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Cloning of SEZ-12 Encoding Seizure-Related and Membrane-Bound Adhesion Protein

Kagemasa Kajiwara,*,† Hideko Nagasawa,† Keiko Shimizu-Nishikawa,‡ Tamiko Ookura,† Minoru Kimura,* and Eiichi Sugaya†,‡,[1]

**Department of Molecular and Developmental Science, Molecular Medicine Research Center, Institute of Medical Science, Tokai University, Kanagawa 259-11, Japan; †Division of Neurobiology, Tsumura Institute for Oriental Medicine, School of Medicine, Keio University, Tokyo, 160, Japan; and ‡Department of Physiology, Kanagawa Dental College, Yokosuka, 238, Japan*

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SEZ-12 is one of the seizure-related cDNAs which was isolated by differential hybridization from primary cultured neurons from the mouse cerebral cortex with or without pentylenetetrazol (PTZ). SEZ-12 expression is transiently down-regulated in the mouse brain by injection of PTZ. To characterize SEZ-12, isolation of full-length cDNA and nucleotide sequence analysis were performed. The deduced amino acid sequence of SEZ-12 revealed that it encodes membrane-bound C-type lectin and has a significant homology to that of human cDNA, DGCR2 and IDD, which were cloned from a balanced translocation breakpoint associated with the DiGeorge syndrome. The isolated cDNA was about 4 kb in length and the message was expressed ubiquitously in various organs with low-abundance. Previously, we also cloned a transmembrane protein which is probably involved in cell-cell interaction by the differential hybridization technique. These findings suggest that transmembrane signaling in neuronal cells may have an important role in PTZ-induced seizure.

During pentylenetetrazol (PTZ)-induced seizure, prolonged and marked depolarization with burst spike discharges (bursting activity) is observed in snail neurons (1, 2) and mouse cerebral neurons (3). We previously demonstrated abnormal intracellular calcium dynamics, protein phosphorylation changes and protein synthesis in bursting neurons (1–6). These observations indicate that seizure activity of neurons influences both rapid responses caused by the modification of cellular substrates such as protein phosphorylation and long-term cellular alterations correlated with gene expressions. However, the precise molecular mechanism of PTZ-induced bursting activity remains unclear. To obtain the molecules associated with PTZ-induced bursting activity, we have recently screened mouse brain cDNA library by differential hybridization and were cloned SEZ clones whose mRNA levels are changed, increased or decreased, with PTZ administration (7). One of the SEZ clones, SEZ-12, hybridized to a single mRNA species of ~4.4 kb in length, is down-regulated by PTZ. In the present study, we analyzed SEZ-12 and found that it has a significant homology to the human cDNA which encodes membrane-bound C-type lectin.

MATERIALS AND METHODS

cDNA cloning. To obtain SEZ-12 clones containing an open reading frame, we screened the 5′-stretched mouse brain cDNA library (CLONTECH) using 1052 bp of the 3′ region of SEZ-12 fragment which was originally isolated by differential hybridization (7) as a probe. Three cDNA clones were isolated from 2×10^5 recombinants. The longest cDNA insert (~4.0 kb) was subcloned into Bluescript II SK+ (Staratagene).

RNA blotting. Primary cultured neurons from the cerebral cortex of mouse embryos were treated with medium containing PTZ (10 mM) for 30 min. At the indicated time after PTZ application, some of these culture media were replaced with

[1] To whom correspondence should be addressed at Department of Physiology, Kanagawa Dental College, 82, Inaoka-cho, Yokosuka, 238 Japan. Fax: +81-422-42-0532.

Abbreviations: PTZ, pentylenetetrazol; SEZ, seizure-related; UTR, untranslated region; DGS, DiGeorge syndrome; DGCR, DiGeorge syndrome critical region.

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normal media and maintained for 30 min. RNA extraction and poly $(A)^+$ RNA selection from these cultured cells were carried out as previously described (7, 8). Each 2 μg of poly $(A)^+$ RNA was denatured with glyoxal, electrophoresed (9) and transferred to a Hybond-N membrane (Amersham). For expression analysis of SEZ-12 mRNA in adult tissues, Multiple Tissue Northern Blot (CLONTECH) was used. These membranes were hybridized with ^{32}P-labeled SEZ-12 probe at 42°C in 50% formamide, 5 × SSPE, 5 × Denhard's solution, 0.5% SDS and 100 mg/ml denatured herring sperm DNA. After washing with 0.1 × SSC and 0.1% SDS at 50°C for 30 min, membranes were autoradiographed with an intensifying screen, and then reprobed with G3PDH cDNA (CLONTECH) as a control.

DNA sequencing. The nucleotide sequences were determined using an Applied Biosystem 373 DNA sequencer with Taq DyeDeoxy terminator cycle sequencing kit (Perkin Elmer). Nucleotide and amino acid sequences were examined by the GENETYX system (Software Development Co.).

In vitro translation. Capped transcripts of SEZ-12 synthesized *in vitro* with T7 RNA polymerase (mRNA capping kit, Stratagene) were translated with [^{35}S] methionine (Amersham) in a rabbit reticulocyte lysate (Amersham) in the presence or absence of canine pancreatic microsomal membrane (Promega). The translation products were subjected to SDS-PAGE (15%) and analyzed by fluorography.

RESULTS AND DISCUSSION

In the previous study, we screened the mouse cerebral cortex cell library by the differential hybridization technique to isolate genes whose expressions are increased or decreased by pentylenetetrazol (PTZ) application and several clones were identified by this screening (7). SEZ-12 is one of these clones and is hybridized to a 4.4 kb low-abundant mRNA which showed down-regulation within 30 min after PTZ application both *in vitro* (Fig. 1A) and *in vivo* (7). The decreased mRNA level was returned to the normal level by rinsing with normal medium (Fig. 1A). The RNA blotting of SEZ-12 demonstrated ubiquitous expression in various organs *in vivo* (Fig. 1B). Since originally screened cDNA was 1 kb in length with no significant homology to any known gene, we rescreened the mouse brain cDNA library using the 1 kb SEZ-12 fragment as a probe. We obtained several clones and analyzed the longest SEZ-12 cDNA. This clone was 3,999 bp in length and contained an open reading frame predicting a protein of 546 amino acids, 198 bp of 5′ UTR preceding the initiation codon and 2162 bp of 3′ UTR followed by a poly(A) tail. Analysis of nucleotide and amino acid sequences of SEZ-12 revealed a significant homology (92% amino acid identity) to those of a human cDNA, DGCR2 (10) or IDD (11), encoding a potential adhesion receptor protein (Fig. 3A). This gene has recently been cloned from a balanced translocation breakpoint associated with the DiGeorge syndrome, and it was mapped the closest to the breakpoint of one patient ever reported (10, 11).

The predicted amino acid sequence of SEZ-12 revealed several structural domains corresponding to those of DGCR2 and IDD as shown in Fig. 2. Hydropathy analysis showed a putative signal sequence for the first 24 amino acids and a single transmembrane domain for amino acids 346 to 364. To confirm the integration of SEZ-12 protein, *in vitro* translation assays with rabbit reticu-

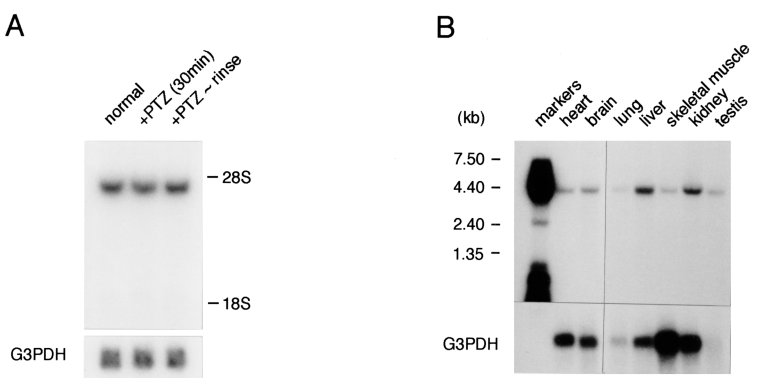

FIG. 1. RNA blot analysis of SEZ-12 in mouse primary cultured neurons (A) and mouse adult tissues (B). G3PDH cDNA was used as a control probe (bottom panel).

A

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mouse  MVPKADSGAFLLLFLLVLTVTEPLRPELRCNPGQFACHGGTIQCIPLPWQCDGWPTCEDK   60
human                                          RS               A  E

       SDEADCPEVTGEARP-YGKETVDLRQGRARGGDPTHFHTVNVAQPVRFSR---KCPSGWH  116
           N       V  HH   A  P          S   A          SFLG   T
                                    *
       HYEGTASCYRVYLSGENYWDAAQTCQRVNGSLATFSTDQELRFVLAQEWDQPERSFGWKD  176
                                   L
                  *
       QRKLWVGYQYVITGRNHSLEGRWEVAFKGSPEVFLPPDPIFASAMSENDNVFCAQLQCFH  236
                     R            S

       FPTLRHHDLHSWHAESCSEKSSFLCKRSQTCVDIKDNVVDEGFYFTPKGDDPCLSCTCHR  298
                      Y

       GEPEMCVAALCERPQGCQQYRKDPKECCKFMCLDPDGSSLFDSMASGMRLVVSCISSFLI  356
                                           N

       LSLLLFMVHRLRQRRRERIESLIGANLHHFNLGRRIPGFDYGPDGFGTGLTPLHLSDDGE  416


       GGTFHFHDPPPPYTAYKYPDMDQPDDPPPPYEASINPDSVFYDPADDDAFEPVEASLPAP  476
                           IG            H                  V

       RDGGIEGALPRHLDQPLPPAETSLADLEDSTDSSSALLVPPDPAQSGSTPATEAPPGGGR  536
       G   S    L R E    T GA        A                    A  L

       LPRASLNTVV                                                    546
       HS S
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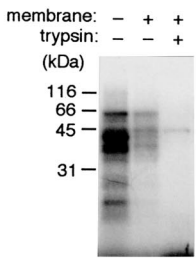
B



C

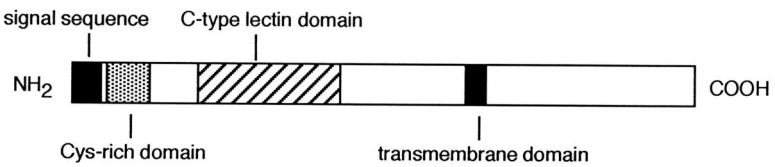


FIG. 2. (A) The deduced amino acid sequence of SEZ-12. The amino acids are numbered in the right margin. The potential signal sequence and transmembrane domain are underlined. The sites of potential N-linked glycosylation are indicated by asterisks. The bottom amino acids indicate the different residues of human DGCR2 (10) and IDD (11) compared to that of SEZ-12. DDBJ accession number; D78641. (B) *In vitro* translation analysis of SEZ-12. (C) Schematic representation of the domain structure of SEZ-12.

locyte lysates were performed. The SEZ-12 translation products migrated with an apparent molecular size of about 61 kDa and were converted to about 47 kDa with trypsin treatment in the presence of microsome membranes (Fig. 2B). Consistent with this finding, there were two sites of potential N-linked glycosylation located in amino acid 145 and 192 in the extracellular region (Fig.

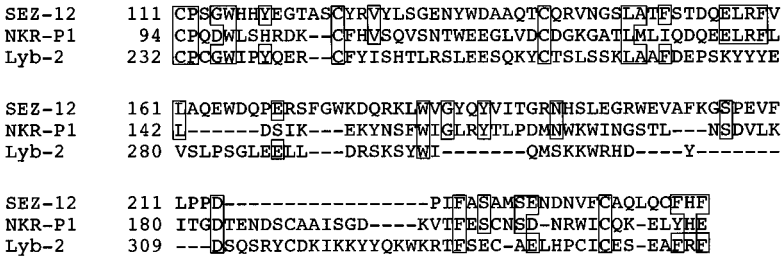


FIG. 3. Sequence alignment of the C-type lectin domain of SEZ-12, NKR-P1 (15) and Lyb-2 (16). The amino acids identical to those of SEZ-12 are boxed.

2A). Furthermore, the SEZ-12 protein sequence contained two extracellular conserved features as shown in Fig. 2C. The first domain for the NH_2-terminal (48 amino acids) shared a ~42% amino acid identity with each of the cysteine-rich repeats of LDL receptor (12, 13). This region contains six conserved cysteins and is considered as a ligand-binding domain (14). The other domain located in the amino acids from 111 to 237 shared a similarity to the C-type lectin domain of NKR-P1 (19% amino acid identity) (15) and Lyb-2 (15% amino acid identity) (16) as shown in Fig. 3. Several members of the C-type lectin family can bind carbohydrates and may influence cell-cell adhesive events (17). Both Lyb-2 and NKR-P1 stimulate intracellular calcium dynamics (18, 19). Furthermore, a recent study has demonstrated that several oligosaccharide sequences of the ganglioside family identified as ligands for NKR-P1 induced dose-dependent elevation of phosphoinositides and free cytoplasmic calcium in natural killer cells (20). It is well known that gangliosides are major components of neural cell membranes. The exogenous gangliosides not only suppress glutamate-induced arachidonic acid release and NMDA receptor-mediated excitotoxcity in cultured neurons (21, 22) but also potentiate NCAM and N-cadherin-dependent neurite outgrowth in PC12 cells (23). On the other hand, our previous study demonstrated that primary cultured neurons from the cerebral cortex of El mice showed spontaneous bursting activity and concomitant changes in both neurite formation and ganglioside pattern (24, 25). These findings suggest that SEZ-12 protein, probably the mouse counterpart of the translation product of the human cDNA, DGCR2 or IDD, may play a key role in delivery of extracellular signals.

In this study, we demonstrated that SEZ-12 encoded a seizure-related membrane-bound adhesion protein and its expression was down-regulated by PTZ in the nervous system. A similar observation has been reported whereby expression of the neural cell adhesion molecule L1 is reduced by low-frequency electrical stimulation in developing sensory neurons (26). Our recent study demonstrated another potential cell adhesion and recognition molecule, SEZ-6, whose expression was up-regulated after PTZ application (27). These findings imply that the gene expression altered by PTZ application may influence transmembrane signaling which is associated with seizure.

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