Long Terminal Repeats of Human Endogenous Retrovirus H Family Provide Alternative Polyadenylation Signals to NADSYN1 Gene

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ABSTRACT

The NADSYN1 gene containing 21 exons was located in human chromosome 11q13.4. Using the computational screening of expressed sequence tag (EST) database, we found alternative transcript of NADSYN1 gene that was related to the HERV-H LTR element. The HERV-H LTR donated the last 22nd exon for NADSYN1 gene and also provided an alternative polyadenylation signal. In the comparison of expression pattern between cellular and alternative transcripts, the cellular transcript has been expressed more frequently than alternative transcript in various normal tissues and cancer cells. The HERV-H LTR element seems to be integrated into our common ancestor genome after the divergence of hominoid primate and Old World monkeys, approximately 25 million years ago. By the integration event of HERV-H LTR in hominoid lineage, specific alternative transcript of NADSY1 gene has been created in the human. The data suggests that HERV-H LTR element of the NADSY1 gene has biological role during primate radiation.

Key words: NADSYN1 gene, HERV-H LTR, polyadenylation, alternative splicing, primate evolution.

INTRODUCTION

Over 45% of human genome was comprised of various transposable elements. Transposable elements include DNA transposons and retroelements. Human genome is estimated to consist of approximately 8% of human endogenous retroviruses (HERV) (International Human Genome Sequencing Consortium, 2001). Probably, HERVs are representing footprints of ancient germ-cell infections by infectious retrovirus. They could influence the genomic instability of host genome through the expression of retroviral genes and genomic rearrangements. Because new integrated HERV elements could disrupt the cellular open reading frame and HERV LTR element could regulate the adjacent gene expression. The majority of HERV families have been inserted into the common ancestor genome after the divergence of New World monkeys and Old World monkeys. They have been subjected to amplification on several times during primate evolution (Sverdlov, 2000). The entire structure of full length HERV is 5′...
LTR-gag-pro-pol-env-3′ LTR. However most of HERVs are defective with multiple stop codons, deletions and insertions mutations (Kim and Crow, 2001; Hong et al., 2003). Nevertheless, structural genes of some HERV families are actively expressed in human placenta and various cancer cell lines (Mi et al., 2000). Several evidences connected to gene regulation are reported, and most of them is related to the LTR elements as a promoter and enhancer for adjacent gene in specific tissues and cell lines (Medstrand et al., 2001; Landry et al., 2003; Landry and Mager, 2003; Dunn et al., 2003).

One of the most abundant HERVs, HERV-H, has appeared in New World monkey, but it has expanded mostly in the Old World monkey lineage (Mager and Freeman, 1995). About 100 copies of full length HERV-H and 1000 copies of solitary HERV-H LTR were scattered in human genome. It has been reported that the LTR of HERV-H family contains potential binding sites for several kinds of transcriptional factor such as Sp1, GC box and TATA box (Nelson et al., 1996; de Parseval et al., 1999). The HERV-H elements are strongly expressed in testicular and lung tumors (Wilkinson et al., 1990; Hirose et al., 1993). Cell-type specific expression and promoter activity of HERV-H LTR have also been reported (Schon et al., 2001). Moreover, the LTR sequences of HERV-H element provided polyadenylation signals to HHLA2 and HHLA3 genes (Mager et al., 1999).

The coenzyme NAD has a role in the majority of metabolic redox reactions and represents an essential component of metabolic pathways in all living cells. NADSYN1 (NAD synthetase 1) catalyzes the conversion of NAD into NAD, and NH3 or glutamine which is used as an amide donor (Emanuelli et al., 2001). Human NADSYN1 and NADSYN2 identified recently catalyze the final step in biosynthesis of NAD (Hara et al., 2003). In this study, we investigated that alternative polyadenylation signal of NADSYN1 gene is provided by HERV-H LTR element during primate evolution.

MATERIALS AND METHODS

Computational screening

The human expressed sequence tags (ESTs), RefSeq mRNAs and non-redundant databases were screened by BLAST version 2.2.9 using HERV-H consensus sequences to identify the novel hybrid transcripts (Altschul et al., 1997). Various transcripts were matched to NADSYN1 to identify the exact splicing pattern. Repeat element included in NADSYN1 transcripts and genomic loci are identified by RepeatMasker program (http://repeatmasker.genome.washington.edu) with various repeat element consensus sequences from the Repbase Update (Jurka, 2000). From the various EST and RefSeq mRNA sequences analysis, we could exactly reconstruct the gene structure of NADSYN1. For identification of alternatively spliced transcripts, alignment analysis using various transcripts of NADSYN1 was performed by Clustalw program (Thompson et al., 2000).

Cell culture

Human cancer cells (NCI-H1915, SNB19, PC3, U031, HepG-2, A549, BeWo, CCHM, Jurkat, 2F7, NIH-OVACAR, Hela, and AZ521) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin at 37°C, and 5% CO2 incubator.

RNA and genomic DNA samples

Total RNA from cancer cells was extracted by high pure RNA isolation kit (Roche), and RNA samples from human tissues (brain, prostate, testis, heart, kidney, liver, lung, placenta, skeletal muscle, spleen, thymus, uterus, and stomach) were purchased by Clonetech. Pure mRNA was subtracted by PolyA Trtract mRNA isolation systems (Promega).

Genomic DNA was isolated from heparinized blood samples by a standard protocol from the following species: (1) hominoids: chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), orangutan (Pongo pygmaeus), and gibbons (Hylobates agilis); (2) Old World monkeys: Japanese monkey (Macaca fuscata), rhesus monkey (Macaca mulatta) and baboon (Papio hamadryas) (3) New World monkeys: night monkey (Aotus trivirgatus), common marmoset (Callithrix jacchus), squirrel monkey (Saimiri sciureus)

PCR and RT-PCR amplification

The genomic DNAs from various primates were examined using PCR amplification. The LTR of HERV-H sequences were amplified by the primer pairs S1 (5′- GTA AGG AGT TGA ATT AAG CA-3′) and AS1 (5′- AGC TTC CTG TCA CAA TCT CA-3′) from Genbank accession NT_033927. The NADSYN1 trans-