## A DOCTORAL DISSERTATION

# Transcription and Chromosomal Location of the Active Retroelements HERV-K and L1Hs in Human Cells



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# 인간 세포에서 Retroelements HERV-K와 L1Hs의 전사와 염색체상의 위치

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이 논문을 이학 박사학위 논문으로 제출함

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# Transcription and Chromosomal Location of the Active Retroelements HERV-K and L1Hs in Human Cells

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### ABSTRACT

Transposable elements play an important role in genome function. They can transpose their chromosome locations using transposition and recombination. The resulting repetitive sequences provide numerous opportunities for genetic recombination. Moreover, the changes in the chromosomal locations of transposable elements induce genomic instability, transformation, and differentiation of cells. The human genome contains several retro-transposable elements. The representative retroelements, human endogenous retroviruses (HERVs) and long interspersed nuclear elements (LINEs), are functionally active. HERVs are estimated to constitute at least 0.5-3% of the human genome; they encode characteristic retroviral proteins, such as gag, pol, and env, and have flanking promoter-like long terminal repeats (LTRs). HERV LTRs and solitary LTRs found in the human genome play an important role in cell biology. LTRs have various elements for regulating the expression of HERVs and neighboring host genes. LINE-1 elements of human (L1Hs) have two open reading frames: one encodes an enzyme for reverse transcription and the other one for RNA-protein complex formation. This study investigated the transcriptions and chromosomal locations of the transcripts of HERV-K and L1Hs in various human cells (two normal lymphocytes, two normal fibroblast, and sixteen cancer cell lines). In addition, this study examined the effects of selected chemical carcinogens on gene expression. RNA transcripts of the retroelements were detected in all cell lines tested, but the relative amounts differed according to cell type. For example, the levels of transcription varied at different developmental stages in fibroblasts as well as in leukemia cells. In addition, the expression patterns of HERV-K genes (HERV-K10 LTR, HERV-K(T47D) LTR, HERV-K(C4) env, and HERV-K10 env), L1Hs p40, and regulatory protein genes (BAX, c-MYC, IL-6, and p53) were

changed after treated with carcinogens. Since cell type-specific activation of HERV-K genes has been reported in various cells, it is thought that HERV-K genes may have functions in proliferation, apoptosis, differentiation, cancer development, and anti-viral protection strategies against exogenous retroviruses. L1Hs transcription was not distinguishable in the cell lines tested as a cell-type independent, but varied in response to carcinogenic and stress factors. Although RNA transcripts of retroelements were detected in the cell lines tested, evidences for a direct association between retroelement expression and human cancers could not find. The results suggest that the altered activity levels of the retroelements contribute to cell transformation, based on changes in the expression of the HERV-K genes and L1Hs p40 in responses to carcinogens. Some carcinogens induced the transposition activity. Genome-scale amplification has shown the altered amplification patterns using transposon-specific ITR and arbitrary random ITR as a amplification primer. After average 5 passages, the distinguishable fragments have seen in an amplification profile. Sequence analysis following a BLAST search and phylogenetic analysis showed that RNA transcripts of the retroelements were transcribed from various locations on most chromosomes. RNA transcripts of HERV-K10 LTR elements were located from the centromere to the telomere in all chromosomes, except chromosomes 21 and Y. HERV-K10 LTRs might be introduced into the human genome several times before and after mammalian speciation. L1Hs element transcripts were found on all but chromosome 21. Active L1Hs elements in at least 140 positions of the haploid human genome were identified by similarity search and putative protein translation of DNA sequences. In addition, the putative active L1Hs elements formed two clusters in the strict consensus tree. Based on chromosomal locations, retroelement transcripts were transcribed from many fragile sites, suggesting their association with chromosome instability, cancer-related breakpoints, and genetic disorder-related loci. After retroelement transcripts were introduced into human genome, they may expand

introns of the genomes and produce many pseudogenes. Consequently, this study suggests that many retroelements are transcribed and transposed in the host genome, and they can respond to various stimuli in the extra- and intracellular environments. In addition, HERV-K and L1Hs retroelements are widely dispersed throughout the human genome, maintaining their retrotransposition potential.

Key words: cancer, carcinogen, chromosomal instability, chromosome, chromosome rearrangement, env, fragile site, gene expression, genetic disorder, genome, genomic instability, HERV-K, HERV-K10 LTR, LTR, LINE-1, L1Hs p40, mutation, recombination, retroelement, retroposon, retrotransposition, retrovirus, transcription, transposable element, transposition, transposon



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### I. INTRODUCTION

The human genome contains a number of repetitive sequences that originated from retroviruses. Recent studies have reported that about one-third of the human genome sequence is derived from different transposable elements (Miki, 1998; Smit, 1999). Of those, endogenous retrotransposable elements have been distributed by retrotransposition during the course of evolution of the genome. The process of retrotransposition is mediated by RNA transcripts, as in human immunodeficiency virus (HIV). This large family of transposable elements can be classified into several groups according to differences in their lengths, structures, and protein encoding genes (Moran *et al.*, 1996; Hohjoh and Singer, 1997; Malik *et al.*, 1999). They contain short- and long interspersed nuclear elements (SINEs and LINEs), such as *Alu* repeats and L1 retroposon, and human endogenous retroviruses (HERVs) (Fig. 1). Although some retroelements are still active and produce deleterious mutations that cause hereditary diseases, most transposon activity in humans seems to be ceased (reviewed in Smit, 1999; Kazazian, 2000).

#### 1. Human Endogenous Retrovirus-K

In 1973, it was discovered that primate placentas produce retrovirus-like particles. These particles not only resemble retroviruses morphologically, but also possess an enzyme activity that is indistinguishable from that of retroviral RNA-dependent DNA polymerase (reverse transcriptase) (Kalter *et al.* 1973; Strickland *et al.*, 1973; Medstrand and Blomberg, 1993).

Vertebrate genomes contain many endogenous retroviral sequences that resulted, in all



Figure 1. Classification of retroelements in human genome.

likelihood, from the integration of exogenous retroviruses into the germ line of a predecessor species. Such the events of inter-genomic integration are thought to be occurred frequently during evolution, and various families of endogenous retroviruses (ERVs) can be found in vertebrate genomes (Wilkinson *et al.*, 1994; Benit *et al.*, 1999). The expression of stably integrated proviral sequences and subsequent retrotranspositions lead to an increase in the copy number of proviral sequences in the host. Probably, HERV-K-like elements may enter into the primate genome about 33-40 million years ago. There are estimated to be ca. 25,000 copies of HERV-K long terminal repeats (LTRs) in the human genome (Leib-Mösch *et al.*, 1993). These integrated proviral sequences have subsequently inherited to a stable Mendelian fashion in subsequent generations and transmitted to newly arising species, and then propagated due to retrotransposition events.

The retroviral genome consists of two, usually identical, ssRNA molecules ranging about 7-10 kb, with 5'-CAP and 3'-poly(A) tail. The order of the genes encoding structural proteins is invariably *gag*, *pol*, and *env*. *Gag* stands for group-specific antigen in the virion core, encoding four separate proteins; matrix protein (Pepinsky and Vogt, 1984), capsid protein (Dickson *et al.*, 1982), nucleocapsid protein, and protease (Coffin *et al.*, 1997). *Pol* encodes three enzymes: reverse transcriptase for synthesis of viral complementary DNA (Varmus, 1987, 1988; Coffin *et al.*, 1997), RNase H, and integrase. *Env* encodes two envelope proteins: surface and transmembrane proteins (Dickson *et al.*, 1982). The viral genome is bounded by LTRs. There is no apparent DNA sequence specificity for host integration sites although some preference has been noted (Wilson and Young, 1975).

Two major characteristics of LTRs make their important features in the genome: they contain regulatory elements and are probably inserted into transcriptionally active regions. Some HERV LTRs recruited during evolution as regulatory elements for gene

control, as they provide sequences for the initiation of transcription, for instance, promoters, enhancers, hormone-responsive elements, transcription-factor binding sites, and polyadenylation signals. Consequently, they can induce significant changes in the expression of neighboring cellular genes (Ting *et al.*, 1992; Feuchter-Murthy *et al.*, 1993; Knössl *et al.*, 1999; Domansky *et al.*, 2000; Schön *et al.*, 2001). Reports suggest that LTRs frequently modulate the expression of nearby genes directly through transcription regulatory signals or regulation of translation (Kapitonov and Jurka, 1999; Kowalski *et al.*, 1999; Mager *et al.*, 1999; Sverdlov, 1998). Note that in the reported examples of LTR involvement in gene regulation, these elements are always incorporated in the 5'-proximal regions of genes. The first clones of HERV sequences were obtained by screening a genomic library with an African green monkey ERV probe (Martin *et al.*, 1981). Since then, a number of HERV elements have been reported, and they are estimated to constitute at least 0.6-3% of the human genome (Wilkinson *et al.*, 1994; Mayer *et al.*, 1998; Zsiros *et al.*, 1998).

The majority of these elements appear to be either rearranged or mutated such that they are in capable of producing proteins. Almost all HERV sequences are too unstable to encode functional proteins. It has been suggested that the HERV-K family is an important exception to this generality because it contains open reading frames (ORFs) and may be involved in tumor development. Full-length ORFs for *gag*, *prt*, *pol*, and *env* have been isolated from the human genome (Wilkinson *et al.*, 1994; Löwer *et al.*, 1996; Kitamura *et al.*, 1996; Mayer *et al.*, 1997).

The HERV-K family of human endogenous retroviral sequences was originally cloned from Syrian hamster intracisternal A type particles (Ono *et al.*, 1986). RNA-transcripts of HERV-K have been detected in distinct cell lines and tissues (Brodsky *et al.*, 1993; Medstrand and Blomberg, 1993; Vinogradova *et al.*, 2001), and HERV-K particles have been identified in the placenta and supernatants of teratocarcinoma cell lines (Boller *et* 



Figure 2. A representative structure of HERV-K and the functions of each gene.

al., 1993; Löwer et al., 1993). Transcripts containing HERV-K pol and env ORFs have been detected in human teratocarcinoma cell lines, and enzymatically active gag and pol gene products have been expressed in vitro (Löwer et al., 1993; Kitamura et al., 1996; Tönjes et al., 1997; Berkhout et al., 1999). Recent reports have suggested the possible involvements of HERV-K sequences in the initiation or progression of germ cell tumors, and Gag protein and gag-encoded retrovirus-like particles are present in tumor cells and derived cell lines, respectively (Sauter et al., 1995; Sauter et al., 1996). Possible roles of HERV-K elements in human diseases have been considered in insulin dependent diabetes mellitus (Conard et al., 1997), seminoma (Sauter et al., 1995), and HERV-K-T47D mammary carcinoma (Seifarth et al., 1998). According to reports of HERV-K elements in several cancer cells, HERV-K LTRs and HERV-W env sequences have been found in RNA transcripts (Zsiros et al., 1998; Kim et al., 2001; Yi et al., 2001). This suggests that HERV-K elements are active in cancer cells and may be involved in carcinogenesis. In addition, HERV-K Gag and Env antibodies have been detected in germ cell tumors and trophoblastic tumors (Müeller-Lantzsch et al., 1993; reviewed in Mayer et al., 1997; Herbst et al., 1999).

#### 2. Long Interspersed Nuclear Elements-1

The LINE-1 element is a retroposon that is found in all mammalian genomes, and belongs to the class of retroposons lacking LTRs. LINE-1 elements account for about 15% of the human genome and have achieved this abundance by retrotransposition (Fanning and Singer, 1987; Hutchison *et al.*, 1989; Singer *et al.*, 1993). Active human L1 sequences (L1Hs) can cause mutations, diseases, genetic variations, and

polymorphisms, and even their remainders can be involved in genetic recombination and chromosomal rearrangements (Gilbert *et al.*, 2002; Kazazian and Goodier, 2002; Sankoff *et al.*, 2002; Chi *et al.*, 2003). At least 3-4% of L1Hs are full-length, while the others are truncated to varying degrees (Hwu *et al.*, 1986; Smit, 1999).

Full-length L1Hs are about 6 kbp in length and contain two nonoverlapping open reading frames (ORFs), a 5' untranslated region (UTR) and 3' poly(A) tail (Fig. 3). ORF1 encodes an RNA-binding protein p40, which forms an RNA-protein (RNP) complex, and has been observed in breast cancer, teratocarcinoma, normal breast tissue, germ cell tumors lines, and in several kinds of tumor cells (Bratthauer and Fanning, 1992; 1993; Bratthauer *et al.*, 1994; Asch *et al.*, 1996). L1Hs p40 has no significant sequence homology to *gag* and *gag*-like proteins observed in endogenous retroviruses (Hohjoh and Singer, 1997). ORF2 encodes a 149-kDa protein, which has endonuclease and reverse transcriptase activities. Examination of the contribution of various protein regions to the RT activity has demonstrated that deletions and mutations in the ORF2 protein domains eliminate or significantly decrease the RT activity (Dombroski *et al.*, 1994; Feng *et al.*, 1996). Furthermore, mutation and deletion analyses of the L1Hs element have shown that both the p40 and the EN and RT regions of the ORF2 protein are required for efficient retrotransposition in cultured cells (Feng *et al.*, 1996; Dhellin *et al.*, 1997; Clements and Singer, 1998).

L1Hs elements are found in all chromosomes including sex chromosomes X and Y. Many L1Hs copies have accumulated in AT-rich regions of human genome (Smit, 1999; Pavlicek *et al.*, 2002). Active L1Hs elements have the capacity to cause mutations, diseases, genetic variations, and polymorphisms, which their inactive copies appear to be involved in chromosome recombination and rearrangement during cell division (Brouha *et al.*, 2002). There are about 100,000 L1Hs elements in the human genome, but only about 30-60 of the full-length L1Hs are capable of autonomous retrotransposition, and

capable of synthesizing new copies at different positions on chromosomes containing the L1Hs integrating target sequence. The transcript is reverse transcribed and integrated into a new genomic location by a process termed 'target-primed reverse transcription' (Luan *et al.*, 1993). On integration, the L1Hs element typically is flanked by target-site duplications of 6-20 bp; the integration site has a weak consensus sequence (5'-Py-Py/AAAA-3') (Cost and Boeke 1998). In addition, L1Hs elements insert at random locations in the genome (Hutchison *et al.*, 1989; Ovchinnikov *et al.* 2001). The haploid human genome has approximately above  $7 \times 10^5$  putative target sites for L1Hs target sequences.

Recently, new retrotranspositions of active human elements have been identified through systematic screening of disease genes (Kazazian et al., 1988; Morse et al., 1988; Miki et al., 1992; Narita et al., 1993; Miki, 1998). These developments have identified new insertions or deletions. For example, insertion in *B-globin* gene in mother and her daughter showing typical \$-thalassemia trait (Kimberland et al., 1999), factor VII and IX genes in the patients with hemophilia A, B (Kazazian et al., 1988; Youssoufian et al., 1988; Woods-Samuels et al., 1989), in the APC gene in a colon cancer patient (Miki et al., 1992), in the retinitis pigmentosa-2 (RP2) gene of a X-linked retinitis pigmentosa and his carrier mother (Kimberland et al., 1999), and in the dystrophin gene of a muscular dystrophy (MD) patient (Holmes et al., 1994); deletions in the PAX gene in aniridia patients (Drechsler and Royer-Pokora, 1996) and in the dystrophin gene in severe muscular dystrophy patients (Sugimoto et al., 2001) have also been identified. The major sequences of L1Hs are integrated in human retinoblastoma (Rb), interleukine 2 (IL-2), factor VIII genes. Since Rb and factor VIII have a high frequency of chromosomal deletions in various cancers and haemophilia A, the universal integration of long, and homologous L1Hs segments in these genes and all chromosomes may promote abnormal DNA rearrangement (Kuo et al., 1998). L1Hs insertions into human



Figure 3. Schematic illustration of LINE1 element.

genes have also been described in cultured mammalian cells transfected with full-length elements, and large deletions due to L1Hs can occur via various pathways *in vitro* and *in vivo* (Moran *et al.*, 1996; Kimberland *et al.*, 1999; Gilbert *et al.*, 2002).

#### 3. Purposes of This Study

While it has been suggested that retroelement activity is involved in genome instability, as repetitive elements are abundant and widespread in the human genome, it is very difficult to elucidate a direct relationship between their activities and cancer development and genome instability. To understand the association between the activity of the autonomous retroelements (HERV-K and L1Hs) and human cancers, this study was performed.

At first, to investigate the difference of gene expression, transcription patterns of HERV-K and L1Hs retroelements were examined in normal fibroblast cells and cancer cells. In addition, to screen the effects on genomic instability, gene expression of these retroelements, and genome scale transposition activities were analyzed in normal fibroblast cells by carcinogen exposure. To determine the active retroelement sequences transcribing and transposing and its chromosomal locations in normal and cancer cells, DNA sequencing of transcripts from various human cells and following analyses on the chromosomal locations were performed. The associations between the activation of these retroelements and genetic disorders including cancers and diseases were investigated. Finally, evolutionary implications were tested by phylogenetic analysis.

## **II. MATERIALS AND METHODS**

#### 1. Cell Lines and Cell Culture

Whole blood samples were collected from peripheral vessel in two healthy individuals. Two normal fibroblasts (Table 1) and sixteen cancer cell lines (Table 2) were used in this study. Cell lines were obtained from Korean Cell Line Bank (KCLB) and American Tissue and Cell Culture Co. (ATCC). Each cell was cultured in Dulbecco's modified Eagle's medium (Gibco-BRL, USA) or RPMI1640 medium, supplemented with 10% fetal bovine serum (FBS) and 1× antimycotic-antibiotics, under constant 37°C and 5%  $CO_2$  condition, as previously described.

#### 2. Chemicals and Treatment

The normal fibroblast cell line CCD-25Lu was treated with benzidine (BZ), dimethylaminoazobenzene (DMBA), dimethyl sulfoxide (Me2SO), ethanol (EtOH), ethidium bromide (EtBr), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). The chemicals were dissolved in the appropriate solvent and diluted at least 100 fold with phosphate-buffered saline (PBS). The dosage of each chemical that corresponded to the maximum resistance concentration was determined by a DNA fragmentation assay and a microscopic observation.

Cell line	Symbol	Age	Gender	Status	Origin
KYJ*	YJ	22	female	normal, lymphocyte	blood
KBG*	BG	30	male	normal, lymphocyte	blood
CCD-25Lu	CC	7	male	normal, fibroblast	lung
HEL-299	HE		·교 중앙	normal, fibroblast	lung

Table 1. Normal human cells used in this study.

\* whole blood from volunteer without clinical symptoms.

Cell line	Symbol	Status	Origin	
CEM-CM3	CE	acute lymphoblastic leukemia	T lymphoblast	
HL-60	HL	leukemia, acute promyelocytic	blood	
Jurkat	JU	acute T cell leukemia	T lymphocyte	
Molt-4	MO	acute lymphoblastic leukemia	T lymphoblast	
U-937	U9	histocytic lymphoma	monocyte	
A-431	A3	carcinoma	skin	
A-498	A4	carcinoma	kidney	
A-549	A5	carcinoma	lung	
HCT-15	HC	adenocarcinoma	colon	
Hep-G2	HG	hepatoblastoma	liver	
MCF-7	МС	adenocarcinoma; pleural effusion	breast, mammary gland	
MIA PaCa-2	MP	carcinoma	pancreas	
SNU-16	SU	adenocarcinoma	stomach; ascites	
SNU-C5	SC	adenocarcinoma	colon; colorectal	
SK-N-SH	SN	neuroblastoma, metastasis to bone brain		
SK-OV-3	SO	adenocarcinoma, malignant ascites	ovary	

Table 2. Human cancer cell lines used in this study.

#### 3. Total DNA Extraction

Total DNA was extracted from cultured cells and whole blood with slightly modified sucrose-proteinase K method. Before application, the whole blood cells were treated red cell lysis buffer (RCLB) to remove the erythrocytes. Leukocytes from whole blood and cultured cells were washed with PBS. All samples were lysed with 5 m $\ell$  of pre-warmed sucrose-proteinase K lysis buffer (27% sucrose, 1× SSC, 1% SDS, 1 mM EDTA, and 100  $\mu$ g/ml proteinase K), separated twice with phenol/chloroform/iso-amylalcohol, centrifuged at 3,000 rpm for 10 min, precipitated in iso-propanol, and washed with ethanol, according to the procedure described by Birren *et al.* (1997). The DNA concentration ranged from 50 ng/ $\mu\ell$  depending on the sample, each DNA was used as a template for PCR amplification, DNA fragmentation assay, and transposition analysis.

#### 4. Cytogenetic Analysis and Fluorescence in situ Hybridization (FISH)

FISH analysis was performed with metaphase chromosomes of peripheral leukocytes, KYJ. The leukocytes were isolated with a gradient centrifugation method using HistoPaque 1770 (Sigma, USA). The cells were cultured in McCoy' 5A medium containing 15% FBS, streptomycin-penicillin, and a mitogen phytohemagglutinin (Sigma, USA). On 3 day, a mitotic blocker, colcemid was treated to prepare the metaphase chromosomes. Harvesting and preparation of slides were performed according to standard cytogenetic procedures. Harvested cells were treated with hypotonic solution (0.075 M KCl) for 20 min at  $37^{\circ}$ C, and fixed at -20 °C in Carnoy's

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fixative. For FISH and G-Banding analyses, cells were dropped on microscope slides and air dried. Slide preparations were allowed to age at room temperature for 1 day or 2 days before the analyses. Slides were stained with Giemsa. Image acquisition and analysis were performed by the cytogenetic analysis software Karyo-G Band ver. 5.0 (Imstar, France). A L1Hs p40 probe was used for FISH analysis of KYJ metaphase. Probes were labelled with purified PCR product using Dig-Labelling and Detection Kit (Roche, Germany). Each labelled probe was precipitated with Salmon sperm DNA and resuspended in hybridization mixture (50% formamide, 10% dextran sulfate, 2× SSC, pH 7.0). The metaphase preparations were pre-treated with RNase A and pepsin. Slides were denatured in 70% formamide,  $2 \times SSC$  for 5 min at 70°C. After probe denaturation at 75  $^{\circ}$ C for 5 min, pre-annealing was performed at 45  $^{\circ}$ C for 30 min, The pre-annealed hybridization mixture was added to the denatured slides and allowed to hybridize for 16 hr. Post-hybridization washing were performed for  $3 \times 5$  min with 50% formamide/  $2 \times$ SSC at 45 °C, and for  $3 \times 5$  min with 50% formamide/0.1× SSC at 60 °C, both with agitation. After blocking with 5% bovine serum albumin in 2× SSC/0.1% Tween-20, the Digoxigenin-labelled probes were detected using anti-digoxigenin Fab fragment conjugated with Rhodamine (Roche, Germany). The air dried slides were counter-stained with DAPI (Sigma, USA) and mounted with antifade (2% DABCO in glycerol). Image acquisition and analysis were performed with the software mFISH ver. 5.0 (Imstar, France).

#### 5. Transposition Analysis

Transposition activity had been screened on each treatment of cell cultures.

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Table 3. List of primers used in transposition analysis.

Name	Gene	Sequence $(5' \rightarrow 3')$	Reference	
HsMar	Hsmar1	TATTAGGTTGGTGCAAAAGT	Demattei et al. (2000)	
Mos1	Mosl	AGCTCGTTTA	Augé-Gouillou et al. (2001)	
Pogo1	Pogol	CAGGCATACCTC	Hugh (1996)	
HSH103	AP*	GTGACGCCGC	This study	
HSH147	AP*	GTGCGTCCTC	This study	

\* is arbitrary primer designed in this study.

Carcinogen treatment had maintained continuously for five culture passages, and total DNA and cellular RNA were extracted as described above.

To amplify the transposition-related region in the genome, two kinds of primers were designed; one is specific primers in flanking region of inverted terminal repeats (ITRs) from three transposons (*Mos1*, *Pogo1* and human *Hsmar1*-like element), and the other is random primers in arbitrary ITR sequences. Nucleotide sequences of the ITR primers were listed in Table 3. Amplification procedures were performed by the method of Misra *et al.* (2001). PCR products were separated on 8% non-denaturing polyacrylamide gels under 100 V constant condition for 4 hr. The bands were stained by EtBr, and then visualized with UV-illumination.

## 6. Extraction of Total RNA 세주대학교 중앙도서관

Total RNA was extracted from cells and whole blood samples using High Pure RNA Isolation Kit (Roche, Germany) according to manufacturer's guide. Whole blood was treated with Red Cell Lysis Buffer to remove the erythrocyte prior to RNA extraction. The harvested cells from non-treated each culture of cell lines directly applied. RNA extraction from each treatment on fibroblast CCD-25Lu was performed on day 3 after treatment. Extracted RNA was dissolved in DEPC-water, and cellular DNA was removed by digestion with RNase-free DNase I (Roche, Germany).

To calculate the amount of RNA, the absorbance was measured at 260 nm and 280 nm wavelength. The RNA samples with  $A_{260}/A_{280}$  ratio over 1.8 were used for cDNA synthesis.

#### 7. cDNA Synthesis and Semi-Quantitative RT-PCR

The first strand cDNA synthesized with 1  $\mu$ g DNase I-digested RNA was reverse-transcribed with the oligo(dT)<sub>15</sub> primer using ImpromII cDNA Synthesis Kit (Promega, USA) according to manufacturer's protocol. After cDNA synthesis, reaction mixture was diluted to final volume of 100  $\mu$ l by addition of 80  $\mu$ l DEPC-water, and the amount of synthesized cDNA was measured with absorbance test. Oligonucleotide primers used in RT-PCR amplification were listed in Table 4.

To amplify the transcripts, 100 ng of cDNA was used as a PCR template to amplify each gene, except for 500 ng of cDNA, HERV-K(T47D) LTR and HERV-K(*C4*) *env*. Amplification was performed with *Taq* DNA polymerase (Promega, USA) and appropriate primer pairs. The PCR mixture contained sterile distilled water, 2.5  $\mu\ell$  10× PCR buffer (10mM Tris-HCl, pH 9.0, 2 mM MgCl<sub>2</sub>, 50 mM KCl, and 0.1% Triton X-100), 10 mM dNTP, 2 units of *Taq* DNA polymerase, 20 nM primers, and cDNA. The PCR was performed with a GeneAmp PCR system 9600 (Perkin-Elmer Cetus, USA). The amplification was performed for 30 cycles with denaturation (45 sec at 94°C), annealing (45 sec at proper temperature according to each primer pair), and extension (1 min at 72°C). The PCR products were electrophoresed on 1.5% agarose gels.

The bands observed on a gel were quantified using Quantity-One image analysis software (Bio-Rad, USA). To examine the relative levels of each gene transcription, the intensity values of observed bands were normalized to those of the human  $\beta$ -actin gene. For cloning and DNA sequencing, RT-PCR product was purified with QIAquick PCR Purification Kit (Qiagen, USA) according to manufacturer's manual.

Table 4. List of primers used in RT-PCR analysis.

Name	Gene	Sequence $(5' \rightarrow 3')$	Source of primer	Reference
KSH45	HERV-K10 LTR	GTATTGTCCAAGGTTTCTCCC	M14123	Kim et al. (2001)
KDS14	IILKV-KIULIK	GTGCTGTGCTTTTGGATATGC	W114125	
K1505	HERV-K(T47D)LTR	TCCCCTTGGAATACTCCTGTTTT	U39936	Patience et al. (1996)
K1506	IIEKV-K(I47D)EIK	CATTCCTTGTGGTAAAACTTTCCA	037730	
KenvS	HERV-K10 env	GTAGAAGTACCTACTGTCAG	AF490464	Sugimoto et al. (2001)
KenvA	IILK V-KIO env	CTCACCAGCAGAATACGGTG	AI 470404	
KC4S	HERV-K(C4) env	ATGTACATCTCTGATCACACTATGG	U07586	Schneider et al. (2001)
KC4AS	IILK V-K(C4) env	TAATTTGGAGTGTTTAGGGCCTA	007580	
Lp40S	L1Hs p40	AGGCCAACGTTCAGATTCAG	U93564	This study
Lp40A		GCTGGTACCGGTTGTTCC	093304	
BAS	β-actin	ATGGGTCAGAAGGATTCCTATG	NM 001101	Gunning <i>et al.</i> (1983)
BAAS	p-actin	CAGCTCGTAGCTCTTCTCCA		
BAXS	BAX	ACCAAGAAGCTGAGCGAGTGT		
BAXA	DAA	ACAAAGATGGTCACGGTCTGCC		
IL6S	IL-6	AATTCGGTACATCCTCGACG		
IL6AS	<i>1L-0</i>	GCGCAGAATGAGATGAGTTG		
MYCS	o 1440	TCTGGATCACCTTCTGCTGG		
MYCA	с-Мус	GCTCCTCTGCTTGGACGGAC		
TP53S	<i>p53</i>	GTGGAAGGAAATTTGCGTGT		This study
TP53A	<i>p</i> 55	TCTGAGTCAGGCCCTTCTGT		11113 3111149

#### 8. Cloning and Sequencing

To determine the nucleotide sequences of transcripts of retroelements, DNA sequencing was performed. The purified PCR product and RT-PCR product were ligated into a pCR2.1-TOPO vector and cloned using TOPO<sup>TM</sup> TA Cloning kit (Invitrogen, USA). Ligation mixture contained 2  $\mu\ell$  sterile distilled water, 2  $\mu\ell$  insert DNA, 1  $\mu\ell$  TOPO vector, and 1  $\mu\ell$  salt solution (1.2 M NaCl, 0.06 M MgCl<sub>2</sub>). Ligation mixture was incubated at room temperature for 15 min. The conditions for transformation and cloning were followed by the manufacturer's manual slightly modified. Plasmid was isolated with Wizard DNA MiniPrep (Promega, USA) and confirmed the insertion of DNA by restriction enzyme digestion or nested PCR. Sequencing reaction was performed in ALFexpress DNA Sequencing System (Pharmacia Biotech, Sweden) with Cy5-labelled vector inner primers (M13-40 and M13 reverse) and Cy5-AutoCycle Sequencing Kit (Pharmacia Biotech, Sweden). The nucleotide sequences of HERV-K10 LTR and L1Hs *p40* obtained by DNA sequencing were compared with previously reported sequences using the BLAST search program in GenBank.

#### 9. SignalScan of the Signal Responsive Sequences of HERV-K LTRs

Analysis of putative signals was screened by SignalScan ver. 4.1 (Prestridge, 1991) with *Mammal and Virus* option using TRANSFAC database (Wingender, 1994). Signal analysis of LTRs of HERV-K10 and HERV-K(T47D) was performed using the sequence from M14123 (Ono *et al.*, 1986) and from U39936 (Patience *et al.*,

1996) previously reported in GenBank, respectively.

# 10. Database Search of the Retroelement Sequences in Human Genome Resources

To analyze the associations between chromosome locations of transcriptionally active retroelements and the human genetic disorders, LOCUSLINK was used to determine the chromosomal map locations in Human Genome Resources. Chromosomal fragile sites previously reported were also screened using LOCUSLINK. OMIM (Online Mendelian Inheritance in Man) was used to search the locus-related genetic disorders and cancers.



#### 11. Protein Sequence Analysis of L1Hs p40

The standard active L1Hs p40 protein sequences retrieved from U93573 (Sassaman *et al.*, 1997) were analyzed using PROSITE database (Bairoch *et al.*, 1997) in Network Protein Sequence (http://npsa-pbil.ibcp.fr). To scan a sequence for sites/signatures PROSCAN carried out against PROSITE database, and a helix-turn-helix motif did HTH (Dodd and Egan, 1990). Phylogenetic analysis on putative protein sequences obtained from the result of translation of L1Hs *p40* nucleotide sequences were carried out using CLUSTAL W (Thompson *et al.*, 1994) for multiple alignment and PHYLIP package (Felsenstein, 1993) for phylogenetic relationship. Protein distance was calculated with the program PROTDIST.

#### 12. Phylogenetic Analysis of Retroelement Transcripts

DNA sequences were multiple aligned using CLUSTAL W (Thompson *et al.*, 1994), and were analyzed using PHYLIP program packages (Felsenstein, 1993). The sequence boundaries of each gene were determined by comparison with those previously reported (Table 3). The multiple alignment of obtained sequences and those previously reported was performed using CLUSTAL W program with the default settings, and then alignment was adjusted manually in order to align several regions of conserved sequences. Genetic distances among sequences were calculated using DNADIST program with the option of two-parameter method (Kimura, 1980) in PHYLIP. A bootstrap analysis was done using 1,000 replicating data sets generated with the SEQBOOT program. Then, CONSENSE program was used to construct a strict consensus tree.



### **III. RESULTS**

Until now, expression of retroelements has been observed in several normal tissues and cancers. To examine the associations between the expression of functionally active retroelements and human cancers, this study has been analyzed the transcription patterns, the effects of carcinogen treatment on gene expression and transposition activity, transcript sequences, and the chromosomal locations of active retroelements HERV-K and L1Hs in human cells.

#### 1. Human endogenous retrovirus-K



#### 1) Cell type-specific transcription of the HERV-K genes

In normal fibroblasts, the pattern of HERV-K transcription was examined using RT-PCR analysis. Different transcription levels were observed (Fig. 4). After standardization to human *β-actin* mRNA, the relative levels of HERV-K *env* genes and HERV-K LTRs transcription were different between CCD-25Lu and HEL-299 cells. The relative transcription of K10 LTR, K10 *env*, and T47D LTR was higher in CCD-25Lu cells than in HEL-299 cells, while that of C4 *env* expression was lower. Lung carcinoma A-549 showed high expression in HERV-K LTRs and C4 *env*, but not of K10 *env*, compared to normal lung fibroblasts (CCD-25Lu and HEL-299 cells). In the leukemia cell lines, T lymphoblast cells (Molt-4 and CEM-CM3) expressed relatively higher levels of HERV-K genes than did HL-60, U-937, or Jurkat. The transcription of K10 *env* gene was relatively higher in several cancer cells and fibroblasts. The C4 *env* gene was also


Figure 4. RT-PCR analysis of the HERV-K genes in human cells. 100 ng of cDNA was used as a PCR template for amplifying the K10 LTR and K10 *env*, and 500 ng of cDNA for T47D LTR and C4 *env*, respectively. M is the DNA size marker, GenRuler DNA ladder.

transcribed in a variety of cells except for SNU-16, but the relative transcription levels were different remarkably (Fig. 5). In the analysis, no differences in the relative transcription levels of normal fibroblasts and cancer cells was detected. While the relative transcription of each gene differed in the cell line tested. In particular, the transcription of HERV-K10 *env*, HERV-K(C4) *env*, and HERV-K(T47D) LTR showed cell-type specificity, as described previously (Majors, 1990; Ting *et al.*, 1992; Kjellman *et al.*, 1999; Sugimoto *et al.*, 2001; Andersson *et al.*, 2002).

#### 2) Various signals can effect on the activation of HERV-K

A conserved region was identified by BLAST search and multiple alignment of HERV-K LTRs. As reported, putative signal responsive elements on LTR include promoter, enhancer, and hormone responsive elements (Baust *et al.*, 2001; Schön *et al.*, 2001). A computer analysis of HERV-K10 LTR sequences was performed using the TRANSFAC database (Wingender, 1994). Figure 6 shows putative signal response sites in HERV-K10 LTR. The viral TATA box and enhancers were found, and the TATA box is the potential region for binding of various transcription factors (TMF, UBP-1, TFIID, TFIIF, TRF, TBP, and NF-E2). In addition, various transcription factor response elements (AP-1, CAC binding protein, GATA-1, Sp1 and TGT3; hormone response elements, GR for glucocorticoid and window 4 and 10 for the human epidermal growth factor) and the oncoprotein c-MYC binding element were also detected.

HERV-K(T47D) LTR has the signal responsive elements, for example, binding sequences for transcription factors (AP-1, AP-2, NF-1, NF-2, NF-E, TFIID, TBP, Sp1, and Pit-1), oncoproteins (v-JUN and c-MYC), and hormone (GR) (Fig. 7).

3) Carcinogens induced the various effects on gene expression of the HERV-K and regulatory proteins in CCD-25Lu

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Figure 5. Relative levels of the HERV-K gene transcription in human cells. 100 ng of cDNA was used as a template for amplifying the K10 LTR and K10 *env*, and 500 ng for T47D LTR and C4 *env*. RT-PCR bands were standardized by comparing the ratio of each HERV-K gene to that of the human  $\beta$ -actin with Quantity One software (Bio-Rad).



Figure 6. Signal scan of the HERV-K10 LTR using the TRANSFAC signal database with human and virus option.

TCAAGTACCCAGGGACACAAAAACTGCGGAGG TA-3 (-)TC-II;_L_strand (+)GR 300 TATGGCCTCGTGGGAAGGGAAAGACCTGACCGT (+)T-Ag (-)NF-1 (-)TGT3 (+)T-Ag (+)AP-1 (+)AP-1
CAAGTACCCAGGGACACAAAAACTGCGGAAGG TA-3 (-)TC-II;_L_strand
= • •
200
-1 (+)PI
n (+)CP1 window_10

Figure 7. Signal scan of the HERV-K(T47D) LTR using the TRANSFAC signal database with human and virus option.

To screen the effects on transcription of HERV-K by carcinogens, CCD-25Lu cells were treated with various chemical carcinogens. The carcinogen treatments had various effects on CCD-25Lu cells. The cytotoxic effects by DNA fragmentation assays also were different. High concentration of MNNG and EtBr induced cell death. Degraded DNA fragments produced in cellular processes of the activated apoptotic pathway were observed in fibroblasts treated with carcinogens, which the pattern of DNA degradation varied according to the carcinogen. Using RT-PCR, the result showed that carcinogens alter the expression of HERV-K genes (Fig. 8) and regulatory protein genes. Cellular mRNA contained more RNA transcripts of HERV-K genes in all treatments, except HERV-K10 LTR in EtOH, EtBr, and MNNG treatment (Fig. 9). Of the regulatory genes, *BAX* mRNA was increased by all the carcinogens, *c-MYC* mRNA reduced by EtBr, and *IL-6* was decreased by MNNG (Fig. 10). The expression of *p53* was reduced by DMBA, EtOH, EtBr, MNNG and TPA in CCD-25Lu fibroblasts (Fig. 11).

#### 4) Carcinogens induced the transposition activity in CCD-25Lu

Screening of transposition events has been examined using random amplification of the genomic DNAs and cDNAs, which extracted from the CCD-25Lu fibroblasts exposed to each carcinogen for 7 passages. After average 5 passages, an distinguishable amplification patterns have been shown (Fig. 12 and 13). Amplification with the arbitrary and transposon specific ITR primers showed that altered patterns were dependent on culture passages. In addition, RT-PCR analysis on cDNA using the same primers has also been shown the differential fragments on gels at 5 passages of carcinogen treatments. Transcription patterns had also been altered by carcinogen treatment in CCD-25Lu fibroblasts. Comparing to saline treated control, carcinogen specific bands were found in the treatments, and relative levels of each amplified band has also been remarkably different (Fig. 14).

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Figure 9. Relative levels of the HERV-K gene transcription in CCD-25Lu cells treated with carcinogens. 100 ng of cDNA was used as a template for amplifying the K10 LTR and K10 *env*, and 500 ng for T47D LTR and *C4 env*. Standardization for the transcription level of each gene was performed as described in Fig. 5.



Figure 10. RT-PCR analysis of the regulatory protein gene transcription in CCD-25Lu cells treated with carcinogens. 100 ng of cDNA was used as a RT-PCR template for amplifying each gene. M is the DNA size marker, GenRuler DNA ladder.



Figure 11. Relative levels of the regulatory gene transcription in CCD-25Lu cells treated with carcinogens. 100 ng of cDNA was used as a RT-PCR template for amplifying each gene. Standardization for the transcription level of each gene was performed as described in Fig. 5.



Figure 12. Transposition analysis on genomic DNAs of CCD-25Lu cells treated with carcinogens for 7 culture passages. Amplification was performed using arbitrary random priming ITR, HSH103. The number on the top of each lane indicates the culture passage. M is the DNA size marker, 1kb ladder plus.



Figure 13. Transposition analysis on the carcinogen treatments after 5 culture passages. A, Amplification patterns on CCD-25Lu cells using HSH103; B, those on HL-60 cells using HSH103; C, those on SK-N-SH cells with *Hsmar1*. M is DNA size marker, 1kb ladder plus.



Figure 14. Transposition analysis on CCD-25Lu cDNAs treated with carcinogens after 5 culture passages using transposon specific ITR primer *Pogo1*. 100 ng of cDNA was used as a template for amplifying. M is DNA size marker, 100 bp ladder plus.

#### 5) HERV-K10 LTRs transcribed from various locations on chromosomes

To investigate active HERV-K10 LTR, the RT-PCR products from the cell lines were sequenced. The characteristics and chromosomal locations of HERV-K10 LTR elements transcribed from human cells are shown in Table 5. The BLAST result confirmed that the HERV-K10 LTR sequences from various human chromosomes were identical. 'K10 LTR groups' have been defined as identical sequences, appearing at more than two loci on different chromosomes or accession numbers. For example, HERV-K10 LTR group 1 occurs in twenty-four identical sequences at fourteen different locations on nine chromosomes (Table 6 and Table 7). The sixty clones sequenced from normal cells were made up of sixteen elements. RNA intermediates of HERV-K10 LTR should be transcribed from as least sixteen positions in the haploid human genome. They had lengths of 297 or 298 bp, and the sequence similarity ranged from 97-100%. From cancer cells, a hundred and fifty clones were sequenced. Their lengths were more vary than those of elements from normal cells, *i. e.*, from 287 to 306 bp, and the sequence similarity ranged from 95-100%. Several HERV-K10 LTR elements were identical to those previously reported by Yi *et al.* (2001).

#### 6) Several locations of HERV-K10 LTR transcripts were specific for cancer cells

The putative chromosomal locations were investigated using a BLAST search of the Human Genome database in GenBank. Forty-four elements were thought to be derived from single location on one chromosome, while the others had multiple origins. The HERV-K10 LTR was found from the centromere to the telomere. Four HERV-K10 LTRs (2F7-1, MC-5, MP-4, and SC-1) might be transcribed from the telomere regions of the four chromosomes (chromosome 4, 8, 9, and 15). Cell-type specific HERV-K10 LTR elements were found, and they were transcribed from different loci of chromosomes. For example, nine clones of HERV-K10 LTRs transcribed from 1q21 were observed in six

Table 5. Chromosomal locations of the HERV-K10 LTR transcripts from the human cells

Cell line	Clone	Length	Similarity (%)	The most similar sequence
КҮЈ	YJ-1	297	99	K10 LTR group 4; AC023162.41(3q21), AL449213.4(3q21)
	YJ-2	297	99	AC114933.2(5p14)
	YJ-3	297	99 99	K114953.2(5)14) K10 LTR group 1; AB023523.1(ND*), AB059364.1(ND), AF276875(ND) AL139421.12(1p22) AC006039.3(7p15) AC100821.2(8q11) AL354855.26(9q34) AK075315.1(11q13), NM_173578.1(11q13), AP003385.2(11c AP000812.4(11q13), AP000776.5(11q22), AC023281.13(12p11), AC048344.44(12p11) AC025420.26(12 AC107977.13(15q25), AC129907.3(15q25), AC109513.14(15 AC140725.3(15q25) AC012175.7(16p13), AC002400.1(16p12), AC008870.8(16q1 AC009132.9(16q23) AL031654(20q12)
	YJ-4	298	99	Group 3
	YJ-5	297	99	Group 3
KBG	BG-1	297	100	AL049570.11(1p35)
1120	BG-2	297	98	BC036118.1(8q24)
	BG-3	297	97	AP005203.3(18p11)
	BG-4	297	7 98 L	Group 1
	BG-5	297	100	AL392107.17(10q24)
	BG-6	297	98	Group 2
CCD-25Lu	CC-1	297	99	Group 3
	CC-2	297	99	K10 LTR group 2; AL049570.11(1p35), AL512637.18(1q44) AC084198(3q12) AF235103.5(8q25), BC036118.1(8q25) AL135901.23(13q14) AC026803.7(19q13)
	CC-3	297	98	K10 LTR group 3; AB023542.1(ND), AB023539.1(ND) AC016839.12(18q11), X54171.1(18q11)
	CC-4	297	99	AL135901.23(13q14)
	CC-5	298	99	AC025674.10(8q11)
	CC-6	297	99	AL135901.23(13q14)
2F-7	2F7-1**	291	100	Z95704.1(4p16)
A-431	A3-1**	297	99	AC019155.4(7q32)
	A3-2**	297	98	AL356019.5(14q11)
	A3-3**	297	99	AC105049.4(8q24)
A-498	A4-1	297	99	K10 LTR group 6; AC079753.7(2q14), BN000002.1(2q14)
	A4-2	298	100	AC025674.10(8q11)
	A4-3	297	98	Group 6
A-549	A5-1	297	100	AL392107.17(10q24)
	A5-2	297	99	AL592284.10(1q21)
	A5-3	294	98	AC020924.8(5q12)
	A5-4	297	99	AC105049.4(8q24)

Table 5 (Continued)

Cell line	Clone	Length	Similarity (%)	The most similar sequence
HCT-15	HC-1	297	97	AC008784.7(5q11)
	HC-2	297	99	K10 LTR group 7; AC104150.6(3q26), AC104640.3(3q26) AC080023.6(11p15), AP003716.4(11q12)
	HC-3	294	95	K10 LTR group 8; AF017229.1(ND), Y17833.1(ND) AC112702.3(19q12), AC010508.8(19q12)
	HC-4	294	96	Group 8
	HC-5	297		AL356019.5(14q11)
Hep-G2	HG-1	297	99	AL392107.17(10q24),
	HG-2	298	99	K10 LTR group 9; BC041646.1(ND), BC011367.1(ND), AF164610.1(ND), K090528.1(ND) AL353807.18(1q22) AC021133.5(6p12), AL121969.12(6p12)
	HG-3	297	99	K10 LTR group 10; BC001407.1(ND)* AL713888.10(10q21)
	HG-4**	297	99	K10 LTR group 11; AF394944.1(Xq26), AL034407.1(Xq26)
	HG-5**	287	100	AC004979(4q31)
HL-60	HL-1	294	100	AL671879.2(6p21)
112 00	HL-2	306	99	AC011447.7(19p13)
	HL-3	299	7 95 E	AC053544.5(4q13)
	HL-4	298	99	AL 592284 10(1 <sub>0</sub> 21)
	HL-5	298	100	AC007283.3(2q33)
Jurkat	JU-1	293	99	AL590543.8(6q25)
Juikut	JU-2	293	98	AC109327.10(17q25)
	JU-3	290	97	K10 LTR group 12; BX546487.5(ND) AC128674.3(1q21) AC127384.4(1q21), AL954711.3(1q21)
	JU-4	298	97	AL049742.8(1q21)
	JU-5	297	98	K10 LTR group 13; AC109322.16(8q24), AC105049.4(8q24) AL445931.29(9q34), AC002103(9q34), AC000387.1(9q34) AC000401.1(9q34), AL591386.18(9q34)
	JU-6**	298	99	AL592284.10(1q21)
	JU-7**	297	99	AL391244.11(1p36)
MCF-7	MC-1**	297	100	AL139404.9(10q11)
	MC-2**	297	100	AC087533.7(8p11)
	MC-3**	297	98	Group 7
	MC-4**	297	98	AC000387(9q34)
	MC-5**	297	100	K10 LTR group 14; AC000387(9q34), AL591386.18(9q34)
MIA-PaCa-2	MP-1**	287	100	AC004979(4q31)
	MP-2**	298	100	AC007283.3(2q33)
	MP-3	298	97	AL592284.10(1q21)
	MP-4	297	99	AC140725.3(15q26)
	MP-5	298	98	AL592284.10(1q21)
	MP-6	288	98	AC011322.29(3q29)
	MP-7	297	99	AL391244.11(1p36)

Table 5 (Continued)

Cell line	Clone	Length	Similarity (%)	The most similar sequence
SNU-C5	SC-1	297	98	Group 2
	SC-2	296	99	AC003976(17q21)
SNU-16	SU-1	297	99	AC019155.4(7q32)
	SU-2	295	100	K10 LTR group 5 AP000346.1(22q11), AP000345.1(22q11)
	SU-3	296	98	Group 6
	SU-4	294	95	AC104301.2(17q21)
	SU-5	298	98	AL592284.10(1q21)
U-937	U9-1	297	99	AL513550.9(6q16)
	U9-2	298	98	AL049742.8(1q21)
	U9-3**	297	100	Group 규 주 아 두 서 과
	U9-4**	297	99	Group 1
	U9-5**	297	99	AC072054.10(7p22)
	U9-6	298	99	AL391244.11(1p36)

\* not determined in Human Genome Resources in GenBank.

\*\* is previously reported by Yi et al. (2001).

Group	No. of identical sequence	No. of location*	No. of chromosome**
K10-1	24	14	10
K10-2	7	6	5
K10-3	4	1	1
K10-4	2	1	1
K10-5	2	1	1
K10-6	2	1	1
K10-7	4	3	2
K10-8	4	1	1
K10-9	7 제주대학:	교 중앙도서관	2
K10-10		UNIVERSITY LIBRARY	1
K10-11	2	1	1
K10-12	4	1	1
K10-13	6	2	2
K10-14	2	1	1

Table 6. HERV-K10 LTR groups found in the similarity search

\* is determined by BLAST search in GenBank database.

\*\* is determined by LOCUS search in Human Genome Resources in GenBank.

	No. of					No.	of	K10	LTF	R gro	oup*					No. of clones
Cell line	clones tested	1	2	3	4	5	6	7	8	9	10	11	12	13	14	found in K10 LTR groups
YJ	20	7	4	1	-	-	2	1	-	-	-	-	-	1	-	16
BG	20	8	3	1	1	-	1	1	-	-	-	-	-	1	-	16
CCD-25Lu	20	6	2	1	-	-	-	2	-	2	-	-	-	1	-	14
A-498	15	6	2	-	-	-	1	-	1	1	-	1	-	-	-	12
A-549	15	3	4	-	1	-	-	1	-	1	-	-	-	1	-	11
HCT-15	15	4	2	1	1	-	-	1	-	-	1	-	-	-	-	10
Hep-G2	15	3	3	1	-	-	-	-	-	2	-	-	1	1	1	12
HL-60	15	4	5	T	조	CH.	하	-	~ (	)-ç	: 1-1	과	1	-	-	10
Jurkat	15	3	3	-JE	JU <del>,</del> N	ATIC	NAL	UNI	VER.	2	LIBR	LARY	-	2	-	10
MIA-PaCa-2	15	3	-	2	-	-	-	2	1	-	-	-	1	1	-	10
SNU-C5	15	6	2	1	-	-	1	-	-	1	-	-	1	1	-	13
SNU-16	15	2	4	1	1	1	-	1	-	1	-	-	-	-	-	11
U-937	15	3	3	1	-	-	-	2	-	1	-	-	-	1	1	12
Total	210	58	37	10	4	1	5	11	2	11	1	1	4	10	2	157

Table 7. HERV-K10 LTR groups and identical sequence clones from the cell lines tested

\* group numbers are given in Table 6.

cancer cells (A-549, HL-60, Jurkat, MIA-PaCa-2, SNU-16, and U-937) and three clones from 1p36 in three cancer cells (Jurkat, MIA PaCa-2, and U-937), but not in normal cells (Table 8). In addition, HERV-K10 LTR transcripts from the eight chromosomal locations (2q14, 2q33, 4q31, 7q32, 8p11, 10q11, 14q11, and 17q21) were shared two kinds of cancers. For example, HERV-K10 LTR clones transcribed from 2q14 were observed in skin carcinoma A-431 and stomach adenocarcinoma SNU-16. The six locations (1q21, 2q14, 9q34, 13q14, 18q11, and 19q13) showed more than two clones from each single cell line. In these, three clones (JU-3, JU-4, and JU-6) transcribed from the same location 1q21 in jurkat. However, some transcripts from the six locations (1p35, 3q31, 5p14, 13q14, 18p11, and 18q11) were specific for normal cells. Those from 8q11 and 10q24 were observed in normal cells as well as in cancer cells. Except for HERV-K10 LTR groups, cell line specific transcripts have not found in chromosomes 11, 12, 16, 20, 21, and Y (Table 8).

#### 7) HERV- K10 LTR elements divided into several clusters

Sixty-two different HERV-K10 LTRs obtained from human cells were subjected to phylogenetic analysis, along with twenty-eight elements, previously reported from the human genome, nine from chimpanzees (Pan troglodytes: AB023912, AB023913, AB023915, AB023916, AB023917, AB023918, AF311937, and M57949), two from macaques (Macaca mulatta: AB024015 and AB024016), two from gorillas (Gorilla gorilla: AB023923 and AB023921), and one from common squirrel monkey (Saimiri sciureus: AB023924). The neighbor-joining (NJ) tree constructed using DNA distances had three main clusters; a branch containing the common ancestral elements of primates. elements shared by human and macaques, and human-specific type I elements (Fi. 15). The human-macaques cluster was further subdivided into human-specific type II elements and other elements. In addition,

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Table 8. Cell line-specific transcripts of the HERV-K10 LTR and itschromosomal locations

Chromosomal	location Symbol of clone*	No. of clone
1p36	JU-7, MP-7, U9-6	3
1p35	BG-1	1
1q21	A5-2, HL-4, JU-3, JU-4, JU-6, MP-3, MP-5, SU-5, U9-2	9
2q14	A4-1, A4-3, SU-3	3
2q33	HL-5, MP-2	2
3q21	YJ-1	
3q29	MP-6	1
4q13	HL-3	1
4q31	HG-5, MP-1	2
5p14	YJ-2	1
5q11	HC-1	1
5q12	A5-3	1
6p21	HL-1	1
6q16	U9-1	1
6q25		1
7p22	U9-5레주대학교 중앙도서관	1
7q32	A3-1, SU-1TONAL UNIVERSITY LIBRARY	2
8p11	A3-2, MC-2	2
8q11	CC-5, A4-2	2
8q24	A3-3, A5-4, BG-2	3
9q34	MC-4, MC-5	2
10q11	HG-3, MC-1	2
10q21	HG-1	1
10q24	BG-4, A5-1	2
13q14	CC-4, CC-6	2
14q11	A3-2, HC-5	2
15q26	MP-4	1
17q21	SC-2, SU-4	2
17q25	JU-2	1
18p11	BG-3	1
18q11	YJ-4, YJ-5, CC-1, CC-3	4
19q12	НС-3, НС-4	2
19q13	HL-2	1
22q11	SU-2	1
Xq26	HG-4	1

\* is given in Table 5.



Figure 15. Neighbor-joining tree based on the sequences of the HERV-K10 LTR transcripts in the human cells. Abbreviations of each HERV-K10 LTR clone were represented in Table 4. Gorilla, *Gorilla gorilla*; MacacaM, *Macaca mulatta*; Pant, *Pan troglodytes*; SaimiriS, *Saimiri sciureus*.

the cluster of common ancestral elements was subdivided into two subclusters: human-chimpanzee-gorilla and human-chimpanzee-common squirrel monkey.

#### 2. Human long interspersed nuclear element-1

#### 1) L1Hs p40 elements were transcribed in various human cells

In RT-PCR analysis, L1Hs *p40* transcripts were found in all cell lines tested, however, the transcripts could not be clearly distinguished among the cell lines tested (Fig. 16). Cellular mRNA for two normal fibroblasts (CCD-25Lu and HEL-299) also contained L1Hs *p40* transcripts, but the relative levels of transcription had not significantly distinguished. The transcription levels of A-549, MCF-7, HCT-15 and SNU-16 were higher than those of leukemic cells and fibroblasts (Fig. 17).

#### 2) Carcinogens changed the level of L1Hs p40 transcription in CCD-25Lu

RT-PCR analysis of mRNAs extracted from cultures treated with carcinogens were shown higher transcription levels, compared to control treated with saline (Fig. 18). Figure 19 shows the relative levels of L1Hs p40 transcription in each carcinogen treatment. Compared to *p*-actin, DMBA treatment caused the highest transcription rate, and EtBr treatment the lowest. Different expression patterns have been observed in the EtBr and MNNG treatments, and resulted in cell death.

#### 3) L1Hs p40 transcribed from various locations on the chromosomes

Using RT-PCR products from four normal cells, fourteen new L1Hs elements were



Figure 16. RT-PCR analysis of the L1Hs p40 transcription in the human cells. 100 ng of cDNA was used as a RT-PCR template for amplifying L1Hs p40. M is the DNA size marker, GenRuler DNA ladder.





Figure 17. Relative levels of the L1Hs p40 transcription in the human cells. 100 ng of cDNA was used as a RT-PCR template for amplifying L1Hs p40. RT-PCR bands were standardized by comparing the ratio of the L1Hs p40 to that of the human *β-actin* with Quantity One software (Bio-Rad) as described in Fig. 5.



Figure 18. RT-PCR analysis of the L1Hs p40 transcription in CCD-25Lu cells treated with carcinogens. 100 ng of cDNA was used as a PCR template for amplifying each gene. M is the DNA size marker, 100 bp ladder plus.



Figure 19. Relative levels of the L1Hs p40 transcription in CCD-25Lu cells treated with carcinogens. 100 ng of cDNA was used as a PCR template for amplifying L1Hs p40. Standardization for the transcription level of each gene was performed as described in Fig. 17.

identified. Length variation was found in several sequences, which ranged from 376 to 382 bp (Table 9). The BLAST analysis showed that many of the determined L1Hs element sequences were identical to previously reported sequences (Table 10 and Table11). Furthermore, several copies of some identical sequences were found on several chromosomes. These were defined as 'L1Hs group', and thirteen distinct L1Hs groups were found (Table 10). For instance, in the human genome, L1Hs group 1 contains sixty-five identical sequences at fifty-one locations on twenty chromosomes excluding chromosomes 17, 18, and 21. Of the L1Hs from normal cells, ten elements are transcribed from a single locus on one chromosome. The chromosome locations were distributed over all chromosomes, excluding chromosome 21, and ranged from the centromere to the telomere. The length of the L1Hs p40 gene, determined from various cancer cells, varied from 363 to 439 bp. Many L1Hs elements had the typical length of 379-bp. Clones HL-1 and JU-4 contained 5' additional sequences, and MP-2 had a 40-bp tandem duplications in the 5' region (Appendix 2). Forty-three sequences from cancer cells were transcribed from a single genome location.

To confirm the chromosomal locations and distributions of L1Hs elements, FISH analysis was performed with normal leukocyte cells. FISH analysis of L1Hs in the metaphase showed that it was a multi-copy retroelement, distributed on all chromosomes. FISH analysis using an L1Hs probe showed that there were over five thousand L1Hs element loci in a single metaphase preparation (Fig. 20)

### 4) Cell line specific L1Hs elements were transcribed from different chromosomal locations

In several cell lines, the specific transcripts of L1Hs *p40* elements were observed. For instance, several L1Hs elements (transcribed from 1p13, 1q42, 3p24, 4p16, 5p13, and Xq13) were transcribed in normal cells but not in cancer cells tested. In these, three

Table 9. Chromosomal locations of the L1Hs p40 transcripts from the human cells

Cell line	Clone	Length SI	milarity (%)	The most similar sequence
CCD-25Lu	CC-1	378	98	AL139400.9(Xq13)
	CC-2	379	98	L1Hs group1; U93564.1(ND*), U93567.1(ND), U93573.1(ND), U93574.1(ND) AL354949.10(1p31), AL683886.8(1p13), AL357045.10(1p11), L021069(1q24), AL162431.17(1q25), AL592494.4(1q31), AC096569.1(2p13), AC015971.4(2p11), AC105762.4(2q14), AC104088.6(2q31), AC104820.4(2q31), AC012486.9(2q33), AF312225(2q34), AC138658.1(2q35) AC099664.2(3p12), AC046144.15(3q13), AC109583.2(3q21), AC011597.27(3q22) AC114806.5(4p15), AC093607.3(4p15), AC013724.8(4q12), AC106884.5(4q13), AC092663.2(4q13), AC004704(4q25), AC096898.6(4q28), AC125376.3(4q32), AC020599(4q32), AC010450.6(5p15), AC091872.3(5p15), AC008948.8(5q13) AL031768.9(6p25), AL512428.13(6p22), AL591051.7(6q22), AL589693.3(6q25), AC004694.1(7p15), AC079855.8(7q11), NG_000004(7q21), AC006992.2(7q35) AC004694.1(17p15), AC025263(12q14), AC095347.6(12q21), AC093925.5(12q21) AL138094.3(12q12), AL025263(12q14), AC095347.6(12q21), AC073940.4(15q15) AC07347(15q15) AC007347(15q12) AL161938.6(20p12), AL137191.5(14q23) AC07347(15q12) AL161938.6(20p12), AL096677.21(20p12) AF421375(22q11), AL078622.7(22q23) AL663118.2(Xp11), AL356317.8(Xq11), Z95325.2(Xq21), AL02308(Xq21), AL590306.7(Xq22)
	CC-3	379	98	L1Hs group 5; AC019201.6(2q24) AC140125.3(5q35) AL354951.7(10q25)
	CC-4	379	99	L1Hs group2; AL133320(1p31), AC104454(1p22) AC092505.2(3p24), AC107028(3p11) AC098597(4p15), AC108516(4q22), AC093729(4q22), AC018680(4q31) AC109494(5p12), AC093255(5q21), AC008467(5q22), AC011413(5q31) AC004200(6p21), AL356601(6q15), AL356438(6q21), AP006196(6q21 AL583853(6q22) AC093775(7p11), AC092595(7q22) AC084257(8q21), AC020783(8q21), AC067844(8q23) AP004242(11q19), AP001876(11q21) AC005885(12q24) AC04907(15q25) AC009063(16q23), AC106814(16q23) AC005939(17q35) AP001028(18p11), AC002980(Xp22), AF148856(Xp22), AC004554(Xp22), AC004000(Xq24)
HEL-299	HE-1	378	98	L1Hs group 11; AC104456(1p22), AL451010(1p21)
	HE-2	379	97	AL596133(1q42)
	HE-3	376	94	AL161450(9p24)
	HE-4	382	100	AC092795.2(3p24)

Table 9 (Continued)

Cell line	Clone	Length	Similarity (%)	The most similar sequence
KYJ	YJ-1	379	100	AL160171.27(1p13)
	YJ-2	378	97	AC096740.3(4p16)
KBG	BG-1	379	98	L1Hs group 4; AC004514.1(8q21) AC040174.5(16q21), AC119049.1(16q21), AC040159.4(16q21)
	BG-2	378	96	L1Hs group 10; AC009319.19(3q27) AC090179.4(15q24), AC090751.9(15q24)
	BG-3	379	94	AL096710.8(6p11)
	BG-4	378	99	AC114967.2(5p13)
	BG-5	377	96	AL160171.27(1p13)
	BG-6	378	97	AL160171.27(1p13)
A-498	A4-1	377	98	AL121772.19(20p11)
	A4-2	379	96	L1Hs group 8; AC068206.10(15q25), AC020687.6(15q25)
	A4-3	380	98	AL096710.8(6p11)
	A4-4	379	100	AL157820.27(13q34)
	A4-5	379	97	AL096710.8(6p11)
A-549	A5-1	380	99	L1Hs group 12; AC079075.6(15q26), AC013787.15(15q26)
	A5-2	379	97	AC007000.2(7q11)
CEM-CM3	CE-1	383	95	AL139320.18(13q14)
	CE-2	378	93	AC079228.7(4q32)
HCT-15	HC-1	378	98	AC034229.4(5p15)
	HC-2	379	99	L1Hs group 3; AC094098.4(5q32), AC016572.6(5q35), AC022413.4(5q35) AL590095.5(10p15) AL117375.12(20p11), AL590285(Xp21), AL161778.19(Xq26)
	HC-3	379	99	L1Hs group 9; AC073326.6(7q31) AL078623.28(20p12)
	HC-4	375	98	L1Hs group 13; AC007992.12(8q22) AC015998.8(8q22), AP003465.2(8q22)
	HC-5	380	98	AL031176.8(Xq23)
HL-60	HL-1	399	98	AC125236.4(4p11)
Jurkat	JU-1	376	97	AC078816.16(3q24)
	JU-2	379	97	AL590131.8(Xq26)
	JU-3	378	100	AL049831.2(14q11)
	JU-4	385	99	AC004140.2(7q21)
	JU-5	378	99	AC093388.4(2q32)
MCF-7	MC-1	376	99	Z72001.3(Xq26)
	MC-2	380	98	AC131151.2(3q11)
	MC-3	379	99	AC131151.2(3q11)
	MC-4	379	99	AC104648.2(2q14)
MIA-PaCa-2	MP-1	380	99	AC106750.3(5q22)
	MP-2	439	96	AC093733.2(4q22)
	MP-3	379	99	AC025477.6(5q12)
	MP-4	379	99	AL356284.11(9p24)

Cell line	Clone	Length	Similarity (%)	The most similar sequence
Molt-4	MO-1	379	97	AC087377.7(11p11)
	MO-2	379	98	AC144523.2(ND)
	MO-3	379	98	AC113385.2(5q21)
	MO-4	379	99	AL159154.16(13q22)
	MO-5	377	98	AL096710.8(6p11)
SK-N-SH	SN-1	379	99	AC022537.8(10q11)
	SN-2	379	99	AC022537.8(10q11)
	SN-3	379	98	AC022537.8(10q11)
	SN-4	379	99	AC004694.1(7p15)
	SN-5	379	99	AC004694.1(7p15)
SK-OV-3	SO-1	379	98	AC073196.3(4q12)
	SO-2	379	99	AC011155.13(18q21)
	SO-3	377	97	AC093783.2(4q28)
SNU-16	SU-1	379	<sup>98</sup> 제주디	L1Hs group 6; AC084264.7(2p22), AC011242.8(2p22) AL356794.10(6q24) AL40052.1((12-22))
	SU-2	379	96	AL160253.16(13q32) AL139136.17(1q31)
	SU-3	385	91	AC079033.12(12g13)
	SU-4	379	97	L1Hs group 7; AC104623.4(2p24) AC091936.2(5q21) AL121852.3(14q12)
	SU-5	384	100	AC097515.5(4p16)
U-937	U9-1	379	100	AC010365.5(19q12)
	U9-2	379	100	AC090060.15(12q21)
	U9-3	379	98	M80340(ND)

### Table 9 (Continued)

\* not determined the chromosomal location in Human Genome Resources in GenBank.

Group	No. of identical sequence	No. of location*	No. of chromosome**
L1Hs-1	65	51	20
L1Hs-2	34	38	14
L1Hs-3	7	6	4
L1Hs-4	4	2	2
L1Hs-5	3	3	3
L1Hs-6	4	3	3
L1Hs-7	3	3	3
L1Hs-8	- 1.SL -	교 중앙도서관	1
L1Hs-9	2	2	2
L1Hs-10	3	2	2
L1Hs-11	2	2	1
L1Hs-12	2	1	1
L1Hs-13	3	1	1

Table 10. L1Hs groups found in the similarity search

\* is determined by BLAST search in GenBank database.

\*\* is determined by LOCUS search in Human Genome Resources in GenBank.

	No. of					Nc	o. of	L1Hs	s grou	ıp*					Total No. of
Cell line	clones tested	1	2	3	4	5	6	7	8	9	10	11	12	13	clones found L1Hs group
KYJ	20	10	4	1		1		1				1			18
KBG	20	9	4				1								14
CCD-25Lu	20	9	3			1	1				1			1	16
HEL-299	20	8	6			2									16
A498	15	5	2		1	1					1				10
A549	15	5	3	2			1						1	1	13
CEM-CM3	15	4	1	2			2	1	1		1			1	13
HCT-15	15	3	1	1	1	1				1			1	1	10
HL-60	15	8	2	1		1		1					1		14
Jurkat	15	3	2	제	주	내흐	교	중	강도	서	관	1			10
MCF-7	15	1	3	2	J NA	2	AL UN	IVER	SITY		ARY				11
MIA-PaCa-2	15	4	1	1	1	1			1			1	1		11
Molt-4	15	5	1			1		1		1		1			10
SK-N-SH	15	4	3	1		2									10
SK-OV-3	15	5	4		1		1				1				12
SNU-16	15	3	3	1	2		1								10
U-937	15	6	1	1	1		1	1	1						12
Total	275	92	44	13	8	14	9	5	5	3	5	4	4	4	210

Table 11. L1Hs groups and the number of identical sequence clones from the cell lines tested

\* group numbers are given in Table 10.



Figure 20. Chromosomal distribution of the L1Hs elements detected by cytogenetic analysis. A, the metaphase chromosomes stained with Giemsa; B, acquired FISH image panel from various metaphases; C, marked chromosomes using mFISH program (Imstar), open circles indicate the centromere of each chromosome, filled-green circles do midsts, and symbol 'D' does un-defined chromosome.

transcripts (transcribed from 1q42, 3p24, and Xq13) were specific for leukocytes. In addition, some elements were specific for each cells, for example, L1Hs elements from 10q11 and 7p15 was specific for neuroblastoma SK-N-SH cells, and those from 3q11 did breast cancer MCF-7 (Table 12). Transcripts from 6p11 were shared a normal leukocyte KBG and two cancer cell lines (A-498 and Molt-4). In addition, four L1Hs clones were located on the chromosome X; a clone from normal fibroblasts and three from cancer cells.

# 5) L1Hs p40 protein sequences may be involved in protein interactions in various cellular metabolism

Putative protein sequences of L1Hs p40 have converted from the DNA sequences. Fourteen from RNA transcripts and twenty-four from GenBank Accessions had active protein sequences. In addition, twelve L1Hs groups possessed the active protein sequences. The motif analysis has shown that the L1Hs p40 protein has two N-glycosylation sites, a cAMP- and cGMP-dependent protein kinase phosphorylation site, seven protein kinase C (PKC) phosphorylation sites, ten casein kinase II phosphorylation sites, an amidation site, a microbodies C-terminal targeting signal, a luecine zipper, and an N-myristoylation site (GKKQNRKTG). In addition, a helix-turn-helix DNA-binding motif (ALKELLKEALNMERNNQYQLLQ) have also found (Fig. 21). Multiple alignment of active L1Hs p40 protein sequences showed various missense mutations including in protein interaction positions (Appendix 3). In addition, nonsense mutations by nucleotide substitution have also been found in the transcripts from various cells (data not shown).

# 6) The sequences of transcripts and proteins of active L1Hs p40 divided into several clusters

Table 12. Cell line-specific transcripts of the L1Hs *p40* gene and its chromosomal locations

Chromosomal location	Symbol of clone*	No. of clone
1p13	YJ-1, BG-5, BG-6	3
1q31	SU-2	1
1q42	HE-2	1
2q14	MC-4	1
2q32	JU-5	1
3p24	HE-4	1
3q11	MC-2, MC-3	2
3q24	JU-1	1
4p16	YJ-2, SU-5	2
4p11	HL-1	1
4q12	SO-1	1
4q22	MP-2	1
4q28	SO-3	1
4q32	CE-2	1
5p15	HC-1	1
5p13	BG-4	1
5q12	MP-3	1
5q21	MO-3	1
5q22	제주대학교 중앙도서관	1
6p11	BG-3, A4-3, A4-5, MO-5 LIBRARY	4
7p15	SN-4, SN-5	2
7q11	A5-2	1
7q21	JU-4	1
8q22	HC-4	1
9p24	HE-3, MP-4	2
10q11	SN-1, SN-2, SN-3	3
11p11	MO-1	1
12q13	SU-3	1
12q21	U9-2	1
13q14	CE-1	1
13q22	MO-4	1
13q34	A4-4	1
14q11	JU-3	1
15q25	A4-2	1
15q26	A5-1	1
18q21	SO-2	1
19q12	U9-1	1
20p11	A4-1	1
Xq13	CC-1	1
Xq23	HC-5	1
Xq26	JU-2, MC-1	2

\* Symbol of clone is given in Table 9.


Figure 21. Protein motifs of the L1Hs p40 protein. AMI, amidation site; CKII, casein kinase II phosphorylation site; cgPK, cAMP-and cGMP dependent protein kinase phosphorylation site; GLY, N-glycosylation site; HTH, helix-turn-helix motif; LZM, leucine zipper; MTS, microbodies C-terminal targeting signal; MYR, N-myristoylation site, PKC, protein kinase C.

Sixty-two L1Hs DNA sequences identified from human cells were subjected to phylogenetic analysis with those previously reported as active forms of L1Hs (Sassaman *et al.*, 1997) and each representative member of L1Hs groups (Appendix 2). The L1Hs elements formed three clusters on a consensus tree, constructed after 1,000 bootstrap replications of the gene sequences. Interestingly, six L1Hs groups were located in the cluster L1C, only L1Hs-11 was in the cluster L1B, and the other six groups were in the L1A (Fig. 22).

Phylogenetic tree based on L1Hs p40 protein sequences showed that three main branches (p40A, p40B, and p40C) appear and resemble those of L1Hs *p40* genes. However, p40A and p40C were human-specific, and p40B contained those of mice, gorilla, chimpanzees (Fig. 23). Active forms of L1Hs p40 have found at least a hundred and sixteen elements in the haploid human genome.





Figure 22. The strict consensus tree derived from the most parsimonious trees based on the L1Hs p40 gene sequences obtained from various human cells and those reported previously in GenBank. The information of each clone is given in Table 9, and that of each group does in Table 11.



Figure 23. The strict consensus tree of active L1Hs p40 proteins from human cells and those converted the DNA sequences previously reported in GenBank. The information of each clone is given in Table 9, and those of groups do in Table 11. Gorg, *Gorilla gorilla*; Mouse, *Mus musculus*; Panp, *Pan paniscus*; Pant, *Pan troglodytes*.

## **IV. DISCUSSION**

#### 1. Transcription and Chromosomal Locations of the HERV-K

HERV-K genes were transcribed in both human cancer cells and normal cells, and the levels of transcription increased in fibroblasts treated with carcinogens. By RT-PCR analysis, RNA transcripts of HERV-K genes were observed as polyadenylated cellular transcripts, and the relative transcription levels varied in each cell line. HERV expression was found in the human cells including various cancers, placenta, and leukocytes of healthy individuals (Medstrand et al., 1992; Brodsky et al., 1993; Simon et al., 1994; Gotzinger et al., 1996; Yi et al., 2001). Cell type-specificity was also observed for K10 LTR, T47D LTR, K10 env, and C4 env. Cell type-specificity and tissue specific expression were reported previously (Majors, 1990; Ting et al., 1992; Medstrand and Blomberg, 1993; Kjellman et al., 1999; Sugimoto et al., 2001; Andersson et al., 2002). The cell-type dependent expression of retroviruses is due to specific regulatory sequences located in the U3 region, which contains a number of transcription-factor responsive sequences (Majors, 1990). For example, T47D LTR can induce expression of a reporter gene displaying distinct activity in different cell lines (Schön et al. 2001). The promoter analysis on HERV-K LTRs using SIGNALSCAN supported the results previously reported. Transcription factor Oct-1 and C/EBP play important roles in the cell-type specific expression. However, neither the Oct-1 binding site nor the CAAT-box was required for such expression of K10 LTR. Instead, the 3' terminus of U3 includes a TATA box sufficient for specificity (Baust et al., 2000; Schön et al., 2001; Vinogradova et al., 2001). The basic mechanery at the TATA box of K10 LTR may be determined the

cell-type specific expression (Lee *et al.*, 2003). In addition, various mutations in K10 LTR sequences have also been observed in signal response elements (Appendix 1). Mutations in TATA box consequently can inhibit the transcription of genes. The other mutations in signal responsive elements can change the affinity of binding to transcription factors such as AP-1, c-MYC, GATA-1, Sp1, TGT3, and others. Since the 5'-LTR of HERV-K plays key roles in HERV-K transcription, this result indicates the sequence variations can also be responsible for the cell-type dependent patterns of HERV-K gene expressions and potential to retrotransposition.

HERV-K LTRs were expressed much more strongly than env genes, suggesting that there are many solitary LTRs distributed throughout the genome. In particular, the transcription patterns of env gene and LTRs were distinct in normal fibroblasts. Depil et al. (2002) suggested that HERV-K relative overexpression in leukemia cells might be specifically associated with tumor development, since there was no significant variation of HERV-K gene expression in normal peripheral blood mononuclear cells after exposure to different factors (PHA, gamma irradiation, 5-azacytidine). Since the CCD-25Lu and HEL-299 cell lines were derived from lung, the expression of HERV-K genes in these lines may be correlated with the stage of cellular development or the degree of cell differentiation. Reports suggested that HERVs expression can be involved in differentiation and morphogenesis of cells (Larsson et al., 1996; Herbst et al., 1999; Lin et al., 1999; 2000; Mi et al., 2000; Feng et al., 2001). Herbst et al. (1999) described the differential expression of HERV-K and an association of HERV-K in pathogenesis of germ cell tumors. HERV-R mRNA increased in cellular differentiation of monocyte U-937 by inducer molecules including TPA, vitamin-D1 and retinoic acid (Larsson et al., 1996), as a consequence of increased immune activity rather than causative of distinct diseases (Johnston et al., 2001). In addition, retinoic acid increased the expression of HERV-K in embryonal carcinoma (Caricasole et al., 2000) and in human

breast cancer (Feng *et al.*, 2001). Sichangi *et al.* (2002) described the expression of HERV in male reproductive tissues speculating possible roles in spermatogenesis, sperm maturation, or tumor formation.

The expression of C4 env in these two cell lines was different, probably due to differences in the expression of *Complement 4* (C4). HERV-K(C4) is located in intron 9 of the C4 gene in the reverse orientation to the C4 coding sequence, and appears as a 6.4-kb insertion in 60% of human C4 gene. The C4 expression leads to production of an anti-sense RNA, which might protect against exogenous retroviral infections (Chu et al., 1995; Schneider et al., 2001).

DNA sequencing and subsequent BLAST search showed that K10 LTR was transcribed from all chromosomes, excluding chromosomes 21 and Y. This supports previous reports of the localization and distribution of entire HERV-K proviruses and its derived solitary LTRs in the human genome (Leib-Mösch *et al.*, 1993; Seifarth *et al.*, 1998; Zsiros *et al.*, 1998; Kim *et al.*, 2001; Sugimoto *et al.*, 2001; Mayer and Meese, 2002). The expression and distribution of multiple HERV elements in various cancers (Feng et al., 2001; 2003; Yi et al., 2001). However, the K10 LTR elements on chromosome 21 and Y were inactive in all cell lines tested. Many K10 LTR elements had functionally active forms and were distributed on several chromosomes or also at fragile sites (Table 13), suggesting associations with chromosomal instability, cancers (Table 14), and genetic disorders (Table 15).

In this study, at least forty-nine HERV-K LTR elements from different loci on the chromosomes transcribed in the cells (Table 6). Except for two loci (8q11 and 10q24), the locations of transcriptionally active K10 LTR elements of normal cells were distinct from cancer cells. The cancer cell-specific and the normal cell-specific loci were observed. In addition, seven of cancer cell-specific loci shared different cancer cells, for example, eight K10 LTR elements transcribed from 1q21 in A-549, HL-60, Jurkat,

Locus	Retroelement detected		Fragile site	
Locus	K10 LTR	L1Hs	Marker name	Class**
1p21		+*	FRA1E	А
1p22	+	++	FRA1D	А
1p36	+++		FRA1A	А
1q21	+++++++++++++++++++++++++++++++++++++++		FRA1F	А
1q25		+	FRA1G	А
1q42		+	FRA1H	Ζ
1q44	+		FRA1I	А
2p11		+	FRA2L	А
2p13		+	FRA2E	А
2p22		++	FRA2K	F
2p24		+	FRA2C	А
2q31		++	FRA2G	А
2q33	+	+	FRA2I	А
3p24		++	FRA3A	А
3q27		+	FAR3C	А
4p15	// TH	즈바하	FRA4D	А
4p16		구네 극포	FRA4A	А
4q12	Har JEJ	U NATIONAL U	FRA4B	B1
4q31	++	+	FRA4C	А
5p13		+	FRA5A	B1
5p14	+		FRA5E	А
5q21		+++	FRA5F	А
5q31		+	FRA5D, B	А
5q35		+++	FRA5G	F
6p22		++	FRA6C	А
6p25		+	FRA6B	А
6q15		+	FRA6G	А
6q21		+++	FRA6F	А
7p11		+	FRA7A	F
7q21		++	FRA7E	А
7q22		+	FRA7F	А
7q31		+	FRA7G	А
7q32	++		FRA7H	А
8q22		+++	FRA8A, B	F, A
8q24	++++		FRA8C, D, E	А
10q11	+	+++	FRA10G	А
10q21	+		FRA10C	B1
10q25		+	FRA10B	B1

Table 13. Chromosomal location of detected retroelement transcripts and fragile sites

Loong	Detection of	retroelement	Fragile site	
Locus	K10 LTR	L1Hs	Marker name	Class
11p15	+		FRA11C, I	А
11q13	++++	+	FRA11A, H	F, A
12q13	+	+	FRA12A	F
12q21		++	FRA12B	А
12q24		+	FRA12C	B2
13q32		+	FRA13D	А
14q23		++	FRA14B	А
16p12	++		FRA16E	D
16p13	+)) = -1		FRA16A	F
16q23		I수네약교 중앙	FAR16D	А
18q21	and JE	+	FRA18B	А
19p13	+		FRA19B	F
20p11		++	FRA20A	F
20p12		+++	FRA20B	А
Xp22		+++	FRAXB	А
Xq22		+	FRAXC	А

Table 13 (Continued)

\* clone detected in LOCUSLINK search.

\*\* is defined by Dhillon *et al.* (2003). Abbreviations: A1, common/aphidicolin; B1, common/BrdU; B2, rare/BrdU; D, common/distamycin-A; F, rare/folic acid; Z, common/ 5-azacytidine.

MIA-PaCa-2, SNU-16, and U-937 cells. 1q21 has a fragile site FRA1F (Table 13); and a chromosomal breakpoint in several cancers including chronic lymphocytic leukemia (CLL), bladder cancer (BC), and rectum cancer (RC) (Table 14). This finding presumes the association between the activation of provirus HERV-K and cancer development, as a transcriptional hotspot. Further studies on the association between them should be performed.

When K10 LTR elements were compared to the sequences of primates (chimpanzee, gorilla, macaques, and common squirrel monkey), three major clusters were made (Fig. 15). Human-specific subclusters and primates shared clusters have also found in the neighbor-joining tree. The time of divergence of the gorilla and human lineages, usually estimated to have occurred between 8 and 11 million years before present (YBP), and that of the chimpanzee and human lineages did about 6 million YBP (Ruvolo *et al.*, 1991; Ovchinnikov *et al.*, 2002). Therefore, this suggests that the proviruses had introduced into the common ancestors of primates at several times before and after speciation. HERV-K10 LTR elements could be classified into 14 classes (noted above as a 'K10 LTR group'), while Mold *et al.* (1997) reported four major classes based on HERV-K U3 region sequences.

### 2. Transcription and Chromosomal Locations of the L1Hs p40

RNA transcripts of the L1Hs elements have been found in human cells, including peripheral leukocytes, fibroblasts, and various human cancer cells, as cell type-independent (Minakami *et al.*, 1992; Becker *et al.*, 1993; Bratthauer and Fanning, 1993).

Locus	Detection of	retroelement	- Concor brook-	Droto opoggana
Locus	K10 LTR	L1Hs	<ul> <li>Cancer breakpoint</li> </ul>	Proto-oncogene
1p11		$+^{1}$	ME	
1p13		++++	ChC*, NE	NRAS
1p21		+	OC, RC, RCC	
1p22	+	++	BC, CLL	
1p31		++	MA, RC	RAB3B, VJUN
1p36	+++		BC, CLL, ML, RC	FGR, SRC, TNFR2
1q21	+++++++++++++++++++++++++++++++++++++++		BC, CLL, RC	
1q22	+			SKI
1q25		+	BC, ChC, PC, RC	ARG, ABL2
1q42		+	BC, NS, OC, PC	RAB4
2p11		+	BC, RC	
2p13		+	CLL	REL
2p22		++	CC, GL	
2p24		+	CLL, RC	MYCN
2q14	++ )	++		LCO
2q31		++ 제수!	내 같고 중앙노서관	
2q33	+	JEJU NA	CLL, RC, NS	
2q34		+	TLL	
3p11		+	RCC	
3p12		+	RCC	
3p24		++	CLL, LC, RCC	RAB5, THRB
3q21	++	+	OC, RCC	,
3q24		+	AML, ME	
3q26	++		RCC	
3q27		+	BC, BLL, NS, OC	
4p11		+	,,,	KIT
4p15		+++	BC, OC	
4p16	+	+	CE	RAF1P1
4q12		++	GIST, TT	
4q31	++	+	CLL	
5p13		+	BC, NS, OC	
5p13	+		BC, INS, CC	M1VI2
5q21		+++	APC, BC, CC, EC, RC	APC
5q21		+++	APC, RCC	0
5q22 5q31		+	BC, CLL	AERB2, CTNNB1, TCT
5q32		+	GC, RCC	
5q32		+++	AML, BC	CSF1R

Table 14. Chromosomal location of retroelement transcripts, human cancer breakpoints and proto-oncogenes

Lagua	Dectection of	f retroelement	- Cancer breakpoint	Droto on company
Locus	K10 LTR	L1Hs		Proto-oncogenes
6p11		++++		PKAS1
6p21	+	+	CLL	PIMI, TNAB, TNFA
6p22		++	BC	
6q15		+	BC, ME	
6q21		+++	BC, ChC, CLL, US**	FYN, MYB, ROS1, SYR
6q25	+	+	LC	
7p11		+	BC	
7p15	+	+++		MYCLK1
7p22	+		CC, PC, RC	
7q11		++	PC	
7q21		++	BC	
7q22		+	BC, CLL	
7q31		+	BC, HCC, OC, RCC	MET
7q32	++ /	/ ■ 제조	BC, CLL	EPHT
7q35				BRAF1
8q11	+++		BC, BR	MOS
8q22		+++	BC, OC, SCC	MYBL1
8q24	++++		BC, BL, OC, RCC	BV1, MYC
9p24		++		OVC
9q34	+++++++++		BC	ABL1
10q11	+	+++	MEN	RET
10q21	+			PTC1
10q24	++	+		HOX11
10q25		+	BC, PC	
11p11		+	PC	SPI1
11p15	+		BC, BR, LC	HRAS, MTACR1
11q12	+			
11q13	++++	+	BC, ChC, CLL, MEN OC, OS	, BCL1, FGF3, FGF4, HSTF1, INT2, SEA
11q14		+		ARHD
11q21		+	NE	
11q22	+		ACC, BR, LC	

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Locus	Dectection of	of retroelement	Cancer breakpoint	Proto-oncogenes
	K10 LTR	L1Hs	Cancer breakpoint	110to-oncogenes
12p11	++		RB	
12q12		+		INT1
12q13	+	+	CLL, BC, ChC, LP	ERBB3, GLI, SAS, WN
12q14		++		RAP1B
12q21		++	BC	
12q24		+	BC, CLL, HA	
13q14	+++	+	BC, BR, RB	BRCA2, RB1
13q22		+	MY	
13q32		+	BC, CLL	
13q34		++	SCC	RAP2A
14q23		at 제주	NS, SC 중앙도서관	
15q25	+++	JEJU NA	BC	FES
15q26	+	++		FPS
16q23	+	++	CLL, EC, MY, RC, US	MAF
17q21	++		ChC, OC, RCC, SC, TLL	BCL5, BRCA1, NE NEU
17q25	+		SC	ERBA2L
18p11		+	APC	
18q11	+++		HCC	
18q21		+	BC, CLL, HCC, NS, RC	BCL2, BCL3, SSAV YES
19p13	+		BC, LL	JUNB, JUND, LPSA, MEL, MELL1, RAB3A, VAV
20p12		+++	BC	
20q12	+			SRC
22q11		+	BC, BR	YESP

Table 14 (Continued)

T a aura	Dectection of retroelement		Concer brooks sint	Desta
Locus	K10 LTR	L1Hs	— Cancer breakpoint	Proto-oncogenes
Xp11		+	RCC, SS	ARAF1
Xp22		+++	US	ELK1
Xq11		+	PC	
Xq22		+	BC, CLL	MYCL2
Xq26	++	+++		HRASP

Table 14 (Continued)

\* childhood cancer not detailed.

\*\* unspecified cancer.

Abbreviations: ACC, anal canal carcinoma; APC, adenomatous polyposis of the colon; AML, acute myeloid leukemia; BC, bladder cancer; BL, Burkitt's lymphoma; BLL, B lymphocytic leukemia; BR, Breast cancer; CC, colon cancer; CE, Cervical carcinoma; CLL, chronic lymphocytic leukemia; EC, esophageal cancer; GC, gastric cancer; GIST, gastrointestinal stromal tumor; GL glioma; HA, hepatic adenoma; HCC, hepatocellular carcinoma; LC, lung cancer; LL, lymphoid leukemia; LS, liposarcoma; MA, melanoma; ME, melanoma; MEN, multiple endocrine neoplasia; ML, myeloid cell leukemia; MTC, medullary thyroid carcinoma; MY, myeloma; NE, neuroblastoma; NS, non-small-cell lung cancer ; OC, ovary cancer; OS, oral squamous cell carcinoma; SC, Schwannoma; SCC, squamous cell carcinoma; SS, synovial sarcoma; TLL, T lymphocytic leukemia; TT, testicular tumor. In cellular RNA transcripts, various mutations led to the loss of function of retrotransposition. Several sequences had a missense mutation in the putative amino acid, or frame-shift mutations affecting their lengths. Length variation was usually caused by insertion/deletion mutations, although MP-2 had a 40-bp tandem duplication in the 5' region, and HE-1 and JU-2 had 5' additional sequences. Sequencing of L1Hs p40 gene showed that 35 elements were the typical length. Sequence analysis provided information on the chromosomal locations of the active elements of the L1Hs. The putative active elements were determined by comparing the similarity, the typical lengths, and the putative protein translation of elements with previously reported active elements and the predicted proteins produced by L1Hs elements. Many L1Hs RNA transcripts appear to be transcriptionally active; twelve L1Hs groups and twenty-five solitary elements had potential activity for retrotransposition.

Protein motif analysis of L1Hs p40 using PROSCAN against PROSITE database showed various motifs for protein interactions (Fig. 20). L1Hs p40 has a luecine zipper motif that contributes to p40-p40 interactions and formation of multimeric complexes (Hohjoh and Singer, 1997). Various protein phosphorylation sites and modification sites had also been found in putative protein sequences. This predicts that activation of L1Hs p40 protein should be necessary to interact with cellular proteins and modify the protein p40 by cellular organelles. In addition, virus-related myristoylation site has been found, suggesting L1Hs p40 can be involved in maturation of the viral proteins and subsequent proteolytic processing (Towler *et al.*, 1988; Grand, 1989). Until now, there are several hypotheses on L1Hs introduction via virus packaging. While, the origin of L1Hs has been predicted based on the comparison analysis of DNA sequences. There are no direct evidences on the origin of L1Hs from retrovirus. Therefore, this finding will support the retrovirus origin or the virus-related introduction (George *et al.* 1996; Malik *et al.* 1999). Moreover, this means that L1Hs activation can be associated to viral pathogenesis in cells. Further study on their relationship will be needed.

Cell line-specific transcripts were observed in many chromosomal locations including three positions on chromosome X. Chromosome 4 had the most transcriptional active locations to transcribe L1Hs elements, but six chromosomes (15, 16, 17, 21, 22, and Y) had not the transcriptionally active locations. Several locations were specific for normal cells and for cancer cells, and shared among them. For instance, locations on 7p15 and 10q11 were specific for the neuroblastoma SK-N-SH cells, and those on 1p13 and 5p13 did for the leukocyte KBG, respectively (Table 12). In addition, mutations of RNA transcripts might lead to changes of L1Hs p40 protein sequences (Appendix 3). Various amino acid substitutions has been shown by the putative translation. Substitutions in protein interaction motifs has also confirmed, supposing the differences of protein activation. For example, since transcript clone MO-1 has two substitutions in PKC phosphorylation motifs, activation of MO-1 L1Hs p40 by PKC phosphorylation will be different efficiency. In addition, four substitutions in HTH motif will change the nucleic acid binding affinity, resulting the altered activity in L1Hs retrotransposition (Pietrokovski and Henikoff, 1997).

In the phylogenetic tree based on L1Hs p40 DNA sequences, these were divided into three main clusters (L1A, L1B, and L1C); L1A was subdivided into two, and L1B into three subclusters (Fig. 22). Protein sequence phylogeny showed that three main branches (p40A, p40B, and p40C) appeared. Both p40A and p40C are human-specific, and p40B shared those of mice, gorilla, chimpanzees (Fig. 23). Of the two human-specific, p40A is more ancestral than others, suggesting L1Hs introduction into mammalian genome might be occurred several times before and after the speciation. Mammalian shared p40B suggested that speciation of mammalian predecessors might have occurred after introduction of almost L1Hs elements. In addition, putative retrotransposable active L1Hs groups scattered over three branches, suggesting that ancestral LINE-1 elements possess the retrotransposition activity even in extracellular environment for a long time.

Up to date, it has been estimated that from thirty to sixty active L1Hs elements reside in the average diploid genome (Sassaman et al., 1997). However, this study suggested that at least a hundred and forty L1Hs elements in the haploid human genome have hypothetical activity potential for RNP complex formation, and they distributed over whole chromosomes. As showed in Figure 22, the active L1Hs elements divided into two clusters, but almost all of them are the members of cluster L1C. This result showed that L1C is the major active form of the L1Hs elements. The non-LTR superfamily is very ancient and LINE-1-like elements are ubiquitous in nature, those were found in plants, fungi, invertebrates, and various vertebrate classes from fish to mammals. L1 elements have been replicating and evolving in mammals for at least the past 100 million years (Furano, 2000). Also, human-specific or primate-specific sequences suggested that multiple introductions of LINE-1 elements, mediated a certain viral genome, had occurred during the mammalian evolution. This result will be contributed to explain the evolution and the speciation of mammals, while more extensive sampling and detailed approaches will be needed to elucidate the relationships between the molecular evolution of LINE-1 and mammals.

#### 3. Transcription and Transposition of Retroelements by Carcinogens

This study showed the differential gene expressions of retroelements and the transposition activity by carcinogens. In the transposition analysis, the altered amplification patterns were also observed, resulting in transposition by carcinogen exposure.

HERV-K gene expression increased in fibroblast exposed to carcinogens along with changes in the expression of regulatory genes. However, the expression patterns of HERV-K env gene and LTRs increased, although this result was not directly associated with changes in the expression patterns of regulatory genes. Since the effects of carcinogens were different, response of the fibroblasts varied. For instance, in cells treated with TPA and DMBA, the expression of BAX, c-MYC, IL-6 mRNA increased, while *p53* mRNA decreased. Although the apoptosis and proliferation pathways were activated, the cells actually proliferated. Levels of BAX and IL-6 in EtBr-treated fibroblasts were more expressed than did non-treated cells, while c-MYC and p53 expression decreased. However, the only HERV-K gene expressed at a lower level was K10 LTR, suggesting that either HERV-K expression is independent of apoptosis or that HERV-K RNA transcripts have longer life span than cellular mRNAs. The expression of active HERV env has been described in various states; enveloping retrovirus-like particles, a transcript of cellular RNA, and a protein of normal human tissues and cell lines (Kitamura et al., 1996; Löwer et al., 1993; Sauter et al., 1995; Berkhout et al., 1999). Induction of IL-6 expression by carcinogens may lead to change of the immune activity and differentiation of lung fibroblast (Miyagi et al., 1991; Samad et al., 1994; Moodley et al., 2003). Cytokines and carcinogens induced the gene expression of HERVs, considering roles in inflammation and carcinogenesis (Larsson et al., 1996; Katsumata et al., 1999).

Under the carcinogen treatments, the expression of L1Hs was the highest in the DMBA treatment, and the lowest in the EtBr treatment. The relative transcription rate of

L1Hs was increased, as compared to the saline control, in all but the EtBr treatment. Since the patterns of relative transcription were different in the EtBr and MNNG treatments, although both EtBr and MNNG eventually induced the apoptosis of CCD-25Lu fibroblasts, the transcription of L1Hs elements is unlikely to be directly related to the induction of cell apoptosis. This means that EtBr and MNNG are contributed to different cellular pathways in CCD-25Lu cells, although the outcome was still apoptosis. In this process, a signal triggered by MNNG might increase the transcription of L1Hs, while EtBr has no effect on this.

The associations between location of repetitive DNA and chromosomal fragile sites were described. Transposon mariner-like elements (Hsmar) were found in human genome, suggesting relationship with fragile site and with genetic disorders (Reiter et al., 1999; Liehr et al., 2001). Hsmar elements are DNA transposons flanking ITR sequences. Chromosomal locations of Hsmar were known as a 'hotspot' to homologous recombination (Koyosawa and Chance, 1996; Reiter et al., 1996; Lupski, 1998). Analysis on transposition using ITR primers of this study showed that the transposition activity was stimulated by exposure to carcinogens, suggesting transposition mediated-genomic instability by carcinogens. Various carcinogens can affect to chromosomal stability. Retroelements and their remainders are involved in homologous recombination, causing chromosome rearrangement, and their expression can facilitate to chromosomal instability (Mager and Goodchild, 1989; Duesberg et al., 2000; Paige et al., 2000; Bardelli et al., 2001; Honma et al., 2002). Since the carcinogenic initiation begins of genomic alteration, chromosomal aberration and the errors in DNA mismatch repair causing to the activation of oncogenes or the inactivation of tumor suppressor genes, it is very important to explain the possible roles of carcinogenic effects on resulting genomic alteration in the cells. Certain stress condition, such as exposure to UV light (Rolfe and Banks, 1986), chemical mutagens (Bradshaw and McEntee,

1989; Morawetz and Hagen, 1990), low temperatures (Paquin and Williamson, 1984) or nitrogen starvation (Santos *et al.*, 1997) can cause an increase in transposition activity (Staleva and Venkov, 2001). Staleva and Venkov (2001) has described the induction of transcription and subsequent retrotransposition of Ty1 retrotransposon by mutagens (methyl methanesulfonate and 4-nitro quinolineoxide) in cultures and cells, and postulated the activation of Ty1 element by DNA damage. Therefore, this finding suggests that the activation of retroelements can lead to transposition activity causing to genomic instability by carcinogens, and can also accelerate the carcinogenesis of the cells.

#### 4. Association between Active Retrolements and Human Genetic Disorders



Active retroelements HERV-K10 LTR and L1Hs were found in the human cells. DNA sequencing and subsequent analyses were used to determine the chromosomal locations of transcripts. Interesting informations were obtained by comparing this result to Human Genome Resources in GenBank and those previously reported.

Many loci of retroelements L1Hs (forty-three) and HERV-K10 LTR (seventeen) were found at fragile sites. Moreover, at several fragile sites, these two retroelements were aligned in a repetitive manner (Table 13 and Appendix 4). Many proto-oncogenes and tumor suppressor genes mapped to these fragile sites, suggesting association with cancers and transformation of cells. For example, tumor suppressor genes *FGR*, *SRC*, and *TNFR2* mapped to 1p36 (FRA1A) associated with BC, CLL, myeloid cell leukemia (ML), and RC. 5q21 (FRA5F) contained an oncogene *APC* and associated with adenomatous polyposis of the colon (APC), BC, colon cancer (CC), esophageal cancer (EC), and RC. Proto-oncogenes *JUNB*, *JUND*, *LPSA*, *MEL*, *MELL1*, *RAB3A*, and *VAV* mapped to cancer breakpoint 18q21 (FRA18B) related to BC, CLL, hepatocellular carcinoma (HCC), non-small lung cancer (NS), and RC (Table 14).

Fragile sites may be unstable regions of the human genome, which might play an important role in the genetic instability associated with cancer predisposition (Sutherland et al., 1998; Ribas et al., 1999). The most fragile sites were associated with cancer breakpoints. For example, the most common fragile sites involved in bladder cancer were FRA1D (1p22), FRA1F (1q21), FRA8C (8q24), FRA9D (9q22), FRA9E (9q32), and FRA11C (11p15) (Moriarty and Webster, 2003). Dhillon et al. (2003) described that the expression of the fragile sites induced by aphidicolin was evaluated from ovarian cancer (OC), breast (BR), and NS patients belonging to different stages of cancer development. The frequency of expression of the fragile sites in cancer patients and their first-degree relatives was found to be statistically significant than those of the controls. In addition, the distribution of fragile sites in BR patients was changed according to different stages of cancer. Fundia et al. (1998) identified the spontaneous breakage and fragile site expression in CLL and suggested that the most of cancer breakpoints were involved in structural abnormalities associated with CLL. FRA16D (16q23) is one of the regions of the long arm of chromosome 16 and has been reported to show loss of heterozygosity (LOH) in various cancers, have identified a over 600 kb homozygous deletion in some cases (Paige et al., 2000; Tunca et a., 2000; Bednarek et al., 2001; Dhillon et al., 2003), and involved in multiple myeloma translocations (Krummel et al., 2000). In addition, chromosomal fragility may be involved in neuro-behavioral disorders (Gericke, 1999), mental retardation (Tükün et al., 2000), senescence and schizophrenia (Morelli et al., 2002). In this study, transcriptional locations of active retroelements have also been found in fragile sites associated with BC (Moriarty and Webster, 2003), leukemia (Sbrana and Musio, 1995; Fundia et al., 1998), CC (Tunca et al., 2000), giant

cell tumors (Nilsson *et al.*, 2002), and BR, OC, and NS (Dhillon *et al.*, 2003) (Table 13). Therefore, this suggests that the location and activation of retroelements HERV-K and L1Hs may be involved in cancer development and genetic disorders.

In addition, a transposition event can result in the deletion, inversion, or translocation of the genes (Youssoufian et al., 1988; Sheen et al., 2000; Gilbert et al., 2002; Sankoff et al., 2002; Chi et al., 2003). Morelli et al. (2002) reported that the nucleotide sequence of FRA6F is rich in repetitive elements; deletion breakpoints within FRA6F are common in several human leukemias and solid tumors, and are associated with hereditary schizophrenia. The locations of the L1Hs elements supported the findings of Morelli et al. (2002), but not the HERV-K10 locations, suggesting that inactive or other types of HERVs are located on FRA6F. Florl and Schulz (2003) described that most deletion breakpoints in cancers were located in or close to L1Hs retrotransposon clusters, and therefore, deletions of CDKN2A in 9q21 (FRA9C) may be facilitated by the presence of L1Hs clusters flanking the locus. A possible mechanism of recombination and transposition of transposable elements might be mediated chromosomal deletion and rearrangement (Lupski, 1998; Kiyosawa and Chance 1996; Reiter et al., 1996; Kolomietz et al., 2002; Toffolatti et al., 2002; Goryshin et al., 2003; Bosch and Jobling, 2003). Kolomietz et al. (2002) suggested Alu repeat clusters as mediators of chromosomal aberrations in tumors. Bosch and Jobling (2003) described that the duplication of the AZFa region of the human Y chromosome is mediated by homologous recombination between HERVs. Double transposition of *Tn5* produced the chromosomal deletion (Goryshin et al., 2003). Huang et al. (1998) found HERV-H and small polydispersed circular DNA (spcDNA) molecules in the midst of FRA7G and FRA3B, and suggested an association with both spcDNA molecules and hotspots for viral integration. HERV-K10 and L1Hs loci are much more common at fragile sites, regardless of whether they are expressed. Fragile sites are hotspots for genomic

instability causing chromosome rearrangement and recombination (Bobe *et al.*, 1993; Fundia *et al.*, 1998; Sutherland *et al.*, 1998; De Mesa *et al.*, 2000; Tükün *et al.*, 2000; Ostertag and Kazazian, 2001; Nowak *et al.*, 2002; Tighe *et al.*, 2002). This study showed the cancer specific transcriptional locations of active retroelements (Appendix 4).

Reports described genomic instability by the activation of interspersed elements such as transposons, by amplification of their flanking inverted terminal repeat sequences, between tumor and normal tissues (Maeda *et al.*, 1999; Ayyadevara *et al.*, 2000; Luceri *et al.*, 2000; Jotwani *et al.*, 2001; Singh and Roy, 2001; Misra *et al.*, 2001). Misra *et al.* (2001) described the alteration of a sequence with homology to HERV-K in primary human glioma, and suggested the viral repeat mediated genomic rearrangement in tumorigenesis. Inverted repeats are as source of genomic instability in yeast (Gordenin *et al.*, 1993). As far as human disease is concerned, recombination in a defective low density lipoprotein (LDL) receptor gene in cases of familial hypercholesterolemia (Horsthemke *et al.*, 1987). Genomic changes by activation of HERVs has been detected in some cancer and cultured cells (Huang *et al.*, 1998; Misra *et al.*, 2001; Goryshin *et al.*, 2003), and hypomethylation of HERV-rich regions is also reported (Florl *et al.*, 1999).

In the cells, repression of retrotransposition activity is very important. Methylation is an important epi-genetic mechanery in the repression of the repetitive transposable elements (Jurgens *et al.*, 1996; Hata and Sakaki, 1997; Woodcock *et al.*, 1997; Yoder *et al.*, 1997; Florl *et al.*, 1999). Florl *et al.* (1999) found the tissue specific hypermethylation of LINE-1 and HERV-K in various human cells and speculated that decreased methylation of retroelements may contribute to genomic instability in specific human tumors such as urothelial carcinoma by rendering these normally repressed sequences component for transcription and recombination. In this regard, hypermethylation of L1Hs has been suggested to inactivate the gene and chromosome. It has been proposed that L1Hs repeats may be mediators for the spread of X chromosome inactivation; in normal somatic cells, X-linked L1Hs elements are hypermethylated on the inactive X (Lyon, 1998; Bailey *et al.*, 2000; Riggs, 2002; Allen *et al.*, 2003; Hansen, 2003). In addition, hypomethylation of L1Hs has been observed in hepatocellular carcinomas, but not in surrounding liver cirrhosis. Differential methylation has also been found in various cancer cells, compared to normal controls. Hypomethylation of the promoters of retroelements has led to increased expression of retroelements and probably to retrotransposition (Alves *et al.*, 1996; Feng *et al.*, 1996; Takai *et al.*, 2000; Lin *et al.*, 2001). In addition, DNA hypomethylation had also reduced genomic instability and can lead to elevated mutation rates (Chen *et al.*, 1998; Rizwana and Hahn, 1999). In this study, transcripts of L1Hs on chromosome X were found in several cell lines (CCD-25Lu, HCT-15, Jurkat, and MCF-7). However, it was impossible to determine the origin of transcripts whether the those derived from inactive or not.

Recent reports described the retrotransposition activity of SINE, LINE-1 and HERVs, resulting in the generation of pseudogenes and multiple genes (Misra *et al.*, 2001; Pavlicek *et al.*, 2002; McNaughton *et al.*, 1998; Cardazzo *et al.*, 2003). Identical sequences and its variants had also been found in this study, which concluded that widespread distributions of the retroelements should be resulted from the retrotransposition. In general, non-LTR element L1Hs and LTR-elements HERV-K also utilize the 'replicative transposition' mechanism of integration, which enables purifying selection to retain active copies in a lineage (Malik *et al.*, 1999). In genomic evolution, intronic expansion and processed pseudogenes might be made by transposition activity. The enlargement of the genome size and the decrease in genome compactness increasing the number and size of introns is a general pattern during the evolution of transposable elements might have important roles in driving intron evolution (Cardazzo *et al.*, 2003;

Salem et al., 2003).

The present study showed transcription and chromosomal location of active retroelements HERV-K and L1Hs in the human cells. These retroelements are multicopy and widespread on chromosomes, and also potentially active. Its activation of transposition and recombination events may be caused to human genetic disorders. Table 15 shows the human genetic disorders related to the transcriptionally active chromosomal locations of these retroelements. Gene expression, germ line and somatic mutations, and insertions of endogenous retroviruses have been found, suggesting a possible role for HERV-K elements in human diseases. Roles in insulin dependent diabetes mellitus (Conard et al., 1997), hypertension (Sirokman et al., 1997), cardiovascular disease (Bing et al., 1998), autoimmune disease (Krieg et al., 1992; Wu et al., 1993; Andersson et al., 1998), multiple sclerosis (Clausen, 2003), neoplastic disease (Suzuki et al., 1986; Bussemakers et al., 1992; Zsiros et al., 1998) and mouse embryonic lethal mutation in  $a_1$  (I) collagen gene (Schnieke *et al.*, 1983) have been postulated (Misra et al., 2001; Florl et al., 1999). Germ line mutations having the retrotransposition of L1Hs elements which abrogate the functions of important genes are known in several diseases, such as the coagulation factor VIII gene in hemophilia A (Kazazian et al., 1988), the dystrophin gene in Duchenne muscular dystrophy (Narita et al., 1993; McNaughton et a., 1998), the *fukutin* gene in Fukuyama-type congenital muscular dystrophy (Kobayashi et al., 1998) and the cytochrome b558 heavy-chain (CYBB) gene in chronic granulomatous disease (Kumatori et al, 1998; Meischl et al., 2000). In addition, somatic mutations caused by retrotransposition of an L1 element had been known in the APC gene in a colon cancer (Miki et al., 1992; Miki, 1998) and in the oncogene c-MYC in a breast cancer (Morse et al., 1988) and in a colon cancer (Naas et al., 1988).

Consequently, it is believed that the locations and expression of retroelements are

Table 15. Chromosomal location of detected retroelement transcripts and the map of human genetic disorders

Locus		lement	- Genetic disorders identified
Locus	K10 LTR	L1Hs	
1p11		+	Adrenal hyperplasia II
1p13		++++	Hypothyroidism; Adrenal hyperplasia
1p21		+	Stargardt MD; Glycogen storage disease III
1p22	+	++	Zellweger S-2; Congenital disorder of glycosylation
1p31		++	Maple-syrup urine disease type III; Corneal dystrophy, gelatinous drop-like; Multiple sclerosis
1p35	++		Hypercholesterolemia, autosomal recessive
1p36	+++		Erythroblastosis fetalis
1q21	++++++++	+	Gaucher's disease type 1
1q22	+		Urate oxidase deficiency
1q24		+	Deafness, nonsyndromic
1q25		+	Nephrotic syndrome, steroid-resistant, autosomal recessive; Short stature, pituitary and cerebellar defects
1q31		++	Cardiomyophathy familial 2; Branchiootic S2; RP 12
1q44	+	2	Fanconi's anemia; Familial cold autoinflammatory S; Lupus erythematosus, systemic
2p11		+	Smith-Magenis S; Methionine adenosyltransferase deficiency
		112	Pakinson disease 3, autosomal dominant;
2p13		+	Lower motor neuron disease, progressive without sensory symptoms
2p22		++	Cáfe-au-lait spots, multiple
2p24		+	NIDDM
2q24		+	Abetalipoproteinemia
2q31		++	Fibromuscular dystrophy of arteries; Syndactyly, type II
2q32		+	Winkle skin S
2q33	+	+	Gracile S; IDDM
2q34		+	Ehlers-Dalnos S, type X
2q35		+	Brachydactyly, type A1; Dermatitis, atopic
3p11		+	Dementia, familial
3p12		+	Spinocerebellar ataxia 7
3p24		++	Thyroid hormone unresponsiveness; Asthma; Marfan S; Moyamoya disease
3q11		++	SCZD 1
3q13		+	Hypoparathyroidism, Familial isolated; Hyperparathyroidism, neonatal severe primary
3q21	++	+	Alkaptonuria, Allergic rhinitis; SCZD2; Dermatitis atopic
3q22		+	Night blindness, congenital stationary
3q24		+	Thyroid hormone resistant, Xenoderma pigmentosa
3q26	++		Sucrose intolerance

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Logue	Retro	element	— Genetic disorders identified
Locus	K10 LTR	L1Hs	- Genetic disorders identified
4p15		+++	Phenylketonuria II
4p16	+	+	Huntington's disease; Ellis van Creveld S;
4010	ŗ		Hypochondroplasia
4q12		++	Limb-girdle MD; Graves disease
4q13	+	++	Dentonogenesis imferpecta-1
4q22		+++	Abetalipoproteinemia
4q25		+	longevity 1; Mental retardation autosomal recessive
4q28		+	Afibrinogenemia, congenital
4q32		+++	Aspartylglucosaminuria
5p13		+	C6, C7, C9 deficiency; SCID
5q11	+		Mucopolyssacharidosis type IV; Polycystic ovary S;
SqII	Ŧ		SCZD 1
5.12	+	+	Stroke (cerebrovascular accident);
5q12	Ŧ	Ŧ	Spinal muscular atrophy 1
5q13		+	Sandhoff disease; Laron dwarfism
5q21		+++	5q- S; Desmoid disease, hereditary
5q22		+++	Corneal dystrophy; MD, limb-girdle, autosomal dominant
5q31		+	Cortisol resistance; Asthma; Corneal dystrophy;
5451		I	Osteoporosis; Graves disease; Carnitine deficiency;
5q32		+	Achondrogenesis type IB; Neonatal osseous dysplasia; IgE
5452		I	responsiveness; Dermatitis, atopic 6
6p11		++++	Autoimmune disease (MHC related)*
6p12	++		NIDDM
			C2, C4 deficiency, Bare lymphocyte S; IDDM; SCZD;
6221	1	+	Polycystic kidney disease, autosomal recessive,
6p21	+	Ŧ	Autoimmune disease; Asthma; Laterality defects, autosomal
			dominant
6222			Fanconi's anemia, complementation group E; Maple syrup
6p22		++	urine disease 3, SCZD
6~16	1		Chorioretinal atrophy, progressive; MD, retinal, California
6q16	+		type I
6q21		+++	Senescence; SCZD; IDDM
6q22		+	Hepatic fibrosis; MD, congenital merosin deficient
6q24		+	Diabetes mellitus, transient neonatal
6q25	+	+	Pakinson disease, juvenile, autosomal recessive
7p11		+	Nystagmus, congenital, autosomal dominant
7-15	т		Spinal muscular atrophy; Hyperinsulinism, autosomal
7p15	+	+++	dominant; RP 9

Table 15 (Continued)

T	Retroelement		Construction in a first first
Locus	K10 LTR	L1Hs	— Genetic disorders identified
7q11		++	Williams S
7q21		++	Erythremia
7q22		+	Chloride diarrhea, familial; Osteogenesis imperfecta congenital
7q31		+	Cystic fibrosis
7q32	++		RP10
7q35		+	Pancreatitis, hereditary; Glaucoma I
8p11	+		Werner S; Pfeiffer S; SCID1
8q11	+++		SCID1; RP1
8q21		+++	Cockane S; Aldesterone deficiency; Hyperaldosteronism Giant cell hepatitis, <i>SCZD</i>
8q22		+++	Cohen S; Hyperlipoproteinemia
8q23		+	Hashimoto thyroiditis; Autoimmune thyroid disease
8q24	++++		Langer-Giedion S; Epilepsy; Myeolcytomatosis
9q31		)+) _	Fukuyama-type congenital MD
9q34	++++++	++	C5 deficiency
10p15		+1.10	JISCZD IONAL UNIVERSITY LIBRARY
10q11	+	+++	Chronic infections
10q21	+		Moebius S; Multiple endocrine neoplasia
10q24	++	+	Congenital adrenal hyperplasia B
10q25		+	IDDM
11p11		+	Dysprothrombinemia
11p15	+		Niemann-Pick disease; Sickle-cell anemia
11q12	+		Atopy
11q13	++++	+	multiple endocrine neoplasia I
11q14		+	Systemic lupus erythematosus Papillon-Lefervre S
11q21		+	SCZD
11q22	+		Ataxia, episodic
12p11	++		Pakinson disease; Alzheimer disease type V
12q12		+	Rickets, vitamin D-resistant
12q13	+	+	Achondrogenesis type II; Stickler S
12q14		++	Tyrosinemia, type III
12q21		++	MD
12q24		+	Alcohol intolerance, acute; Fetal alcohol S, SCZD

Table 15 (Continued)

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Table 15 (Continued)

Loong	Retroe	element	Genetic disorders identified
Locus	K10 LTR	L1Hs	Genetic disorders identified
13q14	+++	+	Wilson's disease; Retinoblastoma
13q22		+	Megacolon
13q32		+	SCZD
13q34		++	Factor VII, X deficiency; SCZD
14q11	++	+	Ichthyosis; RP; Inflammatory Bowel disease
14q12		+	Cardiomyopathy, familial hypertrophic
14q23		++	Cataract, anterior polar
15q15		+	MD, Limb-girdle, autosomal recessive; SCZD
15~24			Mental retardation, severe, with spasticity; Cerebellar
15q24		++	ataxia with mental retardation
15q25	+++	+++	Tay-Sachs disease
15q26	+	++	Bloom S
16p12	++		Batten disease
			Cataract, congenital
16p13	+		Familial Mediterranean fever
1 < 10			Inflammatory Bowel disease I; Amyotrophic latera
16q12		+	sclerosis
16q21		/ +++	Hypodontia; Cataract lamellar
16q23	+ (	++	Pseudohyperkamelia
17q21	++	H.D.	Glanzmann thromboasthemia; Hypertension essential
17q25	+		Rusell-Silver S
18p11		+	Glucocorticoid deficiency
18q11	+++		Amyloid neuropathy, familial
18q21		+	Cholestasis; IDDM
-			IDDM; Hypercholesterolemia, familial; Cerebellar ataxia
19p13	+		Cayman type
			Nephrosis 1, congenital, Finnish type;
19q12	++	+	Pseudoachodroplastic dysplasia; Migraine, familial;
•			Alzheimer disease 2
20p12		+++	Huntington disease-like 1; Kindler S
20q12	+		NIDDM; Immunodeficiency with hyper-IgM, SCZD
22q11		+	Cat-eye S; DeGeorge S; SCZD
Xp11		+	Brunner S; Night blindness, type I
			Becker MD <sup>4</sup> ; MD; Retinitis pigmentosa; Cone dystrophy
Xp21		+	X-linked; Duchenne MD
Xp22		+++	Glycogen storage disease VIII
Xq11		+	Testicular feminization S
Xq13		+	Cleft plate; Turner S
Xq21		++	Agammaglobulinemia type 1; Duchenne MD; Granulomatous disease, chronic

Table 15 (Continued)

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Locus	Retroelement		- Genetic disorders identified	
Locus	K10 LTR	L1Hs	- Genetic disorders identified	
Xq22		+	Agammaglobulinemia type 2; Alagille S; Amelogenesis imperfecta; Alport S and associated diffuse leiomyomatosis	
Xq23		+	Hyperglycerolemia; Cerebellar ataxia 2; Arthrogrypopsis X-linked; Mental retardation X-linked	
Xq24		+	Glycogen storage disease $II b$ ; Albinism-deafness S; Mental retardation X-linked	
Xq26	++	+++	Albinism-deafness S; Immunodeficiency, X-linked, with hyper-IgM	
Yp11		1	Gonadal dysgenesis; Azoospermia factor 1, AZF1	
			데주대학교 중앙도서관 EJU NATIONAL UNIVERSITY LIBRARY	

Abbreviations: IDDM, insulin-dependent diabetes mellitus; MD, muscular dystrophy; NIDDM, noninsulin-dependent diabetes mellitus, SCZD, schizophrenia; SCID, severe combined immunodeficiency; RP, Retinitis pigmentosa; S, syndrome. Italic-lettered names are retroelement-related diseases previously reported. closely related to the chromosomal instability responsible for genetic stability. This study showed that retroelements HERV-K and L1Hs are transcriptionally active in the human cells, as the cell-type specific transcription in HERV-K, and as the cell-type independent in L1Hs, respectively. Many retroelements are still transposing, suggesting the associations between the retrotransposition activity and genomic instability, cancer developments, genetic or epigenetic diseases. In addition, carcinogens may induce the transposition activity of transposable elements in the cells. In the activation of retroelements, various cellular factors may involve in transcription of retroelements and post-translational processes of their proteins. Since the molecular mechanisms that causes the hypomethylation and the activation of L1Hs, HERV-K provirus, and other repetitive sequences are virtually unknown, investigations of this mechanism will be very important. Also, further studies on biological function of retroelements and on the associations between the activation of retroelements and the human genetic disorders will be necessary.

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### APPENDIX I

Multiple aligned sequences of the HERV-K10 LTR from human cells



		_		
1 F	R1 THTE AP- ASTICTSAAATATISECCTCSTERSAASSEAAA		а Алассолькомоското и какана станование и конструкти.	WTA hos: 120 BARBAABBCAT
CC-2 CA			ABCCCCACACCCCCTAAAABBBTCCTBCTBCTBABBABBATTAB	GAGGAAGGAAT
AD 6-20	ASTICTETAATATGECCTOSTGEGAAGEGAAA		ARCCORACACCONTAAAREETCTETECTEAREAREATTAR	BARBAARBCAT
CC-4 CA			-ABCCCBACACCCBTAAABBBTCTBTBCTBABBABBATTAB	BARBAARBCAT
YJ-1 CA			ARCCCRACKCCCRTAAARRETCTRTRCTRAARARRATTAR	GARGAARRYAT
YJ-2 CA BG-1 CA		C-9702222	-ARCCCRACACCCRTAAARRETCTRTRCTGARGARAATTAG -ARCCCRACACCCRTAAARRETCTRTRCTGARGARAATTAG	BARBAARREAT BARBAARREAT
10-1 LA 10-2 CA		C-MILLIP-	- AGE TI DEAL ALLI DE LA AGE DE L'ENDER TE TRANSVERSE LE AGE	SASSAASSAAT
107-X DA		C-STRCCA	-AGCCCGACACCCGTAAAGGCTCTGTGCTGAGGACGACTAG	A GAGGAAGGAAT
BB-4 DA	ASTICTERARATATIGECCTOSTIGEGAAGEGAAA	C-9702222	ARCCORACIONSTAAARRETCTRETRATRAGARGARTTAR	A BARBAARBAAT
2877-0. CA		C-ATODOCT	-АВСССААСАСССАТАААТИЯТСТИТЯСТВАНИАНАТТАЯ	GA GAGGAAGG
A3-1 CA		C-8700000	ARCCCRACKCCCRTAAARRETCTRTRCTRARRARRATTAR	A BARBAARBAAT
A3I-2 CA A3I-3 CA		C-9702222	-ARCCCRACACCCATAAARRETCTSTRCTGARGARBATTAG -ARCCCRACACCCRTAAARRETCTSTRCTGARGARGATTAG	RARRAARRAAT A RARRAARRAAT
A4-1 DA		E-67122222	- Kent Liter Level at Liter Annales II. The Tel. Three Management I Ale	releases
A4-2 DA			AGCCCGACACCCATAAAGGGTCTGTGCTGAGGAGGATTAG	A GARGAAGGAAT
A4-3 DA	ASTETGAAATATGECETCSTGEREGERGAAA	C-9702222	ARCOSACACCOSTAAARRETETTTRETEARCARGARATTAG	CEASEAASEAAC
AS-1 DA			-ARCCCERACACCERTAAARRETETETETETEARRAARRATTAR	R RARRAGECAT
A5-2 DA			-ARCCAGACACCOSTAAABEETCTETECTGAGGAGGATTAG	A GARGAARGAAG
A5-3 CA HC-1 CA			- ARCCCRACACCTRTAAARRETCTRTRCTRARRARRATTAR - ARCCCAACACCCRTAAARRETCTRTRCTRARRARRATTAR	BARBAARRCAT BARBAARRAAT
HC-2 CA			ABOTT SIA CACTOST ANALOST CTUTIS CTUAREAUX TAS	GAGGAAGGAAT
			ATCCCAACACCCRTAAARRETCTRTRCTRAREATTAR	BATBAABBAAT
HC-4 CA	ARCCTGAAATATGROCTCATGRGAAGRGAAA		-ARCCCAACACCCRTAAARRETCTRTRCTRARAATTAG	GATGAAGGAAT
H9-1 CA			-ARCCCRACACCERTAAARRETCTRTRCTRARRARRATTAR	S SASSAASSAT
HE-2 DA			-ARCCCGRACACCCGTAAABRETCTETETETEAREAREATTAG	RARRAARRAAT
HE-3 CA			- ABCCCERACACCCERT AAAABBBTICTISTIGCTIGABBAABBATTAB - ABCCCERACACCCERT AAAABBBTICTISTIGCTIGABBAABBATTAB	RARRAARRCAT A RARRAARRAAC
HL-1 AG			BOTTO DA CACICA CAMAGENTICTO TO AND AND ATTAC	A GAGGGAGG
HL-2 DA			CACCORRELACIONALA ARRENT CTUTINE TRANSPORTATION	GAGGAAGGCAT
HL-3 DA	A ARTCEGAAATTCRECCTCATREGAARETAA	C-8700000C	-ARCCOGACACOCTTAAARRETCTRTRCTGARGARRETTAG	<b>GARGAARGAA</b> C
ACI 17-0.			-ARCCCRACACCERTAAARRETCTRTRCTRARRARRATTAR	
JU-2 CA			ARCCCRACACCORTAAARRETCTETRCTGARGARGATTAG	B BREEAARECAT
JU-3 CA JU-4 CA			- ABCCCERACACCCERT AAAABBBTICTISTIGCTIGABBAABBATTAB - ABCCCERACACCCERT AAAABBBTICTISTIGCTIGABBAABBATTAB	A GARGAARAAR A GARGAARAAR
JU-5 CA			ABOTT SIA CACTOST ANALOST CTUTIS CTUAREAUX TAS	A GAGGAAGGAAC
JU-6 CA			ARCETRACACCERTAAARRETCTRTRCTRARRARRATTAR	A DADDAADDAAT
JU-7 CA	ARTICTERARATATERCCTCRTRRRAARRRAAR		-ARCCCGACACCCGTAAARRETCTGTRCTGARGARGATTAG	GARGAARGAAT
MC-1 CA			-ARCCCRACACCERTAAARRETCTRTRCTRARRARRATTAR	A GAGGAAGGAAT
MC-2 CA			-ARCCCGRACACCCGTAAABRETCTCTRCTGARGARGATTAG	BARTAABBCAT
MC-3 CA MC-4 CA		C-ATTEREDC	- AGACCAACACCOSTAAABBBTCTBTBCTBABBABBATTAA - AGCCCGACACCOSTAAABBBTCTBTBCTBABBABBATTAB	RARRAARRCAT A GARGAARRAAT
MC-5 CA			ABOT DA ACTINITA AND TO THE TRANSPORT	A GAGGAAGGAAT
MP-1 DA			ARCORD CARETRET ANALYSISTIC TO THE TRANSPORTATION	A BAABAABS
MP-2 CA			AGO TEACACCCATAAA EE TOTECTE A EE A EE A TAE	A BARBAARBAAC
MP-3 CA	ASTCTEAAATTCERCCTCATREEAABERTAA	C-STOTOC-WEDG	ABCCCSACACCCSTAAASSETCTSTSCTSASSASSATTAS	A GARGAARGAAR
MP-4 DA	ARTCTRAAATATIRRCCTCRTRRRAAARRAAA		ARCCCRACACCETAAAREETCTETECTEAREAREATTAG	A GAGGAAGGAAT
MP-5 CA MP-6 CA			- ARCCCERACACCCCATAAAARRETCTETECTEARRAARRATTAR - ARCCTERACACCCCERTAAARRETCTETECTEARRAARRAATTAR	A BARBAARBAAR
MP-7 DA			-AGCCCGACACCCGTAAAGGCTCTGTGCTGAGGACGACTAG	A GAGGAAGGAAT
SC-1 CA			ARCCORACIONSTAAARRETCTRETRARARREARTAR	BARBAARBCAT
80-2 DA			-ARCORRAGACIOSTAAARG-TOARTRETRARRARRATTAR	BARBAARBCAT
SU-1 CA		C-9702220	-ARCCCGRACACCCGTAAABRETCTETETEARGARGATTAG	A BARBAARBAAT
51-2 CA 51-3 CA		T-STEEDET	- БЕСССКАСАСССКИТАЛАНИИ СТИТИССКАСКАСКА - АБСССКАСАСССКИТАЛИИ СТИТИССКАСКАСКАСКА - АБСССКАСАССКАТИ АЛАКИИ СТИТИССКАКАКАТАК	A BARBAARBAAR BARBAARBCAT
SU-4 CA		C-ATCCACC	ABO TEACAO TETA AA BEET TETE TEA BARANA TAA	A GAGGAAGG
19-1 DA		C-8700000	ARCCORACACCORTAAARRETCTRETRETRARRARRATTAR	BARBAARRAT
19-2 DA	ARTICTERARATACARCETCATIRRERARBERAR	T-STREET	-ARCCOGACACCOGTAAARGETCTETETEARGAGAATTAG	A GARGAARGAAR
A1 E-91		C-8700000	ABOCTEACACOCOTAAABEETCTETECTEAEEAEEATTAG	
19-4 CA		C-ATCCCCC	-ARCCCRACATICETTAAARRETETETRETEARRAREATTAR	A GAGGAAGGAAT
U9-5 CA U9-6 CA		C-9702222	- ARCCCRACACCCRTAAARRETCTRTRCTGARGARBATTAG - ARCCARGACACCRTAAARRETCTRTRCTGARGARGATTAG	A GARGAARGAAT A GARGAARGAAR
MI4123 DA		C-STOTIC	ABOCCERACACCTRTAAARRETCTRTRCTRARRARRATTAR	A GAGGAAGGAAT
ACTORNIA DA		C-8702222	ABCCCGACACCCCGTAAAABBETCTBTBCTGAAGABGATTAG	RANKAARRAAT
AC114503 CA			-ARCCCERACACCERTAAARRETETETETETEARRAARRATTAR	GAGGAAGGCAT
ALDARSTD. 1. CA			ABCCCBACACCCBTAAABBBTCTBTBCTBABBABBATTAB	EAGERAAGERCAT
ARDONALISI, J. CA ARDONALISI, J. CA			-ASTTERACATION TAAASSTICTSTISTIGASSASSATTAS -ASTTERACATION STAAASSTICTSTICTSTICASSASSATTAS	GAGGAAGGAAT GAGGAAGGAAT
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AL355019, 5, CA			-ARCCCRACKCCCGTAAATIGTCTGTGCTGAGGAGGATTAG	RADIAARDAAT
ACTIDED49, 4, ICA	ASTETGAAATATGGCETCSTGGGATGGGAAA	C-STREETC	Lanneske kennet kalagete te ta ta takadaga ti tag	A DADDAADAAT
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ALSSEZINA, 1. CA ACODERTEA, 7. CA			- AGYYAGAYAYYYYAAAGGTYTTTTTTTTATGAGAAGAATTAG - AGYYYAAYAYAYAAAGGTYTTTTTTTTTAGAGAAGAATTAG	
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ACD11447.7 CA AC105027.1 CA			CARTTTEACATION STAAASSETTETSTERTEASSASSATTAS - ASTTTEACATION STAAASSETTETSTERTEASSASSATTAS	
APODITIS 4 DA			-ABCTTSHALMITTSTAAAABBATCTBTBCTBABBABBATTAB	
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AC140725.01 CA			AGOY GACACYCETTALAGERTCTETETELGAGAGAGATTAG	
ACDI 13522, 2, 12A			ARCHIGACACCOSTAAAGEGETCTISTIGCTGAGAAGEATTAG	
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PanT-2 CA	AGTETGAAATATGGEETCGTGGGAAAGGGAAA	с-ятессес	-ABCCCGACACCCGTAAAGGGTCTGTGCTGAGGAGGATTAG	GARGAARRCAT

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EE-3	BCCTCTTS-	AGACA	AACC	TETETHER			RTETT-BETATAGAACTEGATTE		101-10000
CC-4	BOTTTTE-	AGACA	AAGE	TETTETHE			STOTO-BUTATAAAACCORATTS		127-JULUA
YJ-1	BOCTCTTS-	AGACA	AARD	TETTETHECE			STOTO-BUTATAAAAOOOSATTS		<b>101-11111</b>
YJ-2	BOCTCTTS-	AGACA	AARD	TETTETROT			STOTE-BETATAMANCOCATTS		
89-1	BOCTCTTS-	AGATA	AAGG	TETETHERE			RTCTC-RETATABAACCERATTE		INT-AAAAAA
BB-2	BOTTOTIS-	ABACA	AARS	ETETETETE			STETC-SSTATAMACCESATTS		EEE-AAAAAA
86-3	SCENETIS-	AGACA	AAGG	TETETHOL		CARROAATIRGAATI	RTCTC-RETATABAACCETATTE	TATHETICATETACTH	CC-AAAAA
BB-4	BOCTOTTE-	AGACA	AAGG	TETETERS			ятете-аятатаааассеаатта		EES-AAAAAA
2877-0.	-CONCIDENTS	AGATA	AAGG	TETESTRETE			атетт-аататаааасетаатта		DA-DAAAA
A3-1	BOMONTS-	AGACA	AARS	TETESTREET			RTCTC-BRTATAAAACCEBATTE		EEF-AAAAAA
83-2	<b>SEMINITE</b>	AGACA	AAGG	TETELTRESS			RTCTC-RETATABAACCCRATTE		INT-INTAAA
A31-31	SCENETIS-	AGACA	AAGG	TETETHEORY			RTETC-CATATAAAACETGATTS		IST-AAAAA
M-1	BORDATITE B	AGACA	AAGG	TETTETROSC			RTERE-BETATAMAACCEMITT		GG-GAAAA
A4-2	BOCTOTTS-	AGACA	AASS	TETETHE			втете-вятаталалестватте		AG-AAAAA
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AS-1	BOCTCTTS-	AGACA	AASS	ATCTICTIBLE	Т		STETC-BETATALAACCERATTS		00-11111
A5-2	Southern the	AGATA	AARE	<b>HEILERICE</b>			STOTO-BETETAKAROOOSATTE		DA-DAMA
AS-3	BUTUTUE-	AGACA	AARE	TELEPISED .			STETC-ACTATANAACCORATTS		III-AAAAA
HC-0. HC-2	BUTTUTTE-	AGACA	AAGG AAGA	TETRETREE			STUTC-BETATAAAACCCEATTE		
HD-3	BOCTOTTS-	AGACA	AAGE	TETRETHERE			атете-дататаадаессалтта атета-дататаада теселтта		
HC-4	BOCTOTTS-	AGACA	AACC	TETETHE			атета-автаталал ператта втета-автаталал терратта		127-AAAAA
HG-1	BCCTCTTS-	AGACA	AACC	ATCTICTICC			RTETE-BRIATAAAACCEGATTE		127-AAAAA
HE-2	RETETTER	AGACA	AAGE	TUTUTTAT	-		STOTO-BUTATAAAACCORATTS		107-JULUA
HE-3	BOTTTTE-	AGACA	AAGE	TETTETREE			RICTIC-BUTATAAAACROSATTS		107-JULUA
HG-4	BOCTCTTS-	AGACA	AARD	TETTETHE			STOTO-BUTATAAAAOOOSATTS		<b>101-10000</b>
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HL-2	BOCTCTTS-	AGACA	AAGE -	TETRETREECC			STOTE-BETATALAACCERATTS		
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IU-1	BOCTCTTS-	ABACA	AAGG	TETETHERE			RTCTC-RETATABAACCOMATTR		INT-AAAAAA
JU-2	BOTTETTE-	AGACA	AAGG	TETRETREDE			ITTTE-BITATAWARDSWATTS		IIII-AAAAAA
313-33	REMINISTER	ABATA	AAR	TOTOTOTO			ATTACCORAATTATAR STORE		EA-EAAAA
313-4	REMINISTER	ARATA	AAA	TELEPISE			ATTACCORRECTANT AND CONTRA		EA-EAAAA
30-5	SCENETIS-	AGACA	A.A.MA	TETRETREAC	_		RTCTC-RETATALAACCERATTE		CC-AAAAA
313-6	SCENETIS-	AGACA	AAR	TETIGETROE		TREESAATERAAT	FTCTC-BETATAAAACBCBATTE	TATHETICATETACTH	CC-AAAAA
313-7	BOCTOTTE-	AGACA	AATC:	TODATORA			RTCTC-ARTATAAACCCGATTE		E EE-AAAAA
MC-1	SOUTHTIN-	AGACA	AARS	TETETHE			RTETC-RETATABAACCERATTR		IST-AAAAA
MB-2	SCENETIS-	AGACA	AAGG	TETETHE			RTETC-RETATABAACCERATTR		EX-AAAAA
MI3-31	BOCTOTTE-	AGACA	AASS	TETEETREESC			RTCTC-BRTATAAAACCERATTR		RE-AAAAA
MC-4	BOTTOTTE-	AGACA	AARE	TELEPISEE			STOTO-BETATANAACCORATTS		III-AAAAA
MC-6	BUTUTE-	AGACA	AARE	TELEPISEE			STOTO-BETATANAACCORATTS		III-AAAAA
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MP-5	RETETTE	AGATA	AACC	TETECTNETC			RTCTC-ORTRT&AAARCECOATTC		IIIIIUUU
MP-6	BOCTOTITE C	AAACA	AAGE	TETTETROT		TREETAATRE		TATETTCCATCTACTE	T RE-RAMA
MP-7	BOCTUTTE-	AGACA	AARD	TETTETROE			STOTO-ASTATAAAAOOOSATTS		R RE-AAAAA
507-1	BOCTCTTS-	AGACA	AARD	TETTETROT			STETC-BETATAMANCERATTS		III-MAAA
20-2	BOCTCTTS-	AGACA	AAGG	TETETHOT			RTCTC-RETATABAACCERATTE		C RE-ANAMA
SU-1	BOCTOTTE-	AGACA	AAGG	TETRETREDEC			ITTTE-BITATAAAACCEATTE		INT-AAAAAA
SII-2	REFERENCES	AGATA	AARS	TETRETRETE		THERE AATHERAAT	ettett-getetAAAgeetteAtte	TATATREEATCTACTR	EX-EXAM
503-30	SCENETT-S	AGACA	AAGG	TETTETROSC		TREECAATIRGAATI	RTCTC-RETATABAACCCRATT	TATETTCCATCTACTE	CC-CAAAA
50.1-4	-027777776	AGATA	AMAGA.	TETETTRETE			RTATT-ARTERAAARCCEATTE		
19-1	BOCTOTTS-	AGACA	AAGG	TETRETREEE			STETC-ASTATAAACCERATTS		GG-330303
19-2	RECTENTIS	AGATA	AAGG	TERRETRETE			STETC-SETSTAAASCCCAATTE		GL_GUUU
19-3	BOCTCTTS-	AGACA	AASS	TETECTROSC			STETC-BETATALAACCERATTS		CALINA AND A
119-4	BOTUTIE-	AGACA	AARE	TETETRE			STOTO-BETATANAACCORATTS		III-AAAAA
09-5	BOCTOTICS-	AGACA	AASS	TETECTREE			STETC-BETATALAACCERATTS		<b>100-10000</b>
19-6	Southern the	AGATA	AARE	TEREFORE TO			STOTO-BETETAKAROOOSATTE		DA-DAMA
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	2 BOTTETTE-	AGACA	AASS	TETECTROSC			RTCTC-BRTATAAAACCCBATTE		III AAAAA
	5 BOCTOTITE	AGACA	AARS	TETTETROCC			FTCTC-RETATAAAACCCRATTE		CO-COAAA
	4 BOCTOTTE-	AGACA	AAGG	TETETHERE		TERRICAATERAAT	STETC-CATATAAAACCTGATTS	CATRETICCATETACTR	RE-AAAAA
AL3962107	1 BOCTOTTS-	AGACA	AAGE	ATCTCCTRCCC	Т		RTCTC-BRTATAAAACCCBATTR		SIG-AAAAA
	1 BOCTOTTES	AGATA	AAGG	TETEETBETE		TREECAATERAAT	ETCTC-BETETAAABCCCBATTE	TATATTECATTTACTS	GA-GAAAA
	7 BOCTOTTE-	AGACA	AAGG	TETRETHEE			RTCTC-BRTATAAAACCCBATTE		EE-AAAAA
	a socretts-	AGACA	AAGG	TETRETRESS			RTCTC-BRTATAAAACCCBATTE		EE-AAAAA
	5 BOCTOTTER	AGACA	AAGG	TETECTROE			RTCTC-BRTATAAAACCOBATTE		RG-AAAAA
	1 BOCTOTTS-	AGACA	AAGG	TETECTROE			RTCTC-RETATAAAACCCRATTE		SS-AAAAA
	2 -CCTCTTTS	TEATA	AAGE T				STCTC-BETETAAAACCCBATTE		GL-GUUU
	7 BOCTOTTE-	AGACA	AAGG	TETRETRESS			STOTO-BETATAAAACCOBATTE		BB-AAAAA
	1 BOCTOTTE-	AGACA	AAGG	TETRETREDEC			втете-ветаталалеесскатте		BB-AAAAA
AP003716,	4 SCCTCTTS-	NGACA	NAGE	TCTCCTRCCC		1999CAATSSAAT	RTCTC-BRTATAAAACCCBATTR	TATBCTCCATCTACTS	GG-AAAAA

PanT-5	CA	ASTETSAAATATESECETCSTERSAASSEAAA	C-STOCCCCARCCCGACACCCCSTAAASSET CTGTGCTGASSASSATTAS	BARBAARRAT
PanT-B	CA	ARTCTRAAATATERCCTCRTBRRAARBRAAA	C-STCCCCCARCCCCACACCCCTAAASSETCTSTSCTGASSASSATTAS A	GARGAAGECAT
PanT-9	CA .	AETCTEAAATATERCCTCHTEREAARERAAA	C-BTCCCCCABCCTBACACCCDTAAABBBTCTBTBCTBABBAABATTAB A	RANDAARDAAT
Second	CA .	AETCTEAAATATERCCTCHTEREAARERAAA	C-BTCCCCCABCCCBACACCCDTAAABBBTCTBTBCTBABBAABATTAG A	RANDAARDAAT
Sortilla-1	CA .	AETCTEAAATATERCCTCHTEREAARERAAA	C-BTCCCCCABCCCBACACCCDTAAABBBTCTBTBCTBABBAABATBAB	RADDAADDCAT
Bortilla-2	CA .	AETCTEAAATATERCCTCHTEREAARERAAA	C-BTCCCCCABCCCBACACCEBITAAABBBTCTBTBCTBABBAABATTAB	RANKAARKAT
MacacaN-2	CA	AETCTEAAATATERCCTCCTBREAAGAAAA	TC-ATCACCCARCTTRACACCCRTRARRENTCTRTRCTRARRANDATTAA	RAGGAMAGING

PanT-2 PanT-5	-DETECTING- AGA BEETECTING- AGA BEETECTING- AGA BEETECTING- AGA BEETECTING- AGA BEETECTING- AGA BEETECTING- AGA BEETECTING- AGA	TA AA CA AA CA AA CA AA CA AA CA AA CA AA CA AA		TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE TETESTHESE	THERE WITH THE WATE THE WATE THE WATE THE WATE THE WATE THE WATE THE WATE THE WATE	налагата нестоя на полна	ТИТАТТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ ТИТИТСЕМТЕТИСТВ TUTUTCENTETUCTB TUTUTCENTETUCTB	C 555-AAAAA 555-AAAAA 534-5AAAAA 555-AAAAA 555-AAAAA 555-5AAAA
PanT-9	BOCTOTTE- AGA BOCTOTTE- AGA	20. B.B.	100	TETRETROC	THEREAATER	AATGTETE-GTTATAAAACCCGATTG AATGTETE-GGTATAAAACCCGATTG		85- <i>1</i> 000
SainiriS Sorilla-1			86	TETRETREE		AATGTETE-GGTATAAAACCEGATTG AATGTETE-GGTATAAAACCEGATTG		
	ROCTOTIG- AGA ROCTOTIGE AGA	CA AA TA- AAA	86	TETETREE TETETREE	THEREAATER	АЛТИТСТС- ПИТАТАЛАЛСССВАТТИ МАСИТСТВ-ИНТИТАЛАСССВАТТИ		60-0000 60-60000
PlacacaPI-2	ecciciteee way						JADAIJERATTACTA	1 11
	241	DAC-BP TR	ъ.,	ini .	winde	и4 385		
CC-1	ABRTDERR-ATTORN				TTAAARTS	GATGTTTATGTGTAT GATGTTTATGTGTAT		
CC-31	CORCTTA-GERCIEGA		A C .			EXTERTITATETETAT		
	ASSETTA-BERTERA		A C A C	CAATACTRETTT		GATGTTTATGTGTAT		
	CORCUTA-BERTERA CTROCTTA-BERCTERA		8 E -	CAATACTRETTT		GATETTTATETETAT GATETTTATETETAT		
BG-1	ABRT2688-ATT20820		ьĒ.	CAATACTRETTT	TANAGC	алтатттатытытат алтатттатытытат алтатттатытытат		
	COROCTTA-BERCTERA COROCTTA-BERCTARA		A C .	CAATACTRETTT	WTAAARC	GATETTTATETETAT GATETTTATETETAT		
	COSCUTA-GOOCTAGA		- E	CAATACTRETTT	TATAAART	GATGTTTATGTGTAT		
2877-0.	total and the second second second	A		10 Local Collect Miller Line	TTAATGA C	GATETTTETETAT		
	CORCUTA-BERTERA CTROCTTA-BERATERA		С А. С	CAATACTRETTT CAATACTRETCT		GATGTTTATGTGTAT GATGTTTATGTGTAT		
A31-31	CORCUTTA-GEOCTORIA		E.	CARTACTRETT	TRAAARC	GATETTTATETETAT		
M-1 M-2	CCACCTTA-GENETIESA		8 E-	CAATACTRETET	TTAAGAC	GATETTTATETETAT		
A4-3	COACCTTA-GENETINA	A	ы Сл.	CAATACTRETTT	TTAAGAC	DATETTTATETETAT DATETTTATETETAT		
AS-1	ССАЛССТТА-БЛЯСТВВА ССБОСТТА-БЛЯСТВВА ССАЛССТТА-БЛЯСТВВА ССАЛССТТА-БЛЯСТВВА ССБОСТТА-БЛАСТВВА ССБОСТТА-БЛАСТВВА ССБОСТТА-АЛЯСТВВА ССБОСТТА-БЛАСТВВА ССБОСТТА-БЛЯСТВВА		A CA	CAATACTRETTT	THAAARC	GATETTTATETETAT		
85-2 85-3	CORDETTA-BRACTERA COOPETTA-REACTERA		8 CT. 8 C	CAATACTRETET		GATHTTTCTHTATAT GATHTTTATHTHTAT		
HC-1	CORCTTA-REPORTA		Ē.	CAATACORCTTT	TTAAARC A	GATGTTTATGTGTAT		
	CORCUTA-BERTERA CORCUTA-BERTERA		C C	CAATACTRETTT	WTAAARC	GATGTTTATGTGTAT		
	COSCUTA-GOOCTIGA		- E	CAATACTRETTT	TATAAANE:	БАТЫТТТАТЫТЫТАТ БАТЫТТТАТЫТЫТАТ БАТЫТТТАТЫТЫТАТ БАТЫТТТАТЫТЫТ БАТЫТТТАТЫТЫТ БАТЫТТТАТЫТЫТАТ		
HG-0	AGECTTA-GERTIERA	11.0	A CA	CAATAPTRETT	ISTANAR 🗩	GATELTTATETATAT		
	CORCUTA-BERTERA CORCUTA-BERTERA		С. С	CHATACTRETT	TANAR C	GATET TTATETETAT		
183-4	CORCTTA-GERCTERA		C	CAATACTRETT	BTAAME	GATHTITATHTHTAT OV		
	CARCETTA-AGRETIGA CCACETTAAGGETIGA	A.	A CD	I B CAATATTRETET	TTAATSE E	BRITHTTHTHTATET BATHTTTATHTHTAT		
	COSCUTA-REACTERA		a c. A C.	CAATACTRETET	TTAAGGC	GATETTTATETETAT		
	CORCTTA-ARCTREA			CAATACTRETTT	TTAAAR	EATETTTATET		
	CTROCTTA-BERCTERA CTROCTTA-BEACTERA		A È A CT A CT	CAATACTRETT		GATATTTATGTGTAT		
313-4	CORCETTA-GRACTERA		A CT	CAATACTRETET	TTAAGGC	GATETTTATETATAT		
	CORCUTA-BERTERA CORCUTA-BERTERA		C C	CAATACTRETTT	TANAN T	GATETTTATETETAT GATETTTATETETAT		
313-77	AGECTTA-GERTIERA		E.	CAATACTRETTT	TRAAATC	GATGTTTATGTGTAT		
	CORCUTA-BERTERA CORCUTA-BERTERA		E A CA	CRATACTRETTT		GATGTTTATGTGTAT GATGTTTATGTGTAT		
	COSCUTA-GOOCTIGA		e De E			GATGTTTATGTGTAT		
	CORDITIA-GERCIERA		E	CTATACTRCTTT		EATETTTATETETAT		
	CORCUTA-BERTERA CORCUTA-BERTERA	A.	е 1. ст.	CANTACTRETET		GATETTTATETETAT BETETTTA		
MP-2	CORCUTTA-GEOCTORIA				TTAASTC	GATETTTATETETAT		
	COROCTTA-REACTERA COROCTTA-ARRCTERA		A C A CT C	CAATACTRETET		GATETTTCTETATAT GATETTTATETETAT		
MP-5	CORCETTA-REACTERA		A ET	CARTACTRETET	TTAAGGC	GATGTTTCTGTATAT		
	COROCTTA-BERCTERA COROCTTA-BERCTERA		A R C	CAATACTRETET		GATGTTTATGTGTAT GATGTTTATGTGTAT		
57-1	CORCETTA-BERTIERA		A C	CAATACTRETTT CAACACTRETTT	TRAAATE	BATETTTATETETAT		
	CORDETTA-BERTIERA		8 C -	CAATACTRETTT	TTAAARC	GATETTTETETETAT		
93-1 93-2	CORCUTA-BERTERA CTROCTTA-BERTERA		A CT	CAATACTRETTT B CAATACTRETET	NTAAAGC TTAAGGC	GATGTTTATGTGTATAT GATGTTTATGTATAT		
SIJ-31	AGETTA-BEETEEA		8 C-1	CAATACTRETET	TTAAGAC	EATERTTATETETAT		
511-4 139-1	CTEACTTA-ABECTEEA CCECCTTA-BEECTEEA	A	$b = \frac{1}{2}$	AS BAATACTEATET CAATACTECTT		GATIFTETATETATAT GATIFTETACETISTAT		
19-2	CTECCTTA-REACTERA ACECCTTA-REACTERA		λ ÊΤ	CANTACTRETET	TTAAREC	BATETTTATETATAT		
19-3 19-4	ACROCTTA-BERCTERA COROCTTA-BERCTERA	TS	8 EE.	CAATACTRCTTT CAATACTRCTTT	TAAAARC TAAAARC	GATETTTATETETAT GATETTTATETETAT		
139-5	CORCETTA-BERCIERA		C C	CAATACTRETTT	TTAAARC C	GATETTTATETETAT		
	ABBITTA-BEACTREA		A CT	CAATACTRETET	TTAAREC	BATHTTTTTTTTTTATAT		
	CCROCTTA-BERCTERA CCROCTTA-BERCTERA		A C	CAATACTRETTT	TETAAARC	GATETTTATETETAT GATETTTATETETAT		
ACT 14500	CTROCTTA-GERCTREA		E	CANTACTRETT	TRAAARCS	BATHTTTATHTHTAT		
	COROCTTA-BERCTERA COROCTTA-BERCTERA		A C A C	CANTACTRETTT	TATALAR TALAR	GATETTTATETETAT GATETTTATETETAT		
APDINERIO, 3	CORCETTA-GERETAGA		C	CARTACTRETTT	TAAARC	BATETTTATETETAT		
	CCRCCTTA-BERCTERA CCRCCTTA-BERCTERA		C A C	CAATACTRETTT		GATETTTATETETAT GATETTTATETETAT		
	CORCETTA-REPETINGA		6 C -	CANTACTRETT	TRAAARC	BATHTTTATHTHTAT		
	CTROCTTA-BERATERA COROCTTA-BERCTERA		b = C - i	CANTACTRETET CHATACTRETET		GATHTTTATHTHTAT GATHTTTATHTHTAT		
	CCBCCTTA-BEBCTBBA		$h = \frac{C}{Ch}$	CAATACTRETTT	IN CONTRACT TETANAGE	BATHTTTATHTHTAT BATHTTTATHTHTAT		

А.555284. 1 ССЕССТТА-ВЕАСТИВА АСООНТИА. 7 ССЕССТТА-ВЕЕСТИВА АСПОЛЛАЛ. 2 ССЕССТТА-ВЕЕСТИВА		A	CT C C	СААТАСТВЕТСТІТААВВЕ БАТИТІТЕТИТАТАТ СААТАСТВЕТІТИТААВВС БАТИТІТАТИНІКА СААТАСТВЕТТИТАААВС БАТИТІТАТИНІКТАТ
ACISI133.5 CORCETTA-BERCTERA			C .	CAATACTECTITETAAAEC EATETITATETETAT
AF394944.1 CORCUTA-REPORTERA			C	CAATACTECTITETAAAEC EATETITATETETAT
ALATIETS 2 CARCETTA-ABRITERA		h = h		E CAATATTRETETITAATRE C BETETITETETACET
ACDI1447. 7 ICCACCTTA-GENETRIA		A		CAATACTRETTTETAAARC BATETTTATETETAT
AC105027.1 CTROCTTA-REPOTERA		A	C	CAATACTECTITETAAAEC EATATITATETETAT
APOCOTUS 4 CORCETTA-REPETIESA			C .	CAATACTRETTTETAAARC BATETTTATETETAT
ACODIGET COROCTTA-REPORTERA		A		CAATACTRETTTETAAARC BATETTTATETETAT
AC004979 COROCTTA-GERCTERA		A	CT.	E CAATACTECTCTTTAAGEC ESTETTTA
AC140755.3 COROCTTA-REPOTERA			E	CAATACTECTTTETAAAEC EATETTTATETETAT
ACOLOGIZ 2 COROCTA-REPORTERA		. A	- E	CAATACTRETETRTAAGRE GATETTTATETETAT
AC003976 CCECTTA-REECTERA		. A	- E	CAATACTECTTTETAAAEC EATETTTETETETAT
ACOUNTS & COROCTTA-REPORTERA			E	CAATACTECTTTETAAAEC EATETTTATETETAT
APODIGMS, 1. CTRUCTTA-REPUTIESA		. A		CAATACTRETETITAAGRE GATETITATETATAT
PanT-1 COROCITA-GRECTERA	8	- A-	E	CARTACTECTITETAARE EATETTACETETAT
PanT-2 COROTTA-GRECORDA			ES.	CARTACTECTITETAAREC ERTETTATETETAT
PanT-5 CORCETTA-REPETIESA			E	ATAATACTECTCTETAAGEC GATETTTATETETAT
PanT-8 CORCETTA-REPETIESA			E .	CAATACTECTITETAAAEC EATETTTATETEEAT
PanT-9 CORCETTA-REPORTA			E .	CAATACTECTITETAAAEC EATETTTATETETA-
Satisficial COROCTA-BRACTERA			E .	CAATACTECTITETAAAEC C EATETTTATETETAT
Fortilla-1 CORCTTA-GREETERA			E .	CAATACTECTITETAAASC GATETTTATETETET
Fortilla-2 CORCTTA-GREETERA			C	CAATACTECTITETAAASC GATETTTATETETAT
MacacaM-2 COROTTA-GRECORA		T	ETA	CAATECTECTETTATECAC AA AATETTTATEGAGAT

Abbreviations of each K10 LTR clone were represented in Table 4. Bars in the sequences indicate the gaps, and asterisks under the sequences do the identical sequences. Shadowed sequences are the conserved and corresponding to each signal at the top of the sequences. Gorilla, *Gorilla gorilla*; MacacaM, *Macaca mulatta*; Pant, *Pan troglodytes*; SaimiriS, *Saimiri sciureus*. AP-1, AP-1 binding site; CAC-BP, CAC-binding protein binding site; c-MYC, c-MYC binding site; GATA-1, GATA-1 binding site; GR1, glucocorticoid hormone response element 1, GR2, delayed secondary glucocorticoid response element; Sp1, Sp1 binding site; TGT3, TGT3 binding site; window 4 and window 10, human epidermal growth factor response elements.

#### APPENDIX II

Multiple aligned sequences of the L1Hs p40 gene from human cells



		6D CHARTER	201.001.001.	L companya and			CLOCK CONTRACT LINES
						ICTICRARAA	-GARCAACTCC-AAGA -GARCAACTCC-AAGA
						DETERMENT-	
						CTTTGAGAA	
						TETTEAAGAA	
1						ICCTICAAGAA	
						ICTICAAGAA	
						ICTICANEAA	-GARCAAC-TOCAAGA
						COTORAGA.	- GARCAACTIC - AAGA
						COTTORAGA	- GARCAACTIC- AAGA
			CARAGA	ATROCAC	<b>MAGATACT</b>		-BARCAACTCC-AAGA
						TOTTORAGAM	-สมสารมหา-การมนสม
							- GARCAACTOC-AAGA
						COTTORAGA	-GARCAAC-CC-AAGA
							AGAGCAAC-CONTAGA
1							- GARCAACTEC-AAGA
						COTORAGAG-	-GARCAACTEC-AAGA
						COTOGAGAA	-GARCAAC-TOCATEA
						CONCINCTION OF THE PARTY OF THE	- GARCAACTOC-AAGA
		анылты	<b>NEWGARA</b>	ACRESAC	<b>MAGATACT</b>	TETTEMARAA	-BARCAACTCC-AAGA
						TETTERARA	-GARCAAC-TECAAGA
							-6497447777-4494
						COTTOAGAA	-GARCAAC-TOCAGGA
							-RARCAR-TECARD
							-GARCAACTIC-AAGA -GARCAACTIC-AAGA
						DITIONARY	-GARCAACTIC-AAGA
						LICTICS AGAA	-GARCAACTOC-AAGA
	LIBROWANSTTCASATTC						- GARCAACTOC-AAGA
		GRAATA-	-CARAGA	ACROCES	ANAGATACT		-GARCAACTCC-AAGA
						ISTTEARA.	-GARCAACTICC-AAGA
							-GARCAACTIC-AAGA
	<u>DUNTAR</u>					CARCEAGA	- GARCAAC-TOCAAGA
							-RARCAR-TECARA
							-GARCAACTIC-AAGA -GARCAACTICCAAGA
						DITIONARY	- GAGCAACTIC - AAGA
						COTOSAGAG-	-GARCAACTOC-AAGA
						CONTRACTOR	-GARCATC-TOCAAGA
	ANATACAGAGAACGCCACAAAGATACTCCTCCAGAAGAGAGCCAACGTTCAGATTC						- GARCAACTIC- AAGA
			CARAGA	ATROCAC	AGAGATACT	AARAARITTEE	-BARCAACTCC-AAGA
1						TETTERARA	-BARDARS-TEDAAR
ļ						TELEVISION	-CARCAAC-TOCAAGA
	And an and the set of	- ALATA					
		20101220					-RAACACC-TECAAGA
			CURREN	ACTICAN	AMAGATICT		-GARCATCTCC-AAGA
	JEJU NATIONAL UNI	- GAMATA-	CARARA.	ACRONALS ATROCALS	TOTARAAA		-GARCATCTOC-AAGA -GARCAACTOC-AAGE
	JEJU NATIONAL UNI	- BAATA	CURRENT CURRENT CURRENT	ATROCAC ATROCAC ATROCAC	NARATOT NARATAT NARATAT		-GARCATCTCC-AAGA
	JEJU-NATIONAL UNI	GALATA DUATA GALATA GALATA	2000 2000 2000 2000 2000	ACREENS ATREENS ATREENS ACREENS ACREENS	NAGATOT NAGATAT NAGATAT NAGATAT	естериси естериси стериси стериси	- GARCATCTCC- AAGA - GARCAACTCC- AAGA - GARCAACTCC- AAGA - GARCAAC- TCCCAAGA - GARCAAC- TCCCAAGA - GARCAAC- TCCCAAGA
	JEJU NATIONAL UNI		2000 2000 2000 2000 2000 2000	Arteriae Atteriae Atteriae Arteriae Arteriae Arteriae Briteriae	NAGATOT NAGATAT NAGATAT NAGATAT NAGATAT	19771984644 19771984644 19771984646 19771984644 19771984644	- 6467471772- 4464 - 6467447772- 4464 - 6467447772- 4464 - 6467442- 7727464 - 6467442- 7727464 - 6467442- 77274474
	JEJU NATIONAL UNI		CARAGA CARAGA CARAGA CARAGA CARAGA CARAGA CARAGA	AMERICAN AMERICAN AMERICAN AMERICAN AMERICAN AMERICAN AMERICAN	АЛАСАЛТСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ	TOTTOLAGAA TOTTOLAGAA TOTTOLAGAA TOTTOLAGAA TOTTOLAGAA TOTTOLAGAA TOTTOLAGAA A	- 54524727122- 4454 - 54524427122- 4455 - 54524427122- 4454 - 5452442- 7122454 - 5452442- 71224474 - 54524442- 71224474
	DESU-NATIONAL UNI	GAAATA GAAATA GAAATA GAAATA GAAATA GAAATA GAAATA	CIDIO CIDIO CIDIO CIDIO CIDIO CIDIO CIDIO CIDIO	ATBOLAS ATBOLAS ATBOLAS ATBOLAS ACBOLAS ACBOLAS ATBOLAS	АЛАСАЛТСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ АЛАСАЛТАСТ		- GARCATCTCC- AAGA - GARCAACTTCC- AAGA - GARCAAC-TCC- AAGA - GARCAAC-TCC- AAGA - GARCAAC-TCC- AAGA - GARCAACTTCC- AAGA - GARCAACTTCC- AAGA - GARCAACTTCC- AAGA
	JEJU-NATIONAL UNI	GAAATA DUUATA GAAATA GAAATA GAAATA GAAATA GAAATA GAAATA	-0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101	ACREMAN ATREEAE ATREEAE ACREEAE ACREEAE ATREEAE ATREEAE ATREEAE	AUARATICT AUARATACT AUARATACT AUARATACT AUARATACT AUARATACT AUARATACT AUARATACT		- 545247772-4454 - 5452447772-4455 - 5452447-772-4454 - 5452447-7723454 - 5452447-7723454 - 5452447772-4455 - 5452447772-4455 - 6452447772-4455
	2E3U-WATIONAL UNI	CALATA DALATA DALATA DALATA DALATA DALATA DALATA DALATA DALATA DALATA	-0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101 -0.0101	ACREMAN ATRESAE ACRESAE ACRESAE ACRESAE ACRESAE ATRESAE ATRESAE ATRESAE	AAAGATTEET AAAGATTAET AAAGATTAET AAAGATTAET AAAGATTAET AAAGATTAET AAAGATTAET AAAGATTAET		- SARTATTTY - AASA - SARTAATTY - AASA - SARTAATTY - AASA - SARTAAT - TYYAASA - SARTAAT - TYYAASA - SARTAATTY - AASA - SARTAATTY - AASA - SARTAATTY - AASA - SARTAATTY - AASA - SARTAATTY - AASA
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HZ-4	TAATTETCARATTEACAAATTEAAATTEAAATTEAAATTAAAGEAAATTAAGEAAATTAAGEAAATTAAGEAAATTAAGEAAAATTEACAAATTEAAATTAAAGEAAAATTE	a
HC-5	ТИАТТЕГСИСКИТСКИ СТОЛИТЕКИ ТЕКИТОКИ ПОЛИТИКАТИ СТОЛИТСКИ СТОЛИТСКИ СТОЛИТСКИ СТОЛИТСКИ СКОТИКИ СТОЛИТСКИ СТОЛИ	
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Ju-8	TAATTETCACKAATTEAACTAAATTEAAATTEAAATTAAATT	8
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MD-31	TAATTETEASATTEACAAATTEAAATTEAAATTEAAATTAAASEAAATTAASEAAATTAASEAATTAASEAATTAASEAAATTAASEAATTAASEAATTAASEAATTAASEA	
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511-4	TAATEETOARATEAACAMAETEAAATEAAATEAAATEAAATEATEATEAARAAETAARAAETAARAETAATEATEATAARAETAATEATEATEATEA	
SIJ-5	TAATTETCAGATTEAAAATTEAAAATGAAGAAAAAAATTETTTAAGGGCAGGGAGAGAAAAAAAAAA	8
119-1	TAATTETCARATTEARAT	
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119-31	тактыздаятносомалтакталындынын тыттаддагардарында анадетеринден мартулады мартулартыкандарынан теренен	1
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L1Hz-6	TAATTSTCARATTCACCAAATTSAAATSAAATSAAATAAATTAABERCARCCARAGAA-AAABSTCARATTACCCTCAAABER-AABCCCATCARAETAACARCSRATCTCTCARCA	3
1.1He - 7	TAATTETCAEATTEACCAAAETTEAAATEAAAEAAAAAA-TETTAAEEECAECCAEAEAE-AAAEETEEEETTACCETCAAAEA-AAECCAACAEEEATCETCEEEAA	8
L1He-B	TATTETCASATTCACCAMETTEMATEAAEEAAAAA-TETTAREECASCCARAGA-AAEETCASTTACCCTCAAEEA-AAECCATCASCTACASCEAACTCCCESCA	2
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LibHe-10 LiHe-11	TAATTISTOAGATTOACCAMBITTIGAAATGATGAAAAAATTISTTAAGBECAGCAGAGAAAAAGATCAGGTTAACCCTCAMBIGTAACCATAGACTAACAGCAGAATCTCCCGBCA TAATTATCAGATTCACCAMBITTIGAAATGAAGAAAATTISTTAAGBECAGCCAGAGAGAAAGAAAGACCAGCCAGCAGAAAGAAAAGAAAGAAATCTCCCGBCAG	
Line-iii Line-ii2	TATTET ANT TAXAAN TRAATAAN ANA TITAAN WAA ANT TAXAN AND AND AND AND AND AND AND AND AND A	
L1Hz-13	INSTRUCTOR AND	
MRICHAD	TAATTETCAGATTCACCAAGTTCAAATTAAGGAAAAAA-TETTAAGGGAAGGAAAGGAAAGTCGGGTTACCCTCAAAGGG-AAGCCCAACAGCGGACTAACAGCGGACTCCCCCCCCC	
1906772	TAATTETCAEATTCACCAAAETTEAAATEAAEEMAAAAA-TETTTAEEERCAECCAEAEAE-AAAEETTCECTCAAEEA-AAECCACTAECAECTAECAECTETTEECA	8
1505771	TAATOSTO AGATTO ACCAMENTE ALARGAMANA - TETTA ARROVADO ACCAMENTA ACCONTRA A ARCONTO A ACCAMENTATION OF A A A A A	
150570	TATTETCASATTCACCAMETTEMATEAAEEAAAAA-TETTAREECASCCARAGA-AAAEETCEETTACCCTCAAEEA-AAGCCCATCAGACTAACAECEATCTCEECAE	
150559	TAATTETCASATTOACOAAETTEWA TEWA ENAMANA - TETTA AERECARCOA MARKI - MARKI CAREFORMET A ACCOUNT A ARCON COMPACTA ACCASION ATTO TORSAN TAATTETCASATTOACOA AETTEWA TEWA ENAMANA - TETTA AERECARCOA AURACIA AURECCARE TATACOC AURACTA AURACTA ACCASION A	
CONSUMPTION OF	ТАКТЕКТОКАТТОЛОСИМЕТТЕКАЛЕККИИМА-ТЕТТИКЕВСКЕССИНИКЕ-МАЛЕТСИЛЕТТАКОТОМИЕК-МЕССАТОМИСТАКОНСКИКТОТОВОМ	

– cxvii –

	243	300			360
89-1 89-2	AAAGTCTACAAGCCAGAAG-AGAGTGGGGGGCCAATATTCAAC AAACTCTTCAAGCCAGAAG-AGAGTGGGGGGTAATATTCAAC				
89-3	AAAATATAAAAGACAGAAG-AGAAGTIGGGGGCCAATATTCAAC				
101-4	AMACTETACAADEENGAAG-AGADTAGGGGECMATATTEMAC				
86-5 86-6	AAACTCTATAAGCCAGAAG-AGAGTGGGGGCCAATATTCAAC AAACTCTATAAGCCAGAAG-AGAGTGGGGGCCAATATTCAAC				
YJ-1	AMACTETATAARCCAGAGE-AGACTERERECCAATATTEMAC	ATTETTAAAAAAAAAATTTT-	-CARCON-GARTTCATATONAGE	AMETAIRCTTCTART	
9 <b>3</b> -2	AMACTETACAMICAGAMIC-AGACTIGANGECCAATATTCAAC	ATTETTAAATAAAAAAATTTT-	-CAACCEA-GAATTTCATATCCCCC	AMACTATECTTCATAAST	GAAGG
CC-1. CC-2	АМАСТСТИСИМИССИМИИС-ИСИКТОВИВНСКИ/ТИТСИИС ИМАСССИИСИМССИСИИС-ИСИКТОВОВНССИИТИТСИИС	ATTETTALAGEAAAAAAATTTT_	-CARLES-BARTICATATISALS	-AAALTAAALTTLATAAALT	
CC-31	AMACOCTACGAGOCAGAAG-AGAGTGGGGGCCAATATTCAAC	ATTETTAAAAAAAAAATTTT-	-CAACCE-AGAATTTCATTTCCARCE-	ARACTARCTICATART	
CC-4 HE-1	AMACCETACAAACCACAAAG-AGAATGEBEBECSAATATTCAAC AAACCETACAAACCACAAAG-AGAATGEBEBECSAATATTCAAC				TAAGG
HK-1 HK-2	AGACCETACAAGCCAGAAG-GEAGTGERERCCAATATTCAAC				TAAGG
HIC-31	AMOUTACMBOCAGAG-AGAGTTBOGGCCAACATTCAAC	ATTCTT	-CAACCCA-GAATCTCATATCCATCT	аласталасттеатлаят	
180-4 84-1	GAACTETATAAGECAGAAG-AGAGTGGGGGECGATATTEAAC AAACTECAAGECAGAAG-AGAGTGGGGGECSAATETTEAGE				
M-2	ATACCETACAAGECAGAAG-AGACTTIGGGGCCAATATTCAAC				
A4-31	AMACTETACIDAGENERARG-AGRETERERGENERATATTERRE	ATTETTAMOUNDATTTT-	-CARCEL-ASATTTCATATECASC-	CARCTARGETTCATARGT	
84-4 84-5	AACCTCTATAAACCAGAAG-AGAATGBBBBCCAATATTCAAC AAACTCTCCAAACCAGAAG-AGAATCBBBBCCAATATTCAAC				
A5-1	AAACTICTACAAGCCAGAAG-AGACTIGGGGGTCAATATTCAAC				
AS-2	AMACTETACIMACEMENTER TERRETER ATATTEME	ATTETTAAAAAAAAAATTTT-	-CARCECK-GRATTTCATATECAGES	AUACTAARCTTCATAART	
CE-1. CE-2	ΑλΑΤΤΕΤΑΛΑΑΘΕΥΔΕΑΧΟ-ΑΛΑΑΤΒΕΒΕΒΕΥΔΑΤΑΤΤΕΛΑΟ ΑΑΛΕΤΕΤΑΛΑΑΘΕΥΔΕΑΧΟ-ΑΓΑΕΤΒΕΛΕΒΕΥΔΑΤΑΤΒΕΛΑΕ				-TANAGG GAAGG
HC-1	ALLOTTACIMOTICAL ACCORDING MATATITAL	TTTTUQUUQUTTTT.	CAACCEA BAATTICATATICAGE	-MACTAGETTCATAGT	
HC-2	AAACCETACAAGCEAGAAG AGAGTERGE COAATATTESIAC	ATTETTALOGOULAGAATTTT-	-CARDOC-AGAATTTCATATICAGOS-	AAACTAARCTTCATAART	
HC-3 HC-4	AMOUTING AGE CAGAGE AGAST SECTOR AND A TOTAL - AMOUTING A SECTION AS A				
HC-5	AAACTCTACAAGCCAGAAG-AGACTTGGGGCCAATATTCAAC				
HL-1	AUACTETATAAGEEAGAGAGAGAGAGAGAGEESAATATTEMAC	ATTTTAAAAAAAAAATTTT-	-CAACCE-AGAATTTCATATCCARCE-	AMETAARTTCATAART	
Ju-1 Ju-2	AAACYCTACAAGCCAGAAG-AGAGTGGGGGCCAATATTCAAC ATACTCTACAAGCCAGAAG-AGAGTGGGGGCCAATATTCAAC				
10-2 Ju-3	AAACTCTACAAGCCAGAAG-AGACTGERERCCAATATTCAAC				
Ju-4	AMACTETACIMACEMARAG-AGASTREESERCEMATATTEMAC	ATTETTAAAAAAAAAATTTT-	-CARRESS-GRATTERTATICSEC	AUACTAARCTTCATAART	
Jo-5 MC-1	AMACTETACAAACCACAAA-AGAATTERRETCAATATTEAAC AAACTETACAAACCACAAA-AGAATTERRETCAATATTEAAC				
MC-2	AAACCETACAAGCEAGAAG-AGACTEGGAGCEAATATTCAAC				
M2-3	AMACOCTACAMECCAGAAG-AGACTERGARCCAATATTCAAC	ATTETTAAAGAAAGAATTTT-	-CAACEE-AGAATTTCATATECARCE-	ARACTARCTICATART	
MC-4 MP-1	AMACCETAMAAGCCAGAAG-AGAGTGGGGGCCAATATTCAAC AAACTETAMAAGCCAGAAG-AGAGTGGGGGCCAATATAAAAT				
M2-2	AAACTETACAAGETAGAAG-AGAGTEGEGETEAATATTEAAC				
MP-3	AMACOCTACAMECCAGAAG-ACAGTERERECCAATATTCAAC	ATTETTAAAAAAAAAATTTT-	-CAACEE-AGAATTTCATATECARCE-	ARACTARCTICATART	
MP-4 MD-1	AMACTETACAAACCACAAA-AGAATGEBEBECSAATATTEAAC AGACTETACAAACCACAAA-AGAATGEBEBECSAATATTEAAC				
MD-2			-CALETEA-BAATTTEATATECABLE-		
MD-3i	AMACTETAGAAGCAGAAG-ATAGTGGGGGCAATATTCAAC	ATTETTAAAGAAAGAATTTT-	-CAACCE-CEAATTTCATATCTARCE-	ARACTARCTICATART	
MD-4. MD-5	AMACTETACAAACCACAAA-AGAATTERREECSAATATTEAAC AAACTETACAAACCACAAA-AGAATTERREECSA-TATTEAAC				
SH-1	AAACTICTACAAGCCAGAAG-AGACTIGGGGGCCAATATTCAAC				
SH-2	AAACTETACAAGECAGAAG-AGAGTGGGGGCCAATATTCAAC				
594-3 594-4	AMACTETACAAACCACAAA-AGAATGEBEBECSAATATTCAAC AAACCETACAAACCACAAA-AGAATGEBEBECSAATATTCAAC				
281-5	AAACCETACAAGCEAGAAG AGAGTEGGGGGCEAATATTCAAC				
50-1	AMACTETACMARCAGMAG-AGARTERERERCAATATTCMAC				
50-2 50-3	AMACTETACAAACCACAAA-AGAATGOODDAAAAATATTEAAC AAACTETACAAACCACAAA-AGAATGOODDAACAATATTEAAT				
31-1	AAACHCTACAABCCABAAG-AGAETUBBURCCAATATTCAAC				
SI-2	AMACRETACAARCCAGAAG-AGAGTERERERCCAATATTCAAC				TAAGG
203-3 203-4	AAACTCTACAAGCCAGGAG-AGAGTGGGGGCCAATATTCACCC ATACTCTACAAGCCAGAAG-AGAGTGGGGGCCAATATTCAAC				
31-5	AAACTETACAABCEAGAAG-AGAGTEBEBBBCEAATATTCAAC				
199-1	MACTETACAARCEAGAAG-AGAGTIGEGEGECAATATTEAAC	ATTETTAAAGAAAAGAATTTT-	-CAACCC-ARRATTTCATATCCARCC-	-AAACTAARCTTCATAART	
19-2 19-3	AAACTCTACAAGCCAGAAG-AGAGTUBUBBECCAATATTCAAC AGACCCTACAAGCCAGAAG-AGAGTUBUBBECCAATATTCAAC				GAAGG
LiHe-1	AAACCETACAABCEAGAAG-AGAGTEBEBBBCEAATATTCAAC				
L1He-2	ANACCETACHARCEAGAAG-AGARTIRBERECEAATATTEAAC	ATTCTTAAAGAAAAGAATTTT-	-CAACCC-AGAATTTCATATCCARCC-	AAACTAARCTTCATAART	GAAGG
LiHe-3 LiHe-4	AMACCETACAARCCARAAG-AGAETGREERECCAATATTCAAC AMACTETACAARCCARAAG-AGAETGREERECCAATATTCAAC		-CAACCC-AGAATTTCATATCCARCC- CAACCC-AGAATTTCATATCTGRCC-		GAAGG
Line-5	AAACCETACAABCEAGAAG-AGAGTEBEBBBCEAATATTCAAC				GAAGG
L3Hz-6	ANACCETACHARCEAGAAG-AGARTIRBERECEAATATTEAAC	ATTCTTAAAGAAAAGAATTTT-	-CAACCC-AGAATTTCATATCCARCC-	AAACTAARCTTCATAART	GAAGG
LiHe-7 LiHe-8	AMACTETACAARCEARAAR-ARAFTRERERECEAATATTEAAC AMACEETACAARCEARAAR-ARAFTRERERECEAATATTEAAC				GAAGG
Line-9	AAACCCTACAABCCAGAAG-AGAGTBEERBECCAATATTCAAC				

LINCEDED	TAATTE	TICAGAT	TEACO	WARTER	AATGAAGG		TETTAA	ana A	<b>BCCAGAG</b>	86-88	American	RTTACCC	TOAAAG	8-88B	TEATENEA	CTAACAS	<b>CONATCT</b>	CTICERC	246
LINGERTS	TAATTE	TENENT	TEMES	WARTER	AATGAAGG	MARA -	TRUTAN	and A	accusies	86-88	AGETTER	RTTACCC	TOWAR	8-888	TEATENEA	ETAADAS	<b>CORATET</b>	CTICARE	246
LINGUMAN	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETTAA	eeech	eccheke	86-88	ARCTORE	RTTACCC	TOWAR	a-aan	<b>NRACITACIC</b>	CTAACAS	<b>CORATET</b>	CTICHER	36
LINGUARCE	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETTAA	eeech	eccheke	86-88	ARCTORE	RTTACCC	TOWAR	a-aan	<b>NRACITACIC</b>	CTAACAS	<b>CORATET</b>	CTICHER	36
AL590130	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETTAA	eeech	eccheke	86-88	ARCTORE	RTTACCC	TOWAR	er-AAR	<b>NRACITACIC</b>	CTAACAS	RAGATET	CTTHE	36
AC130.150	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETTAA	eeech	eccheke	86-88	ARCTORE	RTTACCC	TOWAR	er-AAR	<b>NRACITACIC</b>	CTAACAS	<b>CORATET</b>	CTICHER	36
AL096710	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETAAA	eeech	eccheke	86-88	ARCTORA	RTTACCC	NCAAAR	er-AAR	<b>NRACITACIC</b>	CTAACAS	RAGATET	CTICHER	36
Amostory	TAATTE	TENENT	TEACO	WACTER	AATGAAGG	MANAG-	TETTAA	eeech	eccheke	86-88	ARCTORE	RTTACCC	NCAAAR	er-AAR	<b>NRACITACIC</b>	CTAACAS	CARACT	CTICARC	36
AL139136	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETTAA	eeech	eccheke	86-88	ARCTORE	RTTACCC	TOWAR	er-AAR	<b>NRACITACIC</b>	CTAACAS	RAGATET	CTICARC	36
AC099945	TAATTE	TENENT	TEACO	WARTER	AATGAAGG	MANAN-	TETTAA	eeech	eccheke	86-88	AGETAGE	RTTACCC	TOWAR	er-AAR	<b>NRACITACIC</b>	CTAACAS	<b>CORATET</b>	CTICHER	36
ACLD675D	TAATTE	TEAGAT	TEXES	WARTER	AATGAAGG		<b>AATTAN</b>	ana A	anskola	86 - RA	ARCTICES	RTTACCC	araaan	a AAR	NRACITACIC	RTARTAS	<b>CTEATT</b>	CTTREE	144
ALIGH176	TAATTE	TEAGAT	TEXES	WARTER	AATGAAGG		TETTAA	ana A	anniala	86 - RA	ARCTICES	RTTAACC	TOWNE	a AAR	NRACITACIC	станска	<b>EXAMPLE</b>	CTTHE	346
AL157930	TAATTE	TEAGAT	TEXES	WARTER	AATGAAGG		TETTAA	ana A	anniala	86 - RA	ARCTICES	RTTACCC	araaan	a AAR	NRACITACIC	станска	CARATET	CCT HAR	346
ACOSCORE	TAATTE	TEAGAT	TEXES	WARTER	AATGAAGG		TETTAA	ana A	anniala	86 - RA	ARCTICES	RTTACCC	araaan	a AAR	NRACITACIC	TTAACAS	<b>STRATE</b>	CTRACT	346
AP305553Y	TAATTE	TEMENT	TOACG	WARTER	<b>AATGAAG</b>	aaaa-	TETTAA	ATTREE A	accusus	86-88	AGETCHE	STRACCC	TOWNE	a-wa	SCIENTISAGA	сталсая	CHEATCH	CTICAGE	348
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L164e-10	INACCETACIARECARIAG-ARARTHERERERIATATICIARCATTETTAARAAARAATTTCAACCE-ARAATTCATATCARCAACTAARCTTCATAART	-GAAGG
		-GAAGA
	MACTER/CARRENDERS/ ADAPTER/REFECA/ATA/TEA/CATTET/AA/RAA/RAATTTCAACESARACTECA/ATECA/REEAA/ET/AA/RAATA/RAATTECA/REEAA/ET/AA/RAATA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REEAA/ET/AA/RAATTECA/REE	
	AAACCTTACAABCCARAAE-ARAETHRABICCAATATTCAAC-ATTTTTAARAAABAATTTTCAACCT-ARAATTTCATATCCARCC-AAACTAARCTTCATAARC	
MRICHAD	MACCTERCOMBICS/MINING_ADMINING ADMINISTRATION/CKTEETIMAADMAADMATTITCOMCCADMATTICATINISS/005AMACTICATINADTITICATINISS/005AMACTICATINISS/005_	
LINGENTE	MACCENERATE ADMINISTRATING ADMINISTRATICAL AND ADMINISTRATICAL ADMI	
150571	MACCETACARECORDER ADMITHEREECONTATIONS - ATTETNAMENAADMATTT - CANCEL ADMITTCATATEORIS - MACTINEETTOTIARET	
LISCENTE	AMACCETACAMESCARAME ADMITTREBERECOMMATINACE ATTICTIONAGE ADMITTEE CAMOUS ADMATTICATATICS ADMITTECTATION ADMITTECT	CILLING .
	AAACCETACAABCEARAR ARAFTERERECOMMATTOAC ATTELLARRAAAARAATTE CAACTE ARAFTERERECOMMATTOACTAR	
190569		
LEGENE	MACCETACMBCCABME_ABARTHEBBECCAATATTCABCATTCTTMABAAABAATTTCAACCE-ABAATTCATATCCABCMACTABCTTCATARC	
150566	MACCETACMBCCAGAGE AGAITMEENDOCAATATTCMC-ATTETTAAGAAAGAATTT-COACCE-AGAATTCATATCCAGC-MACTAGETTCATAGE	
150565	AMAYYYAAMAYYAAMA AMATHAAMAYAAC AMATHAAMAA AMAA AMAA AMAA AMAA AMAA AMA	
1303563	AMAYYYAAMAYYAAMA AMATHAAMAYAAC AMATHAAMAA AMAA AMAA AMAA AMAA AMAA AMA	
100562	AMAYYYAAMEYYAME AGAFTEREETYAATATYAACATTYTTAAGAAMAAATTTYCAAYYY-AGAATTYATYYAEY-AMAYTAMEYTYAMAAT	
	AMAYYYAAMEYYAAME AGAFTEEREYYAATATTYAACATTYTTAAGAAMAAATTTYCAAYYY-AGAATTYYYARY-AMAYTAARYTAARY	
AC131351	AMALLETACAMELEMAG-AGALLEGAGLESAATATISAACATTETTAAMEMAMMAATTTTCAMLES-AGAATTTSATALESAGLE-AMALLAGAATTSATAAT	
AL09671D	AMACTERAMACEAGAAGEAGAAGEAGAGEAGAATATTEAACEAATTERAAGAAAGAATTTTCAACEE-AGAATTTEATATEEAGEE-AGAACTAAGETTEATAAGT	-60466
	AMACTERACAGENAGAAGENAGAAGENAGAATATTENAACATTETTAAGAAAGAATTTTCAACEN-GAATTTEATATECAGECAMACTAAGETTEATAAGT	
	AMAGETACMAGEYAGAAGE-AGAGTBERREESAATATTSAACATTETTAAGAAAAGAATTTCAACES-AGAATTTSATATESAGES-AMAETAAGETTSATAAGT	
	AAACCCTACAAGCCAGAAG-AGASTGEEERCCAATATTCAACATTCTTAAAGAAAGAATTTCAACCC-AGAATTTCATATCCARCC-AAACTAAGCTTCATAAGT	
	AAACTCTAAAAGCCAGAAG-AGASTGEEERCCAATATTAAATACTCTTAAAGAAAACATTTCAACCC-AGAATTTCATATGEARC-AAACTAAGCTTCATAAGT	
ALIGHT76	AMACTACAMAGONGANG-AGASTTERRECONTATIONACATTCTTANAGANAGASTTTOMOOD-AGASTTCATATOONROC-AMACTARGOTCATAATT	-88469
AL157620	AACTICTATAAGCYGAAG-AGASTIGEEERCCAATATTCAACKTTCTTAAGAAAGAATTTTAACCCA-GAATTTCATATCCARCC-AAACTAAGATTCATAGAT	-GAAGO
	MACTERAMICSAGAAG AGASTGGGGGGAATATTEAACATTETTAAAGAATTTTCAACEA-GAATTTEATATCAGC-AAACTAAGCTTATAAST	
	MARCCTACAAGCAGAAG-AGAETGREGECAATATTCAACATTCTTAAGAAAGAATTTCAACCC-AGAATTTCATATCCAGCC-AAACTAAGCTTCATAAGTTCATAAGT	
	262 420 425	
86-1	AGAATAAATA-CTITA-CAGACAAG-CAAATECTEAG-AGATTTETCACCACA-AGECTERCETAAAGAAGEAGECTAAAGAAGEATAAACATEGAA	
86-2	ADMATAMATE-ETTTA-EMEMORY-EMANTEMENT AND THE THE AND THE ADMATA AND THE ADMATAMATEMENT AND THE ADMATAMATEMENTAMATEMENTAMATEMENTAMATAMATEMENTAMATEMENTAMATAMATEMENTAMATAMATEMATAMAT	
10-3	ADMATAMATA- CTITA- CABACAAE- COATRETENE- ABATTITATOACCAGACROCTRACEMANAGACCTRAARDARCACTAAACATREAAA	
101-4	ADDATADATE CITE: CARAGAS CONTRETENDERTITIETER/CONT. ADDITECTION DARTETERADOR ADDITATE	
86-5	ADARTMANTC-CTTTA-CATACAUR-COMMETTING-ARACT TRITCAC-CATC-ARACTERCTCTALARTMETTCTTRAARRACACTAAACATRAAA	
86-6	ADARTMANTC-CTTTA-CATACAUE-COMMETTING-ARACT TRITCAC-CATC-ARACTERCTCTAAARTIC-CTTTAAARTACACTAAACATRAAA	
YJ-1	ARMATAWATT-CTTTA-CARMONAE-CAMACE-TEME ARATTECHTOXCATC-ARECTERCTERCTERCTERCAREARETCTERUMER-REVIETAACATERIAA	
YJ-2	ADMATAMATANT CO. CAMPANA. COMPRETING ADMITTING C. CONT. ADMITTING ADMITTING ADMITTING ADMITTING ADMITTING ADMIT	
CC-1	ADMATAGATE CETTA CAREFAGE CUMPETIAL AGATTINEAC - CAE- ARECTEDITED AMARCTEDITEMARACIAE AREA AND A	
EE-2	ADMAN AWAN A CITTA TRANSMISSION AND TRANSMISSION CONTRACTOR AND A	
	REPARTAMENTAL CITTAL REACTAGE CAN THE THE TAX CALL - AND CITED CITED CONTRACTOR TO CONTRACT AND CAN THE AND CAN	
CC-3	ARAMEMANATA-CITTA-OARATAAC-OAATRETEAC-ARATTITETCACRACC-ARROTECCETAAARARETCETEAARRAARAACAETAAACATREAAA	
CC-4	ASAMTAMATA-CTTTA-TASACAAC-CAAATATTSAC-ASATTTTATCACCACC-ASSCTSCCTAAAASACTCCTSAASSACACSCTAAACATSAAA	
HE-1	AGMATAMATC-CTTTA-OKRADAR-CMARTERING-AGATTTITRIOCOKCC-AGRCETIACHTAARGETCETIAMATAMBCACTAA-TATBIACA	
HE-2	AGMATAMATA-CITTA-CACACAG-CAMATATINAG-AGATITINTCACCACC-AGENCTICCTICAAGACTCETIGAGENGCEETAAACATBERAA	
HE-31	ACMATAMATC-CTTTA-CAGACAUG-CAMATECTING-ACATETITATCACCACC-AGENETICOFTAMACAERTECTIMACEMECACTAMACATAGAMA	
FIE-4	АЗААТАААТТ-СТТТА-САЛІСИС СААТОСТИС ДОЙТТГАТСІС – СКСС-АЛАСТРОСТТАСАЛІВСТСТААСАЛІЗАЛІСАТІААСАТВИА	
84-1.	AGMATAMATC-CTTTA-CAGACCAG-CAMATACTAAGAAATTTAATCAC-CACC-AGACCAGETTAMAGAAGATCETAMAGAAGAACTA-ACATAMA-	
84-2	AGAAATAAAATA-CTTTA-TAGACAAG- <mark>D</mark> AATRETBAG-AGATTBERCACCACC-AGREETBECTAAAAGACTECTBAAGBAAGEALTAAACATBEAAA	
84-3	AGMATAMATA-CITTA-CAGACAAG-COMATTERIAG-UGATTTREACCACC-AGOCTRECCTAMAGAGOCTRAAGAAGAACAAGAAGAAGAAGAAGAAGAAGAAGAAG	
Ad-4	AGAAATAAAATC-CTTTA-CAGACAAG-CAAATGCTGAG-AGATGTTGTCACCACC-AGGCCTGCCTTACAAGAACTCCTGAAGGAAGCACTAAACATGGAA	
84-6	AGAATAAAATC-CTTTA-CAGACAAG-CAAATACTIAG-AGATTTTATCACCACC-AGECCTRCCCTAAAAGACTCCTGAAGAAGTTCTAAACATGGAAA	
AS-1	AGAAATAAAATA-CTTTA-CAGACAAG-CAAATATTEAG-AGATTTTETCACACCC-AGGETECCCTAAAGACTCCTGAAGGAAGGETAAACATGGAA	
AS-2	АБАААТААЛАТИСТТ-ТА-САБАСАББ-САААТАСТБАБ-АБАТТТТТТСКССААС-АБВССВСССТАААСАБСТССТБААББААБСАСТАААСАТВВАА	
035-0	AGAAATAAAATA-CTTTA-TAGACAAG-CAAATACSAG-AGATTTTGTCACAACSACSAGGC-CGCSSTAAAAGGGGCCTGAAGGAAGSSCTAAACATGGGGA	
CR-2	AGAAATAAAATCACTTTA-CAGACAAG-CAAATGCTGAG-AGATTTTGTCACCACCCAGGACTGCCTTACAAGAGCTCCTGAAGGAAGCACTAAACATGGAAA	
HC-1	AGAAATAAAATC-CTTTA-CAGCCAAGAAATGCTGAG-AGATTTTGTCACCACC-AGACCTGCTGCTGAAGGAAGCACTAAACATGGAAG	
HE-2	AGAAATAAAATA-CITTA-CAGACAAG-CAAATGCTGAG-AGATTTIGTCACCACC-AGGCCTGCCTTACAAGAGCCCCTGAAGGAAGCACTAAGCATGGAAA	
HC-3	AGAAATAAAAATA-CTTTA-CAGACAAG-CAAATGCTGAG-AGATTTTTTCCCCACC-AGGCCTGCCCTAAAAGACTCCTGAAAGAAGCGATAAACATGGAAA	
HC-4	AGAAATAAAAATA-CTTTA-CAGACAAG-CAAATGCTGAG-AGATTTTTTTCACCACC-AGGGCTGCC-TAAAGACTCCTGAAGGACGCCCTAAACATGGAA	
HC-5	AGAAATAAAAATA-CTTTA-CAGACAAG-CAAATGCTGAG-AGATTTTTTCCCCACC-AGGCCTGCCCTAAAGAGCTCCTGAAGGAGTGCTAAACATGGAA	
HL-1	AGAAATAAAAATA-CTTTA-CAGACAAG-CAAATGCTGAG-AGATTTTTTTCACCACC-AGGCTTGCTTTAAAGAGCTCCTGAAGGAGCACTAAACATGGAA	
Ju-1	АБАААТААААТА-СТТТА-САБАСААБ-САААТАСТОАБ-АБАТТТИТСКССАСС-АБВОСТВОССТАААБАВСТОСТБААББААБТВСТАААСАТВВАА	
Ju-2	АБАААТААААТА-СТТТА-САБАСААБ-САААТИСТБАБ-АБАТТТТИТОССАСС-АБВОСТВОССТАААБАВСТОСТБААББААБСАСТАААСАТИВААА	
Ju-3	АБАААТААААТА-СТТТА-САБАСААБ-САААТӨСТӨАБ-АБАТТТТӨТСӨССАСС-АБВССТВСССТАААБАВСТС-ТБААББААБСЫТАААСАТВБАА	
Ju-4	АБАААТААААТА-СТТТА-САБАСААБ-САААТАТТБАА-АБАТТТТКТСКССАСС-АБВССТВСССТАААБАБВСТААББААБСАСТАААСАТБААА	
Ju-5	ADAATAAATACTT-TA-CADACAAB-CAAATACTDAS-ADATTTTISTCACCACC-ADACTTISTCTTAAADARTTCTTBAABAARTACTAAACATBBAA	
MC-1	ADAATAAATA-CTITA-CADACAAB-CAAATTETBAS-ADATTETBAS-CACK-CACK-ADDATTETS-TAAADADATTETBAADAADATAADAAADAA	
MC-2	ADMATAMATA CTITA EMERCIAS COMPACTING ASATTTENTAZ - CAT- ADMITTENTIALMENT TATABASET TATABASET AMAZATEMAA	
MC-3	ADAATAAATA- CTITA- EMERANA- AAATBCTBAG, AGATTITATAA (AGA- ABBCTBASSAAAABABCTACTBAABAABCTAAAATBBAAA	
M2-4	ABMATMAATT-CTTTA-CMEMCAME_CMAATE-TEME_ABATTTENTOXCMT-ABENTERCTENTAMAEMETTETTAMAEMETETAAACMTEMAA	
MD_1	ATAVAATA-CTITA-CAEVASAA-CAVATE:TEME-AEVATTETEVC-CVCC-AEVACTECTTACVAEVACTCTAVAEVACV-TVAACATEVAA	
MD_7	ADMATAMATA CITTA CRACAR AMATRITIA CATTATITA C. C. C. MERCHENTER AMARTICI TRANSMERTE CARACTERIA	
MD_7	ADMATAMATE - ETTA - OMACAME - OMATBETRAE - AMATTERITAL - OVE - AMETERICTIA/MARKETETRA/MARKATATA/ATMAKA	
MP-3 MP-4	ADMATEMENT - CETTA - CARACAME - CAATEMENT - AN ETTERIA - AN ETTERIA - CAE - CAE - AMERICAETTERIA ANALISTICE DAMATA ANALAS A AMARA	
MD-1		
MD-2	ADMAN TAMATA - ETTIA- MARA AMERIKAN TAMATA AMATA	
MD-3	ARMATANANTC-OCTTA-CARCARE-CAATRETERS-REATTITETC/CCACC-ARROTROCTAAARRETOCTEAA-BARRACACTAAATATREAAA	
MD-4	ARMATMAATA-CTTTA-OARACAAR-OAATRETENE-ARATTITETCACCACC-ARROTEACETAAARARETCETEAARBARTRETAACATREAAA	
M0-5	ARMATMAATA-CTTTA-OARACAAR-COAATTCTBAG-ARATTCTRTCACCACC-ARRC-TRODCTAAARARCRCRCTTAARRAARAACACTAAACATRRAAA	
SM-1	ARAWITAWATC-CITTA-OARACAAD-OAATRETEND-BEATTITETCACOACC-ARROTECOCTAWARARETCCTEMARRAROACTAACATREMA	
28-2	ABANATANAATC-CTTTA-CABACAAB-CANATBETERAB-ABATTTTETCACCACC-ABBCCTBCCTRACETCCTBAABBAABCACTAAACATBBAAA	
284-38	ABAWATAWATC-CTITA-CABACAAB-CAWATBCTBAB-ABATTTTHTCACCACC-ABBCCTBCCTBCCTBAABBABCACTABAABCACTABACATBBAAA	
286-4	ABANATAANATA-CTITA-TABACAAB-CAAATBCTBAB-ABATTTTBTCACCACC-ABBCCTBCCTBCCTBAABBABCBCBCTAAACATBBAAB	
286-55	ABAAATAAAATA-CTTTA-TABACAAB-CAAATRCTBAG-ABATTTIRTCACCACC-ABRCCTRCSCTAAAABARCTCCTBAARBAARCSECTAAACATRBAAA	
50-1	ABAAATAAAATA-CTTTA-CABACAAB-CAAATRCTBAG-ABATTTIRTCACCACC-ABRCCTRCCTTACAABABTTCCTBAABBAABCACTAAACATBBAAA	
50-2	ABAAATAAAATA-CITTA-CABATAAB-CAAATBITBAB-ABATTITBICACCACC-ABBCCTBCCCTAAAAAABCTCCTBAABBAABCACTAAAATATBBABA	
50-3	ASAAATAAAATCCTT-TA-CASACAAS-CAAATECTSAS-ASATTTTYTCASCACC-ASBACTECCTAA-AASAETCCTSAASBAASBACTAACATBBAAA	
SU-1	ABAABCAAAATA-CTTTA-CABACAAB-CAAATBCTBAB-ABATTTTBTCACCACC-ABBCCTBCOCTAAAABAABCTCCTBAABBAABCBETAAACATBBAAA	
SE1-2	BABABCAAAATA-CTTTA-CABACABB-CAAATACTBAB-ABATTTTTTCACCACC-ABBRCTBCCCTAAABAABCACCTBAABBAABCACTAAACATBBAAA	
201-20	ASAATAAAAACCETTTAACSEACAASACAAATRETSAS-ASATTTTRTCACCACCEASECETSCCETAAAASARETC-TSAASSAASCEETAARCATSSAAA	
513-4	AGAAATAAAATA-CTITA-TAGACAAG-CAAATECTEAG-AGATTTEECACCACC-AGECCTECCCTAAAAGAETCCTEAAAGEAAGENTACATEGAAA	
SI3-5	AGAACTAAAATACTTCTA-CAGACAAG-CAAATBCTBAG-AGATTTTBTCACCACC-AGBCCTBCOCTAAAAGABCTCCTBAAABBAABCACTAAAACATBBAAA	
139-1	AGAAATAAAATA-CTTTA-CAGACAAG-CAAATBCTGAG-AGATTTTFTCACCACC-AGBCCTTAAAAGAGCTCCTGAAGGAAGTBCTAAACAAGAGAAA	
19-1 19-2	ABWATAWATA-CTITA-CABACAGE-CANATECTERE-REATTITETCACCACC-ABECCTECCTTAWARRECTCCTERARRARDECTAWACACAGAAA ABWATCMATA-CTITA-CABACAGE-CANATECTERE-REATTITETCACCACC-ABECCTERCCTECCCTAWARRECTCCTERARRARDECECTWACATEGAAA	

139-31	AGAAATAAAATA-CTTTA-TAGACAAG	-CAAATECTEAS	-AGATTTTETCA	CCACC-	ARROTTROCTA	WARARCTICCTRAARR	AARCRETAAACATRRAAA
L1Hz-1	AGAAATAAAATA-CTTTA-TAGACAAG	-CAAATECTEAG	-AGATTTTETCA	CCACC-	ARRESTRONTA	WARARCTOCTRAARE	AARCRETAAACATERAAA
LiHe-2	AGAAATAAAATA-CTTTA-TAGACAAG	-CAAATETTEAG	-AGATTTTETCA	CCACC-	ARRETTREACTA	UNSARTEETSUARS	AARENTAAACATERAAA
Little-3	AGAAATAAAATA-CTTTA-CAGACAAG	-CAAATECTEAG	-AGATTTTETCA	CCACC-	ARROTROTTA	WARARCTICCTRAARR	AARCACTAAACATIRGAAA
C.BHe4	AGAAATAAAATA-CTTTA-CAGACAAG	-CAAATECTEAG	-AGATTTTETCA	CCMCA-	ARRESTRONTA	WARARCTOCTRAARE	AARCRETAAACATERAAA
LiHe-5	AGAAATAAAATA-CTTTA-CAGACAAG	-CAAATECTEAG	-AGATTTTETCA	CCACC-	ARRESTRONTA	WARARCTOCTRAARE	AARCACTAAACATIRGAAA
L1Hz-6	AGAAATAAAATA-CTTTA-CAGACAAG	-CAAATECTEAG	-AGATTTTETCA	CCACC-	ARRESTRONTA	WARARCTOCTRAARE	AARCRETAAACATREAAA
L1He-7	AGAAATAAAATA-CTTTA-TAGACAAG	-CANATECTERS	-AGATTTTETCA	CCACC-	ARROTROCTA	WARARCTICCTRAARE	AARCRITAAACATREAAA
C.3Hg-6	AGAAATAAAATA-CTTTA-TAGACAAG	-CAAATECTEAS	-AGATTTERTCA	CCACC	ARRESTREETA	WARACTOTRAKE	AARCACTAAACATERAAA
C.1Hg-9	AGAAATAAAATA-CTTTA-CAGACAAG	-CAAATECTEAS	-AGATETTETCA	CCACC	ARRESTREETA	WARARCTOCTRAARE	AARCRETAAACATERAAA
Lible-10	AGAMATAMATC-CTTTA-CAGACAAG	-CARTERCERS	-AGATTTTETCA	CCMCC-	ARRETHERITAL	UNCLOTTENTION	AMERITANA ATERNA
L1Hz-11	AGAMATAMATOCTT-TA-CAGACAAG	CARATECTERS	-AGATTTTETCA	CCMCC-	ABBOTTBOTTA	<b>WARANTY TRAKES</b>	AMERITANA TERMA
L1Hz-12	AGAMATAMATA-CTTTA-CAGACAAG	CARACTETTERS	-AGATTTTETCA	CACCC-	ARRETHERITAL	UNCLOTTENTION	AMERITANA ATERNA
L1Hz-12	AGAMATAMATA-CTTTA-CAGACAAG	CARATECTERS	-AGATTTTETCA	CCMCC-	ARRETHERITAL	UNCLOTTENTION	AMARTANAAATSEEGA
MRICHAD	AGAAATAAAATA-CTTTA-TAGACAAG	CARACTETTERS	-AGATTTTETCA	CCMCC-	ARRETHERITAL	UNCLOTTENTION	AMERITANA ATERNA
150572	AGAAATAAAATA-CTTTA-TAGACAAG	CARATECTERS	-AGATTTTETCA	CCMCC-	ARRETHERITAL	UNCLOTTENTION	AMERITANA ATERNA
150571	AGAAATAAAATA-CTTTA-TAGACAAG	CARACTERS	-AGATTTTETCA	CCMCC-	ARRETHERITAL	UNCLOTTENTION	AMERITANA TERMA
LINCE/TD	AGAMATAAACTA-CTTTA-TAGACAAG	CARATECTERS	-AGATTTTETCA	CCMCC-	ABBOTTROCATA	UNCLOTTENTION	AMERITERA ATTROUM
150569	AGAAATAAAATC-CTTTA-CAGACAGG	CARATECTERS	-AGATTTTETCA	CCMCC-	ARRETHERITAL	UNCLOTTENTION	AMERITANA ATERNA
130558	AGAAATAAAATA-CTTTA-TAGACAAG	-CARATECTERS	-AGATTTTTRTCA	CCACC	ARRETTROTTA	UNGARTETRAGE	AMERITANAATTEENAA
130556	AGAAATAAAATA-CTTTA-TAGACAAG	-CARATECTERS	-AGATTTTTRTCA	TCMCCT	ARRETTROTTA	UNANTEETENAA	AMERITANAATTEENAA
130565	AGAAATAAAATA-CTTTA-TAGACAAG	-CARATECTERS	-AGATTTTTRTCA	CCACC	ARRETTROTTA	UNANTEETENAA	AMERITANAATTEENAA
130550	AGAAATAAAATA-CTTTA-TAGACAAG	-CARATECTERS	-AGATTTTTRTCA	CCMC9	ARRETTROTTA	UNANTEETENAA	AMERITANAATTEENAA
1005822	AGAAATAAAATA-CTTTA-TAGACAAG	-CARATECTERS	-AGATTTTTRTCA	CCACC	ARRETTROTTA	UNANTEETENAA	AMERITANAATTEENAA
AL590130	AGAAATAAAATA-CTTTA-CAGACAAG	-CARATECTERS	-AGATTTTTRTCA	CCACC	ARRETTROTTA	UNANTEETENAA	AMERICAN AND A TRANSPORT
AC120.151	AGAATAAATA-CTTTA-GAGACAAG	-CARATECTERS	-AGATTTTTRTCA	CCACC	ARRETTROTTA	UNANTEETENAA	AMERITANAATTEENAA
AL096710	AGAAATAAAATA-CTTTA-CAGACAAG	-COMATRCTICAG	-AGATTTTTETCA	CCACC	ACRECTREETA	UUGUARAANTAUUGA	AMERICAN AND A TRANSPORT
APROSPERT	AGAAATAAAATC-CTTTA-CAGACAAG	-CARATECTERS	-AGATTTTTETCA	CCACC	ARRETTROTTA	UNANTEETENAA	AMERICAN AND A TRANSPORT
AL139136	AMMATAMATA-CTTTA-CAGACAAG	-CARATECTERS	-AGATTTORONA	CCACC	ARRESTRACTA	UNANTEETENAA	AMERICAN AND A TRANSPORT
ACD99845	AGAAATAAAATA-CTTTA-CAGACAAG	-CARATRETIGAC	-CRATTETERICA	CCACC	ARRETTROTTA	UNANTEETENAA	AMERITANAATTEENAA
ACLD675D	ATAAATAAAATC-CTTTA-CAGACGAA	-CARATECTERS	-AGATTTTTETCA	CCACC	ARRETROTTA	WARANTTOTAARR	AMERICANUST - REPAIL
ALIX1175	AGUATAMATA-CTTTA-CAGACAAG	-CARATECTERS	-AGATTTTTTTM	CCACC	ARRETTROTTAL	UNGLIGHTETENIGE	AUGETR/AUGT/FETRIAL
AL157620	AGAMTAMATE-CTTTA-CAGACAAG	-CARATECTERS	-AGATETTETCA	CCACC	ARRETTROTTAL	<b>WARANT TOTAL ARE</b>	ALL STREET
ACCESSION	ASUATABATACTT-TA-CASACIAS	-CARATECTERS	-AGATTTTTTTM	CCACC	AGAINTEEDETA	UNGLIGHTETENIGE	ALL STREET
APROXESSO	AGAAATAAAATA-CTTTA-CAGACAAG	-CANATECTERS	-AGATTTTETCA	CCMCC-	ARRECTROCCTA	WARARCTOCTRAARG	AARCRETAAACATREAAA

Abbreviations of each L1Hs p40 clone and each symbol of L1Hs groups were represented in Table 8. Bars in the sequences indicate the gaps, and asterisks under the sequences do the identical sequences.

### APPENDIX III

Multiple aligned sequences of the L1Hs p40 protein from human cells



	1 20	40	50 80
			GC4.
B6-1	EIGRTPORY HITVRF		GRPIRLTADLSAESLOARENGPIFNILKEKNFOA
CC-2	EIGRMPORY HIIVRF	MROKMLRAAREKORV	GKPIRLTADLSAETCOAREMGPIFNILKEKNFOP
CC-3	EIQRTPORY HIIVRF	MKEXMLRAAREKORV	GKPIRLTADLSEETLRAREMGPIFNILKEKNFOP
CC-4	SIGRMPORY HIIVEF	MKEXMLRAAREKORV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
45-2	EIQRMPORC HIIVRF	E MKEXMLRAAREKGOV H	GKPIRLTADLLAETLOARSENGPIFNILKEKNFOP
HC-2	EIQRTPORY HIIVRF	MKEXMLRAAREKORV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
HC-3	EIQRTPORY HIIVRF	MKEXMLRAAREKORV	<b>GKPIRLTAILSAETLOAREMGPIFNILKEKNFOP</b>
Ju-2	EIQRTQORS L HIIVRF	MKEXVLRAAEBKORV	6KPIRLTAELLTDTLQAREM6PIFNILKEXYFQP
MC-3	EIGRTRORY HIIVRF	MKEXMLRAAREKORV	<b>GKPIRLTAILSAETLOARDMEPIFNILKEKNFOP</b>
MC-4	EIQRTPORY 6 HIIVRF	MKEKMLRAAREKORV	<b>GKPIRLTADLSEETLKAREMOPIFNILKEKNFOP</b>
MP-3	EIQRMPORY HIIVRF	MKEEMLRAAREKORV	GKPIRLTEDLSAETLOAROMOPIFNILKEKNFOP
MP-4	EIQRTPORS HIIVRF	MKEEMLRAGREKORV H	6KPIRLTADLLAETLOAREMOPIFNILKEKNFOP
MO-1	EIGRTPORY T NIIVRF	MKEKMLRAATEKORV H	I GRPIRLTTELSAETLOARRENGPIFNRLRENNPHL
MD-2	KIGRTPORY L TTP HIILRF	MKEEILRAAREKORV H	H GKPIRVTALLSAETLOARRVMOPLFNILKEKNFOL
SH-1	EIGRTPORY HIIVRF	MKEKVLRAAREKORV H	I GKPIRLTAELSAETLOAREMGPIFNILKEKNFOP
58-2	EIGRTPORY HIIVRF	MKEKMLRAAREKORV H	I GKPIRLTAELSAETLOAREMGPIFNTLKEKNFOP
SN-3	EIQRAPORY IHIIVRF	KKEKVLRAAREKORV H	I GRPIRLTARLSAETLOARRENGPIFNILKEKNFOP
SI-4	EIQRMPORY HIIVRF	MKEKMLRAARVKORV	<b>GKPIRLTADLSAETLOAREMOPIFNILKEKNFOP</b>
SK-5	EIGRMPORY HIIVRF	MKEKMLRAARVKORV	6KPIRLTADLSAETLOAREM6PIFNILKEKNFOP
SD-1	BIORMPORY HIIVRF	MKEKMLRAVREKEMV	6KPIRLTADLLAETLOAREM6PIFNILKEKNFOP
SD-2	EIGRMPORY HIIVRF	MKEKTLGAAREKORV H	H GKPIRLTADLSAETLOAREMGPIFNILKEKNFOP
SU-1	EIQRTPORYA RIIVRF	MKEKMLRAAREKORV	6KPIRLTADLSAETLOAREM6PIFNILKEKNFOP
09-1	EIGRMPORY M HIIVRF	LOMKEKMLRAAREKEMV	GKSIRLTADLSAETLOAREMOPIFNILKEKNFOP
09-2	EIGRMPORY L V R HTIVRF	MKEKMLRAAREKORV	GKPIRLMADLSAETLOAREMGPIFNILKEKNFOP
09-3	EIGRTPORY HIIVEF	DMKEXMLRAAREKORV	6KPIRLTADLSAETLOARREL6PIFNILKEKNFOP
L1Hs-1	EIGRTPORY HIIVRF	MKEXMLRAAREKORV	<b>EKPIRITALLSAETLOAREMEPIPNILKEKNFOP</b>
L1Hs-2	BIORTPORY HIIVEF	MKEXMLRAAREKERV	<b>EKPIRITALLSAETLOAREMEPIFNILKEKNFOP</b>
L1Hs-3	EIGRTPORY HIIVRF	MKEXMLRAAREKORV	GKPIRLTADLSAETLOARDMGPIFNILKEKNFOP
L1Hs-4	EIGRTPORY HIIVRF	MKEXMLRAAREKOMV	GKPIRLTADLSAETLOAREMGPIFNILKEKNFOP
L1Hs-5	EIGRTPORY HIIVRF	MKEKMLRAAREKGRV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
L1Hs-6	EIGRTPORY HIIVRF	MKEKMLRAAREKGRV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
L1Hs-7	EIQRTPORY HIIVRF	MKEKMLRAAREKGRV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
L1Hs-8	EIGRTPORY HIIVRF	MKEKMLRAAREKGOV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
L1Hs-9	EIQRTPORY HIIVRF	MKEKMLRAAREKORV	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOP
L1HS-10	EIQRTPORY HIIVRF	MMEKMLRAAREKGOV	GKPIRLTADLSAETLOARRENGVIFNILKEKNFOP
L1Hs-11	EIQRMPORY V HIIIRF	MKEKMLRAAREKORV H	GKPIRLTADLSAETLOARRENGPIFNILKEKNFOH
L1Hs-13	EIQRTPRRY HIIVRF	MKEKMLRAAREKORV H	GKPIRLTADLSAETLOARRENRPIFNIFKEKNFOP
M80340	EIQRTPORY HIIVRF	MKEKMLRAAREKORV	GKPIRLTAILSAETLOAFREMGPIFNILKEKNFOP
093572	EIQRTPORY HIIVRF	MKEKMLRAAREKORV	GKPIRLTAILLAETLOAFREMGPIFNILKEKNFOP
093571	EIQRMPORY HIIVRF	MKEKMLRAAREKBCV	GKPIRLTAILSAETLOAFREMGPIFNILKEKNFOP
093570	EIQRTPORY HIIVRF		CKPIRLTAILSAETLOAFREMCPIFNILKEKNFOP
093569	EIQRTPORY HIIVRF	MKEXMLRAAREKORV	GKPIRLTAELSAETLOAFREMGPIFNILKEKNFOP
093568	EIQRTPORY HIIVRF	MKEXMLRAAREKORVN	GKPIRLTADLSAETLOARRENGPIFSILKEKNFOP
093566	EIQRTPORY HIIVRF		GKPIRLTAELSAETLOAFREMGPIFNILKEKNFOP
093565	EIQRTPORY HIIVRF		GKPIRLTAELSAETLOAFREMGPIFNILKEKNFOP
093563	EIQRTPORY HIIVRF		GRPIRLTADLSAETLOARRENGPIFNILKENNFOP
093562	EIGRTPORY HIIVRF		GKPIRLTADLSAETLOARRENGSIFNILKENNFOP
AL590131	EIQRTQORS L HIIVRF		GRPIRLTADLSAETLOAFRENGPIFNILKENNFOP
AC131151	EIQRTPORY HIIVRF		6KPIRLTADLSAETLOAFRDMEPIFNILK6KNFOP
AL096710	EIGRMPORY 6 HIIVRF		H GKPIRLTADLSAETLOARENGPIFNILKENNFOP
AC022537	EIQRTPORY HIIVRF		H GKPIRLTARLSAETLOAREMOPIFNILKENNFOP
AL139136	EIGRTPORY HIIVRF		GKPIRLTADLSAETLOAREMOPIFNILKENNFOP
AC099845	EIGRTPORY HIIVRF	MKEKMLRAAREKGRV	GKPIRLTADLSAETLOAFREMOPIFWILKENNFOP

– cxxii –

	81	100	120
	CX	11	HTH
BG-1	RISYLAKLSFI	IKYPTEKQMLREFVTTRP	
CC-2	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
CC-3	RISFPAKLSFI	RKYFTEKOMLREFVTTRP	1
CC-4	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	1
A5-2	RISYPAKLSFI	IKYFTDRQILRDFFTNRP	P Q
HC-2	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	Q S
HC-3	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	I
Ju-2	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	
MC-3	RISYPAKLSFI	IKYFRDKOMLRDFVTTRP	Y
MC-4	RISYPAKLSFI	IKSPIDKOMLRDFVTTRP	
MP-3	RISYPAKLEFI	IKSPIDKOMLRDFVTTRP	- 뚱앙도서관
MP-4	RFSVPAKLSFI I	R IKSPITKONLRNFVITRH	
MD-1	RISYPAKLSFI	INSPTERHMERESVTTRP	TORSOT LIBRARY
MD-2	RISYPAKLSFI (	R IKYFTDKHMLRDFVTTRH	
SH-1	RISYPAKLSFI	IKSPIDKOMLRDFVTTRP	
36-2	RISYPAKLSFI	IKSPITKQMLREPVITRP	
SH-3	RISYPAKLSFI	IKSPIDKOMLRDFVTTRP	D
SH-4	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
36-5	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
S0-1	RISYPAKLSFI	IKYPTEKOMLREPVTTRP	QF
80-2	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	K
SU-1	RISYPAKLSFI	AKYFTDKOMLRDFVTTRP	
09-1	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	V T
09-2	RISYPAKLSFIN	IKYPTEKOMLREPVTTRP	
09-3	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
L1Hs-1	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
L1Hs-2	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
L1Hs-3	RISYPAKLSFI	IKYPTEKOMLREPVTTRP	Q
L1Hs-4	RISYLAKLSFI	IKYPTEKOMLREPVTTRP	
L1Hs-5	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	
L1Hs-6	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	
L1Hs-7	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	
L1Hs-8	RISYPAKLSFI	IKYFIDKQMLRDLVTTRP	D
L1Hs-9	RISYPAKLSFI	IKYFTDKOMLRDFVTTRP	
L1HS-10	RISYPAKLSFI	IKSPITIKOMPREVITRP	
L1Hs-11	RISYPAKLSFI	E IKSPTDKOMLRDFVTTRP	Q
L1Hs-13	RISYPAKLSFI	IKYPTIKOMLRIFVTTRP	D
M80340	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	1
093572	RISYPAKLSFI	IKYFIDKOMLRDFVTTRP	1

Pant-L1-1	EIGRTPORY	HIIVRF	MKERMLRAAROKORV	GKPIRLTADLSAETLOAREMGPI	FNILKEKNFOP
Pant-L1-2	EIGRTPORY	HIIVRF	MKEKMLRAAROKERV	<b>GKPIRLTADLSAETLOAREMOPI</b>	FNILKEKNFOP
Pant-L1-3	EIGRTPORY	HIIVRF	MKERMLRAAROKERV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Pant-L1-4	EIGRTPORY	HIIVRF	MKEKMLRAAROKERV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Pant-L1-5	EIGRTPORY	HIIVRF	MKERMLRAAROKERV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Pant-L1-6	EIGRTPORY	HIIVRF	MKEKILRAAROKERV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Panp-L1-1	EIGRTPORY	HIIVRF	MKERMLRAAROKERV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Panp-L1-2	ERORTPORY	HIIVRF	MKEKMLRAAROKGOV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Gorg-11-1	EIGRTPORY	HIIVRF	MKEKMLRAAREKORV	<b>GKPIRLTADLSAETLOARRENGPI</b>	FNILKEKNFOP
Nouse-L1-1	EIGRTPORY	HIIVRF	MOEKMLRAAKEKGOV	6KPIRLTADPLAETLOARREMOPI	SNLLKEKNFOP
Nouse-L1-2	EIGRTPORY	HIIVRF	MKEKMLRTAREKORV	<b>GKPIRLTVDLSAETLOARRENGPI</b>	FNILKERNFOP
				*** ** **	

	e e		۰.	* *			
Nouse-L1-2	RISY	PAKLSFI		IKYPTIKOMLRIPVTIRP		Ε	
Nouse-L1-1	RISY	PAKLSFI		IKSPTDROMLRDPVTTRT			
Gorg-L1-1	RISY	PAKLSFI		IKYFTEKOMLREFVTTRP			
Panp-L1-2	RISY	PAKLSFI		IKYPTDKQMLTDFVTTRP			
Panp-L1-1	RISY	PAKLSFI		IKYFTDKQMLTDFVTTRP			
Pant-L1-6	RISY	PAKLSFI		IKYFTDKOMLTDFVTTRP			
Pant-L1-5	RISY	PAKLSFI		IKYPTIKOM_TDFVTTRP			
Pant-L1-4	RISY	PAKLSFI		IKYFTDKOMLTDFVTTRP			
Pant-L1-3	RISY	PAKLSFI		IKYFTDKOMLTDFVTTRP			
Pant-L1-2	RISY	PAKLEFI		IKYFTIKOM.TIFVTTRP V			
Pant-L1-1	RISY	PAKLSFI		IKYFTDKOMLTDFVTTRP			
AC099845	RISY	PAKLSFI		IKYFTDKOMLTDFVTTRP			
AL139136	RISY	PAKLSFI		KIKYFTIKQMLRIFATTRPD			
AC022537	RISY	PAKLSFI		IKSFTEKOMLREFVTTRP			
AL096710	RISY	PAKLSFI		IKYPTIKPMLRIPVTTTP	R		
AC131151	RISY	PAKLSFI		IKYFRDKOMLRDFVTTRP V			
AL590131	RISY	PAKLSFI		IKYFTIKOMLRIFVTTRP			
093562	RISY	PAKLSFK		IKYFIDKOMLRDFVTTRP			
093563	RISY	PAKLSFI		IKYFIDKOMLRDFVTTRP			
093565	RISY	PAKLSFI		IKYFIDKOMLRDFVTTRP			
093566	RISY	PAKLSFI		IKYFIDKOMLRDFVITRP			
093568	RISY	PAKLSFI		IKYFIDKOMLRDFVTTRP			
093569	RISY	PAKLSFI		IKSFTEROMLREFVTTRP			
093570	RISY	PAKLSFI		INVFIDEQUERDFVTTRP			L
093571	RISY	PAKLSFI		IKYFIDKQILRDFVTTRP			

Abbreviations of each L1Hs *p40* clone and each symbol of L1Hs groups were represented in Table 8. Gorg, *Gorilla gorilla*; Mouse, *Mus musculus*; Panp, *Pan paniscus*; Pant, *Pan troglodytes*. HTH, helix-turn-helix motif; PKC, protein kinase C phosphorylation site; CKII, casein kinase II phosphorylation site. Asterisks indicate the identical sequences. Shadowed sequences are the conserved sequences corresponding to each protein interaction motif at the top of the sequences.

## APPENDIX IV

# Chorompsomal locations of each transcript of the HERV-K10 LTR and the

L1Hs *p40* 



















Idiograms of the human chromosomes were obtained from Human Genome Resources in GenBank. Information of each clone was given in Table 5 to HERV-K10 LTR and Table 9 to L1Hs p40. Open rectangles indicate the transcript of HERV-K10 LTR and filled rectangles do that of L1Hs p40.

#### APPENDIX V

Examples of Default Display of L1Hs Sequences Deposited in GenBank Database and the Search Result of the Part of Submissions



```
LOCUS
                                379 bp
            AY392754
                                          DNA.
                                                  linear PRI 15-0CT-2003
DEFINITION Homo sapiens map 7q cell-line CCD-25Lu LINE-1 element, partial
            sequence.
ACCESSION
            AY392754
VERSION
            AY392754,1 GI:37624749
KEYWORDS
SOURCE
            Homo sapiens (human)
  ORGANISM Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia: Butheria: Primates: Catarrhini: Hominidae: Homo,
            1 (bases 1 to 379)
REFERENCE
  AUTHORS
            Han, S.-H., Oh, J.-H., Oh, Y.-S., Kim, J.-H., Song, J.-H. and Oh, M.-Y.
  TITLE
            LINE-1 sequence variations in human lung fibroblasts
  JOURNAL
           Unpublished
REFERENCE
            2 (bases 1 to 379)
  AUTHORS Han, S. -H., Oh, J. -H., Oh, Y. -S., Kim, J. -H., Song, J. -H. and Oh, M. -Y.
  TITLE
            Direct Submission
  JOURNAL
            Submitted (17-SEP-2003) Department of Life Science, College of
            Natural Sciences, Cheju National University, 1 Ara 1-Dong, Jeju,
            Jeju 690-756, Korea
                     Location/Qualifiers 중앙도서관
FEATURES
                     1. 379-JU NATIONAL UNIVERSITY LIBRARY
     source
                     /organism="Homo sapiens"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9606"
                     /chronosone="7"
                     /map="7q"
                     /cell_line="CCD-25Lu"
                     /cell_type="normal fibroblast"
                     /tissue_type="lung"
     repeat_region
                     <1...>379
                     /note="p40"
                     /rpt_family="LINE-1"
                     /rpt_type=dispersed
ORIGIN
        1 ggaatacaga gaatgccaca aagatactcc ccgagaagag caactccaag acacataatt
       61 gtcagattca ccaaagttga aatgaaggaa aaaatgttaa gggcagccag agagaaaggt
      121 cgggttaccc tcaaaggaaa gcccatcaga ctaacagcgg atctctcggc agaaacccta
      181 caagccagaa gagagtgggg gccaatattc aacattetta aagaaaagaa ttttcaacce
      241 aqaatttcat atccagccaa actaagcttc ataagtgaag gagaaataaa atactttata
      301 gacaagcaaa tgttgagaga ttttgtcacc accaggcctg ccctaaaaga gctcctgaag
```

361 gaagegetaa acatggaaa

1: <u>AY392754</u> Homo sapiens map 7q cell-line CCD-25Lu LINE-1 element, partial sequence gi 37624749 gb AY392754.1 [37624749]	<u>Links</u>
<b>2:</b> <u>AY392753</u> Homo sapiens map 10q cell-line CCD-25Lu LINE-1 element, partial sequence gi 37624748 gb AY392753.1 [37624748]	<u>Links</u>
<b>3:</b> <u>AY392752</u> Homo sapiens map 7p cell-line CCD-25Lu LINE-1 element, partial sequence gi 37624747 gb AY392752.1 [37624747]	<u>Links</u>
<b>4:</b> <u>AY392751</u> Homo sapiens map Xq13.1 cell-line CCD-25Lu LINE-1 element, partial sequence gi 37624746 gb AY392751.1 [37624746]	<u>Links</u>
5: <u>AY392750</u> Homo sapiens map Xq23 cell-line HCT-15 LINE-1 element, partial sequence gi 37624745 gb AY392750.1 [37624745]	<u>Links</u>
6: <u>AY392749</u> Homo sapiens map 8q cell-line HCT-15 LINE-1 element, partial sequence gi 37624744 gb AY392749.1 [37624744]	<u>Links</u>
7: <u>AY392748</u> Homo sapiens map 7q cell-line HCT-15 LINE-1 element, partial sequence gi 37624743 gb AY392748.1 [37624743]	<u>Links</u>
8: AY392747 Homo sapiens map 5q cell-line HCT-15 LINE-1 element, partial sequence gi 37624742 gb AY392747.1 [37624742]	<u>Links</u>
<b>9:</b> <u>AY392746</u> Homo sapiens map 5p cell-line HCT-15 LINE-1 element, partial sequence gi 37624741 gb AY392746.1 [37624741]	<u>Links</u>

초 록

전위인자 (transposable element)는 유전체 기능에 중요한 역할을 담당하며 전위 와 재조합을 통해서 염색체의 다른 위치로 전위할 수 있다. 그 결과로 생성된 반복 서열들은 많은 유전적 재조합의 기회를 제공하게 된다. 뿐만 아니라, 염색체상에서 전위인자의 위치 변화는 유전체 불안정성, 세포의 형질전환과 분화를 초래하기도 한다. 인간 유전체에서 몇 종류의 역전위인자 (retro-transposable elements)들이 알려 져 있다. 대표적 역전위인자인 인간내생성레트로바이러스 (HERVs)와 긴 산재형 고반복 인자 (LINEs)들은 기능적인 전위활성 보유하고 있다. HERV는 인간 유전 체의 0.5%-3% 정도로 추정된다; 전형적인 레트로바이러스 단백질인 gag, pol, env 를 암호화하고 있으며, 양 끝에 유사 프로모터로 작용하는 장만달반복배열 (LTR) 들이 위치한다. 인간 유전체에서 발견된 HERV LTR과 독자적인 LTR들은 세포생 물학적으로 중요한 역할을 수행하고 있다. LTR들은 HERV 유전자 및 인접한 숙주 유전자의 발현을 조절하는 다양한 요소들을 보유하고 있다. 인간 LINE-1 (L1Hs)은 두 개의 열린 해독틀 (ORF)이 내재하고 있다: 그 중 하나는 역전사를 담당하는 역 전사효소를 암호화하고, 다른 하나는 RNA-단백질 복합체 형성을 담당한다. 본 연 구는 다양한 인간 세포주들 (2종의 정상 림프구와 2종의 정상 섬유아세포, 16 종의 암세포주)을 대상으로 HERV-K와 L1Hs의 전사 및 전사체의 염색체 위치를 조사하 였다. 또한, 이들 역전위인자들의 유전자 발현에서 몇 가지 화학적 발암물질의 효 과를 시험하였다. 역전위인자들의 RNA 전사체는 사용된 모든 세포주에서 발견되 었으나, 확인된 전사체의 상대적인 양은 세포 유형에 따라 서로 다르게 나타났다. 예를 들어, 섬유아세포들과 백혈구 세포들에서 전사 수준이 세포의 발생단계에 따 라 상이하게 나타났다. 또한, HERV-K 유전자들 (HERV-K10 LTR, HERV-K(T47D) LTR, HERV-K(C4) env, and HERV-K10 env)과 L1Hs p40 유전자, 조절 단백질 유전 자들 (BAX, c-MYC, IL-6, and p53)의 발현 양상은 처리된 발암물질에 따라 변화하였

다. 다양한 세포에서 HERV-K 유전자의 활성이 세포유형 특이적으로 보고 되었기 에, HERV-K 유전자들이 세포의 증식, 사멸, 분화, 암 발생 및 외생성레트로바이러 스의 유입에 대항하는 항바이러스 방어 전략 등에 관여함을 암시한다. L1Hs의 전 사는 세포유형적 특이성을 보이지는 않았으나, 스트레스나 암 발생 인자에 대한 반 응에서는 다양하게 나타났다. 비록, 역전위인자 RNA 전사체가 연구에 사용한 모 든 세포주에서 발견되었지만, 역전위인자의 발현과 인간 암 사이에서 직접적인 관 련성에 대한 증거를 찾기에는 불충분하였다. 이상의 결과는 발암물질에 대한 반응 에서 HERV-K와 L1Hs p40 유전자 발현의 변화를 근거하여, 역전위인자의 활성 수 준의 변화가 세포의 형질전환에 기여함을 암시하고 있다. 몇몇 발암물질은 전위활 성을 유도하였다. 트랜스포존 특이적 ITR과 인위적인 무작위 ITR을 증폭 시발체로 사용하여 유전체 수준의 증폭을 수행한 결과, 증폭산물은 변화된 양상을 보였다. 평균 5세대 배양 후, 증폭산물 중에서 구별되는 절편들이 관찰되었다. 염기서열 분 석에 수반된 BLAST 검색, 계통유전학적 분석을 통해 역전위인자의 전사체들이 거 의 모든 염색체의 다양한 좌위로부터 전사됨을 보여주었다. HERV-K10 LTR 인자 의 전사체들은 21번 염색체와 Y 염색체에서는 나타나지 않았으며, 전사좌위는 염 색체의 동원체 부근부터 말단소립에 이르기까지 전 영역에서 걸쳐 나타났다. HERV-K10 LTR은 포유류 종분화 이전과 이후에 수차례에 걸쳐 인간 유전체로 유 입된 것으로 보인다. L1Hs 전사체들은 21번 염색체를 제외한 모든 염색체에서 확 인되었다. 유사도 검색과 DNA 서열에 대한 가상적인 단백질 서열 해독은 인간 반 수체 유전체 내에 적어도 140개의 전사활성 좌위가 확인되었다. 또한 절대성 원칙, 단순통합분지도 (majority rule, strict consensus tree)에서 활성 L1Hs 인자들이 두 집 단 (cluster)을 형성하였다. 염색체상의 위치를 살펴보면, 역전위인자 전사체들이 다수의 염색체취약부위 (fragile site)로부터 전사되며, 이와 같은 사실은 역전위인 자의 염색체상 위치와 염색체 불안정성 (chromosomal instability)과 암관련 절단부 위 (cancer-related breakpoint), 유전병관련 좌위 사이의 관련을 암시하고 있다. 역전 위인자들이 인간유전체로 유입된 이후, 유전체의 인트론을 확장시키고, 많은 유사 유전자 (pseudogene)들을 생성하였다. 결론적으로, 본 연구는 많은 역전위인자들이 숙주 유전체 내에서 전사, 전위되고 있을 뿐만 아니라, 세포내외 환경으로부터 다 양한 자극에 대해서도 반응할 수 있음을 제안하고 있다. 또한, 활성형 HERV-K와 L1Hs 역전위인자들은 인간 유전체에서 자신의 전위 활성이 잠재성을 유지한 채 광 범위하게 분포하고 있다.

주요어: 돌연변이, 레트로포손, 레트로바이러스, 발암물질, 암, 역전위, 역전위인자, 염색체, 염색체 불안정성, 염색체 재배열, 유전병, 유전자 발현, 유전체, 유전체 불 안정성, 전사, 전위인자, 전위, 재조합, 취약부위, 트랜스포존, env, HERV-K, HERV-K10 LTR, LINE-1, L1Hs p40, LTR



감사의 글

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학문 연구와 논문 작성에 이르기까지 모든 면에서 부족하기만 했던 저를 위해 끊이 없는 관심과 사랑으로 보살펴주셨고, 학문에 임하는 마음가짐을 흩어지지 않게 인 도해주신 오문유 교수님께 진심으로 감사의 말씀을 드립니다. 지난 6년, 대학원 석 사, 박사 과정을 거치는 동안 항상 학문에 정진할 수 있게 가르쳐주셨고, 언제나 강 직하고 올곧은 학자의 상을 보여주셨기에, 아직도 많이 모자라지만 오늘 이 자리에 설 수 있게 되었습니다. 감사하다는 말로는 이루 다 보답할 수 없는 은혜를 입고 떠 나는 제자를 아껴주시니, 은혜에 감사하고 항상 올바른 학문탐구에 매진할 것을 다 짐하며, 다시 한 번 머리숙여 감사드리며 항상 건강하시길 기원합니다.

부족한 제 논문에 대한 심사를 맡아 바뿌신 와중에도 세세히 다듬어 주시고, 더불 어 더 나온 연구자의 길을 보여주신 제주대 생명과학과 김세재 교수님, 의학과 강 희경 교수님, 제주고대 홍승호 교수님, 제주하이테크산업진흥원 김기옥 박사님께 도 깊은 감사의 말씀을 드립니다. 그리고 생물학에 관한 많은 가르침을 주신 김원 택 교수님, 이용필 교수님, 오덕철 교수님, 이화자 교수님, 고석찬 교수님께도 감사 의 말씀을 드립니다. 석사논문 연구때부터 많은 가르침을 주셨던 김세재 교수님께 재삼 감사드립니다.

철부지같던 시절 대학원 생활의 두려움을 없애 주었고, 학문이라는 험한 길을 걸어 갈 힘을 더해 준 고미희 박사님, 정용환 박사님께 감사드립니다. 실험과정에서 같 이 밤새며 부푼 라면으로 허기를 달래도 불평 한 번 않던 유전학실험실 가족들. 오 유성 선생님과 오주형 선생님 고맙습니다. 김재환, 송지훈 대학원 후배들, 이제 대 학원생이 될강민철, 오대주 후배들 너무 고맙고, 황준호, 강성일, 김윤정, 김동철 후 배들도 너무너무 고맙다. 더불어 그동안 유전학실험실을 거쳐간 선후배들에게도 감사의 뜻을 전합니다. 특히, 대학원 입학에 대한 용기를 일깨워주었고, 때로 형보 다도 더 친근한 모습으로 다독이며 앞서간 어느 자리에서도 후배에게 격려와 함께 많은 도움을 준 정용환 박사님께 진심으로 고마움을 전합니다.

지난 6년간의 대학원 생활에 많은 도움을 준 분자생물학실험실 박지권 박사님, 강

신해 박사님, 정형복, 진영준, 최진영 대학원생들, 미생물학실험실 이동헌 박사님, 이정배 선배님, 생태학실험실 송관필선배님, 문명옥 선배님과 생물학과 모든 대학 원생들에게 감사드립니다.

의과대학 해부학교실 이봉희 교수님, 김명주 교수님, 조직학교실 이영기 교수님, 박덕배 교수님, 병리학교실 강현욱 교수님, 생확학교실 조문제 교수님, 기생충학교 실 정영배 교수님께도 감사드립니다. 특히, 실험을 위해 많은 도움을 주신 약리학 교실 강희경 교수님, 유은숙 교수님, 박수영 박사님, 이혜자, 김상철, 현재희, 윤원 중 대학원생, 미생물학교실 고영상 교수님과 윤지현 후배에게도 진심으로 감사드 립니다. 임희경, 김영미, 강윤석, 이정희 선생에게도 감사의 뜻을 전합니다.

생물학에 대한 제 관심과 연구에 대한 의욕을 키워낼 수 있었던 사범대학 과학교육 과 생물교육 박행신 교수님, 정충덕 교수님, 오홍식 교수님, 이순동 교수님께도 감 사드립니다. 제주도 자연사박물관 정세호 박사님, 김완병 선배님께도 감사드립니 다. 농업시험장 김성철 박사님께도 감사드립니다. 그리고 쉽지 않은 연구 소재에 애타고 있을 때, 멀리서 힘을 실어준 부산대학교 김희수 교수님, 이주미 대학원생 에게도 진심으로 감사드리며, 격려해주신 해양과학대학 이제희 교수님께도 감사 드립니다.

고등학교를 졸업하고 지금껏 못난 친구 둔 덕에 마음고생 많으면서도, 어느날 불쑥 나타나도 반겨주던 친구 최종철에게 감사드립니다. 짧지만은 않았던 1년여의 시간 동안 내 곁을 지켜주며 때로 나보다 더 몸과 마음을 걱정해 주며, 옷음과 기쁨을 건 네준 고윤미에게도 진심으로 감사의 뜻을 전합니다.

바쁜 생활 속에서도 언제나 동생에 대한 관심을 늦추지 않았던 하나뿐인 누님과 넉 넉한 매형, 웃음이 예쁜 귀염덩어리 오하나양에게도 감사드립니다.

언젠가 어린시절, "대학원 가겠다"는 말을 들으시고는 많이 놀라시고도 그러마 하 셨고, 없는 형편에 홀로 한숨과 눈물로 살아오시면서도 너무나 당당한 모습으로, 오늘의 저가 있기까지 밤낮으로 비바람 가리지 않고 어려움 막아주시고 보살펴 주 신 사랑하는 나의 어머님. 지난 30년간 드릴 것 하나없던 제가 오늘 비로소 어머님 께 감사하다는 말씀과 함께 미약하나마 이 논문을 드릴 수 있어 더없이 기쁩니다. 부디 오래오래 건강하십시오.