FEBS 30101 FEBS Letters 579 (2005) 6333–6337

The N-terminal cleavage of cellular prion protein in the human brain

Isabelle Laffont-Proust[a,b], Baptiste A. Faucheux[a,c], Raymonde Hässig[b], Véronique Sazdovitch[a], Stéphanie Simon[d], Jacques Grassi[d], Jean-Jacques Hauw[a], Kenneth L. Moya[b,e], Stéphane Haïk[a,*]

[a] *INSERM Avenir Team – Human Prion Diseases, IFR70, Neuropathology, Salpêtrière Hospital, F-75013 Paris, France*
[b] *CEA-CNRS URA2210, Service Hospitalier Frédéric Joliot, F-91406 Orsay, France*
[c] *DRCD, Assistance-Publique – Hôpitaux de Paris, Paris, France*
[d] *CEA, Pharmacology and Immunology Department, F-91191 Gif-sur-Yvette, France*
[e] *CNRS UMR8542, Ecole Normale Supérieure, F-75231 Paris, France*

Received 12 September 2005; revised 4 October 2005; accepted 10 October 2005

Available online 19 October 2005

Edited by Jesus Avila

Abstract Human brain cellular prion protein (PrP^c) is cleaved within its highly conserved domain at amino acid 110/111 ↓ 112. This cleavage generates a highly stable C-terminal fragment (C1). We examined the relative abundance of holo- and truncated PrP^c in human cerebral cortex and we found important inter-individual variations in the proportion of C1. Neither age nor postmortem interval explain the large variability observed in C1 amount. Interestingly, our results show that high levels of C1 are associated with the presence of the active ADAM10 suggesting this zinc metalloprotease as a candidate for the cleavage of PrP^c in the human brain.

Keywords: Cellular prion protein; Cerebral cortex; Truncation; ADAM10

1. Introduction

Prion diseases are a group of devastating brain diseases, that includes Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler–Scheinker syndrome in humans [1]. The human prion diseases can be sporadic, inherited or transmissible in origin, and sporadic CJD comprises a broad spectrum of clinicopathological variants [2].

According to the protein only hypothesis, the mechanism for prion propagation is thought to involve conversion of host encoded cellular prion protein (PrP^c) into conformers that are insoluble and resistant to proteinase K (PrP^{res}) and that accumulates in prion-targeted tissues. In humans, the primary sequence of PrP^c (through a methionine–valine polymorphism at codon 129) modulates the susceptibility to sporadic [3] and variant CJD [4], the incubation period in iatrogenic CJD [5], and the phenotype of the disease in sporadic CJD [6] and in some genetic forms of the disease [7]. Thus, a better characterization of the biology of the PrP^c is essential for the elucidation of prion propagation in humans.

PrP^c is expressed in most tissues and in particular in the central nervous system [8]. Mature human PrP^c spans residues 23–231. The protein has an amino-terminal domain with a set of octarepeats, a central hydrophobic domain, and a carboxy-terminal region containing two Asn-linked glycosylation sites. PrP^c can be cleaved to generate a C-terminal fragment (C1). In the brain, the normal processing of PrP^c includes proteolysis at residues 110/111 ↓ 112 and generates a membrane-associated fragment called C1 [9] that is highly stable and that accumulates at the plasma membrane [10].

Several mechanisms for PrP^c truncation have been investigated in vitro or in cells in culture including oxidation [11,12] and a role for metalloproteases [13,14]. However, the in vivo processing cellular machinery remains to be clarified. Here, we examined PrP^c truncation and putative mechanisms of the C1 fragment formation in a large number of non-CJD human cases.

2. Materials and methods

2.1. Preparation of human brain homogenates

The postmortem parietal isocortex was obtained from individuals unaffected by prion diseases. Patient families gave informed consent for the autopsy and tissues were collected according to the protocol of the Neuropathology Department of the Salpêtrière Hospital (Paris, France). Brain tissues were obtained from patients with the following neuropathology confirmed diagnoses: senile dementia of the Alzheimer type ($n = 8$), fronto-temporal dementia ($n = 2$), adenocarcinoma with metastasis and carcinomatous meningitis ($n = 1$), carcinomatous meningitis ($n = 1$), and amyotrophic lateral sclerosis ($n = 11$). After the autopsy, tissues were stored at −80 °C.

Brain tissues were homogenized (30 mg tissue/ml) in ice-cold 10 mM Tris, pH 7.4 containing protease inhibitors (P8340, Sigma, Saint Louis, MO). Protein concentration was measured by the method of Lowry et al. [15]. The absence of spontaneous in vitro cleavage of PrP^c was controlled, in a preliminary experiment, with the incubation of 20 μg of proteins at 20 °C for 0–4 h.

2.2. Protein deglycosylation

Twenty μg of proteins were denatured and incubated with 0.125 U of peptide *N*-glycosidase F (PNGase F; P0704L, New England Biolabs, Beverly, MA), at 37 °C, for 2 h, according to the manufacturer's instructions. The reaction was stopped by adding an equal volume of 2× denaturing buffer (0.125 M Tris, pH 6.8; 4% wt/vol sodium dodecyl sulfate, 10% vol/vol 2-mercaptoethanol, and 20% vol/vol glycerol).

*Corresponding author. Fax: +33 1 42 16 18 99.
E-mail address: haik@solar.chups.jussieu.fr (S. Haïk).

Abbreviations: ADAM10, a disintegrin and metalloproteinase domain 10; C1, C-terminal fragment; CJD, Creutzfeldt–Jakob disease; DNP, dinitrophenyl; PNGase F, peptide, *N*-glycosidase F; PrP^c, cellular prion protein; PrP^{res}, protease resistant PrP; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

0014-5793/$30.00
doi:10.1016/j.febslet.2005.10.013

2.3. Detection of free carbonyl groups of proteins

The protein free carbonyl content was measured by forming protein hydrazone derivatives using 2,4 dinitrophenyl (DNP) hydrazine (42210, Fluka, Buchs, Switzerland) as described by Jolivalt et al. [16]. Proteins were derivatized with an equal sample volume of 0.5 mM of 2,4 DNP hydrazine (pH 6.3) for 1 h at room temperature. The reaction was stopped by adding an equal volume of 2× denaturing buffer.

2.4. Western blot immunoassay

Proteins were separated by 12%, or 5–15% linear gradient, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred onto a poly(vinylidene difluoride) membrane (Millipore, Billerica, MA). Western-blotting was performed using anti-PrP, anti-ADAM10 or anti-DNP antibodies and chemiluminescence (RPN-2209; Amersham Biosciences, Freiburg, Germany) for the detection of peroxidase activity. The well-characterized SAF60 antibody recognizes an epitope at human PrP residues 157–161 (Pharmacology and Immunology Department, CEA/Saclay, France). Rabbit polyclonal anti-ADAM10 was from Chemicon (AB19026, Temecula, CA). Polyclonal anti-DNP antibodies used for detection of free carbonyl groups of proteins were from Molecular Probes (A-6430, Eugene, OR). Secondary antibody anti-mouse-HRP used was from Amersham Biosciences (NA931) and secondary antibody anti-rabbit-HRP used was from Sigma (A0545).

2.5. Densitometry analysis

Western blot films were scanned (600 dpi; ImageScanner, Amersham Pharmacia Biotech.) and regions of interest corresponding to full-length PrP^c, truncated PrP^c, mature disintegrin and metalloproteinase ADAM10 and immature ADAM10 were manually delimited using ImageMaster Labscan v 3.00 (Amersham Pharmacia Biotech.). The total signal intensity in the region of interest was corrected for background density. Values for C1 PrP^c were calculated as a percentage of total (full-length plus C1) PrP^c. Values for mature ADAM10 were calculated as a percentage of total (mature plus immature) ADAM10. For the DNP derivatized proteins, all bands corresponding to stained proteins were quantified.

2.6. Statistical analysis

Linear regression and correlation between C1 as a percent of total PrP^c and postmortem delay or age were carried out using StatView v 4.0 (Abacus Concepts, Berkeley, CA). The correlation analysis of C1 as a percentage of total PrP^c with DNP derivatized protein, or mature ADAM10 as a percentage of total ADAM10, was carried out similarly. A P value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. C1 fragment formation does not occur in vitro

SAF60 detected several bands representing heterogeneous glycosylation of PrP^c (27–34 kDa; Fig. 1A). After PNGase F treatment, SAF60 recognized a band at 27 kDa (full-length form of PrP^c) and another band at 18 kDa (C1 fragment described earlier by Chen et al. [9], corresponding to N-terminally truncated form of PrP^c). Since PNGase F treatment allowed a clear separation of full-length and truncated forms of PrP^c, all subsequent analyses were carried out after enzymatic treatment.

To investigate whether the C1 fragment of PrP^c was the result of an in vitro lysis, we incubated a human isocortex homogenate at 20 °C. Fig. 1B shows that there was no variation in C1 amount even after 4 h incubation. This result clearly indicates that there was no detectable in vitro formation of C1 fragment of PrP^c in our experimental conditions.

3.2. Correlation between detection of C1 fragment and age, postmortem delay and oxidation state of proteins

To study further the mechanism possibly involved in PrP^c cleavage in human brain, we examined a large series of cases ($n = 21$) that varied in terms of age and postmortem interval. To investigate the correlation between the relative amount of C1 and age or postmortem interval, densitometric data from Western blotting experiments were analyzed using simple linear regression (Fig. 2A and B). There was no significant correlation between the presence of C1 fragment and age ($R^2 = 0.028$; Fig. 2A). Similarly, when the relative abundance of C1 was analyzed with respect to postmortem interval

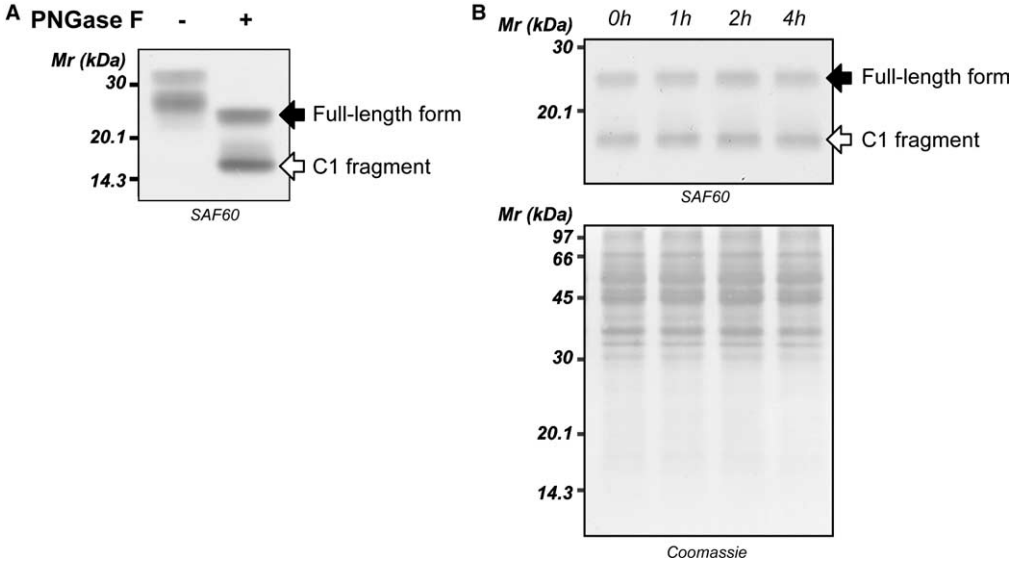

Fig. 1. C1 fragment in the human brain. Electrophoretic pattern of PrP^c in the human brain (A). Twenty μg of parietal isocortex homogenate proteins pretreated or not with PNGase F were separated by SDS–PAGE. PrP^c was detected using SAF60 antibody. PrP^c C1 and absence of spontaneous in vitro lysis (B). Parietal isocortex homogenate was incubated for 0–4 h at 20 °C. Twenty μg of isocortex homogenate protein was treated with PNGase F and separated by SDS–PAGE. PrP^c was detected using SAF60 antibody. Coomassie staining of the poly(vinylidene difluoride) filter is shown as a loading control.

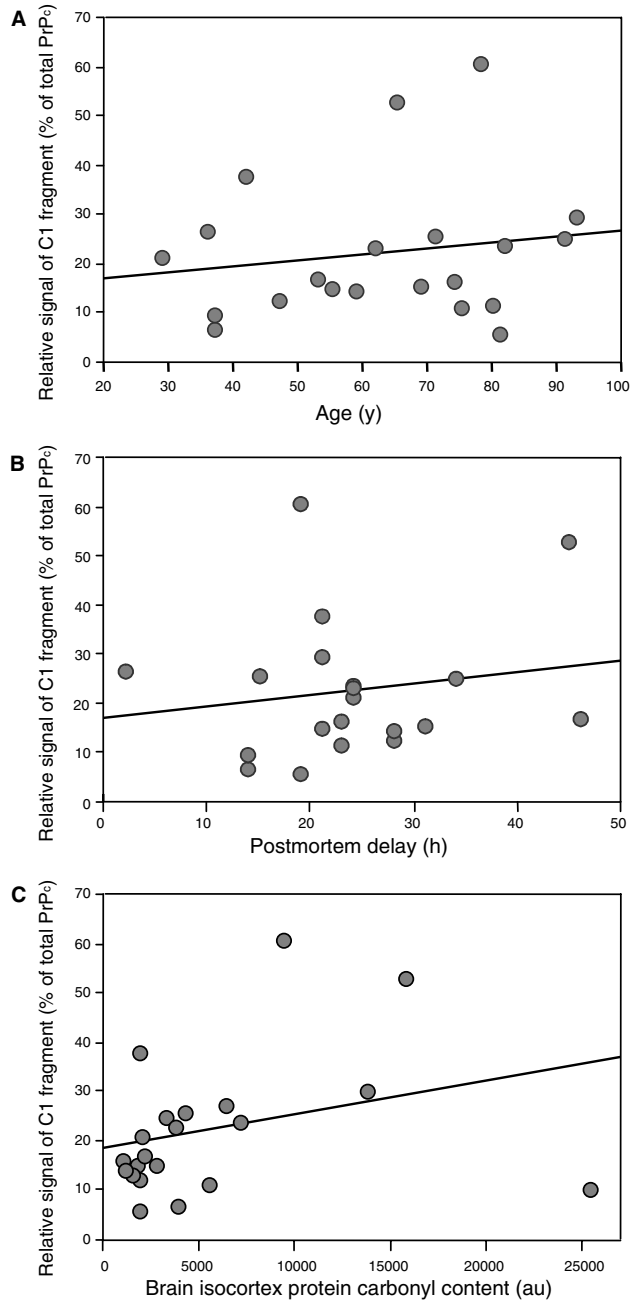

Fig. 2. Level of PrP^c C1 fragment and age, postmortem delay or brain isocortex protein carbonyl content of the cases (A–C). Full-length and truncated PrP^c were vizualized by Western blotting of cerebral isocortex from 21 cases. PrP^c signal was quantified by densitometry. The relative signal of C1 fragment is expressed as a percentage of total PrP^c (sum of full-length PrP^c and C1 fragment) versus age (A), postmortem delay (B) or brain isocortex protein carbonyl content (C). Regression lines are shown.

(Fig. 2B) no correlation was observed ($R^2 = 0.027$). In addition, when the results were analyzed with respect to pathologies no significant differences were found ($P > 0.10$; Kruskal–Wallis test). The value of P was superior to 0.35 when comparing senile dementia of the Alzheimer type group to other diseases group, and superior to 0.70 when comparing amyotrophic lateral sclerosis group to other diseases group (Mann–Whitney test).

Since McMahon et al. [11] have reported the cleavage of PrP^c in cells in culture on exposure to reactive oxygen species, we used a DNP hydrazone detection assay as a measure of oxidative modifications in proteins. Regression analysis of the total dinitrophenylated protein signal with C1 in the 21 human samples showed no correlation between oxidation state of proteins and amount of PrP^c fragment ($R^2 = 0.039$; Fig. 2C).

3.3. Expression of the metalloprotease ADAM10 and PrP^c cleavage

The relative abundance of C-terminal PrP^c fragment versus total PrP^c varied considerably between subjects as illustrated in Fig. 3A for two representative cases. Because the metalloprotease ADAM10 has been reported to cleave PrP^c in vitro [14], we examined the presence of the enzyme in brain homogenates. In samples treated with PNGase F (Fig. 3B), the anti-ADAM10 antibody detected a prominent form of about 80 kDa representing the deglycosylated immature precursor and a shorter and less abundant form of 55 kDa corresponding to the active enzyme processed by furin [17]. The presence of the detectable active form varied between cases but was not related to the intensity of the latent form. Interestingly, active ADAM10 was readily detected in the sample that contained the highest level of C1 fragment (case 2; Fig. 3A and B). Western blot analyses for active ADAM10 were therefore carried out in brain homogenates and combined with the analyses of the relative abundance of C1. The mean of C1 fragment as a percentage of total PrP^c among individuals with detectable mature ADAM10 ($n = 4$) was 42% whereas this average was only 17% among those without detectable ADAM10 ($n = 17$; $p < 0.01$, Mann–Whitney test; Fig. 3C). The amount of C1 fragment was significantly correlated with mature ADAM10 measured in the 21 human samples (Spearman rank- order correlation coefficient: $r_s = 0.63$, $P < 0.005$; Fig. 3D).

4. Discussion

In the present study, we demonstrated that in the human brain: (i) the proportion of the C-terminal C1 fragment of PrP^c was highly variable between individuals; (ii) high amount of cleaved PrP^c was associated with the presence of detectable active zinc metalloprotease ADAM10.

The N-terminal region of PrP^c is involved in the pathogenic conversion of the molecule: (i) in vitro, PrP^{res} formation is significantly reduced by deletion of residues 34–113 [18]; (ii) dominant negative experiments showed that N-terminal truncations of PrP^c are less effective inhibitors of PrP^{res} formation than full-length PrP^c [19]; (iii) work from Flechsig et al. [20] suggests that a PrP^c C-terminal fragment is effective in reversing the resistance of PrP^c null mice to prion diseases, but, incubation times are longer and PrP^{res} is about 30-fold lower than in wild-type mice. It seems that even if the absence of the N-terminal part of PrP^c might not completely prevent transmissible spongiform encephalopathy diseases, it may slow down the infection and therefore, PrP^c truncation could be considered as protective against prion disease propagation. We can speculate that interindividual variations of PrP^c truncation, like those we observed, contribute to interindividual variations in the incubation period and the disease duration within genotype subgroups of cases with sporadic CJD and transmitted forms of the disease.

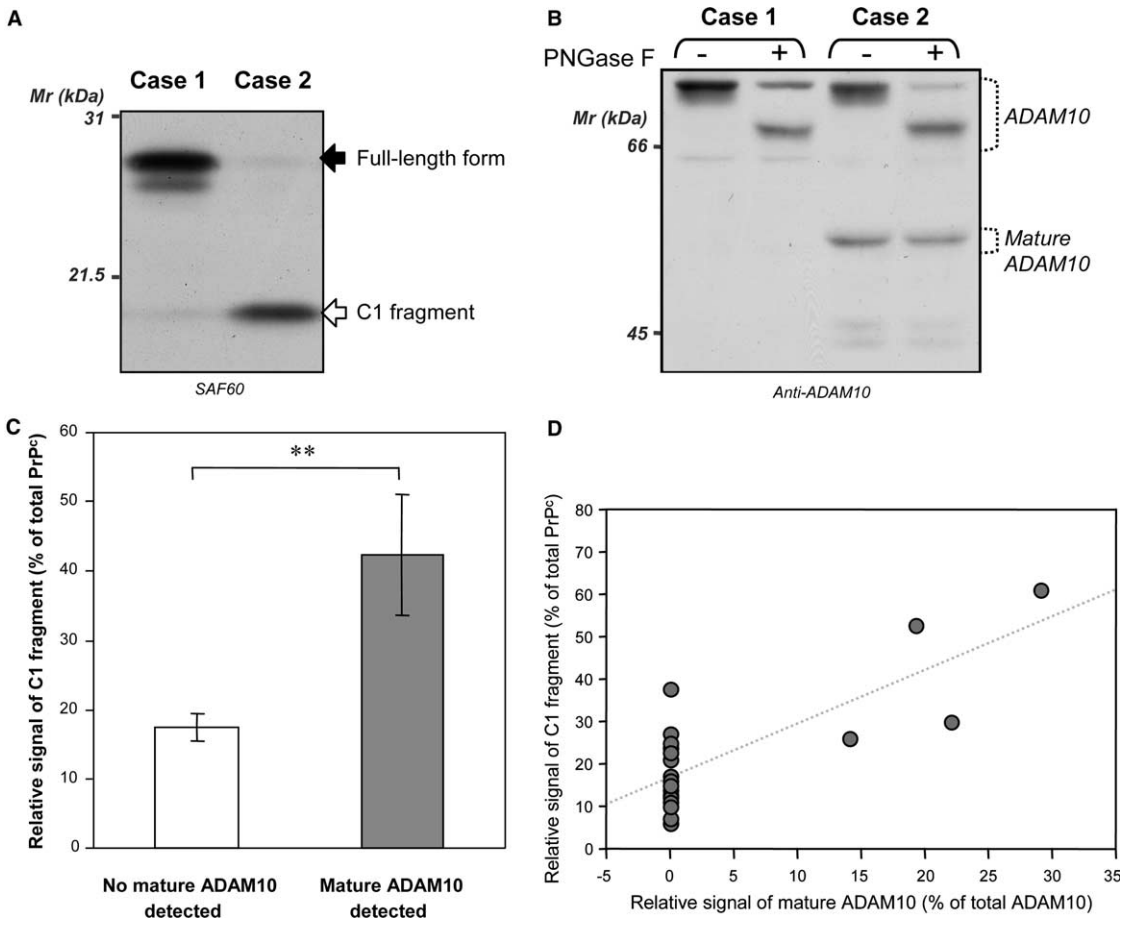

Fig. 3. Expression of the metalloprotease ADAM10 and PrP^c cleavage. Electrophoretic pattern of PrP^c (A) and ADAM10 (B) in human brain. Forty-five μg of protein of human parietal isocortex homogenates from 2 cases were separated by SDS–PAGE. PrP^c was detected using SAF60 antibody (A). ADAM10 was detected using rabbit polyclonal antibodies (B). Search for an association between C1 fragment level and active ADAM10 detection (C,D): The relative signal of C1 fragment expressed as a percentage of total PrP^c (sum of full-length PrP^c and C1 fragment) in 21 cases was statistically different (**, $P < 0.01$) between cases in which active ADAM10 was detected ($n = 4$) and the others ($n = 17$; C). The relative signal of C1 fragment in 21 cases correlated with the relative signal of mature ADAM10 expressed as a percentage of total ADAM10 (sum of mature and immature forms of ADAM10; D).

A variety of structurally and functionally unrelated cell surface proteins can be proteolytically processed by the action of a group of zinc metalloproteases. A previous study has shown that C1 formation in brain homogenates could be blocked with inhibitors of metalloproteases [13]. Further supports for a metalloprotease-mediated mechanism in C1 formation have come from studies of human embryonic kidney 293 transfected cells in which the formation of C1 fragment was induced by stably overexpressing human ADAM10 [14]. ADAM10, a mammalian disintegrin metalloprotease, processes many molecules such as ephrin-A2, a protein with a glycosylphosphatidylinositol anchor. We tested the hypothesis that ADAM10 may participate in C1 formation in vivo. When we used the presence or absence of the active form of ADAM10 as a criterion for separating the patients into two groups, we found that the groups differed significantly in the levels of C1. Namely, in brain homogenates from individuals in which mature ADAM10 was detected, level of PrP^c C1 was more than twice as abundant as compared to individuals in which we did not detect ADAM10. Taken together, our results are consistent with a role for ADAM10 in PrP^c cleavage in the human brain.

In summary, our results demonstrate an important interindividual variability in the abundance of truncated PrP^c in the human brain. We did also observe that the mature form of ADAM10 was detected in individuals with higher levels of the PrP^c fragment supporting the notion that this metalloprotease contributes to PrP^c cleavage. Finally, given that expression of truncated and full-length PrP^c influences prion propagation, this pathway may contribute to interindividual variations in prion diseases progression.

Acknowledgements: This work was supported in part by the GIS "Infections à prions". B.A. Faucheux acknowledges funding from the Assistance Publique-Hôpitaux de Paris (Délégation à la Recherche Clinique et au Développement).

References

[1] Prusiner, S.B. (1996) Molecular biology and pathogenesis of prion diseases. Trends Biochem. Sci. 21, 482–487.
[2] Parchi, P., Giese, A., Capellari, S., Brown, P., Schulz-Schaeffer, W. and Windl, O., et al. (1999) Classification of sporadic Creutzfeldt–Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann. Neurol. 46, 224–233.
[3] Palmer, M.S., Dryden, A.J., Hughes, J.T. and Collinge, J. (1991) Homozygous prion protein genotype predisposes to sporadic Creutzfeldt–Jakob disease. Nature 352, 340–342.

[4] Wadsworth, J.D., Asante, E.A., Desbruslais, M., Linehan, J.M., Joiner, S. and Gowland, I., et al. (2004) Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science 306, 1793–1796.
[5] Brandel, J.P., Preece, M., Brown, P., Croes, E., Laplanche, J.L. and Agid, Y., et al. (2003) Distribution of codon 129 genotype in human growth hormone-treated CJD patients in France and the UK. Lancet 362, 128–130.
[6] Hauw, J.J., Sazdovitch, V., Laplanche, J.L., Peoc'h, K., Kopp, N. and Kemeny, J., et al. (2000) Neuropathologic variants of sporadic Creutzfeldt–Jakob disease and codon 129 of PrP gene. Neurology 54, 1641–1646.
[7] Goldfarb, L.G., Petersen, R.B., Tabaton, M., Brown, P., LeBlanc, A.C. and Montagna, P., et al. (1992) Fatal familial insomnia and familial Creutzfeldt–Jakob disease: disease phenotype determined by a DNA polymorphism. Science 258, 806–808.
[8] Prusiner, S.B. (1998) Prions. Proc. Natl. Acad. Sci. USA 95, 13363–13383.
[9] Chen, S.G., Teplow, D.B., Parchi, P., Teller, J.K., Gambetti, P. and Autilio-Gambetti, L. (1995) Truncated forms of the human prion protein in normal brain and in prion diseases. J. Biol. Chem. 270, 19173–19180.
[10] Shyng, S.L., Huber, M.T. and Harris, D.A. (1993) A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J. Biol. Chem. 268, 15922–15928.
[11] McMahon, H.E., Mange, A., Nishida, N., Creminon, C., Casanova, D. and Lehmann, S. (2001) Cleavage of the amino terminus of the prion protein by reactive oxygen species. J. Biol. Chem. 276, 2286–2291.
[12] Mange, A., Beranger, F., Peoc'h, K., Onodera, T., Frobert, Y. and Lehmann, S. (2004) Alpha- and beta-cleavages of the amino-terminus of the cellular prion protein. Biol. Cell 96, 125–132.
[13] Jimenez-Huete, A., Lievens, P.M., Vidal, R., Piccardo, P., Ghetti, B. and Tagliavini, F., et al. (1998) Endogenous proteolytic cleavage of normal and disease-associated isoforms of the human prion protein in neural and non-neural tissues. Am. J. Pathol. 153, 1561–1572.
[14] Vincent, B., Paitel, E., Saftig, P., Frobert, Y., Hartmann, D. and De Strooper, B., et al. (2001) The disintegrins ADAM10 and TACE contribute to the constitutive and phorbol ester-regulated normal cleavage of the cellular prion protein. J. Biol. Chem. 276, 37743–37746.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
[16] Jolivalt, C., Leininger-Muller, B., Drozdz, R., Naskalski, J.W. and Siest, G. (1996) Apolipoprotein E is highly susceptible to oxidation by myeloperoxidases, an enzyme present in the brain. Neurosci. Lett. 210, 61–64.
[17] Anders, A., Gilbert, S., Garten, W., Postina, R. and Fahrenholz, F. (2001) Regulation of the alpha-secretase ADAM10 by its prodomain and proprotein convertases. FASEB J. 15, 1837–1839.
[18] Lawson, V.A., Priola, S.A., Wehrly, K. and Chesebro, B. (2001) N-terminal truncation of prion protein affects both formation and conformation of abnormal protease resistant prion protein generated in vitro. J. Biol. Chem. 276, 35265–35271.
[19] Zulianello, L., Kaneko, K., Scott, M., Erpel, S., Han, D. and Cohen, F.E., et al. (2000) Dominant-negative inhibition of prion formation diminished by deletion mutagenesis of the prion protein. J. Virol. 74, 4351–4360.
[20] Flechsig, E., Shmerling, D., Hegyi, I., Raeber, A.J., Fischer, M. and Cozzio, A., et al. (2000) Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. Neuron 27, 399–408.