrocytes and in the cerebral cortex, corpus callosum, frontal lobe, parietal lobe, temporal lobe, medulla oblongata, hippocampus, pons,

Table 3 *YWHAE/14-3-3ε* genetic alterations in human neurological disorders

Genetic alteration	Human condition/disease	Effect/symptoms	References
Deletion along with TUSC5, MYO1C, CRK, LIS1	Miller–Dieker syndrome	Severely reduced intellectual abilities, developmental delay, seizures	Bi et al. 2009
Duplication with TUSC5, MYO1C, CRK, LIS1	Developmental delay	ADHD, autism	Bi et al. 2009
Deletion with TUSC5	Developmental delay	Learning difficulties	Bi et al. 2009
Microdeletion with CRK, LIS1	Miller–Dieker syndrome with ILS	Severe brain malformations, cortical thickening	Cardoso et al. 2003
Microduplication with TUSC5	Miller–Dieker syndrome with autism	Autistic behavior	Bruno et al. 2010
Microduplication with LIS1	Miller–Dieker syndrome	Moderate psychomotor retardation, speech delays, behavioral problem	Hyon et al. 2011
Deletion with CRK but not LIS1	Miller–Dieker syndrome with epilepsy	Generalized epilepsy, developmental delay, and non-specific white matter changes	Shimojima et al. 2011; Tenney et al. 2011
Polymorphisms	Schizophrenia	Frequency of SNPs different in cases vs. controls	Ikeda et al. 2008
Polymorphisms	Schizophrenia, bipolar disorder	No association	Liu et al. 2011
Polymorphism (rs34137556)	Schizophrenia	No association	Moens et al. 2011

ILS isolated lissencephaly, *CRK* viral oncogene causes increased tyrosine-phosphorylated proteins, *TUSC5* tumor suppressor candidate 5, *MYO1C* myosin-1C, *LIS1* encodes subunit of platelet-activating factor acetylhydrolase 1B (PAFAH1B1), *ADHD* attention deficit hyperactivity disorder

and cerebellum (Mignon-Ravix et al. 2010). In animal models, 14-3-3ε is present homogeneously throughout brain neuropil areas and in high levels in synapses, co-localizing with synaptosomes (Baxter et al. 2002b; Bi et al. 2009; Martin et al. 1994), suggesting that 14-3-3ε may serve as a good biomarker in the CNS for degenerating synapses and by extension neurodegeneration in general. But what happens in the brain if there are genetic alterations of *YWHAE*?

In humans, Miller–Dieker syndrome is caused by a deletion or duplication of genes on the 17p13 chromosome including YWHAE (Table 3) (Bi et al. 2009; Bruno et al. 2010; Cardoso et al. 2003; Hyon et al. 2011; Shimojima et al. 2011; Tenney et al. 2011). *YWHAE* appears to be the crucial gene, depending on which other genes are affected, whose loss leads both to neurocognitive deficits including learning disabilities, autism, epilepsy, and attention deficient hyperactivity disorder (ADHD), and to lissencephaly (Table 3) (Bi et al. 2009; Bruno et al. 2010; Cardoso et al. 2003; Hyon et al. 2011; Shimojima et al. 2011; Tenney et al. 2011). In animal models, both the duplication and the deletion of *YWHAE* lead to anomalous neuronal migration, which likely underlies the lissencephaly phenotypes (Table 4) (Bi et al. 2009; Spalice et al. 2009; Toyo-oka et al. 2003; Yingling et al. 2003). Heterozygous mice present reduced learning and memory and heightened anxiety (Table 4) (Ikeda et al. 2008), suggesting that YWHAE is essential for normal neuronal development and function.

Given the apparent importance of *YWHAE* in neuronal structure and function, are there polymorphisms in the gene associated with pathologies? Single nucleotide polymorphisms (SNP) in *YWHAE* were assayed for a possible relationship with schizophrenia, which is a complex mental disorder with a fairly high degree of heritability (Table 3) (Ikeda et al. 2008). Only one study has found SNPs associated with schizophrenia and others apparently associated with reduced risk for the condition (Table 3) (Ikeda et al. 2008). This suggests that perhaps increased *YWHAE* expression in humans carrying the identified SNP is protective.

Table 4 *YWHAE/14-3-3ε* genetic alterations in models of neurological impairments

Alteration	Effect	References
Deletion along with TUSC5, MYO1C, CRK, PAFAH1B1 in mice	Mild to severe migration abnormalities	Bi et al. 2009
Duplication along with TUSC5, MYO1C, CRK, PAFAH1B1 in mice	Mild brain anomalies	Bi et al. 2009
Deletion along with TUSC5 in mice	Mild to severe migration abnormalities	Bi et al. 2009
Ywhae$^{-/-}$ mice	Defect in brain development and neuronal migration	Toyo-oka et al. 2003
Ywhae$^{+/-}$ mice	Impaired working memory in radial arm maze and enhanced anxiety in plus maze	Ikeda et al. 2008

However, two other studies indicate no association between *YWHAE* SNPs in schizophrenia or bipolar disorders (Table 3) (Liu et al. 2011; Moens et al. 2011). Another study examined YWHAE SNPs from individuals who committed suicide and proposed that it is a potential suicide susceptibility gene (Yanagi et al. 2005). The effects of polymorphism (if any) in rodent models have not been reported yet. We propose that studies should examine if there is an association between HIV and HCV neurodegeneration and the YWHAE SNPs and whether there are other polymorphisms in other 14-3-3 isoforms related to HAND and/or other neurodegenerative disorders.

Is YWHAE/14-3-3ε a biomarker for HIV-dependent neurodegeneration?

Can 14-3-3ε protein levels be used to track disease progression? This is supported by studies indicating that 14-3-3ε is present in the CSF in those with HIVE and/or HAD (Gelman and Nguyen 2010). 14-3-3ε does interact with Vpr, modulating G(2)/M cell-cycle arrest via Cdc25C phosphorylation-dependent association (Fig. 2) (Matsuda et al. 2006). Also, it directly interacts with GPR15 HIV accessory protein to modulate receptor stability (Shikano et al. 2005). In addition, gp120 levels, which regulate cell cycle and apoptosis, are inversely related to those of 14-3-3ε (Fig. 2) (Kapasi et al. 2001). In human 293T cells, cleavage of 14-3-3ε releases BAD, facilitating its translocation and subsequent interaction with Bcl to promote cell death (Fong et al. 2010; Won et al. 2003). 14-3-3ε also interacts with the core protein of HCV a commonly co-infecting virus in HIV patients an interaction which regulates apoptosis. There is also evidence that in normal cells, 14-3-3ε is necessary for maintaining neuronal integrity by promoting both survival and neuronal regeneration (Berg et al. 2003a; Datta et al. 2000). Therefore, the collective evidence clearly indicates that 14-3-3ε is involved in multiple processes implicated in HIV pathogenesis and disease progression.

Conclusion

14-3-3s are present in the CSF of those with HIV. Many of the isoforms, including YWHAE/14-3-3ε, either directly or indirectly interact and modulate HIV-related proteins involved in BBB trafficking, stability of receptors, apoptosis, and cell-cycle arrest. Taking that into consideration, we propose that 14-3-3ε would be an appropriate biomarker for HIV-related neurodegeneration and that, additionally, it may also offer a target for therapeutic intervention.

We propose that the presence of 14-3-3 proteins in the CSF of HIV seropositive patients is likely the consequence of apoptotic or necrotic lysis of neurons and their release in the CSF of HIV-infected patients. In humans, CSF volume is about 150 ml, and the rate of CSF production is about 550 ml/day, indicating that 14-3-3 proteins in CSF are turned over about 3.7 times per day (Thomson and Bertram 2001; Wakabayashi et al. 2001). Hence, 14-3-3 proteins in CSF might be a biomarker reflecting the state of neuronal destruction and neurodegeneration. However, further studies looking at the prognostic significance of specific antibodies against 14-3-3 isoforms are required.

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