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Molecular Cloning and Phylogeny of the Human Endogenous Retrovirus HERV-W LTR Family in cDNA Library of Human Fetal Brain

Joo-Mi Yi[1], Jae-Won Huh[1], Kyung-Mi Shin[1], Ji-Won Lee[1], Young-Choon Lee[2], In-Ho Paik[3], Kyung-Lib Jang[1] and Heui-Soo Kim[1]*

[1]*Division of Biological Sciences, College of Natural Sciences, Pusan National University, Pusan 609-735, Korea*
[2]*Division of Biotechnology, Faculty of Natural Resourses and Life Science, Dong-A University, Pusan 604-714, Korea*
[3]*Department of Psychiatry, Catholic University of Korea, College of Medicine, Seoul 137-701, Korea*

Abstract

Long terminal repeats (LTRs) of the human endogenous retrovirus (HERV) have been found to be coexpressed with genes located nearby. It has been suggested that the LTR elements have contributed to the genetic variation of human genome connected to various diseases. Recently, HERV-W family was identified in the cerebrospinal fluids and brains of individuals with schizophrenia. Using cDNA library derived from human fetal brain, we performed PCR amplification and identified seven new HERV-W LTR elements. Those LTR elements showed a high degree of sequence similarity (98~99%) with HERV-W LTR (AF072500). A phylogenetic tree obtained by the neighbor-joining method revealed that seven new HERV-W LTR elements (FB-1, 2, 4, 8, 9, 10, 12) were closely related to the AX000960, AF072504, and AF072506 from GenBank database. Our data suggest that several copy numbers of the HERV-W LTR elements are expressed in human fetal brain and may contribute to an understanding of biological function connected to neuropsychiatric diseases.

Key words – HERV W, LTR elements, Human fetal brain, cDNA library, Phylogeny

Introduction

Human endogenous retroviruses (HERVs) are footprints of ancient germ-cell retroviral infections [24]. Retroviral sequences may interact with cellular oncogenes [25] and long terminal repeat (LTR) elements have the capacity to exert a regulatory influence as promoters and enhancers of cellular genes. Most HERV families encompass a relatively low copy number of per haploid genome [19], compared with others that are either high copy number or single copy retroviral elements [21]. These different copy numbers could represent either multiple integration events or provirus amplified after the integration by retrotransposition. Comparative analysis of the HERV LTR elements in human genome could help us to understand the possible impact of HERVs on evolution and genome regulation.

Retroviral particles have been recovered from monocyte cultures from patients with multiple sclerosis [22] and virion-associated MSRV (multiple sclerosis associated retrovirus)-RNA has been reported in serum of patients with the disease [8]. Expression of MSRV sequences in normal placenta allowed the reconstruction of a 7.6kb putative genomic retroviral RNA with RU5-gag-pol-env-U3R organization, with a polypurine binding site (PBS)

*To whom all correspondence should be addressed
Tel : +82-51-510-2259, Fax : +82-51-581-2962
E-mail : khs307@hyowon.cc.pusan.ac.kr

showing similarity with avian retrovirus PBS used by tRNATrp [4]. Southern blot hybridizations using MSRV probes allowed characterization of a copy MSRV-related human endogenous retrovirus family named HERV-W [4]. We examined HERV-W *pol* and *env* gene sequences in human monochromosomes, and found multiple frame-shift and termination codons by deletion/insertion or point mutation [11,14]. The HERV-W LTR elements were detected in hominoids, Old and New World monkeys, suggested that they have inserted in the primate genome approximately 55 million years ago [15]. The expression, structure and promoter activity of HERV-W LTR elements were examined in human cell lines [23]. The HERV-W family was identified in the cerebrospinal fluids and brains of individuals with schizophrenia [10]. Here we identified the HERV-W LTR family in cDNA library of human fetal brain and phylogenetically analyzed with those sequences derived from GenBank database.

Materials and Methods

PCR amplification for HERV-W LTR elements

The cDNA synthesized from mRNA of human fetal brain (Clontech) was used as a template for PCR amplification. New 416-bp LTR elements of HERV-W family were amplified by the primer pair HS47 (5'-TGGTCCATGTTTCTTACGGCT-3', bases 127-147) and DS16 (5'-AAGATGGTGGTGAACCACTTC-3', bases 521-541) from the HERV-W (GenBank, accession no. AF072500). The PCR conditions were used as described by Kim et al. [13] with an annealing temperature of 56°C.

Molecular cloning of PCR products

PCR products were separated on 2% agarose gel, purified with the QIAEX II gel extraction kit (Qiagen) and cloned into the T-khs307 vector [12]. The cloned DNA was isolated by the alkali lysis method using the High Pure plasmid isolation kit (Roche). Individual plasmid DNAs were screened for inserts by PCR using the original primers designed for the locus.

DNA sequencing and data analysis

Positive samples were subjected to sequence analyses on both strands with T7 and M13 reverse primers using an automated DNA sequencer (Model 373A) and the DyeDeoxy terminator kit (Applied Biosystem). Nucleotide sequence analysis was performed using the GAP and PILEUP programs of the GCG software (Genetics Computer Group, University of Wisconsin). The neighbor-joining phylogenetic analysis was performed with the MEGA program [18]. Nucleotide sequences of HERV-W LTR elements were retrieved from the GenBank database with the aid of BLAST network server [2].

Results and Discussion

Retroviruses have been known as one of the infectious agents involved in the pathogenesis of schizophrenia. Karlsson et al. [10] reported the identification of retroviral sequences in cerebrospinal fluids obtained from individuals with recent-onset schizophrenia, and the differential transcriptional up-regulation of members of the HERV-W family of endogenous retroviruses in the postmortem frontal cortex of individuals with schizophrenia. We performed PCR amplification and identified seven new HERV-W LTR elements using cDNA library of the human fetal brain. Those LTR elements showed a high degree of sequence similarity (98~99%) with that of HERV-W LTR (AF072500) (Table 1). To understand the phylogenetic relationship among HERV-W LTR elements, we retrieved the LTRs from the GenBank database and analyzed them with new HERV-W LTR elements. A phylogenetic tree obtained by the neighbor-joining method revealed that seven new HERV-W LTR elements (FB-1, 2, 4, 8, 9, 10, 12) were closely related to the AX000960, AF072504, and AF072506 from GenBank database (Fig. 1). They were also aligned with the HERV-W LTR element (Fig. 2). One or two bp deletions

Table 1. Percentage similarity of nucleotide sequences of HERV-W LTR elements

	1	2	3	4	5	6	7	8	9	10	11	12
1. W-LTR	-											
2. AX000960	98.4	-										
3. AF072504	98.9	98.7	-									
4. AF072506	99.5	98.9	99.5	-								
5. AC007244-2	83.9	83.6	84.1	84.4	-							
6. FB-1	98.9	98.4	98.9	99.5	83.9	-						
7. FB-2	98.9	98.4	98.9	99.5	83.9	98.9	-					
8. FB-4	98.2	98.7	99.2	99.7	84.1	99.2	99.5	-				
9. FB-8	99.2	98.7	99.2	99.7	84.7	99.2	99.2	99.5	-			
10. FB-9	99.2	98.7	99.2	99.7	84.1	99.2	99.7	99.7	99.5	-		
11. FB-10	98.1	97.6	98.1	98.7	83.1	98.1	99.7	99.7	98.4	98.9	-	
12. FB-12	99.5	98.9	99.5	100	84.4	99.5	99.5	99.7	99.7	99.7	98.7	-

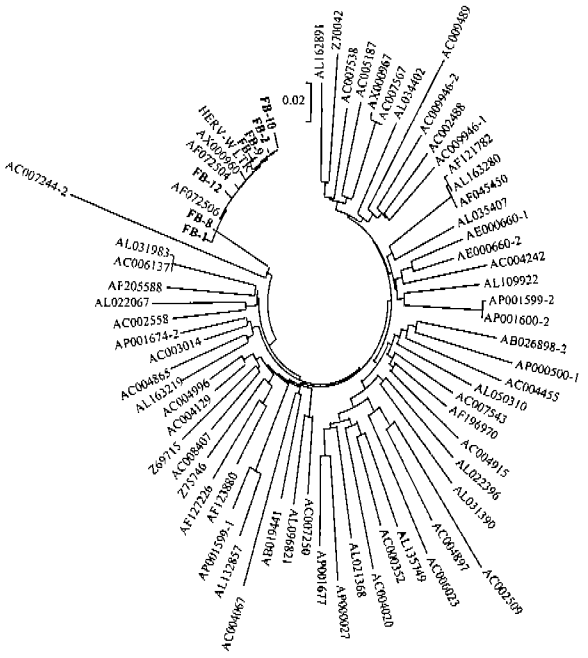

Fig. 1. Phylogenetic tree obtained by neighbor-joining method for the LTR elements of the HERV-W family in cDNA library of the human fetal brain. Branch lengths are proportional to the distances between the taxa.

or additions were notified in aligned sequences. Recently, several copy numbers of the HERV-W LTR elements were isolated from the human mammary carcinoma cell line T47D [23]. In an analysis of promoter activity, the W8 LTR element showed highest promoter activity in LC5 cells, while the W23 LTR element did not show the activity in any cell lines. Expression patterns of the HERV LTR elements varied in various cell lines (epidermal keratinocytes, liver cells, kidney cells, pancreatic cells, lymphocytes, and lung fibroblasts), in some cases showing strict cell type specificity [23]. Transcription of RNA homologous to members of the HERV-W family of retroviruses was also found to be up-regulated differentially in the frontal cortex regions of brains obtained postmortem from individuals with schizophrenia [10].

The HERV LTR elements could be useful for obtaining tissue-specific promoters. Akopov et al. (1998) have noted that such sequences have the capacity to modify the expression of neighboring genes, and suggested that such modifications may have been acquired in the course of human evolution. The HERV-K-T47D-related LTR element has mediated polyadenylation of cellular transcripts [3]. Such phenomenon was very recently demonstrated in nucleosomal binding protein NSBP1 in Xq13.3 [16]. In case of another retro-element (the HERV-F LTR element), a similar phenomenon was observed in relation to the Krppel-related zinc finger gene ZNF195 [17]. A solitary HERV-K LTR element in the HLA DQ region (DQ-LTR3) resulted in suffering from the type I diabetes mellitus in

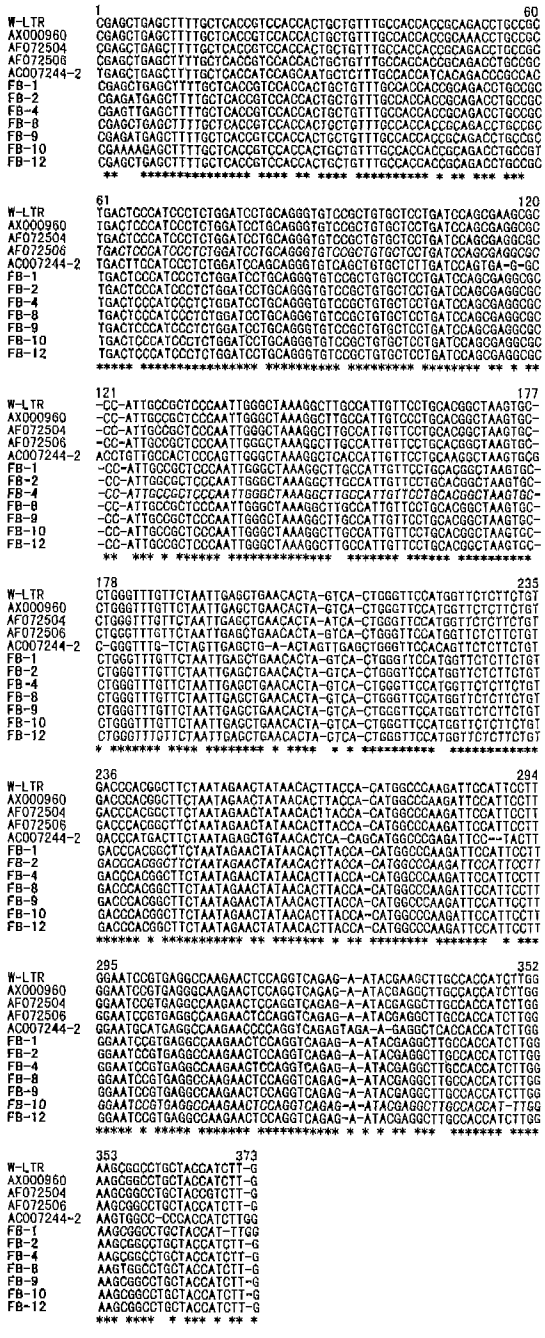

Fig. 2. Sequence alignments of the HERV-W LTR elements that identified in human fetal brain with AX000960, AF072504, and AF072506 from GenBank database. New HERV-W LTR elements (FB-1, 2, 4, 8, 9, 10, and 12) are shown in bold letter. Consensus sequences are shown by an asterisk on the bottom row. AC007244-2 was used as outgroup. The nucleotide seguence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide seguence databases under accession numbers AB066653~AB066659.

246 German and Belgian families [5]. The retroviral LTR element (DQLTR3) was human-specific insertion [6]. This type of the retroviral elements also induced alternative splicing in the human leptin receptor [9]. The solitary HERV LTR elements showed that they retained detectable activity in human carcinoma cells, and could direct the transcription in both orientations relative to the reporter gene [7]. Medstrand et al. [20] reported that LTR elements were used as alternative promoters for the endothelin B receptor and apolipoprotein C-I genes in humans. In this report, our new sequence data of the HERV-W LTR elements in cDNA library of the human fetal brain may be of great use in future studies for understanding of biological function of the HERV-W LTRs connected to neuropsychiatric diseases in human brain.

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초록 : 인간 태아의 뇌로부터 만들어진 cDNA library에서 내생 레트로바이러스 HERV-W LTR의 클로닝 및 분자계통분류

이주미 · 허재원 · 신경미 · 이지원 · 이영춘 · 백인호 · 장경립 · 김희수
(부산대학교 생명과학부)

인간 내생 레트로바이러스의 LTR엘리먼트는 그들의 근처에 있는 유전자와 함께 발현하고 있는 것으로 알려졌다. 그들은 또한 인간의 게놈 내에서 유전적 변이를 유발시켜 다양한 질병과 연루되어 있는 것으로 제안되어졌다. 최근, 정신분열증 환자의 뇌척수액 및 뇌에서 내생 레트로바이러스 HERV-W 패밀리가 동정되었다. 본 연구에서는, 인간 태아의 뇌로부터 만들어진 cDNA library 에서 새로운 내생 레트로바이러스 HERV-W LTR 를 동정하고 분석하였다. 이들 LTR 엘리먼트는 이미 밝혀진 HERV-W LTR (AF072500)과 높은 염기서열의 유사성(98~99%)을 보여 주었다. NJ법에 의한 분자계통 분류도에 의하면, 새로 동정된 HERV-W LTR 엘리먼트 (FB-1, 2, 4, 8, 9, 10, 12)는 GenBank의 데이터 AX000960, AF072504, AF072506과 가까운 유연관계에 있음을 알 수 있었다. 다수의 HERV-W LTR 엘리먼트가 인간의 태아 뇌에서 발현하고 있음이 확실히 밝혀졌으며, 그들의 염기서열은 신경정신분열증과 관련된 생물학적 기능을 이해하는데 기여할 것이다.