Review paper

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New light on prions: putative role of co-operation of PrP^c and Aβ proteins in cognition

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Abstract

A seminal article of Takahashi et al. reporting concomitant accumulation of cellular prion protein (PrP^c) and β-amyloid (Aβ) in dystrophic neurites, within neuritic plaques raised an exciting issue that is important for our understanding of mechanisms of neurodegeneration. The mentioned authors interpreted their findings rather cautiously, however since the time of their publication, several reports representing different approaches and methods have seemed to indicate that both proteins appear to co-operate more intrinsically than it could have been imagined earlier. The goal of the review is to sum up the accruing research data with special attention to evidence pointing to the co-operative role of PrP^c and Aβ in cognitive impairment.

Key words: *prion protein, β-amyloid, cognitive impairment, Alzheimer disease, tau protein.*

Introduction

Though Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD) are characterized by distinct neuropathological changes, they share common pathological features. They are both conformational diseases, related to accumulation of altered proteins, which results in a loss of global cognitive functions. Alzheimer's disease is a predominant neurodegenerative disorder characterized by two major pathological changes: amyloid plaques and neurofibrillary tangles. Amyloid plaques are extracellular formations consisting of β-amyloid (Aβ) and cellular material outside and around neurons. Neurofibrillary tangles are intracellular aggregates of microtubule-associated tau protein, which has become hyperphosphorylated and misfolded. Creutzfeldt-Jakob disease is a rapidly progressive brain disease caused by infectious-like self-perpetuating mechanism leading to conversion of physiological cellular prion protein (PrP^c) to its Scrapie conformation (PrP^{Sc}) [52]. PrP^{Sc} creates extremely stable forms, which accumulate in infected tissue resulting in its spongiform degeneration [53]. There are little data concerning interactions between prion proteins and Aβ, both in their physiological and conformationally changed form with regard to cognitive functions and dementia. Takahashi *et al*. reported concomitant accumulation of PrP^c with Aβ in dystrophic neurites within one of the amyloid plaque types, called neuritic plaques [82]. Also Aβ was found to be deposited with PrP^{Sc} in CJD [26]. Due to the suspicious findings of those proteins within the same individual, speculations arise

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around the possibility of connection between the pathophysiological processes occurring in these two neuropathological conditions [9,70]. These interactions may also have an impact on cognitive performance. Therefore, the following question arises: do prions and Aβ co-operate in cognitive impairment? Here, we review recent findings at the crossroads of cognitive neuroscience and neuropathology in order to expose the independent role of these proteins in cognition and their possible interactions, and to seek the answer to this question.

Prion protein

PrP^c is a highly conserved protein, which may be found in most of vertebrates, at every stage of their development and in all types of tissues, especially in the nervous system [79]. PrP^c is a glucolipid-anchored cell membrane sialoglycoprotein, which is localized in raft-like microdomains [57]. It is presented on the presynaptic and postsynaptic membranes [91] of neurons, of many brain areas including hippocampus and the cortex [73]. Membrane-anchored PrP^c passes internalization and recycling through the endosomal pathway [58]. It becomes internalized and degraded in lysosomes [68] or is released into the extracellular space [77]. Proposed roles in the physiology of this protein are related to its localization on the cell surface. PrP^c may act as a cellular adhesion molecule which plays a part in neurite outgrowth, neuronal differentiation and survival [54]. These functions are consistent with a finding that PrP^c can associate with surface proteins like laminin, laminin receptor precursor, and the neural cell adhesion molecule (NCAM) [6]. Animal studies have also revealed that absence of PrP^c is connected with disrupted olfactory physiology and behaviour [79], altered circadian clock, modification of sleep pattern [37], and increased sensitivity to seizures [85]. New reports bring new data indicating various potential features of this protein, making it difficult to find its one consistent function.

A growing number of works on mice models and on humans indicate a possible role of prion protein in the area of cognitive functions. The most common source of those reports comes from mice models of PrP^c gene ablation or over-expression. PrP^c null mice presented hippocampus-dependent spatial learning and memory consolidation impairment [23]. Interestingly, those changes were reversed by re-expression of PrP^c [12]. Additionally, deficiency of this protein may cause attention deficits [56]. Knockout mice were also more vulnerable to age-related decline in memory [20] and motor processes [64]. Moreover, PrP^c over-expression leads to hyperactivity, increased preference for visual, tactile and olfactory stimuli associated novelty [73]. Also it is noteworthy that a five-fold increase in PrP^c expression in comparison to wild type mice results in an increased resistance against age-related cognitive decline [72]. Due to the plenty of PrP^c functions there are many possible molecular mechanisms in which this protein affects cognitive processes. PrP^c is localized in the synaptic area, and it may interact with other proteins in this structure, playing a role in the synaptic plasticity [91]. An *in vitro* study has revealed that recombinant PrP (rPrP) induces rapid processing of both axons and dendrites as well as it increases the number of new synapses [40]. Consistently, deletion of PrP^c resulted in impaired LTP together with a decrease in fast GABA-A receptor-dependent inhibition [21,22]. Perhaps changes in LTP are associated with the ability of PrP^c to make physical interaction with receptors for the glutamate. It has been shown that PrP^c is able to co-immunoprecipitate with NR2D subunit of the NMDA, suggesting its possible direct modulation [6]. Transgenic (Tg) mice lacking PrP^c showed enhanced NMDA-induced currents, which became reversed after its over-expression [42]. PrP^c also affects kainate receptors [71] and metabotropic glutamate receptors [4] suppressing neuronal excitability through many different mechanisms. Noteworthy, it has been proved that the range of synaptic responses increased with the (higher) level of the PrP^c expression within glutamatergic synaptic transmission in the hippocampus, but the overall probability of transmitter release appeared unchanged [12]. This example shows that PrP^c-dependent modulation of glutamatergic stimulation and plasticity is more complex than just the control of the level of neurotransmitter. Regulation of glutamate signalization within synapses is important not only because of plasticity, but also due to the fact that abnormal Ca^{2+} currents, caused by aberrant activation of the NMDA receptor, results in excitotoxicity [32], which leads to neurodegeneration. Experiments mentioned above indicate that PrP^c modulates this process. This neuroprotective mechanism may be the second way in which PrP^c is involved in cognitive functions. Tg mice expressing modified PrP (with-

out its central region residues 105-125 – ΔCR PrP), resulted in massive excitotoxic degeneration of cerebellar granule neurons [15,51]. There is evidence that PrP^c also plays a role in neuroprotection through the regulation of intracellular signalling cascades, mediating cellular survival [55], and its over-expression protects cell lines from apoptosis. Protective activity has been also proved in glia cells. PrP^c acted as a radical scavenger in both ROS-rich solution and astrocytes cultures *in vitro*, and its activity was essential in their protection against oxidative stress. This feature may reflect its protective functions in conditions similar to those observed during neurodegeneration and ischemia [5]. Another defensive mechanism is associated with the ability of PrP^c to bind co-chaperon molecule, called stress-inducible protein 1 (STI1). They create a complex that acts as a survival and differentiation promoter [88]. Intriguingly, blocking the connection between PrP^c, STI1 and laminin adversely influences memory [18,19]. There are only scarce data indicating cognitive functions of PrP^c in humans, but they bring promising results. One epidemiological study of 1322 elderly participants revealed that subjects in higher serum PrP^c quintiles appeared to have lower cognitive functioning scores than those in the lowest PrP^c quintile. There are two proposed mechanisms of serum PrP^c elevation. Either there is reduced nerve cell integrity, or a higher serum PrP^c level reflects the abundance of PrP^c in neuro-cellular membranes [8].

Exploratory analysis of 335 healthy volunteers revealed that even SNP of the PrP^c gene might influence cognitive functions in humans, especially a common polymorphism at codon 129, which results in the translation of methionine or valine on a short β-sheet region in the C-terminal domain of the protein [77]. Methionine at codon 129 is associated with lower scores on several subscales of HAWIE-R subscales (German version of the Wechsler Adult Intelligence Scale Revised), especially with the Digit Symbol subtest. Interestingly, PrP-IQ association was the strongest in the less educated individuals; as opposed to other studies showing that the genetic influence on IQ is higher among higher educated families [76]. The same polymorphism is associated with the reduction of white matter in a group of healthy volunteers and patients with schizophrenia [78]. However studies on long-term memory revealed that healthy subjects presenting the same Met129 yielded the highest effect size, recalling 17 percent more words twenty four hours after the list-learning task than carriers of Val129 gene type, but there was no significant difference between those groups in short-term memory. Authors of this study hypothesize that despite the fact that Met129 allele may facilitate self-perpetuating conformational changes of the human prion protein, it may have a beneficial effect on long-term memory by hypothetical prion-based mechanism [66]. Studies mentioned above revealed that PrP^c and its gene may aspire to the role of a potential biomarker of cognitive measurements. The scarcity of investigations in humans and a plenty of possible mechanisms limit possibilities to draw a definite conclusion as for the existence of one causative relation between PrP^c and cognition. In spite of divergence of its functions, subserving somewhat unrelated processes, there is a prospect to indirectly indicate common ground of its activity. PrP^c may act as a protein, involved in global protection of the organism in a micro- and macroscopic perspective: at the microscopic (cellular) level – protecting the cell against apoptotic factors, ROS, excitotoxicity, toxins and at a macroscopic level – affecting the whole organism, by playing a significant role in cognition, especially in defensive attention, spatial and long-term memory and also olfactory physiology and behaviour (crucial for the survival chances of an animal).

β-amyloid

β-amyloid is a peptide consisting of 36-43 amino acids which originates from the cleavage of the transmembrane glycoprotein called amyloid precursor protein (APP), by the proteolytic activation of α-, β- and γ-secretase [80]. Generation of Aβ may occur in the neuronal axonal membranes and is preceded by APP-mediated axonal transport of β-secretase and presenilin-1 [39]. Amyloid precursor protein is cleaved by β-secretase, producing soluble and a cell-membrane bound fragment of APP [46], which is then cleaved by γ-secretase. This reaction produces APP intracellular domain (AICD) associated with the regulation of gene transcription and Aβ, which is released to plasma, cerebrospinal fluid and brain interstitial fluid [30,90]. It has been established that $Aβ_{39-42}$ are hydrophobic self-aggregating peptides, of which Aβ42 is a major component of senile plaques observed in AD, but it still remains controversial how those peptides are involved in the

cognitive decline observed during this disease [36]. The "Aβ cascade hypothesis" suggests the major role of amyloid plaques, especially fibrillar Aβ ones in the aetiology of AD, reporting a correlation between the amount of those formations and cognitive dysfunctions [3,24,25,35,67]. Recent studies with the use of detailed measures of Aβ pathology suggest an opposite explanation. Research on APPswe/PS1ΔE9 double transgenic mice (well-established model of AD) has revealed that hippocampal soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were highly correlated with spatial learning and long-term contextual memory impairments. Also, hippocampal soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were strongly correlated with spatial memory impairments, but no correlations were observed between mentioned cognitive functions and amyloid plaque formations such as: total Aβ plaque load, fibrillar Aβ plaque load and also insoluble Aβ levels. Authors of this study revealed that a tiny fraction of soluble peptides in the hippocampus and cortex is an independent factor in predicting cognitive impairments in this transgenic mice model, suggesting "soluble Aβ hypothesis" as a major mechanism of cognitive decline in AD [89]. Consistently with this hypothesis, experiments on young domestic chicks show that an injection of soluble Aβ 5 minutes prior to training caused memory loss, due to the consolidation failure 35 minutes later [31]. Also, reduction of soluble $A\beta_{42}$ or $A\beta_{42}$ and $A\beta_{40}$ by γ-secretase modulators (GSMs) ameliorated cognitive deficits in Tg2576 plaque-free mice model of AD. However, a later study suggests that newly synthesized soluble $A\beta_{42}$ may play a more significant role in cognitive impairments than plaque-associated soluble Aβ [61]. Injections of Aβ to rats result in rapid cognitive disruption, showing a direct interference with the cognitive functions not only through neurodegeneration, but also through interruption of their cellular mechanisms [17,50,69]. It has been shown that Aβ impairs hippocampal long-term potentiation (LTP) by deterioration of tetanus-induced activation of guanylate cyclase and increase of cGMP. Those changes prevent protein kinase G activation and phosphorylation of GluR1, finally resulting in impaired translocation of AMPA receptors to synaptic membranes [62]. Interactions of Aβ with nicotinic, insulin and glutamatergic receptors may also have an impact on synaptic plasticity and spine formation [17,50,63]. These mechanisms explain why injection of Aβ oligomers before the acquisition of new information disrupts the process of the consolidation without affecting its retrieval, when the information was properly stored [28]. Furthermore, it has been shown that Aβ may have an impact on the cognitive function through its influence on NADPH oxidase enzyme (NOX) activation. NOX is responsible for production of free radicals, and also it plays a role in neuronal physiology, particularly in hippocampal electrophysiology [43,83]. Data show a significant direct linear relationship between NOX activity, cognitive impairment and age-depended increase in $A\beta_{1-42}$. This correlation suggests that oxidative stress caused by NOX-associated redox pathway may be another possible mechanism in which Aβ is involved in cognitive decline [10]. Oxidative stress associated with membranes is a possible mechanism in which Aβ may cause synaptic dysfunction and disruption of cellular ion homeostasis [59]. Interestingly, mounting research show intraneuronal accumulation of Aβ as a possible mechanism of cognitive dysfunction, especially in the early stages of the AD [34,45,87]. This accumulation is associated with morphological alterations of synapses [81] and with a decrease in synaptophysin around the affected neurons in AD patients [38]. Studies on 3xTg-AD mice showed a correlation between the cognitive and synaptic dysfunction with the accumulation of intraneuronal Aβ which occurred before formation of amyloid plaques [7,65]. Also hypercholesterolemia accelerates intraneuronal accumulation of Aβ oligomers, resulting in synapse loss and memory impairment [84]. This view of the complexity of the mechanisms and forms by which Aβ affects cognitive functions will be helpful for proper understanding of its possible interactions with PrP^c.

Cooperation?

Tellingly, comprehensive studies have shown that out of 225 000 proteins screened in a cell model, only those with PrP^c expression were strongly binding soluble $A\beta_{42}$ [49]. It has been proposed that PrP^c exhibits receptor affinity to β-sheet-rich conformers due to its ~95-110 region and the cluster of basic residues within the *N*-terminal 23-27 segment [14]. As to the protective functions of PrP^c mentioned before, one may say that this protein will also protect the cell against Aβ, but a growing number of research comes with opposite findings and also with new controversies. Lauren et al. proposed that synthetic oligomeric forms of Aβ impair LTP through

their interactions with PrP^c [49], but other studies did not confirm this result [1,11]. However, more novel findings showed that antibodies against 94-104 domain of PrP^c blocked inhibition of LTP caused by soluble extracts of AD brain [2,29]. Also hippocampal slices lacking PrP^c were resistant to LTP inhibition by Aβ. Similar relationships can be observed in studies on Aβ-dependent neurotoxicity. Prnp 0/0 mice are more resistant to neurotoxic effects of Aβ oligomers [44] and accordingly, over-expression of PrP^c in neuronal cell lines increases vulnerability to such effects. It has been also shown that deletion of PrP^c expression in APPswe/PSen1ΔE9 rescues 5-HT axonal degeneration, loss of synaptic markers and early death, and interestingly, Tg mice containing Aβ plaques, but lacking PrP^c show no spatial learning and memory impairments [33]. APP/PS1 Tg mice, treated for 2 weeks with intraperitoneal injections of 6D11 anti-PrP antibodies, recovered in cognitive learning tasks behaving the same as wild-type mice [16]. Surprisingly, mentioned studies revealed not only that PrP^c is not neuroprotective against Aβ, but even it may be necessary for its neurotoxicity and its impact on cognitive functions. Nevertheless, some authors have reached opposite conclusions. Rial *et al.* showed that Tg-20 mice characterized by a five-fold increase in PrP^c expression was resistant to a single intracerebroventricular injection of 400 pmols/mouse of aggregated $Aβ_{1-40}$, revealing no impairments of memory and spatial learning in comparison to the wild type and PrP^c knockout mice. This resistance was accompanied with a decrease in activated caspase-3 protein and Bax/Bcl-2 ratio and reduced hippocampal cell damage [73]. Calella *et al*. [11] showed that the PrP^c level had no effect on LTP impairment in APP/PS1 mice, and those results were also confirmed by Kessels *et al*.'s studies [41]. Also participation of PrP^c in mediation of Ab neurotoxicity had been challenged by results of studies on isolated hippocampal cells from Prnp 0/0 and Prnp +/+ mice. Authors concluded that PrP^c in specific conditions may exert a relevant role in neurotoxicity because of sequestration of Aβ oligomers rather than a functional activity associated to the protein (for review [28]). A more recent study shed a light on the interactions of these proteins adding some important premise to proper understanding of the controversies and the confounding results mentioned above. Larson *et al*. revealed that AD brain-purified Aβ dimers are specifically binding PrP^c. This complex triggers Src Tyrosine Kinase Fyn, which activates the kinase and leads towards abnormal phosphorylation of Fyn and tau. This reaction occurs in neuronal dendritic spines and leads to aberrant tau missorting and hyperphosphorylation. Authors also revealed that dosage of Prnp regulates these changes. This comprehensive study made *ex vivo*, *in situ*, and *in vitro* indicates that this PrP^c-mediated process may play an important role in late stages of AD, when Aβ dimers reach their highest level [47].

Proper understanding of this finding is facilitated by Chen *et al*.'s study on human neuroblastoma cells. They found that over-expression of PrP^c downregulates tau protein transcription level through Fyn, Fyn kinase and MEK pathway. β-amyloid oligomers reverse this pathway by binding to PrP^c, probably by inducing its surface retention that interferes with caveolae-mediated PrP^c endocytosis and Fyn activation. Phosphorylated Fyn level increased in a dose-dependent manner 2 hours after Aβ oligomer treatment and interestingly it became decreased 1 day after this treatment. Surprisingly, the murine PrP^c M128V, which correspond with the high AD risk polymorphic human PrP^c M129V [74] allele was able to bind Aβ oligomers, but it was unable to reverse the tau reduction [13]. This may be another explanation why this polymorphism was associated with lower IQ and white matter reduction in the study mentioned earlier in this article.

The above authors (see ref. [13, 47], and also Larson and Lesne [48]) suggest that confounding results about PrP^c-mediated impairments, as those described previously, may be attributed only to a subset of Aβ oligomers that are mediated through PrP^c. For example, no dependence in LTP impairments in studies of Calella *et al*. [11] may be a result of low levels of Aβ dimers in young aged Tg-mice. A protective effect of PrP^c over-expression against intracerebroventricular injection of Aβ may be a result of a low concentration of its assemblies. It has been proven that picomolar concentrations of the Aβ did not trigger Fyn activation [47]. In our opinion, if PrP^c downregulates tau protein, and Aβ binds to PrP^c, reverting this process, it is quite possible that effects of Aβ will vary due to Aβ and PrP^c ratio. Noteworthy, PrP^c is not only connected in aetiology of AD by its direct interactions with Aβ, but also due to its negative modulation of BACE1 activity. PrP^c declines with age, and is decreased in sporadic AD, but there are no alterations in familial AD cases. In sporadic

AD, the PrP^c level is inversely correlated with BACE1 activity, Aβ load, soluble Aβ, and insoluble Aβ. It is also inversely correlated with the stage of disease, as indicated by Braak tangle stages, distinguished according to the distribution of the tau pathology, especially the neurofibrillary tangles. Authors of this study point out that a decreased level of PrP^c results in a decreased zinc uptake within the synapses. Such condition results in an elevated synaptic zinc level, which favours binding Aβ oligomers to the NMDA receptors, and mediates the excitotoxicity [86]. In our opinion, it is more probable that an inverse correlation with Braak stage assessed tauopathy was caused by the elevation of Aβ and PrP^c ratio, and its direct impact on tau expression. A biophysical examination of recombinant PrP revealed that this protein represents a unique intrinsic feature to form multiple non-native isoforms rich in β-sheets, which may result in a large spectrum of PrP^c *in vivo*. It has been proved that in uninfected human brains PrP is presented also in the form of oligomers, and even large aggregates, called insoluble PrP^c ($iPrP^c$) which stand for ~5-25% of total PrP. It is proposed that if soluble PrP^c can bind to soluble $A\beta_{42}$, also $iPrP^c$ will bind insoluble Aβ, modulating its deposition [91]. It is therefore consistent with Takahashi *et al.*'s findings [82] (confirming the prior report of Ferrer *et al.* [27]) indicating that PrP^c is present in amyloid deposits. Many other related issues remain seemingly untouched. For example, amyloid Aβ deposits are formed within vessels and amyloid angiopathy is not only limited to arteries, but also affects veins [60]. It is interesting whether there is also an interaction between Aβ and PrP^c in this compartment of brain tissue. To summarize, a growing number of research indicate new possible functions of PrP^c in the field of cognition, both in its physiological and pathophysiological aspects. Considering new data, we are compelled to stronger appreciate the role of soluble forms of Aβ in the pathomechanism of AD, which possibly even prevails over its insoluble deposits. This may be regarded as a "paradigm shift" in an understanding of cognitive decline in AD. Despite the remaining controversies, recent findings prompt us to consider that roads of PrP^c and Aβ meet at the point of tauopathy and moreover, formation of PrP^c-Aβ complexes may result in the consumption of PrP^c, what modulates Aβ neurotoxicity, possibly depriving nervous tissue of the neuroprotective function of PrP^c.

References

1. Balducci C, Beeg M, Stravalaci M, Bastone A, Sclip A, Biasini E, Tapella L, Colombo L, Manzoni C, Borsello T, Chiesa R, Gobbi M, Salmona M, Forloni G. Synthetic amyloid-β oligomers impair long-term memory independently of cellular prion protein. Proc Natl Acad Sci U S A 2010; 107: 2295-2300.
2. Barry AE, Klyubin I, Mc Donald JM, Mably AJ, Farrell MA, Scott M, Walsh DM, Rowan MJ. Alzheimer's disease brain-derived amyloid-β-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. J Neurosci 2011; 31: 7259-7263.
3. Bartoo GT, Nochlin D, Chang D, Kim Y, Sumi SM. The mean A beta load in the hippocampus correlates with duration and severity of dementia in subgroups of Alzheimer disease. J Neuropathol Exp Neurol 1997; 56: 531-540.
4. Beraldo FH, Arantes CP, Santos TG, Machado CF, Roffe M, Hajj GN, Lee KS, Magalhães AC, Caetano FA, Mancini GL, Lopes MH, Américo TA, Magdesian MH, Ferguson SS, Linden R, Prado MA, Martins VR. Metabotropic glutamate receptors transduce signals for neurite outgrowth after binding of the prion protein to laminin gamma1 chain. FASEB J 2011; 25: 265-279.
5. Bertuchi FR, Bourgeon DM, Landemberger MC, Martins VR, Cerchiaro G. PrPC displays an essential protective role from oxidative stress in an astrocyte cell line derived from PrPC knockout mice. Biochem Biophys Res Commun 2012; 418: 27-32.
6. Biasini E, Turnbaugh JA, Unterberger U, Harris DA. Prion protein at the crossroads of physiology and disease. Trends Neurosci 2012; 35: 92-103.
7. Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 2005; 45: 675-688.
8. Breitling LP, Müller H, Stegmaier C, Kliegel M, Brenner H. Association of prion protein with cognitive functioning in humans. Exp Gerontol 2012; 47: 919-924.
9. Brown P, Jannotta F, Gibbs CJ Jr, Baron H, Guiroy DC, Gajdusek DC. Coexistence of Creutzfeldt-Jakob disease and Alzheimer's disease in the same patient. Neurology 1990; 40: 226-228.
10. Bruce-Keller AJ, Gupta S, Knight AG, Beckett TL, McMullen JM, Davis PR, Murphy MP, Van Eldik LJ, St Clair D, Keller JN. Cognitive impairment in humanized APP×PS1 mice is linked to Aβ(1-42) and NOX activation. Neurobiol Dis 2011; 44: 317-326.
11. Calella AM, Farinelli M, Nuvolone M, Mirante O, Moos R, Falsig J, Mansuy IM, Aguzzi A. Prion protein and Abeta-related synaptic toxicity impairment. EMBO Mol Med 2010; 2: 306-314.
12. Carleton A, Tremblay P, Vincent JD, Lledo PM. Dose-dependent, prion protein (PrP)-mediated facilitation of excitatory synaptic transmission in the mouse hippocampus. Pflugers Arch 2001; 442: 223-229.
13. Chen RJ, Chang WW, Lin YC, Cheng PL, Chen YR. Alzheimer's amyloid-β oligomers rescue cellular prion protein induced tau reduction via Fyn pathways. ACS Chem Neurosci 2013; 4: 1287-1296.
14. Chen S, Yadav SP, Surewicz WK. Interaction between human prion protein and amyloid-beta (Abeta) oligomers: role of N-terminal residues. J Biol Chem 2010; 285: 26377-26383.

15. Christensen HM, Dikranian K, Li A, Baysac KC, Walls KC, Olney JW, Roth KA, Harris DA. A highly toxic cellular prion protein induces a novel, nonapoptotic form of neuronal death. Am J Pathol 2010; 176: 2695-2706.
16. Chung E, Ji Y, Sun Y, Kascsak RJ, Kascsak RB, Mehta PD, Strittmatter SM, Wisniewski T. Anti-PrP C monoclonal antibody infusion as a novel treatment for cognitive deficits in an Alzheimer 's disease model mouse. BMC Neurosci 2010; 11: 130.
17. Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat Neurosci 2005; 8: 79-84.
18. Coitinho AS, Freitas AR, Lopes MH, Hajj GN, Roesler R, Walz R, Rossato JI, Cammarota M, Izquierdo I, Martins VR, Brentani RR. The interaction between prion protein and laminin modulates memory consolidation. Eur J Neurosci 2006; 24: 3255-3264.
19. Coitinho AS, Lopes MH, Hajj GN, Rossato JI, Freitas AR, Castro CC, Cammarota M, Brentani RR, Izquierdo I, Martins VR. Short-term memory formation and long-term memory consolidation are enhanced by cellular prion association to stress-inducible protein 1. Neurobiol Dis 2007; 26: 282-290.
20. Coitinho AS, Roesler R, Martins VR, Brentani RR, Izquierdo I. Cellular prion protein ablation impairs behavior as a function of age. Neuroreport 2003; 14: 1375-1379.
21. Colling SB, Collinge J, Jefferys JG. Hippocampal slices from prion protein null mice: disrupted Ca(2+)-activated K+ currents. Neurosci Lett 1996; 209: 49-52.
22. Collinge J, Whittington MA, Sidle KC, Smith CJ, Palmer MS, Clarke AR, Jefferys JG. Prion protein is necessary for normal synaptic function. Nature 1994; 370: 295-297.
23. Criado JR, Sánchez-Alavez M, Conti B, Giacchino JL, Wills DN, Henriksen SJ, Race R, Manson JC, Chesebro B, Oldstone MB. Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. Neurobiol Dis 2005; 19: 255-265.
24. Cummings BJ, Cotman CW. Image analysis of beta-amyloid load in Alzheimer's disease and relation to dementia severity. Lancet 1995; 346: 1524-1528.
25. Cummings BJ, Pike CJ, Shankle R, Cotman CW. β-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. Neurobiol Aging 1996; 17: 921-933.
26. Debatin L, Streffer J, Geissen M, Matschke J, Aguzzi A, Glatzel M. Association between deposition of beta-amyloid and pathological prion protein in sporadic Creutzfeldt-Jakob disease. Neurodegener Dis 2008; 5: 347-354.
27. Ferrer I, Blanco R, Carmona M, Puig B, Ribera R, Rey MJ, Ribalta T. Prion protein expression in senile plaques in Alzheimer's disease. Acta Neuropathol 2001; 101: 49-56.
28. Forloni G, Balducci C. β-amyloid oligomers and prion protein: Fatal attraction? Prion 2011; 5: 10-15.
29. Freir DB, Nicoll AJ, Klyubin I, Panico S, Mc Donald JM, Risse E, Asante EA, Farrow MA, Sessions RB, Saibil HR, Clarke AR, Rowan MJ, Walsh DM, Collinge J. Interaction between prion protein and toxic amyloid β assemblies can be therapeutically targeted at multiple sites. Nat Commun 2011; 2: 336.
30. Ghiso J, Frangione B. Amyloidosis and Alzheimer's disease. Adv Drug Deliv Rev 2002; 54: 1539-1551.
31. Gibbs ME, Maksel D, Gibbs Z, Hou X, Summers RJ, Small DH. Memory loss caused by β-amyloid protein is rescued by a b3-adrenoceptor agonist. Neurobiol Aging 2010; 31: 614-624.
32. Gillessen T, Budd SL, Lipton SA. Excitatory amino acid neurotoxicity. Adv Exp Med Biol 2002; 513: 3-40.
33. Gimbel DA, Nygaard HB, Coffey EE, Gunther EC, Laurén J, Gimbel ZA, Strittmatter SM. Memory impairment in transgenic Alzheimer mice requires cellular prion protein. J Neurosci 2010; 30: 6367-6374.
34. Gouras GK, Tampellini D, Takahashi RH, Capetillo-Zarate E. Intraneuronal β-amyloid accumulation and synapse pathology in Alzheimer's disease. Acta Neuropathol 2010; 119: 523-541.
35. Haroutunian V, Perl DP, Purohit DP, Marin D, Khan K, Lantz M, Davis KL, Mohs RC. Regional distribution of neuritic plaques in the nondemented elderly and subjects with very mild Alzheimer disease. Arch Neurol 1998; 55: 1185-1191.
36. Hiltunen M, van Groen T, Jolkkonen J. Functional roles of amyloid-beta protein precursor and amyloid-beta peptides: evidence from experimental studies. J Alzheimers Dis 2009; 18: 401-412.
37. Huber R, Deboer T, Tobler I. Prion protein: a role in sleep regulation? J Sleep Res 1999; 8 (Suppl 1): 30-36.
38. Ishibashi K, Tomiyama T, Nishitsuji K, Hara M, Mori H. Absence of synaptophysin near cortical neurons containing oligomer Aβ in Alzheimer's disease brain. J Neurosci Res 2006; 84: 632-636.
39. Kamal A, Almenar-Queralt A, LeBlanc JF, Roberts EA, Goldstein LS. Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. Nature 2001; 414: 643-648.
40. Kanaani J, Prusiner SB, Diacovo J, Baekkeskov S, Legname G. Recombinant prion protein induces rapid polarization and development of synapses in embryonic rat hippocampal neurons in vitro. J Neurochem 2005; 95: 1373-1386.
41. Kessels HW, Nguyen LN, Nabavi S, Malinow R. The prion protein as a receptor for amyloid-beta. Nature 2010; 466: 3-4.
42. Khosravani H, Zhang Y, Tsutsui S, Hameed S, Altier C, Hamid J, Chen L, Villemaire M, Ali Z, Jirik FR, Zamponi GW. Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. J Cell Biol 2008; 181: 551-565.
43. Kishida KT, Pao M, Holland SM, Klann E. NADPH oxidase is required for NMDA receptor-dependent activation of ERK in hippocampal area CA1. J Neurochem 2005; 94: 299-306.
44. Kudo W, Lee HP, Zou WQ, Wang X, Perry G, Zhu X, Smith MA, Petersen RB, Lee HG. Cellular prion protein is essential for oligomeric amyloid-β-induced neuronal cell death. Hum Mol Genet 2012; 5: 1138-1144.
45. LaFerla FM, Green KN, Oddo S. Intracellular amyloid-β in Alzheimer's disease. Nat Rev Neurosci 2007; 8: 499-509.
46. Lahiri DK, Farlow MR, Sambamurti K, Greig NH, Giacobini E, Schneider LS. A critical analysis of new molecular targets and strategies for drug developments in Alzheimer's disease. Curr Drug Targets 2003; 4: 97-112.
47. Larson M, Sherman MA, Amar F, Nuvolone M, Schneider JA, Bennett DA, Aguzzi A, Lesné SE. The complex PrP(c)-Fyn cou-

ples human oligomeric Aβ with pathological tau changes in Alzheimer's disease. J Neurosci 2012; 32: 16857-16871a.
48. Larson ME, Lesne SE. Soluble Aβ oligomer production and toxicity. J Neurochem 2012; 120 (Suppl 1): 125-139.
49. Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. Nature 2009; 457: 1128-1132.
50. Lesné S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 2006; 440: 352-357.
51. Li A, Christensen HM, Stewart LR, Roth KA, Chiesa R, Harris DA. Neonatal lethality in transgenic mice expressing prion protein with a deletion of residues 105-125. EMBO J 2007; 26: 548-558.
52. Liberski PP. Historical overview of prion diseases: a view from afar. Folia Neuropathol 2012; 50: 1-12.
53. Liberski PP, Sikorska B, Wells GA, Hawkins SA, Dawson M, Simmons MM. Ultrastructural findings in pigs experimentally infected with bovine spongiform encephalopathy agent. Folia Neuropathol 2012; 50: 89-98.
54. Linden R, Martins VR, Prado MA, Izquierdo I, Brentani RR. Physiology of the prion protein. Physiol Rev 2008; 88: 673-728.
55. Lo RY, Shyu WC, Lin SZ, Wang HJ, Chen SS, Li H. New molecular insights into cellular survival and stress responses: neuroprotective role of cellular prion protein (PrPC). Mol Neurobiol 2007; 35: 236-244.
56. Lobão-Soares B, Walz R, Prediger RD, Freitas RL, Calvo F, Bianchin MM, Leite JP, Landemberger MC, Coimbra NC. Cellular prion protein modulates defensive attention and innate fear-induced behaviour evoked in transgenic mice submitted to an agonistic encounter with the tropical coral snake Oxyrhopus guibei. Behav Brain Res 2008; 194: 129-137.
57. Madore N, Smith KL, Graham CH, Jen A, Brady K, Hall S, Morris R. Functionally different GPI proteins are organized in different domains on the neuronal surface. EMBO J 1999; 18: 6917-6926.
58. Magalhães AC, Silva JA, Lee KS, Martins VR, Prado VF, Ferguson SS, Gomez MV, Brentani RR, Prado MA. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J Biol Chem 2002; 277: 33311-33318.
59. Mattson MP. Pathways towards and away from Alzheimer's disease. Nature 2004; 430: 631-639.
60. Mendel T, Wierzba-Bobrowicz T, Stępień T, Szpak GM. β-amyloid deposits in veins in patients with cerebral amyloid angiopathy and intracerebral haemorrhage. Folia Neuropathol 2013; 51: 120-126.
61. Mitani Y, Yarimizu J, Akashiba H, Shitaka Y, Ni K, Matsuoka N. Amelioration of cognitive deficits in plaque-bearing Alzheimer's disease model mice through selective reduction of nascent soluble Aβ42 without affecting other Aβ pools. J Neurochem 2013; 125: 465-472.
62. Monfort P, Felipo V. Amyloid-β impairs, and ibuprofen restores, the cGMP pathway, synaptic expression of AMPA receptors and long-term potentiation in the hippocampus. J Alzheimers Dis 2010; 22: 795-809.
63. Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L. High-level neuronal expression of abeta 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci 2000; 20: 4050-4058.
64. Nazor KE, Seward T, Telling GC. Motor behavioral and neuropathological deficits in mice deficient for normal prion protein expression. Biochim Biophys Acta 2007; 1772: 645-653.
65. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. Neuron 2003; 39: 409-421.
66. Papassotiropoulos A, Wollmer MA, Aguzzi A, Hock C, Nitsch RM, de Quervain DJ. The prion gene is associated with human long-term memory. Hum Mol Genet 2005; 14: 2241-2246.
67. Parvathy S, Davies P, Haroutunian V, Purohit DP, Davis KL, Mohs RC, Park H, Moran TM, Chan JY, Buxbaum JD. Correlation between Abetax-40-, Abetax-42-, and Abetax-43-containing amyloid plaques and cognitive decline. Arch Neurol 2001; 58: 2025-2032.
68. Peters PJ, Mironov A Jr, Peretz D, van Donselaar E, Leclerc E, Erpel S, DeArmond SJ, Burton DR, Williamson RA, Vey M, Prusiner SB. Trafficking of prion proteins through a caveolae-mediated endosomal pathway. J Cell Biol 2003; 162: 703-717.
69. Poling A, Morgan-Paisley K, Panos JJ, Kim EM, O'Hare E, Cleary JP, Lesné S, Ashe KH, Porritt M, Baker LE. Oligomers of the amyloid-beta protein disrupt working memory: confirmation with two behavioral procedures. Behav Brain Res 2008; 193: 230-234.
70. Powers JM, Liu Y, Hair LS, Kascsack RJ, Lewis LD, Levy LA. Concomitant Creutzfeldt-Jakob and Alzheimer diseases. Acta Neuropathol 1991; 83: 95-98.
71. Rangel A, Burgaya F, Gavín R, Soriano E, Aguzzi A, Del Río JA. Enhanced susceptibility of Prnp-deficient mice to kainate-induced seizures, neuronal apoptosis, and death: role of AMPA/kainate receptors. J Neurosci Res 2007; 85: 2741-2755.
72. Rial D, Duarte FS, Xikota JC, Schmitz AE, Dafré AL, Figueiredo CP, Walz R, Prediger RD. Cellular prion protein modulates age-related behavioral and neurochemical alterations in mice. Neuroscience 2009; 164: 896-907.
73. Rial D, Piermartiri TC, Duarte FS, Tasca CI, Walz R, Prediger RD. Overexpression of cellular prion protein (PrP(C)) prevents cognitive dysfunction and apoptotic neuronal cell death induced by amyloid-β ($A\beta_{1-40}$) administration in mice. Neuroscience 2012; 215: 79-89.
74. Riemenschneider M, Klopp N, Xiang W, Wagenpfeil S, Vollmert C, Müller U, Förstl H, Illig T, Kretzschmar H, Kurz A. Prion protein codon 129 polymorphism and risk of Alzheimer disease. Neurology 2004; 63: 364-366.
75. Rodolfo K, Hässig R, Moya KL, Frobert Y, Grassi J, Di Giamberardino L. A novel cellular prion protein isoform present in rapid anterograde axonal transport. Neuroreport 1999; 10: 3639-3644.
76. Rowe DC, Jacobson KC, Van den Oord EJ. Genetic and environmental influences on vocabulary IQ: parental education level as moderator. Child Dev 1999; 70: 1151-1162.
77. Rujescu D, Hartmann AM, Gonnermann C, Möller HJ, Giegling I. M129V variation in the prion protein may influence cognitive performance. Mol Psychiatry 2003; 8: 937-941.

78. Rujescu D, Meisenzahl EM, Giegling I, Kirner A, Leinsinger G, Hegerl U, Hahn K, Möller HJ. Methionine homozygosity at codon 129 in the prion protein is associated with white matter reduction and enlargement of CSF compartments in healthy volunteers and schizophrenic patients. Neuroimage 2002; 15: 200-206.
79. Sagdullaev BT, Aguzzi A, Firestein S. Olfactory behavior and physiology are disrupted in prion protein knockout mice. Nat Neurosci 2009; 12: 60-69.
80. Šerý O, Povová J, Míšek I, Pešák L, Janout V. Molecular mechanisms of neuropathological changes in Alzheimer's disease: a review. Folia Neuropathol 2013; 1: 1-9.
81. Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK. Intraneuronal Alzheimer Aβ42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am J Pathol 2002; 161: 1869-1879.
82. Takahashi RH, Tobiume M, Sato Y, Sata T, Gouras GK, Takahashi H. Accumulation of cellular prion protein within dystrophic neurites of amyloid plaques in the Alzheimer's disease brain. Neuropathology 2011; 31: 208-214.
83. Tejada-Simon MV, Serrano F, Villasana LE, Kanterewicz BI, Wu GY, Quinn MT, Klann E. Synaptic localization of a functional NADPH oxidase in the mouse hippocampus. Mol Cell Neurosci 2005; 29: 97-106.
84. Umeda T, Tomiyama T, Kitajima E, Idomoto T, Nomura S, Lambert MP, Klein WL, Mori H. Hypercholesterolemia accelerates intraneuronal accumulation of Aβ oligomers resulting in memory impairment in Alzheimer's disease model mice. Life Sci 2012; 91: 1169-1176.
85. Walz R, Amaral OB, Rockenbach IC, Roesler R, Izquierdo I, Cavalheiro EA, Martins VR, Brentani RR. Increased sensitivity to seizures in mice lacking cellular prion protein. Epilepsia 1999; 40: 1679-1682.
86. Whitehouse IJ, Miners JS, Glennon EB, Kehoe PG, Love S, Kellett KA, Hooper NM. Prion protein is decreased in Alzheimer's brain and inversely correlates with BACE1 activity, amyloid-β levels and Braak stage. PLoS One 2013; 8: e59554.
87. Wirths O, Multhaup G, Bayer TA. A modified β-amyloid hypothesis: intraneuronal accumulation of the β-amyloid peptide – the first step of a fatal cascade. J Neurochem 2004; 91: 513-520.
88. Zanata SM, Lopes MH, Mercadante AF, Hajj GN, Chiarini LB, Nomizo R, Freitas AR, Cabral AL, Lee KS, Juliano MA, de Oliveira E, Jachieri SG, Burlingame A, Huang L, Linden R, Brentani RR, Martins VR. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. EMBO J 2002; 21: 3307-3316.
89. Zhang W, Hao J, Liu R, Zhang Z, Lei G, Su C, Miao J, Li Z. Soluble Aβ levels correlate with cognitive deficits in the 12-month-old APPswe/PS1dE9 mouse model of Alzheimer's disease. Behav Brain Res 2011; 222: 342-350.
90. Zlokovic BV, Frangione B. Transport-clearance hypothesis for Alzheimer's disease and potential therapeutic implications. Landes Bioscience 2003; 114-122.
91. Zou WQ, Zhou X, Yuan J, Xiao X. Insoluble cellular prion protein and its association with prion and Alzheimer diseases. Prion 2011; 5: 172-178.