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Citation for published version:
Lewis, V, Whitehouse, IJ, Baybutt, H, Manson, JC, Collins, SJ & Hooper, NM 2012, 'Cellular Prion Protein Expression Is Not Regulated by the Alzheimer's Amyloid Precursor Protein Intracellular Domain' PLoS One, vol 7, no. 2, ARTN e31754. DOI: 10.1371/journal.pone.0031754

Digital Object Identifier (DOI):
10.1371/journal.pone.0031754

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
PLoS One

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Cellular Prion Protein Expression Is Not Regulated by the Alzheimer’s Amyloid Precursor Protein Intracellular Domain

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Abstract

There is increasing evidence of molecular and cellular links between Alzheimer’s disease (AD) and prion diseases. The cellular prion protein, PrPC, modulates the post-translational processing of the AD amyloid precursor protein (APP), through its inhibition of the β-secretase BACE1, and oligomers of amyloid-β bind to PrPC which may mediate amyloid-β neurotoxicity. In addition, the APP intracellular domain (AICD), which acts as a transcriptional regulator, has been shown to control the expression of PrPC. Through the use of transgenic mice, cell culture models and manipulation of APP expression and processing, this study aimed to clarify the role of AICD in regulating PrPC. Over-expression of the three major isoforms of human APP (APP695, APP751 and APP770) in cultured neuronal and non-neuronal cells had no effect on the level of endogenous PrPC. Furthermore, analysis of brain tissue from transgenic mice over-expressing either wild type or familial AD associated mutant human APP revealed unaltered PrPC levels. Knockdown of endogenous APP expression in cells by siRNA or inhibition of γ-secretase activity also had no effect on PrPC levels. Overall, we did not detect any significant difference in the expression of PrPC in any of the cell or animal-based paradigms considered, indicating that the control of cellular PrPC levels by AICD is not as straightforward as previously suggested.

Introduction

Alzheimer’s disease (AD) and prion diseases fall within the spectrum of neurodegenerative diseases which are causally linked to misfolded and aggregated proteins. Due to similarities in various structural elements and proteolytic processing events involving the major proteins involved in these diseases, potential links and parallels in both disease mechanisms and possible therapeutic avenues have been proposed [1,2,3,4]. Increasingly, recent studies have shown more direct molecular links between AD and prion diseases. A substantive molecular link was provided when PrPC expression was shown to control the expression of PrPC. Through the use of transgenic mice, cell culture models and manipulation of APP expression and processing, this study aimed to clarify the role of AICD in regulating PrPC. Over-expression of the three major isoforms of human APP (APP695, APP751 and APP770) in cultured neuronal and non-neuronal cells had no effect on the level of endogenous PrPC. Furthermore, analysis of brain tissue from transgenic mice over-expressing either wild type or familial AD associated mutant human APP revealed unaltered PrPC levels. Knockdown of endogenous APP expression in cells by siRNA or inhibition of γ-secretase activity also had no effect on PrPC levels. Overall, we did not detect any significant difference in the expression of PrPC in any of the cell or animal-based paradigms considered, indicating that the control of cellular PrPC levels by AICD is not as straightforward as previously suggested.

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major neuronal splice variant. Recently, we reported that only the AICD produced from the β- and γ-secretase cleavage of APP695, and not that produced from the other two isoforms, is transcriptionally active as assessed by its ability to upregulate neprilysin expression [19]. This transcriptionally active AICD was only produced in neuronal (SH-SY5Y and N2a) cell lines and was not functional in non-neuronal human embryonic kidney (HEK293) cells [19]. Further, AICD produced from the familial AD associated Swedish mutant form of APP695, known to be subject to increased BACE1 cleavage compared to wild type APP695 [21], was more transcriptionally active relative to wild type APP695 [19].

The molecular and cellular links between APP and PrPC were extended recently when PrP C expression was reported to be regulated by AICD [22]. Overexpression of APP751 in HEK cells triggered a significant increase in PrP C immunoreactivity, while a reduction in PrP C was observed in APP deficient fibroblasts. The γ-secretase inhibitor DAPT significantly reduced PrP C levels in primary neurons, implicating a role for AICD in controlling the expression of PrP C [22]. The aim of the present study was to clarify the role of AICD in the regulation of PrP C and to specifically determine whether, similar to the control of neprilysin expression [19], there was an APP isoform effect.

Results

Over-expression of APP does not alter endogenous PrP C protein expression

Initially we sought to replicate the findings of Vincent et al. [22] by expressing APP751 in HEK cells. In addition, we looked to advance this research by determining whether the control of PrP C expression by AICD was specific to a particular APP isoform. HEK cells stably over-expressing either APP695, APP751 or APP770, alongside a vector only control were assessed for total cell associated PrP C and APP protein levels by western blotting (Fig. 1A and B). Surprisingly, in contrast to previously published results [22], although there was a significant 2-3-fold increase in APP in the cells transfected with any of the three APP isoforms, there was no significant difference in PrP C level in any of the APP isoform expressing cells when compared to the Hyg vector-only controls.

We have recently shown that transcriptionally active AICD is only produced by the BACE1 and γ-secretase cleavage of the APP695, APP751, and APP770 isoforms, and not by the other two isoforms [19]. Further, we also showed that the transcriptionally active AICD produced from the APP695, APP751, and APP770 isoforms was not functional in non-neuronal human embryonic kidney (HEK293) cells [19]. Therefore, we hypothesized that the control of PrP C expression by AICD was specific to a particular APP isoform. The aim of the present study was to clarify the role of AICD in the regulation of PrP C and to specifically determine whether, similar to the control of neprilysin expression [19], there was an APP isoform effect.

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Figure 1. Over-expression of APP isoforms in HEK cells does not alter endogenous PrP C. (A) Representative western blot of APP and PrP C (antibody 3F4) in HEK cells stably transfected with either the vector alone (Hyg) or one of the APP isoforms (APP695, APP751, APP770), and subsequent β-actin staining to allow adjustments for equal protein loading. Approximate molecular weights (kDa) are indicated. (B) Quantification of APP and PrP C protein levels expressed relative to Hyg control cells (dashed line). Data from 4 independent experiments. Statistical analysis by one way ANOVA with Dunnett’s post test comparison to the Hyg cells, ***p<0.001, **p<0.01, n.s. not significant.

doi:10.1371/journal.pone.0031754.g001

PrPC Is Not Regulated by AICD

Figure 1. Over-expression of APP isoforms in HEK cells does not alter endogenous PrP C. (A) Representative western blot of APP and PrP C (antibody 3F4) in HEK cells stably transfected with either the vector alone (Hyg) or one of the APP isoforms (APP695, APP751, APP770), and subsequent β-actin staining to allow adjustments for equal protein loading. Approximate molecular weights (kDa) are indicated. (B) Quantification of APP and PrP C protein levels expressed relative to Hyg control cells (dashed line). Data from 4 independent experiments. Statistical analysis by one way ANOVA with Dunnett’s post test comparison to the Hyg cells, ***p<0.001, **p<0.01, n.s. not significant.

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APP<sub>695</sub> isoform in neuronal cells [19]. In light of this, and the negative result observed in the non-neuronal HEK cells, we utilized mouse neuronal N2a cells over-expressing human APP<sub>695</sub> or APP<sub>751</sub> to again assess total PrP<sup>C</sup> and APP protein levels (Fig. 2A and B). Despite a significant 2.5-fold increase in APP expression in the N2a cells transfected with the cDNAs encoding either APP<sub>695</sub> or APP<sub>751</sub>, there was no difference in endogenous PrP<sup>C</sup> levels when comparing the APP over-expressing cells with each other or the vector only controls.

To further examine the effect of APP over-expression on PrP<sup>C</sup> levels, two transgenic mouse models were investigated. PrP<sup>C</sup> and APP protein levels were evaluated in brain homogenates from I5 mice which over-express wild type human APP, J20 mice which over-express human APP containing the Swedish/Indiana familial AD mutations [23] and non-transgenic matched genetic background control mice (Fig. 3A and B). Despite a significant 2.8-fold increase in APP in the transgenic I5 mice, as compared to the non-transgenic mice, there was no difference in brain PrP<sup>C</sup> levels.
Analysis of the J20 mice, although only involving two animals, reinforced this conclusion. Collectively these over-expression experiments indicate that control of PrPC expression does not appear to involve AICD in either cell-based or transgenic animal paradigms.

Reduction of AICD production through APP gene silencing or γ-secretase inhibition does not alter expression of endogenous PrPC

In light of the above results, we considered whether the level of AICD required to regulate PrPC in the cell lines or the transgenic mice were already maximal from the endogenous APP, such that the AICD produced from the over-expressed APP was not having any additional affect on PrPC expression. Thus we sought to investigate a possible role for endogenous AICD in the control of PrPC expression. First, to reduce endogenous APP levels and thereby remove the substrate for AICD production, N2a cells were treated with siRNA against murine APP. Cells were harvested, lysed and PrPC and APP levels measured by western blotting (Fig. 4A and B). After directed siRNA treatment there was a significant 70% decrease in total APP levels (endogenous AICD level is below the limits of detection by immunoblot; data not shown). However, the amount of PrPC remained unchanged following siRNA knockdown of endogenous APP.

In order to test further for a possible involvement of endogenous AICD in controlling PrPC expression, both HEK and N2a cells were treated with DAPT, a cell permeable γ-secretase inhibitor. Again, whole cell lysates were assessed for PrPC and APP expression, as well as the levels of C83 and C99, by western blotting (Fig. 5A and B). Although DAPT treatment inhibited γ-secretase activity, as shown by the significantly increased C83 and C99 levels (9.4-fold in the HEK cells and 17.8-fold in the N2a cells), there was no difference in endogenous PrPC protein levels in the DAPT treated cells as compared to the untreated cells (Fig. 5C and D). Together these results indicate that in both a neuronal and a non-neuronal cell line, endogenous AICD is also not involved in the control of PrPC protein expression.
Discussion

Similarities in the pathogenesis of the protein-misfolding neurodegenerative illnesses, especially AD and prion diseases, and possible connections between these diseases have long been contemplated [1,2,3,4]. Elucidation of any functional links between these diseases is an important research goal, with determination of the most appropriate protein or process to target for development of therapeutics being paramount. Links in the pathologies of AD and prion diseases have been determined, with various reports of AD features in prion disease brains [24,25,26], and PrPC localized in Aβ plaques in AD brain [27,28]. In addition, a polymorphism at codon 129 of the prion protein gene, known to influence susceptibility to sporadic and iatrogenic human prion disease [29,30], may also influence susceptibility and the pathophysiology of AD [31,32,33]. Interestingly there is some indication of a more direct interaction between Aβ and PrPC, with the finding of an acceleration and exacerbation of both AD and prion disease pathologies in animals engineered to have both of these diseases, and enhanced protein misfolding due to cross-seeding events stimulating oligomerization in vitro [34]. This propensity for cross-seeding highlights the importance for a more complete understanding of interactions between these key proteins and any resultant downstream consequences.

Recent studies have provided evidence of direct interactions between the proteins central to AD and prion diseases. Various studies have determined that the cellular prion protein can act as a receptor for Aβ, with Aβ oligomers binding to PrPc with high affinity, although there are conflicting views as to the physiological significance of this binding. Some results suggest that Aβ synaptic toxicity is mediated through its binding to PrPc [7,11,12], which specifically impacts on spatial learning and memory in vivo [35], whereas others have reported that Aβ oligomer neurotoxicity occurs independently [8,9]. Confounding the relationship between these key proteins, and in apparent contrast to PrPc mediating Aβ neurotoxicity, PrPC has been shown to decrease production of Aβ from wild type APP through its interaction with the β-secretase BACE1 [5]. This interaction, mapped to the BACE1 pro-domain, leads to slowed BACE1 trafficking following exit from the ER, thereby increasing its localization in the trans-Golgi network and reducing levels at the cell surface and consequently in endosomes where APP β-cleavage occurs [6]. Importantly, these studies also ascertained links in the pathology of AD and prion diseases. It was found that human prion disease-associated mutations in PrPc did not inhibit BACE1, and scrapie infected mice brains contained dramatically higher Aβ levels [5], suggesting a loss of PrPc function perhaps as a result of PrPc→PrPSc conversion during prion disease progression.

PrPc may be a key therapeutic target for sporadic AD, and the recent report that PrPc expression was controlled by AICD in a γ-secretase dependent manner [22] presented a potential avenue for achieving this. Further, a possible feedback model reconciling the control of APP processing and PrPc expression in both normal conditions and in the presence of increased Aβ such as that seen in AD was proposed [36]. Therefore our study was carried out to further understand the relationship between AICD production and PrPc expression. However, utilizing a range of experimental approaches we found no evidence for AICD involvement in PrPc expression. This is despite using a cellular system (N2a cells expressing APP695) in which we have proven that AICD is transcriptionally active [19]. If AICD is involved in regulating the transcription of PrPC, then the mechanism underlying this is more complex than that involved in regulating the expression of neprilysin and is not readily reproduced in cultured cells or transgenic mice over-expressing human APP. Our findings have implications for the continued investigation and design of possible AD therapeutics.

Materials and Methods

Ethics statement

All experimental procedures performed on mice were approved by the Roslin Institute (University of Edinburgh) Ethical Review Process Committee and carried out under the UK Home Office License 60/3478. Human embryonic kidney (HEK) cells were obtained from the European Collection of Cell Cultures and

Figure 4. Knockdown of APP expression in N2a cells has no effect on PrPc protein levels. (A) Representative western blots of APP and PrPc (antibody 6H4) in N2a cells treated with APP directed siRNA, non-coding control siRNA, and a no RNA transfection control (H2O control), and subsequent β-actin staining. Approximate molecular weights (kDa) are indicated. (B) Quantification of APP and PrPc protein levels expressed relative to the H2O control cells. Data from 3 independent experiments. Statistical analysis by one way ANOVA with Dunnett’s post test comparison to the H2O control cells, **p<0.01, n.s. not significant.

doi:10.1371/journal.pone.0031754.g004
murine neuroblastoma (N2a) cells were obtained from Dr Lehmann, Université Montpellier, France [37].

**Cell culture**

HEK cells and N2a cells stably over-expressing the human APP isoforms (APP695, APP751 and APP770), alongside the vector-only (Hyg), were generated by electroporation as described previously [19]. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Lonza, Basel, Switzerland) containing 10% (v/v) fetal bovine serum (FBS; Biosera, East Sussex, UK) and 1% penicillin/streptomycin (Lonza), in a humidified incubator at 37°C, 5% CO2.

**APP gene silencing**

To ablate endogenous APP expression in the N2a cells, cells were grown to 80% confluency in growth medium prior to treatment with 50 nM final concentration of murine APP directed siRNA, non-coding siRNA or siRNA-free controls following the manufacturer’s instructions (Thermo Fisher Scientific, Lafayette, CO, USA). Briefly, sub-confluent cell monolayers were washed gently with OptiMEM (GIBCO, Invitrogen, Glasgow, UK), before further incubation in OptiMEM for approximately 30 min (37°C, 5% CO2) during siRNA preparation. A 10 µM siRNA solution in 1x siRNA buffer of either murine APP directed siRNA (ON-TARGETplus SMARTpool) or non-coding siRNA control (ON-
TARGETPlus Non-targeting pool was prepared, and diluted to 1 μM in OptiMEM. For the RNA-free control, sterile RNase-free water was diluted 1:10 in OptiMEM. DharmaFECT Transfection Reagent-1 was diluted 1:40 in OptiMEM, mixed gently and incubated for 5 min at room temperature. Equal volumes of the diluted siRNA/control and DharmaFECT solutions were then mixed and incubated for 20 min at room temperature, prior to the addition of 4× volumes of OptiMEM containing 10% (v/v) FBS. The OptiMEM was then removed from the cells, and replaced with the OptiMEM/FBS/siRNA complexes (or control) for 72 h.

Inhibition of γ-secretase

To inhibit endogenous γ-secretase activity, HEK or N2a cells were grown to 90–95% confluence prior to treatment. The cell monolayer was then washed twice with PBS prior to incubating the cells in serum-free OptiMEM containing a final concentration 10 μM N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT; Sigma-Aldrich, Dorset, UK), or an equal volume of dimethyl sulfoxide (DMSO, as the control) for 24 h.

Cell lysis, SDS-PAGE and immunoblotting

When confluent and/or after appropriate treatments as described above, cells were washed twice in phosphate-buffered saline (PBS with Ca2+ and Mg2+; Lonza), harvested by scraping into PBS, and pelleted at 500 g for 3 min. Cell pellets were lysed for 30 min on ice in cold lysis buffer (25 mM Tris/HCl, pH7.5, 150 mM NaCl, 5 mM EDTA, 1% (v/v) Triton X-100) containing Complete, a protease inhibitor cocktail (Roche, West Sussex, Switzerland), as indicated in the figure legends. For detection of PrPC the membrane was incubated with either the monoclonal antibody 22C11 (Millipore, Billerica, MA, USA), or the secondary antibody described above. All chemiluminescent images were captured by a Fujifilm LAS-3000 Intelligent Dark Box.

 western blotting as described above for cell lysates.

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(Sigma-Aldrich) was used. After washing off non-specifically bound primary antibody with PBST, membranes were incubated in peroxidase-conjugated rabbit-anti-mouse or goat-anti-rabbit secondary antibodies (Sigma-Aldrich), before further washes with PBST and detection using enhanced chemiluminescence (Fierce ECL substrate; Thermo Fisher Scientific). To assess and correct for protein loading, membranes were stripped at low pH [1% (v/v) aqueous HCl] for approximately 30 min, re-blocked and probed with an anti-β-actin antibody (clone AC-15, Sigma-Aldrich) and the secondary antibody described above. All chemiluminescent images were captured by a Fujifilm LAS-3000 Intelligent Dark Box.

Transgenic mice and tissue homogenisation

Animals were obtained from The Jackson Laboratory (Bar Harbor, ME, USA), and all care was carried out in strict accordance with institutional guidelines. Transgenic I5 mice [Line B6.Cg-Tg(PDGFβ-APP)5 J20 (5–9 weeks old) and age-matched non-transgenic littermate controls were homogenized in 2% (w/v) SDS solution containing protease inhibitors, and homogenates centrifuged at 100,000 g for 1 h at 4°C. The resultant supernatant was assayed for total protein and assessed for PrPβ and APP protein by SDS-PAGE and western blotting as described above for cell lysates.

Densitometry and statistical analysis

Quantification and densitometric analyses were carried out using Image J v1.42q. Within each experiment, data was normalised to β-actin, and expressed relative to the control samples. Statistical analyses were performed in GraphPad Prism v5.03. All quantitative data are expressed as the mean ± SEM, unless stated otherwise.

Author Contributions

Conceived and designed the experiments: VL IJW HB. Analyzed the data: VL IJW HB JCM NMH. Wrote the paper: VL IJW NMH.

References

1. Winiarski T, Sigurdsson EM (2007) Therapeutic approaches for prion and Alzheimer’s disease. FEBS J 274: 3784–3798.
2. Checler F, Vincent B (2002) Alzheimer’s and prion diseases: distinct pathologies, common proteolytic denominators. Trends Neurosci 25: 616–620.
3. Taylor DR, Hooper NM (2007) Role of lipid rafts in the processing of the pathogenic prion protein and Alzheimer’s amyloid-β-betas. Semin Cell Dev Biol 18: 638–646.
4. Barnham KJ, Cappai R, Beyreuther K, Masters CL, Hill AF (2006) Delineating common molecular mechanisms in Alzheimer’s and prion diseases. Trends Biochem Sci 31: 465–472.
5. Parkin ET, Watt NT, Hussain I, Eckman EA, Eckman CB, et al. (2007) Cellular prion protein regulates beta-secretase cleavage of the Alzheimer’s amyloid precursor protein. Proc Nat Acad Sci USA 104: 11062–11067.
6. Grifhins HH, Whitehouse JJ, Baybutt H, Brown D, Kellett KA, et al. (2011) PrP protein interacts with BARCE1 protein and differentially regulates its activity toward wild type and Swedish mutant amyloid precursor protein. J Biol Chem 286: 35499–35509.
7. Laurent J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. Nature 457: 1129–1132.
8. Baldacci C, Heg M, Stravalaci M, Bastone A, Sicil A, et al. (2010) Synthetic amyloid-β oligomers impair long-term memory independently of cellular prion protein. Proc Nat Acad Sci USA 107: 2295–2300.
9. Galella AM, Farinelli M, Nuvolone M, Miraute O, Moos R, et al. (2010) Prion protein and Abeta-related synaptic toxicity impairment. EMBO Mol Med 2: 306–314.
10. Chen S, Yadav SP, Surewicz WK (2010) Interaction between human prion protein and amyloid-beta (Abeta) oligomers: role of N-terminal residues. J Biol Chem 285: 26377–26383.
11. Barry AE, Klyubin I, Mc Donald JM, Mably AJ, Farrell MA, et al. (2011) Alzheimer’s disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. J Neurosci 11: 464–472.
12. Freir DR, Nicoll AJ, Klyubin I, Panico S, Mc Donald JM, et al. (2011) Interaction between prion protein and toxic amyloid beta assemblies can be therapeutically targeted at multiple sites. Nat Commun 2: 336.
13. Vardy ER, Catto AJ, Hooper NM (2005) Proteolytic mechanisms in amyloid-beta metabolism: therapeutic implications for Alzheimer’s disease. Trends Mol Med 11: 464–472.
14. Cao X, Sudhof TC (2001) A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. Science 293: 115–120.
15. Belyaev ND, Nalivaeva NN, Makova NZ, Turner AJ (2009) Neprilysin gene expression requires binding of the amyloid precursor protein intracellular domain to its promoter: implications for Alzheimer disease. EMBO Rep 10: 94–100.
16. Pardossi-Piquard R, Petit A, Kasaizai T, Sunyach C, Abes da Costa C, et al. (2005) Presenilin-dependent transcriptional control of the Abeta-degrading enzyme neprilysin by intracellular domains of betaAPP and APLP. Neuron 46: 541–554.

17. Goodger ZV, Rajendran L, Trutzel A, Kohli BM, Nisch RM, et al. (2009) Nuclear signaling by the APP intracellular domain occurs predominantly through the amyloidogenic processing pathway. J Cell Sci 122: 3703–3714.

18. Hoyer SE, Williams RJ, Perkinton MS (2009) Synaptic NMDA receptor activation stimulates alpha-secretase amyloid precursor protein processing and inhibits amyloid-beta production. J Neurosci 29: 4442–4460.

19. Belyaev ND, Kellett KA, Beckett C, Makova NZ, Revett TJ, et al. (2010) The transcriptionally active amyloid precursor protein (APP) intracellular domain is preferentially produced from the 695 isoform of APP in a beta-secretase-dependent pathway. J Biol Chem 285: 41443–41454.

20. Tanaka S, Nakamura S, Ueda K, Kameyama M, Shiojiri S, et al. (1988) Three types of amyloid protein precursor mRNA in human brain: their differential expression in Alzheimer’s disease. Biochem Biophys Res Commun 157: 472–479.

21. Citron M, Oltersdorf T, Haas C, McConlogue L, Hung AY, et al. (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer’s disease increases beta-protein production. Nature 360: 672–674.

22. Vincent B, Sunyach C, Orzechowski HD, St George-Hyslop P, Checler F (2009) p53-Dependent transcriptional control of cellular prion by presenilins. J Neurosci 29: 6752–6760.

23. Nishida N, Harris DA, Vilette D, Laude H, Frobert Y, et al. (2000) Successful transmission of three mouse-adapted scrapie strains to murine neuroblastoma cell lines overexpressing wild-type mouse prion protein. J Virol 74: 320–3.