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Antibody to a Zinc Finger Protein in a Patient with Paraneoplastic Cerebellar Degeneration

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In some patients with paraneoplastic cerebellar degeneration (PCD), autoantibodies against neural components have been identified. Here, we demonstrate a major 58 kd protein antigen in an immunoblot of human cerebellum by serum from a patient with PCD. Immunohistochemically, the serum recognized neural cells especially Purkinje cells in a human brain. To identify the details of the target antigens for the antibody, we isolated a cDNA clone from a human cerebellar library. Homology searches revealed a similarity with the zinc finger proteins. PCD related proteins reported here may be important to maintain neural cells especially those in the cerebellum, and further studies on this molecule may help us elucidate the causes of degenerative or autoimmune diseases in the cerebellum.

Although etiological mechanisms of paraneoplastic syndrome have not been revealed, antibodies to common molecules between nervous tissues and cancers have been detected in the serum and cerebrospinal fluid (CSF) from patients with malignant tumors [1-5]. Therefore, these antibodies may damage nervous tissues.

In paraneoplastic cerebellar degeneration (PCD), antibodies against cerebellar Purkinje cells have been detected in the serum or CSF from patients with neoplasms of the lung [3,4,6], gynecological organs [1,7] or lymphnodes [8]. Especially, in PCD associated with gynecological cancers, an antibody against consistent cerebellar antigens, Yo, has been frequently observed [1,4].

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Here, we have isolated a cDNA clone (cerebellar zinc finger: CZF) from a human cerebellar cDNA library by immunoscreening using the serum from a patient with PCD. Its deduced amino acid sequence has not been reported previously and homology searches have revealed similarity with functional molecules for protein synthesis as a transcriptional factor.

MATERIALS AND METHODS

Case report: At age 77, a woman noticed ascites and cytological examination revealed mucinous adenocarcinoma in 1984. After chemotherapy by intraperitoneal injection of mitomycin C and subsequent administration of 5-fluorouracil, the ascites disappeared and no relapse has been observed so far. However, the origin of the mucinous carcinoma has remained unknown. In 1988, she developed cerebellar ataxia, which progressed rapidly and symptoms completed within a week. Laboratory findings revealed abnormal increase of lactate dehydrogenase (LDH), CA-125 in the serum, and slight increase of cell count (30/cmm) and protein (64 mg/dl) in the CSF. She was diagnosed as PCD by characteristic onset and course of cerebellar ataxia and underlining carcinoma.

Immunoblot and immunohistochemistry: The immunoblotting procedure was performed as previously described with minor modifications [9]. Lyophilized tissue homogenates were electrophoresed on 11% polyacrylamide/SDS slab gel and transferred to nitrocellulose papers. Immunostaining was carried out by the avidin-biotin complex peroxidase (ABC) procedure [10].

Cryostat sections of a human brain were immunostained by the ABC method [10]. The sections were pretreated with the normal porcine serum diluted to 1:20 with phosphate-buffer saline (pH 7.4) and then incubated with diluted patient serum (1:200).

Northern blot analysis: Total RNA was extracted from the human cerebrum, cerebellum and liver by the guanidine isothiocyanate/cesium chloride method [11]. Northern blot analysis was performed as reported previously [12]. The filters were hybridized with an EcoRI fragment of the cloned cDNA.

Screening of lambda gt11 library by antiserum: A lambda gt11 cDNA expression library constructed from human cerebellar poly(A)+ RNA (Clontech,USA) was immunologically screened by the serum from the patient. The recombinant phages ($2x10^6$) were plated on E.coli strain Y1090 and incubated at 42°C for 5 hrs. Then they were induced by overlaying nitrocellulose filters (Advantec,Tokyo) saturated with 10mM isopropylthiogalactoside (IPTG). The filters were washed with Tris-HCl,saline and incubated at room temperature for 1 hr with diluted serum (1:200) as the first antibody. Incubations at room temperature for 1 hr with diluted biotinylated anti-human IgG (1:200) and avidin-peroxidase complex (1:200) were followed by staining with chloronaphthol. The positive signals were purified by several rounds of antibody screening until 100% of the plaques gave positive signals. The obtained cDNA clone was subcloned into pUC118 (pCZF) and double strand DNA was prepared [13], and then the DNA sequence was obtained by the dideoxy method [14].

RESULTS

Immunoblotting and immunohistochemical studies: The serum from the patient detected in a consistent and specific manner a major protein antigen with a molecular mass of about 58 kd and a few minor protein bands in immunoblots of human cerebellar homogenate (Fig.1).

The serum reacted with the nucleus and the cytoplasm of Purkinje cells in the cerebellum (Fig.2). Some neurons and glial cells in the cerebrum were also stained but faintly by the serum (data not shown).

Northern blot analysis: Northern blot of the human cerebellum demonstrated three major bands migrated from 2 kb to 4.5 kb,

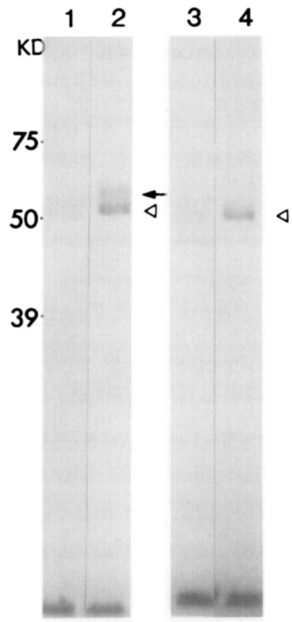

Figure 1. Immunoblot analysis of human tissues with the serum from a patient with paraneoplastic cerebellar degeneration (PCD). Western blots of cerebral (lane 1 and 3) and cerebellar homogenate (lane 2 and 4) were incubated with diluted serum (1:200) from a patient with PCD (lane 1 and 2) and a control (lane 3 and 4). The serum from the patient with PCD recognizes one distinct antigen (arrow) and a few higher bands, and intrinsic heavy chain of immunoglobulin (open triangle) which is also seen in blots incubated with the control serum. Molecular weights (KD) are shown on the left.

Figure 2. Immunohistochemical analysis of human cerebellum with the serum from the patient with paraneoplastic cerebellar degeneration (PCD). Frozen sections of human cerebellum incubated with diluted serum (1:200) from the patient with PCD by the avidin-biotin immunoperoxidase method. Note that both the cytoplasm and the nucleus of all Purkinje cells are stained.

while that of cerebrum and liver seemed to show a faint single band and no reaction, respectively (Fig.3).

Cloning of cDNA and sequence analysis: The antibody screening of a human cerebellar cDNA library by the serum from the PCD patient yielded a single positive clone , CZF. The clone was found to contain a 300 bp cDNA insert. The characteristic structure of the sequence was a pattern of tandem repeats consisting of units of 84 nucleotides; 28 amino acids. Homology searches of the cloned cDNA sequence with the GeneBank, EMBL, or NBRF databases have revealed the highest similarity with ZNF7 and ZNF8 [15], which are members of the human zinc finger proteins. Translation of the cloned cDNA sequence yielded a multifingered protein containing 2 complete and 2 incomplete finger repeats (Fig.4). Each unit

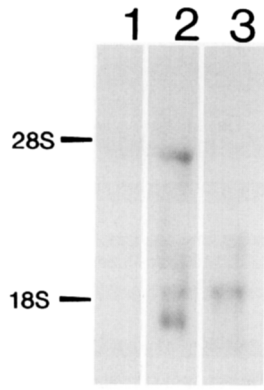

Figure 3. Northern blot analysis of CZF mRNA in human liver (lane 1), cerebellum (lane 2) and cerebrum (lane 3). Total RNA (10 μg each) was electrophoresed and hybridized with an EcoRI fragment of pCZF.

conforms exactly to the consensus sequence $CX_2CX_3FX_5LX_2HX_3H$ found in ZNF7, ZNF8, Sp1 [16] and Kruppel [17] zinc finger motifs.

DISCUSSION

In PCD patients with gynecological cancers including ovarian and breast cancers, autoantibodies to neural tissues are specific to Purkinje cells [1,7]. The antigenic proteins recognized by the

C x e C g K a F x x x s x L x x H Q r i H t G e k p y x	Consensus ZNF7
C x x C G k x F x x x x x L x x H x r x H t g e k p y x	Consensus ZNF8
C x x C x x x F x x x x x L x x H x x x H T G E K P Y x	Consensus Kruppel
C x x C g K r F x r s d h L x x H x r t H T G E x x F x	Consensus Sp1
K A F N W S S S L T K H K R I H T R E K P F K	CZF Sequence
C K E C G K A F I W S S T L T R H K R I H T G V S P Y K	
C E E C G K A F S R S S T L T K H K T I H T G E K P Y K	
C K E C G K A F K H S S A L	

Figure 4. Comparison of amino acid sequence of CZF with ZNF7, ZNF8, Sp1 and Kruppel zinc finger motifs. The sequences are given in one-letter code and aligned to show the repeated units. Amino acids matching the zinc finger consensus sequence are boxed. A consensus for the repeated motifs of ZNF7, ZNF8, Kruppel and Sp1 is displayed, uppercase letters correspond to strictly conserved amino acids, and lowercase letters to the amino acids of which more than 60% are conserved, x indicates no conservation. Amino acid sequence of CZF consists of four repeated units, two complete units and two incomplete units, and it is given in uppercase letters.

autoantibody in serum from these patients are homogeneous. Two kinds of proteins with different molecular weights, one with 34 kd (CDR34) [18] and the other 62 kd (CDR62), are recognized by the sera from the PCD patients. In Japanese PCD patients with ovarian or uterine cancer, a 52 kd protein characteristically expressed in Purkinje cells is frequently detected by their sera [19]. Only two cDNA clones encoding the CDR34 [18] and the 52 kd protein [19] have been analyzed, and complete amino acid sequence has been revealed. No homology in nucleotide sequence is observed between the CDR34 and the 52 kd protein. The molecular structures of those proteins had not been reported and had no similarities with any previously described proteins. So far, experimental cerebellar degeneration has not been successfully provoked in animals by active immunization of the CDR34 or the 52 kd protein and passive transfer of those antibodies. Therefore, the category and functions of these proteins have not been known yet.

Here, we demonstrated a cDNA clone encoding a 58 kd protein that was recognized by the serum from the patient with PCD. The deduced amino acid sequence of our 58 kd protein was different from those of the CDR34 and the 52 kd protein. Homology searches, interestingly, revealed high similarity in nucleotide and amino acid sequences with the zinc finger sequence motifs (Fig.4). Northern blotting and immunoblotting analyses for the cDNA clone we described here revealed high expression in the cerebellum. Therefore, we call it cerebellar zinc finger protein.

The zinc finger motif has been identified as a cDNA binding domain in transcriptional regulatory proteins [20]. To date, three types of DNA binding structures are known; the first well characterized structure is a helix-turn-helix motif [21,22], the

second type is the zinc finger motif and the third type is a recent reported leucine zipper motif [23]. The proteins with such DNA binding motifs are thought to regulate gene expression as trans-acting factors. The zinc finger motif has been initially identified in the RNA polymerase III transcription factor, TFIIIA [20,24]. It consists of nine tandemly repeated sequence of approximately 30 amino acid residues and has two cystines and two histidines residues (C_2H_2) in each repeat binding a zinc ion. Similar zinc finger motifs have been subsequently identified in other genes of various species including human [16]. However, transcriptional regulatory factors specific for the human nervous system have not been well characterized. If the CZF is a transcriptional regulatory factor specific for the central nervous system (CNS), further examination for its function may reveal expression mechanisms of the CNS specific proteins.

Although the functions of the CDR34 and the 52 kd protein remain unknown, antibodies against these proteins may play a primary role in Purkinje cell degradation, because similar antibodies are not seen in the sera from normal controls, neurologically normal patients with cancers, nor patients with only neurological disorders, and because cerebellar symptoms and signs are improved after successful treatment of the cancer in a PCD patient with the anti-Yo antibody [4]. In case of our CZF protein, it may play an important role as a transcriptional regulatory factor for expression of essential proteins in neuronal cells, especially in Purkinje cells. Therefore, degeneration of CZF by its antibody may affect the synthesis of these proteins in Purkinje cells and may cause cerebellar degeneration. So far, the etiological causes of spinocerebellar degeneration or antigens responsible for autoimmune cerebellar ataxia after viral infection have not been identified. Further

analyses for the CZF may help us elucidate the causes of degenerative or autoimmune diseases in the cerebellum.

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