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Gene Structure and Phylogenetic Analysis of Cytohesin Family

Kyung-Mi Shin, Ji-Won Lee, Joo-Mi Yi and Heui-Soo Kim†

Division of Biological Sciences, College of Natural Sciences, Pusan National University, Pusan 609-735, Korea

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Abstract Cytohesin family has been thought to participate in inside-outside signaling linking growth factor receptor stimulation of PI 3-kinase to cell adhesion and stimulate nucleotide exchange of ARF through its Sec7 domain. The genomic structure of the cytohesin family was analyzed by BLAST search using cDNA and genomic DNA sequences from the GeneBank database. The cytohesin-2 was encoded by 12 exons, while the cytohesin-4 was encoded by 13 exons. The Sec7 and PH domains were not encoded by separate exons. In an analysis of retroviral integration, those two families did not contain any retroviral elements in introns or exons. The phylogenetic tree calculated by the neighbor-joining method suggests that the cytohesin-1 family was closely related to cytohesin-3 (ARNO3) family. These data could be of great use in further studies for resolving the exact function and evolution of the cytohesin family.

Key words: BLAST search, cytohesin family, gene structure, phylogeny

Introduction

Cytohesin family has been isolated using a yeast two-hybrid system with the CD18 (intracellular portion of the integrin β2 chain) [1]. It has been proposed to be a human homolog of the yeast Sec7 gene product, which is crucial component in protein transport [2]. Cytohesin-1 has been implicated in the induction of integrin-binding to cell adhesion molecules [1]. It has been also demonstrated to have guanine nucleotide-exchange factor activity with ADP-ribosylation factor (ARF) [3].

A closely related protein, ARNO (ARF nucleotide binding-site opener), has been shown to contain a central Sec7 domain that promotes guanine-nucleotide exchange on ARF1. It also contains a PH (pleckstrin homology) domain that mediates an enhancement of the ARNO exchange activity by negatively charged phospholipid vesicles supplemented with phosphatidylinositol bispholipid vesicles supplemented with phosphatidylinositol bisphosphate [4]. ARNO is almost identical with human cytohesin-2 (GenBank accession no. U70728). ARNO3, a Sec7-domain guanine nucleotide exchange factor for ARF1, has been involved in the control of Golgi structure and function [5]. It has been assigned to human chromosome 7p21 by radiation hybrid mapping [6]. Recently, cytohesin-4 was cloned from a human brain cDNA library [7].

Cytohesin like molecules (CLM-1, 2, 3) have been isolated from mouse ES cells derived from embryoid bodies. Their transcriptional alternatives and tissue specific expressions have been implicated in complex and multiple regulations in early embronic cells and specific organ of the adult mouse [8]. In this report, we analyzed the structure of the human cytohesin-2, 4 genes and their phylogenetic relationships among cytohesin family.

Materials and Methods

Nucleotide sequences [human cytohesin-1 (M85169), human cytohesin-2 (U70728), human ARNO3 (AJ223957), human cytohesin-4 (Z94160), monkey C1MON (AB022021), monkey C2MON (AB023376), mouse CLM-1 (AB013464), mouse CLM-2 (AB013466), mouse CLM-3 (AB013470)] were retrieved from the GenBank database and genomic structure of the cytohesin family was analyzed with the aid of BLAST network server [9]. Sequence analyses of the cDNA and deduced amino acid were performed using the GAP, PILEUP, and PRETTY from the GCG program (University of Wisconsin) and GENETYX system (SDC). The neighbor-joining phylogenetic analysis [10] was performed with the MEGA program. Statistical significance evaluation of the branching pattern was performed with 100 replications.

Results and Discussion

The cytohesin-2 (ARNO) has been shown to contain a central Sec7 domain and PH domain (Fig. 1). A similar genects18.1 was identified to be most closely related to the cytohesin-2 gene and mapped on chromosome 19 [11]. Using

†Corresponding author
Phone: 82-51-510-2259, Fax: 82-51-581-2962
E-mail: khs307@hyowon.cc.pusan.ac.kr

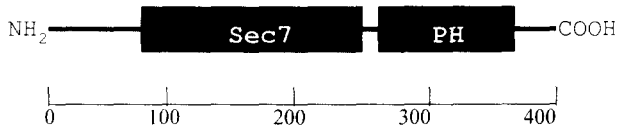

Fig. 1. Schematic diagram showing the domain (Sec7 and PH) structure of human cytohesin-2

cytohesin-2 cDNA sequences (GenBank accession no. U707 28), we analyzed the genomic structure of cytohesin-2 gene by BLAST search. The cytohesin-2 gene was also located on chrosome 19 and matched with accession no. NT_011190. As shown in Fig. 2, the Sec7 domain contains part of the exon3 and exon7, exon4, exon5, and exon6, whereas PH domain contains part of the exon7 and exon11, exon8, exon9, and exon10 in structure analysis of cytohesin-2 gene. The exon1 was identified from accession no. AC026803, while the others were identified from accession no. AC 008403. The first intron showed 499024 bp in length, while the exon10 of the PH domain showed 726 bp. In comparative analysis with mouse cytohesin-2 (CLM-2), the CLM-2 was composed of six exons [8]. The Sec7 and PH domains were not encoded by separate exons. The Sec7 domain was encoded by exon2 and exon3, whereas the PH domain was encoded by exon3, exon4, exon5, and exon6. The human cytohesin-2 shared 99.8% amino acid sequence identity with the mouse CLM-2. This high level of sequence similarities of amino acids suggests that they have very similar biological functions.

Recently, cytohesin-4 was cloned from a human brain cDNA library and resembles other cytohesins [7]. Using cytohesin-4 cDNA sequences (GenBank accession no. AF 075458), we analyzed the genomic structure of cytohesin-4 gene by BLAST search. The cytohesin-4 gene was also located on chromosome 22 and matched with accession no. NT_011520. As shown in Fig. 3, the Sec7 domain contains part of the exon4 and exon9, exon5, exon6, exon7, and exon8, whereas PH domain contains part of the exon9 and exon13, exon10, exon11, and exon12 in structure analysis of cytohesin-4 gene. The exon1 was identified from accession no. AC074203, while the others were identified from accession no. Z94160. The first intron showed 16780924 bp in length, while the exon12 of the PH domain showed 155 bp. Average sequence similarity of amino acid was 84.6% between cytohesin-4 and ARNO3 and 82.8% between cytohesin-4 and cytohesin-2.

To understand the evolutionary relationships among cytohesin gene family, a phylogenetic tree was constructed with the neighbor-joining method using the cDNA sequences. Cytohesin-1 family and cytohesin-3 family (ARNO3) were found to be more closely related to each other than either of them was to the cytohesin-2 family. The cytohesin-4 was appeared as a primitive (Fig. 4).

In our previous study, the mouse CLM-2 is a single-copy gene [8] as also shown in the human cytohesin-1 gene.

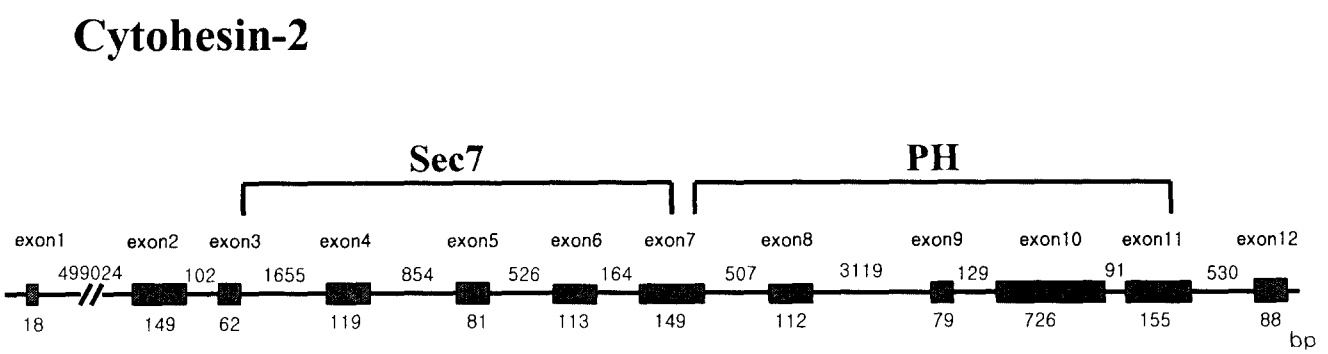

Fig. 2. Gene structure of cytohesin-2. The cytohesin-2 gene was composed of twelve exons.

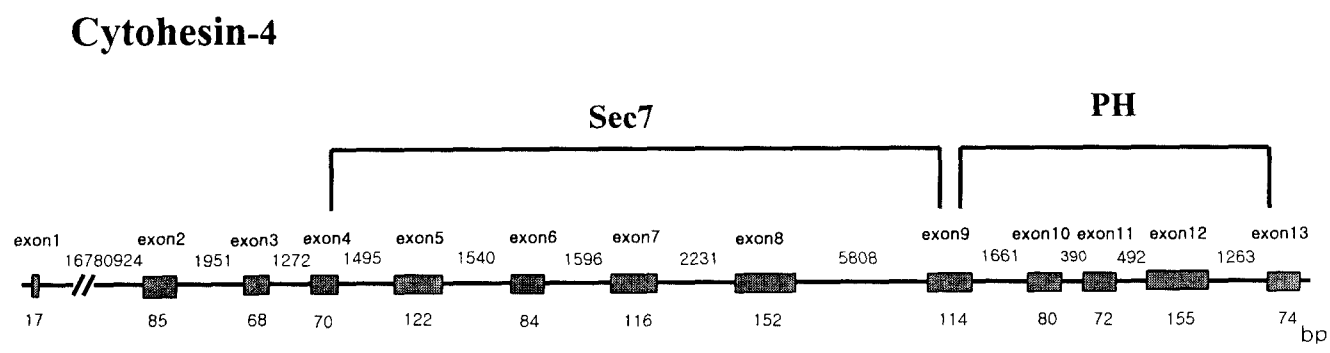

Fig. 3. Gene structure of cytohesin-4. The cytohesin-4 gene was composed of thirteen exons.

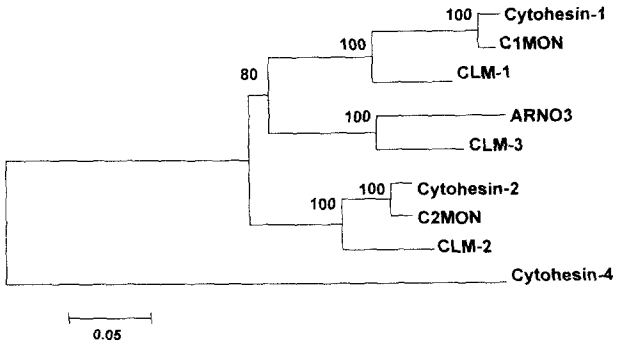

Fig. 4. Phylogenetic tree comparing cytohesin family of human, monkey, and mouse. The values at branch-points indicate the percentage support for a particular node after 100 bootstrap replicates.

The size of the mRNA showed different pattern in humans. The transcripts of 2.1, 4.4, 9.7 kb in length detected in human cytohesin-1, whereas the two transcripts of 2.1 and 6.5 kb were revealed in human cts18.1 [1]. In mouse CLM-2, two different size of transcripts, 1.8 and 4.0 kb, were detected [8]. Human pleckstrin-Sec7 domains gene (PSD) has showed three different tissue specific transcripts (1.8, 2.3, 4.3 kb) as shown in mouse CLM-2. These phenomena allow us to speculate that different transcripts of cytohesins might be implicated in integration of the retroviral elements. Using the GCG and GENETYX programs, therefore, we examined retroviral elements, HERV, SINE, LINE, Alu, and solitary LTR elements from the introns and exons sequences of the cytohesin family. However, such an elements did not identified in those families.

In summary, we determined the gene structure of cytohesin-2 and 4 using the genomic and cDNA sequences from the GenBank database. The cytohesin-2 gene contained 11 introns and 12 exons, while the cytohesin-4 gene contained 12 introns and 13 exons. Interestingly, the first intron showed large fragments in both cytohesin. Phylogenetic analysis among cytohesin family indicated that cytohesin-1 and cytohesin-3 families showed sister relationship. These data could be very important in further research for understanding the function and evolution of cytohesin family.

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