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Recombinant Neural Protein PrP Can Bind with Both Recombinant and Native Apolipoprotein E *In Vitro*

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Abstract The most essential and crucial step during the pathogenesis of transmissible spongiform encephalopathy is the conformational change of cellular prion protein (PrP^{C}) to pathologic isoform (PrP^{Sc}). A lot of data revealed that caveolae-like domains (CLDs) in the cell surface were the probable place where the conversion of PrP proteins happened. Apolipoprotein E (ApoE) is an apolipoprotein which is considered to play an important role in the development of Alzheimer's disease and other neurodegenerative diseases by forming protein complex through binding to the receptor located in the clathrin-coated pits of the cell surface. In this study, a 914-bp cDNA sequence encoding human ApoE3 was amplified from neuroblastoma cell line SH-SY5Y. Three human ApoE isomers were expressed and purified from *Escherichia coli*. ApoE-specific antiserum was prepared by immunizing rabbits with the purified ApoE3. GST/His pull-down assay, immunoprecipitation and ELISA revealed that three full-length ApoE isomers interact with the recombinant full-length PrP protein *in vitro*. The regions corresponding to protein binding were mapped in the N-terminal segment of ApoE (amino acid 1–194) and the N-terminal of PrP (amino acid 23–90). Moreover, the recombinant PrP showed the ability to form a complex with the native ApoE from liver tissues. Our data provided direct evidence of molecular interaction between ApoE and PrP. It also supplied scientific clues for assessing the significance of CLDs on the surface of cellular membrane in the process of conformational conversion from PrP^{C} to PrP^{Sc} and probing into the pathogenesis of transmissible spongiform encephalopathy.

Key words prion disease; PrP; apolipoprotein E; protein interaction; caveolae-like domain

PrP is a cell surface glycoprotein that exists in neurons and other tissues in mammals. Numerous evidences implied that PrP plays an important role in copper metabolism, signal transduction and other biological processes in the central nerve system [1]. In a group of rare and fatal neurodegenerative diseases, such as transmissible spongiform encephalopathy (TSE) or prion diseases, the normal cellular membrane protein PrP^{C} conformationally changes to its abnormal pathogenic form PrP^{Sc} by exposure to extraneous PrP^{Sc} or other unknown pathways [2].

Prion diseases have been described in numerous mammalian species, including sheep and goat scrapie, bovine spongiform encephalopathies (BSE) and human Creutzfeldt-Jakob disease (CJD). The main neuropathology changes of these diseases are spongiform degeneration and the abnormal deposit of PrP in central nerve tissues [3]. Although it has been widely recognized that the most essential step is the conformational conversion from normal

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PrP^{C} to abnormal PrP^{Sc}, the exact sites in cells and the mechanisms still remain unknown. Several reports indicated that the conformational change of prion protein might take place in cell membrane. Recent studies suggested that the pathogenic conformational change possibly occurs in the CLDs within the plasma membrane [4].

Apolipoprotein E (ApoE) is a lipoprotein that exists widely in various tissues. Three isoforms have been mapped in the population, ApoE2, E3 and E4, which are encoded by ε2, ε3 and ε4 alleles, respectively, differing only in their amino acid sequences at positions 112 and 158 [5]. ApoE forms a protein complex through binding to its low-density lipoprotein receptor (LDLR), located in the clathrin-coated pits and CLDs of the cell surface. The ligand-receptor complex can be taken up by the cells via clathrin-mediated endocytosis, mediating the clearance of cholesterol particles from blood [6]. Some other physiological functions of ApoE have also been described, such as signal transduction, cellular nutrition, cell generation and development [7].

ApoE is considered to play an important role in the pathogenesis of some neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease [8, 9]. In AD, ApoE can affect the clearance and deposit of β-amyloid by binding with it [10]. Some studies suggested that ApoE is related to prion diseases. In a squirrel monkey TSE animal model, ApoE has been found to be co-localized with PrP in brain tissue [11]. Increased transcription of the specific ApoE mRNA was observed in mouse brains infected by TSE agent [12]. In the cerebral spinal fluids of BSE infected cattle, remarkable increases in ApoE were repeatedly found [13]. These evidences highlighted that ApoE might somehow participate in the pathogenesis of TSE.

In order to address the possible molecular interaction between PrP and ApoE, the two proteins were employed into the assays for protein-protein interaction. We found that recombinant PrP was able to form complexes with both recombinant and native ApoE *in vitro*. Our findings supplied scientific clues to the hypothesis that the molecular interaction between PrP and ApoE may help PrP, even PrP^{Sc}, enrich in CLDs, where the PrP pathological conformational change may take place.

Materials and Methods

Cell culture and RNA extraction

Human neuroblastoma cell line SH-SY5Y was maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, USA) containing 10% fetal cattle serum, 50 U/ml penicillin and 50 μg/ml streptomycin. Total cellular RNA was extracted with a commercially supplied Trizol agent (Invitrogen, Carlsbad, USA) and stored at −70 ºC.

Plasmid construction

To obtain cDNA of human ApoE, 1 μg SH-SY5Y cellular RNA was mixed with 5 U AMV reverse transcriptase (Invitrogen), 10 U RNasin, 20 mM dNTP and 20 pM oligo(dT) in a total volume of 20 μl at 42 ºC for 1 h. Two microliters of product was mixed with 2.5 U LA *Taq* polymerase (TaKaRa, Dalian, China), 20 mM dNTP, 2×GC buffer II, and human *ApoE* gene specific primers ApoE-F (5′-AGGATCCAAGGTGGAGCAAGCG-3′, *Bam*HI site underlined) and ApoE-B (5′-AGAATTCGTGATTGTCGC-TGGG-3′, *Eco*RI site underlined) for PCR at following conditions: 94 ºC for 30 s, 58 ºC for 30 s, 72 ºC for 60 s, 30 cycles. The 914 bp PCR product was ligated with commercially supplied pMD18-T vector (TaKaRa) generating pT-ApoE3. After being verified by sequencing, the insert was cleaved from pT-ApoE3 with *Bam*HI and *Eco*RI, and cloned into a His-tag fusion expression vector pET32a (Novagen, San Diego, USA) generating pET-ApoE3. The plasmids pET32-E2 and pET32-E4 containing full-length human ApoE2 and E4 cDNA respectively were kindly provided by Prof. K. H. WEISGRABER [14].

To construct the expression recombinant plasmids for N- and C-terminal of ApoE, the segment encoding amino acid 1–194 of ApoE was amplified with the primers ApoE-F and ApoE194-B (5′-AAGCTTTCAAGTGGCG-GCCCGC-3′, *Hin*dIII site underlined), and the segment encoding ApoE peptide from amino acid 195 to 299 was amplified with the primers ApoE299-F (5′-GGATCCACTG-TGGGCTCCCTG-3′, *Bam*HI site underlined) and ApoE-B, both using pT-ApoE3 as the templates. The amplified ApoE fragments were separately cloned into expressing plasmid pQE30-GST containing both His-tag and GST-tag [15], generating pQEG-ApoE-N and pQEG-ApoE-C.

To generate C-terminus truncated human *prnp* gene that encodes 68 amino acids (amino acid 23–90), PCR was carried out with primers HuPrP-F (5′-GGATCCATGAA-GAAGCGGCCAAAGCCTGG-3′, *Bam*HI site underlined) and HuPrP-B-90 (5′-GAATTCCTGACTGTGGGTGC-CACCTTATTGA-3′, *Eco*RI site underlined) at following conditions of 94 ºC for 50 s, 58 ºC for 50 s and 72 ºC for 60 s, 30 cycles, using pT-HuPrP [16] as the template. The 216 bp fragment of PCR product was ligated to pMD18-T vector generating pT-HuPrP23–90, and then subcloned into a GST fusion expression vector pGEX-2T (Amersham

Pharmacia, Uppsala, Sweden) generating pGST-HuPrP23–90.

Protein expression and purification

Three isoforms of His-ApoE, GST-ApoE-N and GST-ApoE-C, as well as HuPrP23–90, HuPrP91–231 and HuPrP23–231 [16] were expressed in *Escherichia coli* strain BL21(DE3) or JM109, respectively. Briefly, transformed bacteria were grown to an A_{600} of 0.5–0.6 and induced by isopropyl-β-*D*-thiogalactoside at final concentration of 0.5 mM. Cells were harvested by centrifugation. Then cells were resuspended in PBS (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) as protease inhibitor for His-tagged protein expression; or resuspended in PBS containing 1 mM EDTA, 300 mM NaCl and 30 mM Tris-HCl, pH 8.0, 1 mM PMSF for GST-fusion protein expression. Lysozyme was added to a final concentration of 20 μg/ml, and cells were lysed by incubation for 30 min and sonication for 24×10 s with a 10 s interval at 400 W. The His-tagged proteins were purified with Ni-NTA agarose (Qiagen, Hilden, Germany), and GST-fusion proteins were purified with glutathione-Sepharose 4B (Amersham Pharmacia), according to the manufacturers' protocols. Protein concentrations were determined using the BCA kit (Qiagen).

Western blot

Various purified ApoE proteins, ApoE N- and C-terminal proteins were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% defatted milk in PBST (phosphate buffered saline, pH 7.6, containing 0.05% Tween-20) overnight at 4 ºC, the membranes were incubated with 1:2000 rabbit anti-ApoE antibody (Santa Cruz, Santa Cruz, USA) for 2 h at room temperature and then further incubated with 1:2000 horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Santa Cruz). The protein bands were visualized by ECL kit (PE Applied Biosystems, Foster City, USA).

Antibody preparation

Five hundred micrograms of purified ApoE protein was mixed with complete Freund's adjuvant and injected hypodermically into SPF-level rabbits at multi-points. Ten days later, the rabbits were boosted by 200 μg of ApoE protein mixed with incomplete adjuvant. Total five boosting were done at a 10 d interval. Two weeks after the fifth boosting, rabbits' blood was collected using a carotid intubation under anesthesia with ether. For the purification of IgG, the collected sera were precipitated with 50% and 33% ammonium sulfate in sequence, and furthermore, purified with Sepharose G chromatography.

ELISA

Polyclonal antibodies of Doppel [17] and Tau [18] were described elsewhere. Anti-GST polyclonal antibodies were purchased from Santa Cruz. An ELISA protocol was established to screen the potential interactions between ApoE and other proteins. His-ApoE in 0.05 M sodium bicarbonate buffer, pH 9.6, was coated onto a 96-well plate at 100 ng/well at 4 ºC overnight. All wells were blocked with 5% bovine serum albumin (BSA) in PBST at room temperature for 2 h. Various testing proteins of the same molar concentration in PBS containing 2% BSA were transferred to the wells. After 2 h incubation, the plates were washed with PBST three times, and polyclonal antibodies against the corresponding proteins were used at a dilution of 1:4000 and incubated for 45 min. Bound antibodies were detected using horseradish peroxidase conjugated secondary antibody and developed with 3,3′,5,5′-tetramethylbenzidine (Sigma, St. Louis, USA). Absorbance at 450 nm was measured using a microplate reader after the reaction was terminated by addition of 2 M H_2SO_4. An equal amount of GST protein was used as the control.

To screen the potential interactions of various PrP segments with different isoforms of ApoE, 50 ng of each PrP protein was coated onto wells of a 96-well microplate and subsequently incubated with various ApoE proteins. The bound ApoE was measured with the same protocol described above.

Immunoprecipitation

Ten micrograms of three isoforms of ApoE were respectively mixed with 5 μg of HuPrP23–231 or HuPrP91–231 in binding buffer (50 mM Tris-HCl, 100 mM NaCl, pH 8.0) in a volume of 500 μl at 4 ºC for 2 h. After incubation with 1:2000 diluted monoclonal antibody 3F4 (DakoCytomation, Cambridgeshire, UK) for 2 h, 10 μl of protein G Sepharose beads pre-equilibrated with binding buffer were introduced into the reaction mixture and incubated for another 2 h with vibrant shaking. The Sepharose beads were precipitated by centrifugation at 500 *g* for 5 min and washed with 500 μl of washing buffer (50 mM Tris-HCl, 200 mM NaCl, pH 8.0) for three times. The bound antibody-antigen complexes were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. The bound ApoE proteins were detected with 1:2000 diluted anti-ApoE polyclonal antibodies. To address the interaction between ApoE and PrP N-terminal segments, 10 μg of each of three isoforms of ApoE protein was

incubated with 5 μg of HuPrP23–90 respectively, and subsequently precipitated with anti-PrP polyclonal antibodies [19]. The bound ApoE proteins were detected according to the protocol described above.

GST fusion protein pull-down assay

To identify interactions between HuPrP23–231 and ApoE N- or C-terminal fragment, 5 μg of purified HuPrP23–231 protein was incubated with 10 μg of ApoE N- or C-terminal fragment in 500 μl of binding buffer containing 20 mM Tris-HCl, 200 mM NaCl, 10 mM aprotinin, pH 8.0, at 4 ºC for 4 h, while an equal amount of GST protein was used as a control. Fifteen microliters of glutathione agarose beads were added to the reaction solution and incubated at 37 ºC for 30 min with end-over-end mixing. After centrifugation at 500 *g* for 2 min, the supernatants were discarded and beads were washed three times with 500 μl of binding buffer. The complex was separated on 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. To visualize the bound PrP protein, a Western blot assay was carried out, using 3F4 antibody at 1:2000 as the primary antibody and HRP-conjugated anti-mouse IgG (Santa Cruz) at 1:4000 as the secondary antibody.

One gram of liver tissue from healthy hamsters was prepared to 10% homogenates in lyses buffer [20]. The homogenate was centrifuged at 20,000 *g* for 90 min, removing the debris of the tissue. Ten microgrammes of HuPrP23–90 (with GST-tag) protein was added to the homogenate in a volume of 2 ml at 4 ºC for 4 h. The bound ApoE was detected as described above.

His-tagged protein pull-down analysis

Five microgrammes of HuPrP23–231 (with His-tag) and 2 ml of 10% hamster liver homogenate were incubated at 4 ºC for 4 h. Ni-NTA agarose (10 μl) pre-equilibrated with binding buffer were introduced into the mixture and incubated for 2 h with vibrant shaking. The mixture was centrifuged at 500 *g* for 2 min, and the supernatant was discarded and beads were washed three times with 500 μl of binding buffer. The complexes were separated on 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The bound ApoE was detected as described above.

Results

Expression of various ApoE proteins in *E. coli*

A 914 bp cDNA fragment corresponding to the full length human ApoE was amplified from cell line SH-SY5Y, with A mutated to G at nt 787. Sequence of Cys112/Arg158 indicated that it was the E3 isoform. Using affinity chromatography of Ni-NTA agarose, an approximately 54 kDa His-ApoE fusion protein was purified from the lysate of *E. coli* BL21(DE3) cells transformed with the recombinant plasmid pET-ApoE3 [**Fig. 1(A)**]. Western blot analysis revealed that the 54 kDa protein was specifically recognized by the commercial anti-ApoE antibody [**Fig. 1 (D)**, lane 2].

N- and C-truncated ApoE proteins containing GST were expressed in the *E. coli* strain JM109 and purified by the affinity chromatography of Ni-NTA agarose. As expected, two fusion proteins, at approximately 48 kDa (ApoE-N) [**Fig. 1(B)**] and 37 kDa (ApoE-C) [**Fig. 1(C)**], were specifically recognized by anti-ApoE antibody in Western blot [**Fig. 1(D)**, lanes 1 and 3].

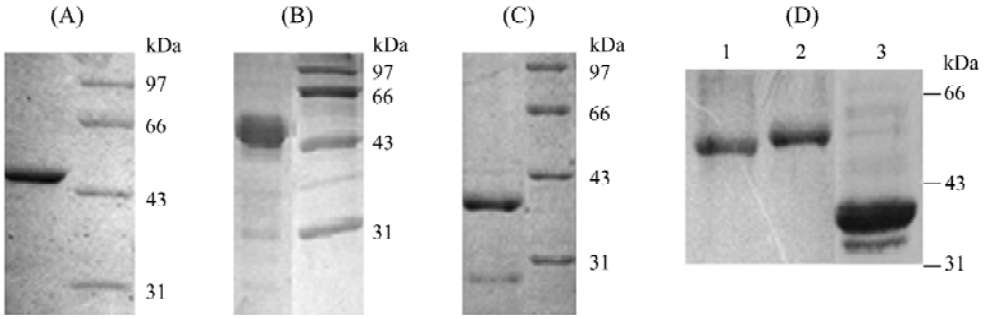

Fig. 1 Purification and identification of various ApoE proteins in *Escherichia coli* and 12% SDS-PAGE analyses of the purified proteins

(A) His-ApoE3. (B) GST-ApoE-N. (C) GST-ApoE-C. (D) Western blot analysis with ApoE specific antibody. 1, GST-ApoE-N; 2, His-ApoE3; 3, GST-ApoE-C. The protein molecular markers were illustrated on the right.

Three ApoE isoforms interacted with PrP protein *in vitro*

It has been described that ApoE is involved in the growth and other activities of neuron cells [21]. To demonstrate the interactions of ApoE3 and PrP proteins, an ApoE3-coated ELISA was established to capture the possibly bound protein, using GST protein as a negative control. At the same mole ratios as coated ApoE, the full-length human PrP (HuPrP23–231) showed obvious binding activity (**Fig. 2**, column 3), while the truncated PrP (HuPrP91–231) did not show any binding capacity with the coated ApoE (**Fig. 2**, column 4) compared with the negative control. To address the potential interactions of ApoE and other neuroproteins, recombinant Tau and Doppel were tested in the ApoE-coated ELISA. Both Tau and Doppel did not show any activity in binding with the coated ApoE (**Fig. 2**, columns 1 and 2) compared with the negative control. It implied that ApoE might specially interact with PrP.

Immunoprecipitation tests revealed that all ApoE2, E3 and E4 could be precipitated with anti-PrP antibody in the presence of HuPrP23–231 (**Fig. 3**), whereas none of the three ApoE proteins showed any detectable interaction with HuPrP91–231 (**Fig. 3**). Quantitative analyses of the immunoblot images did not show a remarkable difference between the three ApoE proteins and HuPrP23–231 (data not shown). These results implied a molecular interaction between ApoE and PrP *in vitro*, probably in the N-terminal region of PrP.

To test whether PrP protein had different binding activities with various ApoE isoforms, ApoE2, E3 and E4 were incubated in the wells coated with HuPrP23–231 respectively, and GST was used as a negative control. Obviously, with the increasing amounts of ApoE in the preparations, the *A* values increased, showing a dose-dependant manner (**Fig. 4**). No notable difference in binding activity of HuPrP23–231 was observed among three isoforms, when mole ratios of ApoE to PrP were 2:1, 1:1, 1:2 and 1:4. Only in the preparations with more ApoE molecules (ApoE to PrP was 5:1 and 10:1), did ApoE3 show relatively stronger binding ability.

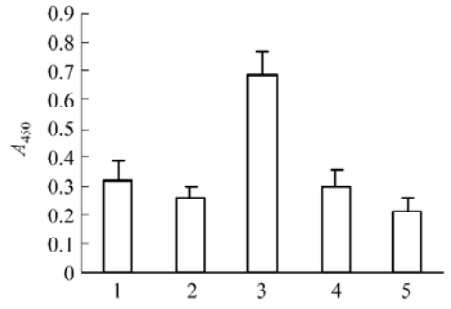

Fig. 2 Screening of the potential molecular interactions of ApoE with several neural proteins using an ApoE coated ELISA
1, Doppel; 2, Tau; 3, HuPrP23–231; 4, HuPrP91–231; 5, GST. The values of A_{450} are averaged by three independent assays.

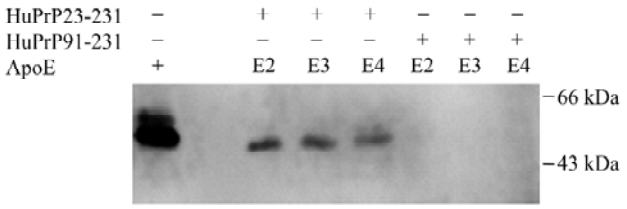

Fig. 3 Immunoprecipitation test for molecular interactions of three ApoE isomers with HuPrP23–231 and HuPrP91–231
The first lane from left is directly input His-ApoE used as a marker. Protein molecular weight markers are shown on the right.

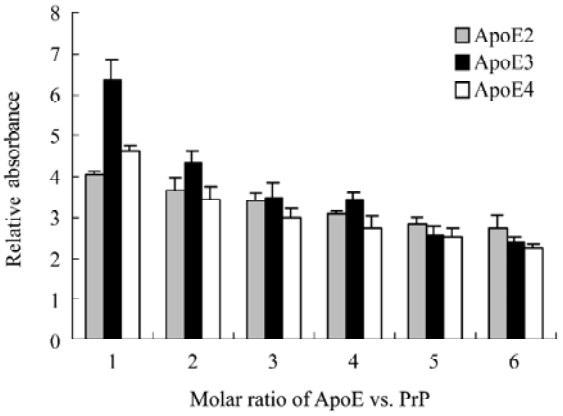

Fig. 4 Comparison of the interaction activities of three individual ApoE isomers with HuPrP23–231 in ELISA
1–6, the molar ratios of ApoE versus PrP are 10:1, 5:1, 2:1, 1:1, 1:2 and 1:4, respectively. Data were represented as mean±SD (n=3).

Binding position of PrP to ApoE located at amino acid 23–90 of PrP

The failure for HuPrP91–231 to bind with ApoE in immunoprecipitation and ELISA indicated that the region in PrP that interacts with ApoE might locate in its N-terminal. To confirm this possibility, a 204-bp human *prnp* sequence that encodes amino acid 23–90 was inserted into pGEX-2T and transformed into *E. coli* BL21(DE3). An approximately 30-kDa protein in GST-fusion form was purified by the affinity chromatography of glutathione agarose and verified by Western blot with anti-PrP

polyclonal antibodies. An immunoprecipitation test showed that all ApoE isoforms formed detectable complexes with HuPrP23–90, and the bound ApoE was recognized by anti-ApoE antibody in Western blot [**Fig. 5(A)**]. Furthermore, full-length (HuPrP23–231), N-terminal (HuPrP23–90) and C-terminal (HuPrP91–231) PrP segments were tested in ApoE3-coated ELISA, in which GST-coated wells were used as negative controls in parallel. **Fig. 5(B)** showed that HuPrP23–231 and HuPrP23–90 have remarkable binding activities with the fixed ApoE3, whereas HuPrP91–231 failed. At molar ratios of 1:1 and 1:2 (ApoE3 vs. PrP), HuPrP23–90 showed comparable binding ability as the full-length HuPrP23–231. These results indicated that the interacting region of PrP with ApoE is located at the N-terminal.

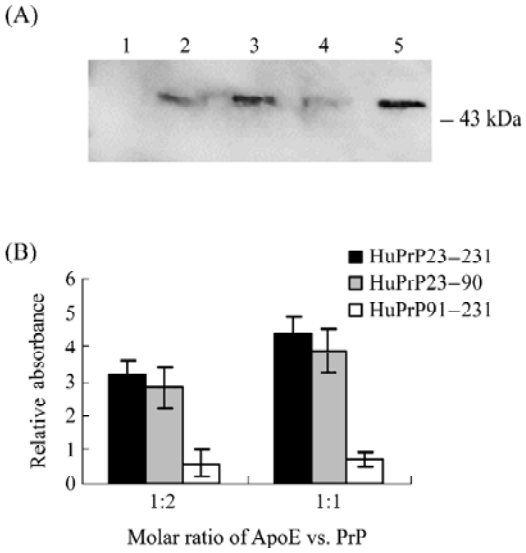

Fig. 5 Molecular interaction of three ApoE isoforms with N-terminal segment of PrP

(A) Immunoprecipitation assay. 1, GST; 2, ApoE2; 3, ApoE3; 4, ApoE4; 5, direct input ApoE3 as positive control. Protein molecular weight marker is shown on the right. (B) ELISA. Data were represented as mean±SD (n=3).

N-terminal of ApoE binds with PrP protein

To map the region within ApoE interacting with PrP protein, same mole amount of full-length ApoE protein, ApoE N- and C-terminal fragments were incubated with the HuPrP23–231 and precipitated with anti-PrP-specific antibody respectively. The bound ApoE molecules were visualized by Western blot with anti-ApoE specific antibody. The protein complexes were detected clearly in the reactions containing full length and N-terminal ApoE proteins and HuPrP23–231 [**Fig. 6(A)**, lanes 2 and 3], but not in the reactions of C-terminal ApoE (lane 4) and GST protein (lane 5). Since the expressed ApoE N- and C-terminal proteins had GST-tag, GST pull-down tests were also conducted with the same amount of HuPrP23–231. After eluted from glutathione-Sepharose 4B, the bound PrP was detected by Western blot with anti-PrP-specific antibody. A very clear PrP signal was detected in the preparation of ApoE N-terminal protein [**Fig. 6(B)**, lane 2], but not in that of ApoE C-terminal protein (lane 3), the control GST (lane 4) or in that of GST-CAT (lane 5), indicating that ApoE N-terminal peptide formed a complex with the input of PrP protein. It also indicated that the region within ApoE responsible for interaction with PrP might locate at N-terminal region.

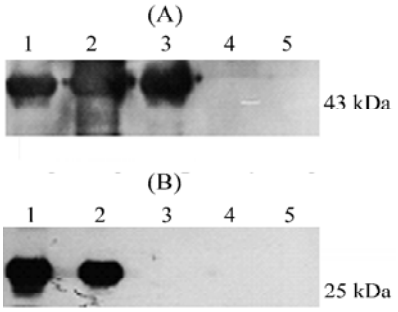

Fig. 6 Molecular interaction of HuPrP23–231 with various ApoE segments

(A) Immunoprecipitation assay. 1, direct input ApoE as positive control; 2, ApoE; 3, GST-ApoE-N; 4, GST-ApoE-C; 5, GST. (B) GST pull-down assay. 1, directly input PrP as positive control; 2, GST-ApoE-N; 3, GST-ApoE-C; 4, GST; 5, GST-CAT. Molecular weight markers are shown on the right.

PrP proteins interact with the native ApoE from liver tissues

ApoE is remarkably expressed in liver and brain tissues. To find out whether PrP protein could form a complex with native ApoE *in vitro*, hamster liver homogenates were prepared. After incubation with recombinant HuPrP23–231, the mixture was incubated with Ni-NTA agarose and the possible bound ApoE signals were visualized by Western blot with anti-ApoE antibody. **Fig. 7(A)** showed a 34 kDa ApoE-specific band in the reaction of HuPrP23–231 with liver tissue extracts (lane 1), whereas there was no positive signal in the preparation containing only HuPrP23–231 or liver extracts (lanes 2 and 3), indicating that the recombinant PrP was able to react with the native ApoE in the liver homogenate. Furthermore, GST fusion protein

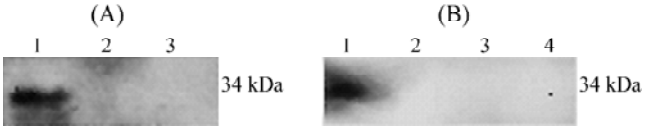

Fig. 7 Binding of native ApoE in liver tissues with PrP proteins

(A) His pull-down assay of HuPrP23–231. 1, HuPrP23–231 with liver tissue extracts; 2, extracts of liver tissues without HuPrP23–231; 3, HuPrP23–231 without extracts of liver tissues. (B) GST pull-down assay of HuPrP23–90. 1, GST-HuPrP23–90 with liver tissue extracts; 2, GST; 3, extracts of liver tissues without GST-HuPrP23–90; 4, GST-HuPrP23–90 without extracts of liver tissues. Molecular weight markers are shown on the right.

HuPrP23–90 was mixed with liver extracts and GST pull-down assay was conducted. Subsequent immunoblot with anti-ApoE antibody revealed that a 34 kDa band in the preparation of HuPrP23–90 with liver extract [**Fig. 7(B)**, lane 1], but not in GST control (lane 2) or in the preparations either containing only HuPrP23–90 (lane 3) or liver extracts (lane 4). The results suggested that the N-terminal PrP peptide could bind the native ApoE.

Discussion

The data in this study provided direct evidence that recombinant PrP can bind to both recombinant and native ApoE proteins *in vitro*. ApoE, a 299-amino acid protein (34 kDa), plays a significant role in lipoprotein metabolism, as it is the major ligand in receptor-specific lipoprotein uptake. ApoE is a ligand for all members of the LDLR family and a constituent of lipoprotein particles that transport lipids throughout the circulation and between cells. In the nervous system, non-neuronal cell types, most notably astroglia and microglia, are the primary producers of ApoE, while neurons preferentially express the receptors for ApoE [22]. Increased transcription of ApoE mRNA, remarkable co-deposits of ApoE with PrP and disease progressive-related increase of ApoE in the brain tissues from naturally and experimentally infected animals indicate that ApoE might participate in the pathogenesis of TSE [12]. Our study suggested a novel molecular basis that other proteins in nerve tissues, i.e., ApoE may participate in the pathogenesis of prion diseases.

In humans, ApoE exists in three major isoforms, E2, E3 and E4. Among them, E4 isoform is at greater risk for developing late-onset Alzheimer's disease [23]. A French research group has even suggested that the ApoE alleles are major susceptible factors for CJD, in which ε4 allele of the ApoE gene is taken as a risk factor [24]. However, subsequent researches with more samples proved that the difference is not statistically significant [25,26]. Our protein interaction tests *in vitro* did not reveal a significant difference in the binding activity with PrP among the three ApoE isoforms. Although the influence of different ApoE isoforms on TSE sensitivity and pathogenesis still remains unclear, similar binding activities of ApoE proteins to PrP suggest that ApoE isoforms may not have differences, at least, in recognizing PrP molecules.

It is generally accepted that the conformational change of prion is the most important and crucial step in TSE pathogenesis. The region responsible for interaction with ApoE within PrP protein was assigned to residue 23–90 at the N-terminus. There are four proline/glycine rich octarepeats (PHGGGWGQ) between amino acid residues 51 and 90. Structural analyses of PrP protein reveal that the N terminal is highly flexible and lacks identifiable secondary structure under the experimental conditions. Several biological activities have been confirmed in this region, including binding Cu^{2+}, interacting with sGAG proteoglycan and several neuron proteins [27]. However, the region correlating with neural toxic lies in the middle region of PrP protein (amino acid 106–126), while C terminus segment corresponds to the conformational change [4,28]. It indicates that PrP protein may bind to target proteins or receptors through its N-terminal segment, and afterwards, displays its physiological or pathological activities through exposing its middle and C terminal domains.

Our results showed that the fragment of residue 1–194 at N-terminus within ApoE protein region is responsible for interaction with PrP protein. Human ApoE N-terminal domain (amino acid 1–191) bears low-density lipoprotein receptor-binding sites, which locates at the domain of amino acid 136–158. Its C-terminal domain (amino acid 210–299) is a lipoprotein-binding site with supercoil structure. ApoE is the ligand for several receptors, including the apolipoprotein low-density lipoprotein receptor (LDL receptor), lipolysis-stimulated receptor (LSP) and human ApoE receptor 2, which participated in the signal transduction, cell nutrition during brain development [7]. One might think that the interaction between PrP and ApoE, especially accumulation of PrP^{Sc} during the pathogenesis of TSE, would block cell nutrition and signal transduction processes, leading to neuron death. Actually, in AD, the direct binding of ApoE with amyloid peptide impairs ApoE receptor-dependent protective signals that promote neuronal survival and synaptic plasticity that may influence the amyloid clearance and fibril formation [10,29].

Our research only proposes the data of molecular interaction between ApoE and PrP protein, however, the biological significance is still unknown. The floating characteristic of ApoE in body fluids and between the cells, wide distribution of ApoE receptors among various tissue cells make it possible to be a carrier for extraneous PrP^{Sc} transferring from peripheral tissues to central nerve system. Wide distribution of various ApoE receptors in caveolae-like domains on the surface of neuron cells correspond well with the newly proposed domain in which conversion from PrP^{C} to PrP^{Sc} occurs. It is reasonable to hypothesize that PrP, even PrP^{Sc}, is enriched in caveolae-like domains through interacting with ApoE. Highly concentrated PrP molecules in the special room may help PrP^{Sc} contact with its normal isoform PrP^{C}, leading to conformational conversion. In fact, presence of potential receptors of PrP^{C} in CLDs has been already supposed that it might be a trans-membrane protein recognizing PrP with its extracellular portion [30]. More detailed studies are needed to clarify whether the receptors of ApoE correlate with or even are the hypothesized receptors for PrP.

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