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

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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).

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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

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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

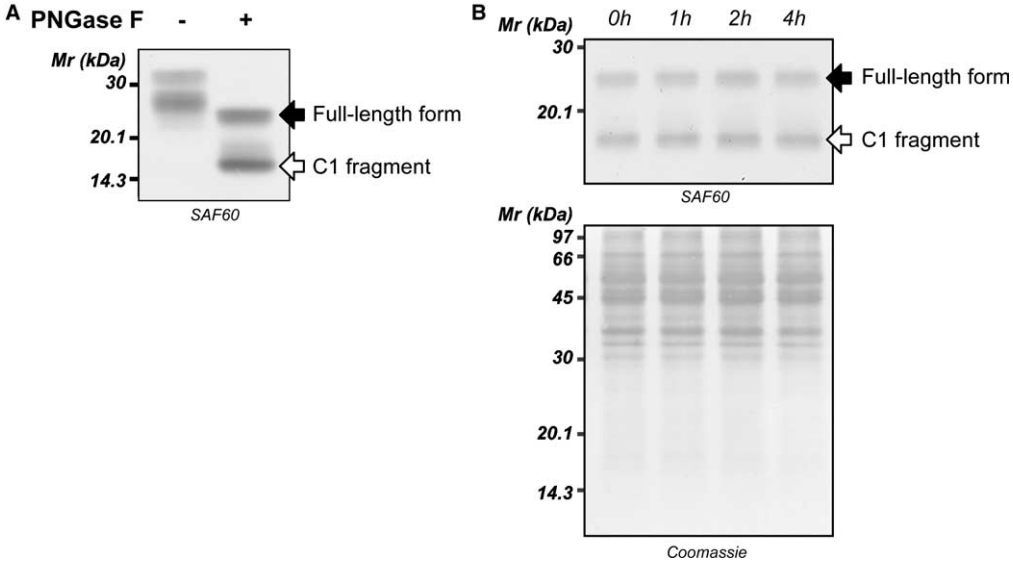

Fig. 1. C1 fragment in the human brain. Electrophoretic pattern of PrP^c in the human brain (A). Twenty μg of parietal isocortex homogenate proteins pretreated or not with PNGase F were separated by SDS–PAGE. PrP^c was detected using SAF60 antibody. PrP^c C1 and absence of spontaneous in vitro lysis (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.