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Cutting Edge: Primary Structure of the Light Chain of Fusion Regulatory Protein-1/CD98/4F2 Predicts a Protein with Multiple Transmembrane Domains That Is Almost Identical to the Amino Acid Transporter E16

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The CD98 light chain (CD98LC) was copurified from HeLa S3 cells by an affinity chromatography using a mAb specific for the fusion regulatory protein-1 (FRP-1) which is identical to the CD98 heavy chain. On the basis of the N-terminal sequence (63 amino acids) of purified CD98LC polypeptide, we have cloned a PCR fragment (155 bp) from a HeLa S3 cDNA library and finally obtained a full cDNA clone encoding the CD98LC. Fluorescence in situ hybridization analysis using the cDNA assigned the CD98LC gene to the long arm of human chromosome 16 (16q24). The predicted amino acid sequence suggested that CD98LC is a protein with multiple transmembrane domains and is almost identical to the amino acid transporter E16. Resting monocytes and lymphocytes expressed CD98LC as analyzed by a newly isolated anti-CD98LC mAb, which showed cross-reactivity with insect Sf9 cells as well as with various mammalian cell lines. The Journal of Immunology, 1999, 162: 2462–2466.

Fusion regulatory protein-1 (FRP-1) is a glycosylated 80-kDa protein and expressed in most cell lines of human origin (1, 2). Two mAbs (mAbs 6-1-13 and 4-5-1), recognizing the FRP-1, enhanced cell fusion induced by viruses including Newcastle disease virus, measles virus, and HIV (1, 2).

Furthermore, peripheral blood monocytes can be fused in the presence of mAb 6-1-13 or 4-5-1, and the resulting polykaryocytes exhibit osteoclast-like cell phenotypes (3, 4). Human CD98/4F2 is a disulfide-linked complex composed of a glycosylated heavy subunit, the heavy chain of CD98 (CD98HC; 85 kDa) and a nonglycosylated light subunit, the light chain of CD98 (CD98LC; 41 kDa) (5). We identified the FRP-1 80-kDa polypeptide to be the same molecule as the CD98HC molecule (6). The CD98HC is a type II transmembrane (TM) glycoprotein with 529 amino acids (7, 8), whose biological functions other than fusion regulation have been proposed, such as an amino acid transporter (9) or an Na+/Ca2+ exchanger (10, 11). Recently, CD98HC has been reported to be an important regulator of integrin-mediated cell adhesion (12). However, little is known about the structure and function of CD98LC.

Because mAb 6-1-13 coprecipitated a nonglycosylated 37-kDa polypeptide which was linked to FRP-1 via disulfide bondage (2), we regarded the 37-kDa polypeptide as identical to CD98LC molecule (6). Accordingly, we tried to isolate and characterize CD98LC molecule, which could be copurified with FRP-1 through an affinity chromatography using mAb 6-1-13.

In the present study, we determined the N-terminal amino acid sequence of CD98LC polypeptide. Subsequently, a full cDNA clone encoding CD98LC was obtained that enabled mapping of the gene on human chromosome. Furthermore, for the first time, a hybridoma clone secreting mAbs against human CD98LC has been isolated.

Materials and Methods

Cells and immunofluorescence analysis

HeLa S3, HeLa, BHK, L929, MDCK, and ESK cells were cultured at 37°C in MEM fortified with 5% calf serum. Aedes albopictus S9 cells were cultured in MEM supplemented with 10% fetal bovine serum.
FIGURE 1. Analysis of CD98LC polypeptide. A, A Coomassie blue-stained SDS-PAGE gel separating affinity-purified CD98 complex under reducing conditions. B, Western blot analysis of affinity-purified CD98 complex. Diluted CD98 complex was run on an SDS-PAGE gel under reducing conditions, blotted onto a nitrocellulose membrane, and immunostained with mAbs specific for human parainfluenza type 2 virus F protein (negative control, mAb 117-1A, lane 1), CD98LC (mAb L-2, lane 2), and CD98HC (mAb 6-1-13, lane 3). C, N-terminal amino acid sequence of purified CD98LC polypeptide. Amino acids from 51 to 55 (shown as xxxxx) could not be identified because of a mechanical accident. Arrows indicate the positions of amino acid sequences to which the degenerate primers, M-N1 and M-RV2, correspond. Shown in the parentheses are the nucleotide sequences of the degenerate primers (N = A/G/C/T, R = A/G, M = A/C, D = A/G/T).

at 27°C in Grace's medium with 10% FCS. PBMCs were isolated from the heparinized whole blood of human volunteers by Ficoll-Hypaque density-gra

Purification of CD98LC

Plasma membranes were separated from HeLa S3 cells according to the method described by Maeda et al. (14). The plasma membranes were solubilized as described (2) and subjected to a gel filtration chromatography. The fractions were screened for CD98HC Ag by dot blot immunostaining using mAb 6-1-13, and the Ag-positive fractions were applied to an im

Fluorescence in situ hybridization (FISH) analysis

A cDNA fragment (nucleotides 5–1571 in Fig. 2) encoding the CD98LC was amplified by PCR from the HeLa S3 library. The PCR product was cloned in pCR1-TOPO vector, biotinylated by nick-translation with biotin-16-deoxyuridine triphosphate (Boehringer Mannheim, Indianapolis, IN), and hybridized to R-banded chromosome spreads from a normal human lymphocyte as described before (16). Hybridized probe was detected via fluorescein-conjugated avidin (Boehringer Mannheim). Chromosomes were counterstained with 0.2 μg/ml propidium iodide for the R-banding. Digital images for each fluorescence were taken by a cooled charge-coupled device (CCD) camera (PentaMax-1317-K1; Princeton Instruments, Trenton, NJ) equipped on a Zeiss Axiosplan 2 MOT epifluorescence microscope (Carl Zeiss, Jena, Germany).

Isolation of hybridoma cell line for CD98LC

Affinity-purified CD98 complex was separated on a PAGE gel as described above. Then, superfically stained CD98LC band was cut out and elec
troeluted with the aid of Centricon Microelectroeluter (Amicon, Beverly, MA). After dialysis against water, the purified CD98LC was added with 100 μg of LPS and injected i.c. onto a BALB/c mouse. The mouse was boosted twice, and hybridoma cells were produced as described previously (17). Screening of the culture fluid was performed by ELISA using fixed HeLa cell monolayers. Hybridoma cells of interest were further screened by Western blot analysis (18) in which purified CD98 complex was used.

Results and Discussion

The N-terminal amino acid sequencing of purified CD98LC polypeptide

CD98 complex was affinity purified from HeLa S3 membranes by using mAb 6-1-13, an anti-CD98HC Ab (1, 2, 6). Fig. 1A shows the separation of purified CD98 complex in SDS-PAGE, revealing major broad bands of CD98HC and CD98LC together with faint bands of Ig (heavy and light chains) and FRP-1-related molecules (19). Then, CD98LC was purified from a preparative PAGE gel and used to immunize a mouse. Subsequently, a hybridoma clone was isolated that secreted specific Ab for CD98LC (Fig. 1B, lane 2). From another preparative PAGE gel, CD98LC polypeptides were blotted onto a polyvinylidene difluoride membrane and sub-jected to N-terminal sequencing. As shown in Fig. 1C, alanine residue was identified at the N terminus of the identified 63 amino acids. It should be noted that this alanine was detected with an amount of 157 pmol in the first sequencing cycle, whereas other amino acids in the same cycle gave signals of ≤14 pmol (that of methionine was 5.8 pmol).

CD98LC is a membrane protein with multiple TM domains

On the basis of the identified N-terminal amino acid sequence, a pair of degenerate primers (M-N1 and M-RV2) was arranged (Fig. 1C). Subsequently, by employing degenerate PCR, nested vector-PCR and 5′-RACE-PCR procedures, cDNA fragments encoding CD98LC were obtained. The cDNA sequence and deduced amino acid sequence are shown in Fig. 2. Because the cDNA sequence did not contain a stop codon at the 5′ region, the position of the initiation codon (nucleotides 16–18) for methionine is tentative. If translation starts with this methionine, the cDNA can encode a 507-amino acid protein with 11 TM domains which has no N-terminal signal sequence as analyzed by the PSORT prediction program, indicating that the N terminus of CD98LC may locate in

cDNA cloning from HeLa S3 mRNA library

First, two primers which corresponded to the identified amino acid se-quences, EARERK and GIEVFTP (Fig. 1C), were used for degenerate PCR, which was performed by using a Agt11 CDNA library for HeLa S3 mRNA (Clontech Laboratories, Palo Alto, CA). Then, cDNA fragments of ex-pected size (155 bp) were cloned in pCR1-TOPO vector (Invitrogen, Carlsbad, CA), and sequenced by the ABI PRISM 310 genetic analyzer (Applied Biosystems Division, Perkin-Elmer, Foster City, CA). Subse-quently, oligonucleotide primers were arranged within the 155-bp cDNA and within the Agt11 vector, and nested PCR was performed successively in both the 5′ and 3′ directions by using the HeLa S3 cDNA library as the

template (nested vector-PCR). 5′-RACE-PCR was performed by using freshly prepared HeLa S3 mRNA with the aid of the Marathon CDNA Amplification Kit (Clontech Laboratories). The nucleotide and amino acid sequence analyses were aided by SDC-GENETYX computer programs (Software Development, Tokyo, Japan) and by using the following pro-

psort.rib.nibb.ac.jp/form.html), and ISREC Tmpred (http://www.isrec.isbbs.ch/software/TMPRED_form.html).
the cytoplasm and consequently that the C terminus may be extracellular. Homology search analysis using the advanced BLAST indicated that the primary structure of CD98LC is nearly identical with that of an amino acid transporter E16 (GeneBank accession number AF077866) (20). Differences in the nucleotide sequences between CD98LC and E16 were found at nucleotide positions 546(C-G), 675(G-A), 705(C-G), and 1023(C-T); the nucleotide difference at position 705 contributed to a single amino acid difference at residue 230(Asn vs Lys). Therefore, our present report seems to provide direct evidence for the assumption that the amino acid transporter E16 is one of the light chains of human 4F2/CD98 (20).

Rattus LAT1 (514 amino acids) and Xenopus IU12/ASUR4 (507 amino acids) are also amino acid transporters and considered to be rat and frog homologues of human E16, respectively (20–22). Partial or incomplete cDNA sequences of LAT1 and E16 molecules were already reported (23, 24). Although LAT1, E16, and IU12/ASUR4 are all assumed to have 12 TM domains (20–22), our analysis using the PSORT program predicted that CD98LC is a protein with 11 TM domains as described above. Interestingly, when analyzed using ISREC TMpred program, CD98LC was also estimated to have 12 TM domains (data not shown). This was because an additional TM domain (TM5) was postulated by the ISREC TMpred program (Fig. 2). Importantly, however, the positions of the other 11 TM domains predicted by the ISREC TMpred program were similar to those predicted by the PSORT program. Therefore, these 11 domains may actually function as TM segments. If CD98LC has 11 TM domains, then the putative six extracytoplasmic regions of CD98LC include two cysteine residues at positions 164 and 496. In this context, it should be remembered that the human CD98HC has two cysteine residues (at residues 109 and 330) in the extracytoplasmic domain (7, 8). We have previously shown that the Cys330 is not involved in the disulfide linkage to murine CD98LC (25). In addition, a truncated form of CD98HC, which consisted of residues 111–529 and thus did not contain Cys109, was secreted into the culture fluid when expressed in Cos cells (unpublished data). Taken together, we assume that Cys330 of human CD98HC is involved in the disulfide linkage with CD98LC (via Cys164 or Cys496). Noteworthily, it has recently been
described that a cysteine residue located between TM3 and TM4 domains of E16, possibly Cys\(^{164}\), is involved in the disulfide bridge with CD98HC (20).

As the protein sequencing data indicated (Fig. 1C), the N terminus of purified CD98LC could be the Ala\(^{10}\) (Fig. 2). Thus, the presumptive N-terminal nine residues (MAGAGPKRR) may be removed by processing in the cell or degraded during the purification process.

**Detection of CD98LC Ag in cells**

We previously showed that CD98HC was well expressed in resting monocytes and activated lymphocytes (6). Therefore, we examined whether CD98LC could also be expressed in these cells. As shown in Fig. 3, A and C, flow cytometric analyses indicated that the expression level of CD98HC in resting lymphocytes was low but evidently increased by Con A stimulation, whereas CD98LC was expressed in resting lymphocytes as abundantly as in stimulated cells. On the other hand, the expression level of CD98HC in resting monocytes increased upon cultivation (Fig. 3, B and D), whereas CD98LC was abundantly expressed in monocytes irrespective of cultivation (Fig. 3, B and D). Thus, CD98LC is expressed on the cell surface of lymphocytes and monocytes independently of the expression level of CD98HC, suggesting that expression levels of CD98HC and CD98LC are controlled under different regulatory mechanisms in PBMCs. This finding is contradictory to the observation by Mastroberardino et al. (20), in which SPRM1 (a platyhelminth homologue of E16) was surface localized in Xenopus oocyte only when expressed with human CD98HC. Such discrepancy may arise from the difference(s) in the cell type (human PBMC or Xenopus oocyte) and/or in the origin of light chain (human or nematode). One possibility is that CD98LC may associate with other unidentified molecules in PBMCs that may enable surface expression of CD98LC as efficiently as CD98HC. On the other hand, the CD98LC-specific mAb reacted with hamster (BHK), mouse (L929), dog (MDCK), pig (ESK), and mosquito (Sf9) cells as well as with human HeLa cells (data not shown), revealing that possible homologues of CD98LC in these animals share a common epitope(s). In this context, it should be pointed out that CD98HC can form a functional disulfide linkage even with a platyhelminth homologue of E16 (20). Taken together, putative CD98LC homologues seem to be structurally and functionally conserved among mammals, amphibians, arthropods, and platyhelminthes.

**Chromosomal localization of CD98LC gene by FISH**

Fig. 4 shows a typical example of the FISH image for human CD98LC cDNA: fluorescein (A) and R-banding (propidium iodide) (B) from the same spread. Comparisons between the fluorescence signals and the banding patterns of chromosomes indicated that consistent hybridization signals were detected on the chromosome bands 16q24. The gene for human CD98HC was previously assigned to the long arm of chromosome 11 (26). Thus, the loci for human CD98LC and CD98HC do not link to each other. Accordingly, the above observation that the expression levels of CD98HC and CD98LC in PBMCs are controlled under different regulatory mechanisms may, in part, reflect the difference in the loci of their genes.

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