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Long Terminal Repeat of an Endogenous Retrovirus HERV-K Family from Human Liver and Kidney cDNA

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Abstract Long terminal repeat (LTR) of human endogenous retrovirus K family (HERV-K) has been found to be coexpressed with sequences of closely located genes. We examined the transcribed HERV-K LTR elements in human liver and kidney tissues. Using the cDNA synthesized from mRNA of human liver and kidney, we performed PCR amplification and identified six HERV-K LTR elements. Those LTR elements showed a high degree of sequence similarity (93.3~96.6%) with human-specific LTR. A phylogenetic tree obtained by the neighbor-joining method revealed that HERV-K LTR elements were mainly divided into two groups through evolutionary divergence. New isolated HERV-K LTR elements (Liv-1, 2, 3 and Kid-1, 2, 3) were belonged to group 1. Our data suggests that HERV-K LTR elements are active on human liver and kidney tissues and may represent a source of genetic variation connected to human disease.

Key words: HERV-K LTR elements, human cDNA, liver and kidney, PCR, phylogeny

Introduction

The HERV-K family of human endogenous retroviral sequences was originally cloned from Syrian hamster intracisternal A type particles [15], has homology to mouse mammary tumour virus, and includes sequences that are expressed in normal placenta and leukemic cells [19]. HERV-K elements probably entered the primate genome after the split of New World monkeys in the Oligocene era, 33-40 million years ago [20]. It is estimated that there are now approximately 25,000 copies of HERV-K LTRs in the human genome, and they have randomly transposed across the chromosomes in the course of human evolution [13]. The possibility that some of this increase has occurred recently is suggested by the presence in HERV-K sequences of a functional integrase [9]. The ratio of synonymous to nonsynonymous substitutions suggests that evolutionary selection has recently been or is still operating [21]. Medstrand and Mager [14] have demonstrated that some clusters of HERV-K LTRs show low (average 1.5%) divergence; in comparisons across primate species members of their cluster 9 are specific to the Homo sapiens. HERV-K LTRs bind host cell nuclear proteins and have the potential to activate neighboring genes [1]. A possible role of HERV-K elements in disease has been considered in relation to insulin dependent diabetes mellitus [4], seminoma [16] and in the case of HERV-K-T47D mammary carcinoma [18]. Here we isolated six HERV-K LTR elements that were transcribed in human liver and kidney tissues and analyzed them phylogenetically with other HERV-K LTR family.

Materials and Methods

The cDNA synthesized from mRNA of human liver and kidney (Clontech) was used as a template for PCR amplification. New HERV-K LTR elements were amplified by the specific primer pair HS45 (5′-GTATTGTCCAAGGTTTCTCCC-3′, bases 8541-8561) and DS14 (5′-GTGCTGTGCTTTTGGATATGC-3′, bases 8859-8879) from the human endogenous retroviral element, HERV-K10 (GenBank, accession no. M14123). PCR products were separated on 2% agarose gel, purified with the QIAEX II gel extraction kit (Qiagen) and cloned into the T-khs307 vector [7]. The cloned DNA was isolated by the alkali lysis method using the High Pure plasmid isolation kit (Boehringer Mannheim). Individual plasmid DNAs were screened for inserts by PCR using the original primers designed for the locus. Positive samples were subjected to sequence analyses on both strands with T7 and M13 reverse primers using an automated DNA

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sequencer (Model 373A) and the DyeDeoxy terminator kit (Applied Biosystem). Nucleotide sequence analyses were performed using the GAP and PILEUP programs from the GCG software (Genetics Computer Group, University of Wisconsin). The neighbor-joining phylogenetic analysis was performed with the MEGA program [12]. Nucleotide sequences of HERV-K LTR elements were retrieved from the GenBank database with the aid of BLAST network server [2].

Results and Discussion

Using cDNA from human liver and kidney as a template, PCR approach with HERV-K LTR specific primers resulted in the amplification of the expected 341 bp fragments (Fig. 1). We cloned and determined nucleotide sequences of HERV-K LTR elements from the PCR products. Six HERV-K LTR elements (Liv-1, 2, 3 from the liver cDNA and Kid-1, 2, 3 from the kidney cDNA) were identified. The nucleotide sequences of the HERV-K LTR elements from the cDNA libraries are presented in alignment with those of human-specific LTR elements (Fig. 2). One or two bp insertions were noted in new identified HERV-K LTR elements. They have a high degree of sequence similarity (93.3~96.6%) with human-specific LTR elements (Table 1). In human genome, several thousand copies of HERV-K LTR elements are distributed randomly. We already identified some of the LTR elements using human monochromosome [6,8]. Medstrand and Mager [14] also identified human-specific HERV-K LTR elements (AC002400, AC002508, AC002350) from the GenBank database. Those all LTR elements were used to construct a phylogenetic tree with six new HERV-K LTR elements. As shown in Fig. 3, HERV-K LTR elements were mainly divided into two groups through evolutionary divergence. Human-specific HERV-K LTR element (AL034407), proliferated in human Xq26 recently, was belonged to group II. Likewise, other human-specific HERV-K LTR elements (AC002400, AC002508, AC002350) were also belonged to group II. New isolated HERV-K LTR elements (Liv-1, 2, 3 and Kid-1, 2, 3) from the cDNA of human liver and kidney in this study were belonged to group I. The data allow us to speculate that new HERV-K LTR elements are active on human liver and kidney tissues

Fig. 1. PCR amplification of HERV-K LTR elements from human liver and kidney cDNA. The lanes are numbered for the human cDNA. M: marker (ϕX174/*Hae* III), 1: liver cDNA, 2: kidney cDNA.

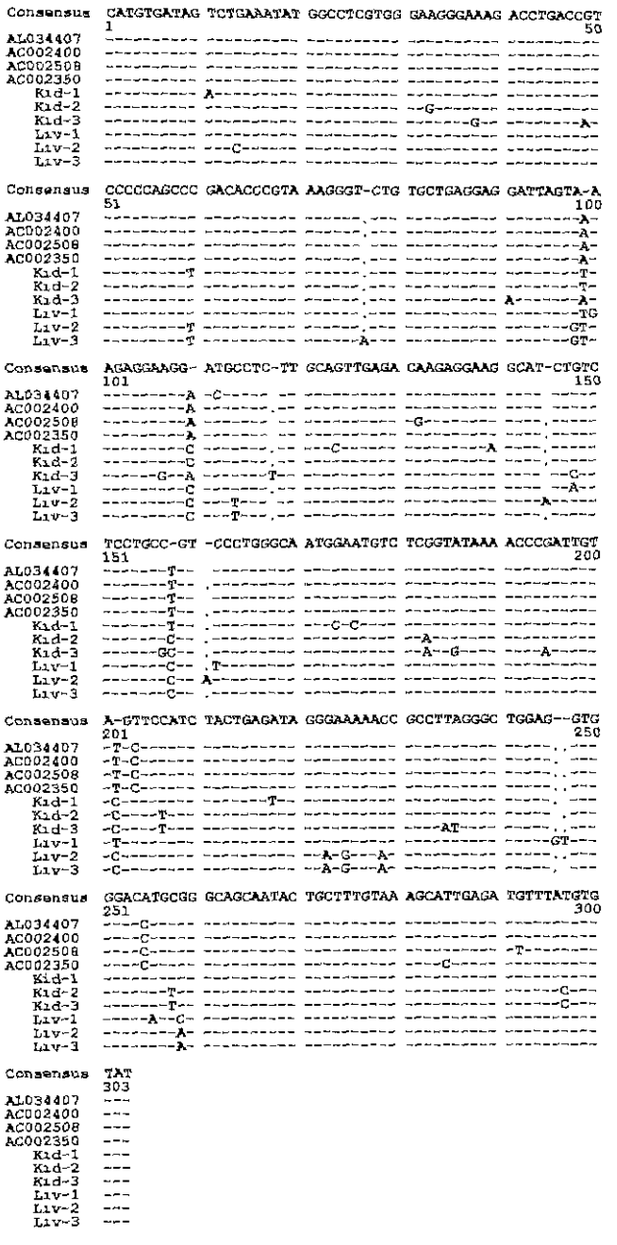

Fig. 2. Nucleotide sequence alignments of the HERV-K LTR elements from human liver and kidney cDNA with human-specific HERV-K LTRs, AL034407, AC002400, AC002508, AC002350. Consensus sequences are shown on the top row. Dashes indicate no change to the consensus sequence.

and may represent a source of genetic variation connected to human disease. Apokov et al. [1] have noted that such sequences have the capacity to modify the expression of neighboring genes. HERV-K-T47D-related LTR element has mediated polyadenylation of cellular transcripts [3]. Two HERV-K LTR elements have been detected in the human histocompatibility complex locus HLA-DQ [5]. One LTR element (DQ-LTR3) of the HERV-K family at the

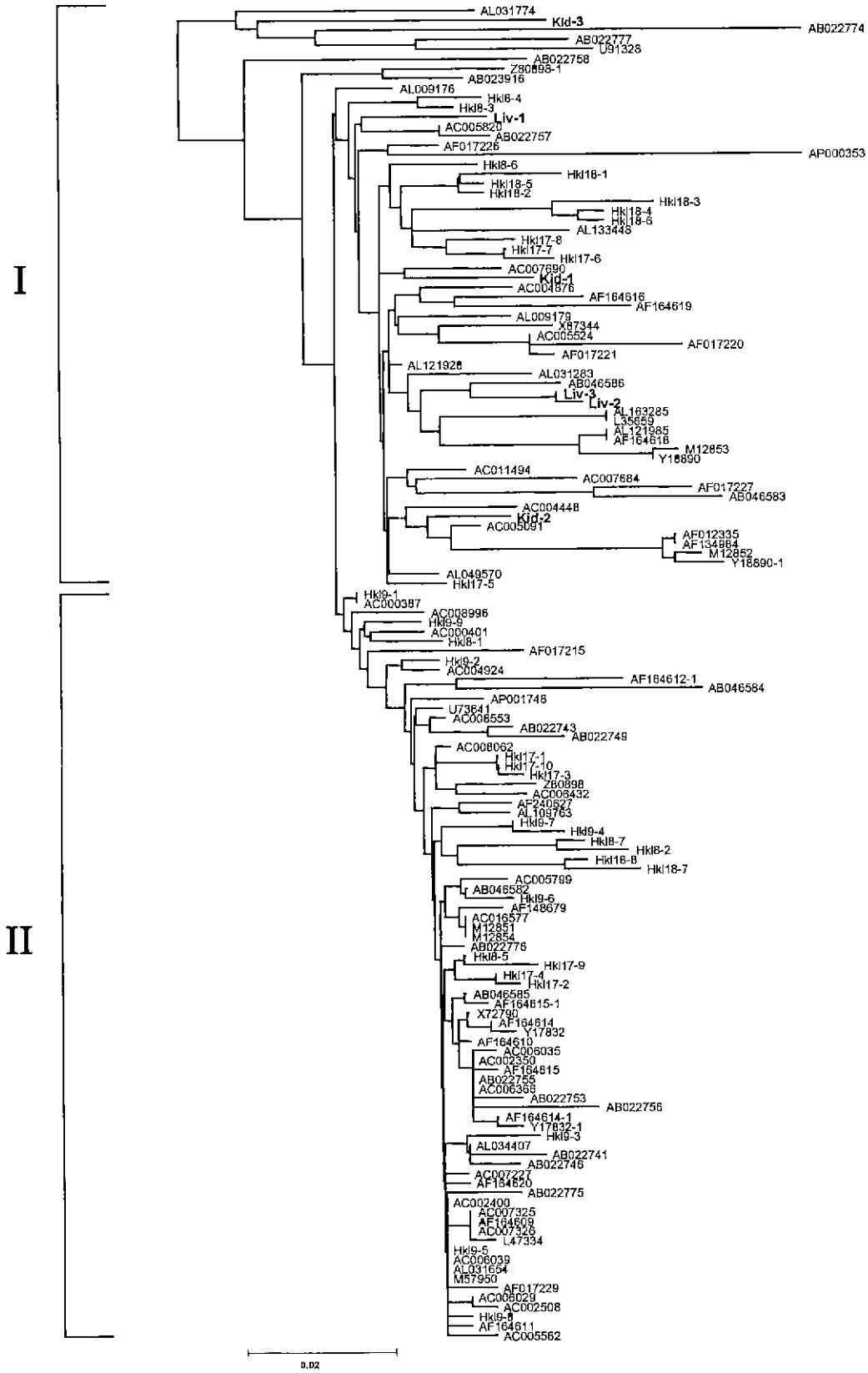

Fig. 3. Phylogenetic tree obtained by neighbor-joining method for the HERV-K LTR family. Branch lengths are proportional to the distances between the taxa. The accession numbers of HERV-K LTR elements were obtained from the GenBank database.

Table 1. Percentage similarity of nucleotide sequence of HERV-K LTRs

	1	2	3	4	5	6	7	8	9	10
1. AL034407	–									
2. AC002400	99.7	–								
3. AC002508	99.0	99.3	–							
4. AC002350	99.3	99.7	99.0	–						
5. Kid-1	95.6	96.0	95.3	95.6	–					
6. Kid-2	96.0	96.3	95.6	96.0	95.6	–				
7. Kid-3	93.6	93.9	93.3	93.6	91.9	95.6	–			
8. Liv-1	96.3	96.6	96.0	96.3	95.3	96.3	92.9	–		
9. Liv-2	94.9	95.3	94.6	94.9	95.3	95.6	91.9	95.6	–	
10. Liv-3	95.3	95.6	94.9	95.3	95.6	96.0	92.3	96.0	99.7	–

HLA-DQB1 locus has been associated with rheumatoid arthritis [17]. An element described as almost identical to SINE-R.C2 that is derived from the HERV-K LTR element is cause of Fukuyama-type muscular dystrophy [11]. HERV-K LTR elements are expressed with neighboring gene. Such phenomenon seems to be assimilated by the neighboring gene during human evolution. A HERV-K-T47D LTR element showed such event with FLT4 gene [3]. HERV-F LTR element was also expressed with Krppel-related zinc finger gene ZNF195 [10]. Therefore we suggest that six new HERV-K LTR elements (Liv-1, 2, 3 and Kid-1, 2, 3) isolated from cDNA of the human liver and kidney deserve further investigation as potential leads to the tissue-specific diseases.

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