

The diversity of the structure and genomic integration sites of HTLV-1 provirus in MT-2 cell lines

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Abstract A human T-lymphotropic virus Type 1 (HTLV-1) positive cell line, MT-2, derived from human cord leukocytes co-culturing with adult T cell leukemia/lymphoma (ATL) cells is commonly used in HTLV-1 research; however, the details of provirus integrated in MT-2 genome have not yet been characterized. In this study, five types of HTLV-1 proviral sequences were detected in 11 different sites of the genome in a reference MT-2 cell line. The five types of HTLV-1 proviral sequences were one complete proviral genome, two types of proviruses with deletion of large internal viral sequences (5.3 and 3.9 kB), one provirus with a large deletion (6.2 kB) from 5′LTR to position 6257, and one provirus of LTR only. The provirus with identical deletion of large internal viral sequence (5.3 kB) was found to be integrated into six different sites (chromosomes). A complete provirus and three of four types of defective provirus were consistently detected in two other MT-2 cell lines cultured in different laboratories. Not only *Tax/Rex* RNA and *HBZ* RNA, but also the transcriptional product for a specific defective provirus, were detectable in all three MT-2 cell lines. Because it has been reported that defective provirus is frequently detected in ATL cells, these results may be important in understanding the mechanism of HTLV-1 proviral polymorphism, which may be related to leukemogenesis. In addition, the large

variation in integrated HTLV-1 proviruses makes it important for researchers to exercise caution in their assessment and interpretation of results using MT-2 cell lines.

Keywords HTLV-1 · Defective virus · MT-2 cell line

Introduction

Human T-lymphotropic virus Type 1 (HTLV-1) is a causative agent of adult T-cell leukemia/lymphoma (ATL) and a progressive neurological disease known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1, 2]. HTLV-1 integrates into the host genome of T-lymphocytes as a provirus [3]. Currently, HTLV-1 positive cell lines are frequently used for the analysis of HTLV-1 infection and the mechanism of leukemogenesis. Miyoshi et al. established an HTLV-1 positive cell line, MT-2, from human cord leukocytes co-culturing with ATL cells [4–6]. Using southern blotting analysis, Kobayashi et al. found that at least eight HTLV proviruses were integrated into the chromosomes of MT-2 cells [7]. Subsequent to this, however, few detailed analyses of HTLV proviruses in MT-2 cells have been presented. It has been reported that HTLV-1 provirus is integrated into the host genome as complete or defective provirus. Two types of defective proviruses have been reported in ATL cells [8–10]. One retains both LTRs but lacks internal sequences, such as the *gag* and *pol* coding regions. The other has deletion of 5′LTR and its flanking internal sequence of provirus. Because ATL cells frequently harbor defective provirus, researchers have suspected that it plays a role in the development of ATL [11]. However, the mechanism by which HTLV-1-infected cells harbor complete provirus or

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defective provirus remains unclear. Analysis of proviruses integrated into the genome of HTLV-1 positive cell line may help clarify the mechanism. Therefore, in the current study, the genomic structure and sites of integration of HTLV-1 provirus in MT-2 cell line, one of the most frequently used cell lines, was analyzed in depth.

Materials and methods

MT-2 cell lines

An MT-2 cell line (MT-2J in this report) was purchased from the Japanese Collection of Research Bioresources Cell Bank (JCRB1210, Lot 09142007). Two other MT-2 cell lines (MT-2A and MT-2B) have been independently passaged in University of Miyazaki and University of Nagasaki.

Chromosomal analysis

Chromosomal analysis of MT-2J cells was performed utilizing a method described elsewhere [12]. Briefly, cells were treated with colcemid (Roche Diagnostics, Mannheim, Germany) to arrest the cell cycle at M phase. After hypotonic treatment with 0.075 M KCl solution, the cells were fixed with acetic acid: methanol (3:1) and stained with Giemsa. The number of chromosomes in 25 cells was counted using light microscopy.

Quantification of HTLV-1 provirus

Genomic DNA was isolated from MT-2 cells using a QIAamp[®] DNA Mini kit (QIAGEN, Tokyo, Japan) according to the instructions provided in the kit. Approximately 100 ng of genomic DNA was used as the template for polymerase chain reaction (PCR). The nucleotide position number of HTLV-1 provirus was according to Seiki et al. (accession no. J02029) [13]. The copy number of HTLV-1 provirus (5'LTR-*gag*, *gag*, and *pX* coding regions) in MT-2J cells was measured by the real-time PCR based on the method described by Ueno et al. [14]. The gene for albumin was used as an internal control.

Analysis of genomic structure and sites of integration of HTLV-1 provirus in MT-2J cells

To detect the integration sites of HTLV-1 provirus, inverse-long-PCR (IL-PCR) was first performed using a method described previously [15]. Briefly, genomic DNA was digested with *EcoRI* and self-ligated by T4 ligase. This was followed by digestion with *MluI*. The primers used in

this analysis were a forward primer in the U5 region of the LTR (HTLV-8856F 5'-TGCCTGACCCTGCTTGCTCAACTCTACGTCTTTG-3': positions 8856–8889) and a reverse primer, (HTLV-123R 5'AGTCTGGGCCCTGACCTTTTCAGACTTCTGTTTC-3': positions 123–90). PCR was performed using LA Taq DNA polymerase (TAKARA BIO, Shiga, Japan). Yeast RNA (ThermoFisher, CA, USA) was added to PCR reaction mixture at a final concentration of 100 ng/μL to increase amplification efficiency.

Because IL-PCR may be unable to detect the entire provirus integrated into MT-2 cells due to sequence variation, inverse-PCR (I-PCR) using *Sau3AI*, *AluI* and *PstI* as restriction enzyme was performed, with slight modifications, according to a method described previously [16]. Briefly, the genomic DNA was digested with *Sau3AI* or *AluI*, and self-ligated by T4 ligase. After digestion with *SacII*, nested PCR was performed using the Ex taq DNA polymerase (TAKARA BIO). Primers for the first PCR were as follows: forward primer (IPCR-2bF 5'-AAGTACCGGCGACTCCGTTG-3': positions 8978–8997), and reverse primer (IPCR-1R 5'-AAGCCGGCAGTCAGTCGTGA-3': positions 8966–8947). Primers for second PCR were as follows; forward primer (IPCR-4bF 5'-CCAGCGACAGCCCATCCTAT-3': positions 9006–9025), and reverse primer PCR-1R. In case of *PstI* digestion, digested DNA of MT-2J was self-ligated by T4 ligase. This was followed by digestion with *MluI*. PCR of the resultant DNA was performed using forward primer HTLV-8856F and reverse primer (HTLV-7400R 5'-TCGCTTGACACAGTCTCCAAACACGTAGAC-3': positions 7400–7369).

PCR products were subjected to sequence assay using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and ABI Prism 310 DNA Sequencer (Applied Biosystems), according to the instructions provided with the assay kits. DNA sequence of provirus and the adjacent human genome was referred to the information by BLAT search (<http://genome.ucsc.edu/cgi-bin/hgBlat>).

Based on the genome DNA sequence adjacent to each provirus, we then performed the integration-site-specific PCR to identify the whole structure of HTLV-1 provirus [17]. The primer, which was specific to the MT-2J genome adjacent to the integration site of each provirus, was synthesized. Three primers for the HTLV-1 proviral sequence were used for the integration-site-specific PCR (HTLV-7002R: 5'-AGTATTTGAAAAGGAAGGAAGAGGAGAAGGCA-3': positions 7002–6971, HTLV-1206TF: 5'-AAGTCCCTTCCAGTCATGCATCCACATGGTG-3': positions 1206–1235, and HTLV-7150F: 5'-CTTCTAAGGATAGCAAACCGTCA-3': positions 7150–7172). The resultant PCR products were subjected to sequencing assay as described above.

Comparison of HTLV-1 provirus among MT-2J, MT-2A and MT-2B

To determine whether the HTLV-1 provirus found in MT-2J could also be detected in MT-2A and MT-2B, PCR was performed using primers, which are integration site specific for each provirus in the MT-2J cell line. DNA extracted from three cell lines (MT-2J, MT-2A and MT-2B) was excised for PCR using Ex Taq DNA polymerase. The PCR products were electrophoresed on 0.8 % agarose gel, and visualized by ethidium bromide staining.

Detection of transcriptional products of HTLV-1 *Tax/Rex*, *HBZ* and a defective provirus

There was a question as to whether RNA could be transcribed from the complete and defective provirus found in MT-2 cells. Reverse transcription-real time PCR (RT-real time PCR) to detect HTLV-1 *Tax/Rex* RNA, *HBZ* RNA, and RNA transcribed from a defective HTLV-1 provirus (described later) was performed. MT-2 cells (MT-2J, MT-2A and MT-2B) and Jurkat cells were excised to the RNA extraction using TRIzol[®] Reagent (Life Technology Japan, Tokyo, Japan) according to the instructions provided. Resultant RNA was reverse transcribed to complementary DNA (cDNA) using an ImProm-II Reverse Transcription System (Promega, Madison, WI, USA) with oligo dT primer according to the instructions. The primers of PCR for *Tax/Rex* cDNA were as follows; forward primer (Tax-F 5'-CCCGCCGATCCCAAAGAAA-3': positions 5169–5187), reverse primer (Tax-R 5'-GGGTATCCGAAAAGAAGACTCTG-3': positions 7345–7367). The primers of PCR for *HBZ* cDNA were as follows; forward primer (HBZ-F 5'-GGCAGAACGCGACTCAACC-3': positions 8728–8710), reverse primer (HBZ-R 5'-CGGGCATGACACAGGCAAG-3': positions 7259–7277). In case of defective provirus, which was most common in MT-2J in this study (described later), specific primers for both adjunctive sites of the deficiency of provirus (positions 1333–6658) were prepared as follows; forward primer (MT-2 B-G def-F 5'-AGCAAGAAGTCTCCCAAGCAG-3': positions 1284–1304), reverse primer (MT-2 B-G def-R 5'-GGAGGCGATGTGGTTGCA-3': positions 6704–6687). *GAPDH* RNA was used as an internal control. The primers for the PCR of *GAPDH* cDNA were as follows; forward primer (GAPDH-F 5'-GATGCTGGCGCTGAGTACG-3'), reverse primer (GAPDH-R 5'-GCAGAGATGATGACCCTTTTGG-3'). Based on the amount of PCR products, the cycles of PCR was adjusted as 30, 37, 25 and 25 for *Tax/Rex*, *HBZ*, a defective provirus and *GAPDH* cDNA, respectively. Plasmid containing these RT-PCR products for *Tax/Rex*, *HBZ*, a defective provirus and *GAPDH* was constructed using pGEM T-Easy Vector (Promega Corporation, Madison, WI, USA) and was used as a control template for real-time PCR. Primers for real-

time PCR for *Tax/Rex*, *HBZ*, a defective provirus and *GAPDH* were the same as those described above. The FAM-labeled probes for *Tax/Rex*, *HBZ*, a defective provirus and *GAPDH* were as follows; Tax-P: 5'-FAM-CCAACACCATGGCCCCCTTCCCAGG-TAMRA-3: positions 5195–5205, 7324–7337; HBZ-P: 5'-FAM-CGAAACAGCCCTGAGGCCGCCATC-TAMRA-3: positions 8703–9693, 7293–7281; MT-2 B-G def-P: 5'-FAM-CCAGTTTATGCAGACCATCCCTGTAAACCA-TAMRA-3: positions 1318–1337, 6664–6673; GAPDH-P: 5'-FAM-TGGAGTCCACTGGCGTCTTACCACC-TAMRA-3. Real-time PCR was performed in a duplicate manner using Light Cycler 2.0. Relative quantification of transcriptional products of HTLV-1 *Tax/Rex*, *HBZ* and a defective provirus was performed using *GAPDH* RNA as an internal control.

Results

Quantification of HTLV-1 provirus

Chromosomal analysis showed the number of chromosomes in MT-2J cells distributed to be between 81 and 97 (data not shown). Although this analysis showed heterogeneity of chromosomes in this cell line, cells with 95 chromosomes were most common. Therefore, the MT-2J cell line was considered to have a tetraploid genome, basically, and the copy number of HTLV-1 provirus per 4 copies of albumin coding genome was assumed to be the number of proviral copies per one MT2J cell. Real-time PCR showed that the copy number of HTLV-1 provirus (5'LTR-*gag*, *gag*, and *pX* coding regions) per one MT-2J cell (4 copies of albumin coding region) was 11, 2, and 12, respectively. These data suggested the existence of many defective proviruses in MT-2J cells.

Genomic structure and sites of integration of HTLV-1 provirus in MT-2J cells

To identify the integration sites of HTLV-1 provirus and its DNA sequence, IL-PCR using *EcoRI* digestion followed by DNA sequencing was first performed (Fig. 1a). Six proviral DNA sequences were found to be integrated into six different sites of the MT-2J genome (B, C, D, E, H, and J) (Table 1). To identify the other integration sites, I-PCR using *Sau3AI*, *Alu I* and *Pst I* digestion followed by DNA sequencing, was performed (Fig. 1b–d). As a result, five additional proviral DNA sequences were found to be integrated into five different sites of MT-2J genome (A, F, G, I, and K) (Table 1).

To identify the whole structure of provirus at each integration site, integration-site-specific PCR was performed. Based on the information of the MT-2J genomic

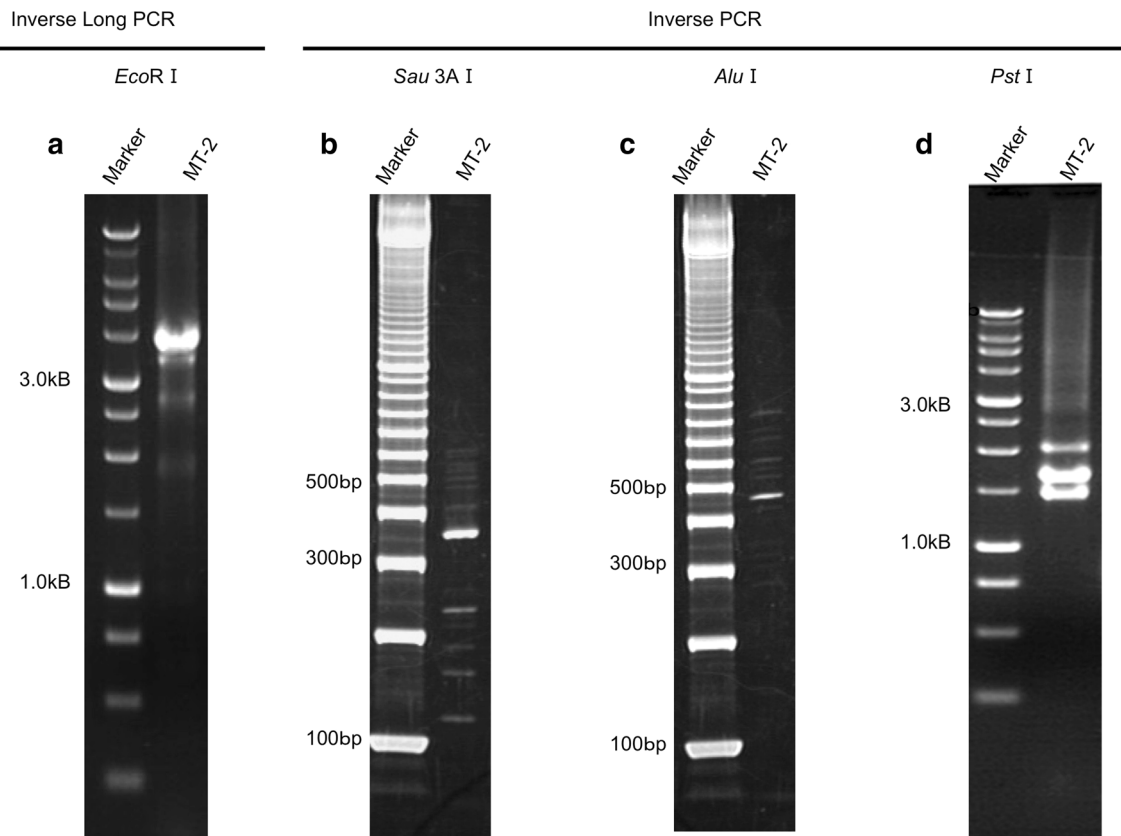


Fig. 1 Inverse-long polymerase chain reaction(IL-PCR)and inverse polymerase chain reaction (I-PCR)of human T-lymphotropic virus Type 1 provirus in MT-2J cells. **a** IL-PCR products using *EcoRI* was electrophoresed on 0.8 % agarose gel. **b** I-PCR products using

Sau3AI was electrophoresed on 10 % polyacrylamide gel. **c** I-PCR product using *AluI* was electrophoresed on 10 % polyacrylamide gel. **d** I-PCR product using *PstI* was electrophoresed on 0.8 % agarose gel

Table 1 Detection of human T-lymphotropic virus Type 1 (HTLV-1) provirus by inverse-long polymerase chain reaction (IL-PCR) using *EcoRI* and by inverse polymerase chain reaction (I-PCR) using *Sau3AI*, *AluI*, and *PstI*

HTLV-1 provirus in MT-2	IL-PCR	I-PCR		
	<i>EcoRI</i>	<i>Sau 3AI</i>	<i>AluI</i>	<i>PstI</i>
A		+	+	
B	+	+		
C	+	+		
D	+	+	+	+
E	+			
F			+	+
G				+
H	+			
I			+	
J	+	+	+	+
K		+	+	

DNA sequence adjacent to each HTLV-1 provirus, primers, which anneal to the expected genomic DNAs on the upstream or the downstream of the HTLV-1 provirus of

each integration site was synthesized. PCR was then performed using these primers in combination with primers (HTLV-7002R, HTLV-1206TF, or HTLV-7150F), which anneal to the HTLV-1 proviral genome. The DNA sequence of integration-site-specific PCR products was analyzed, and the whole structure of HTLV-1 provirus was identified. The structure and integration sites of HTLV-1 proviruses in MT-2J are summarized in Fig. 2. Only one complete HTLV-1 provirus was found to be integrated into MT-2J (Fig. 2a). A provirus of identical 5.3 kB deletion (positions 1333–6658) of internal sequence was found to be integrated into six different sites (Fig. 2b–g). A provirus with a deletion of at least 3.9 kB of internal sequence was detected (Fig. 2h); however, the detailed internal DNA sequence between positions 6842 and 8846 could not be identified. One provirus was found to have a large deletion (6.2 kB) of HTLV-1 sequence from 5' LTR to position 6257 (Fig. 2i). The other provirus was found to have only the DNA sequence of single LTR. This type of defective provirus was integrated into two different chromosomes (Fig. 2j, k). Therefore, five different types of HTLV-1 proviruses were detected in 11 different sites of the MT-2J

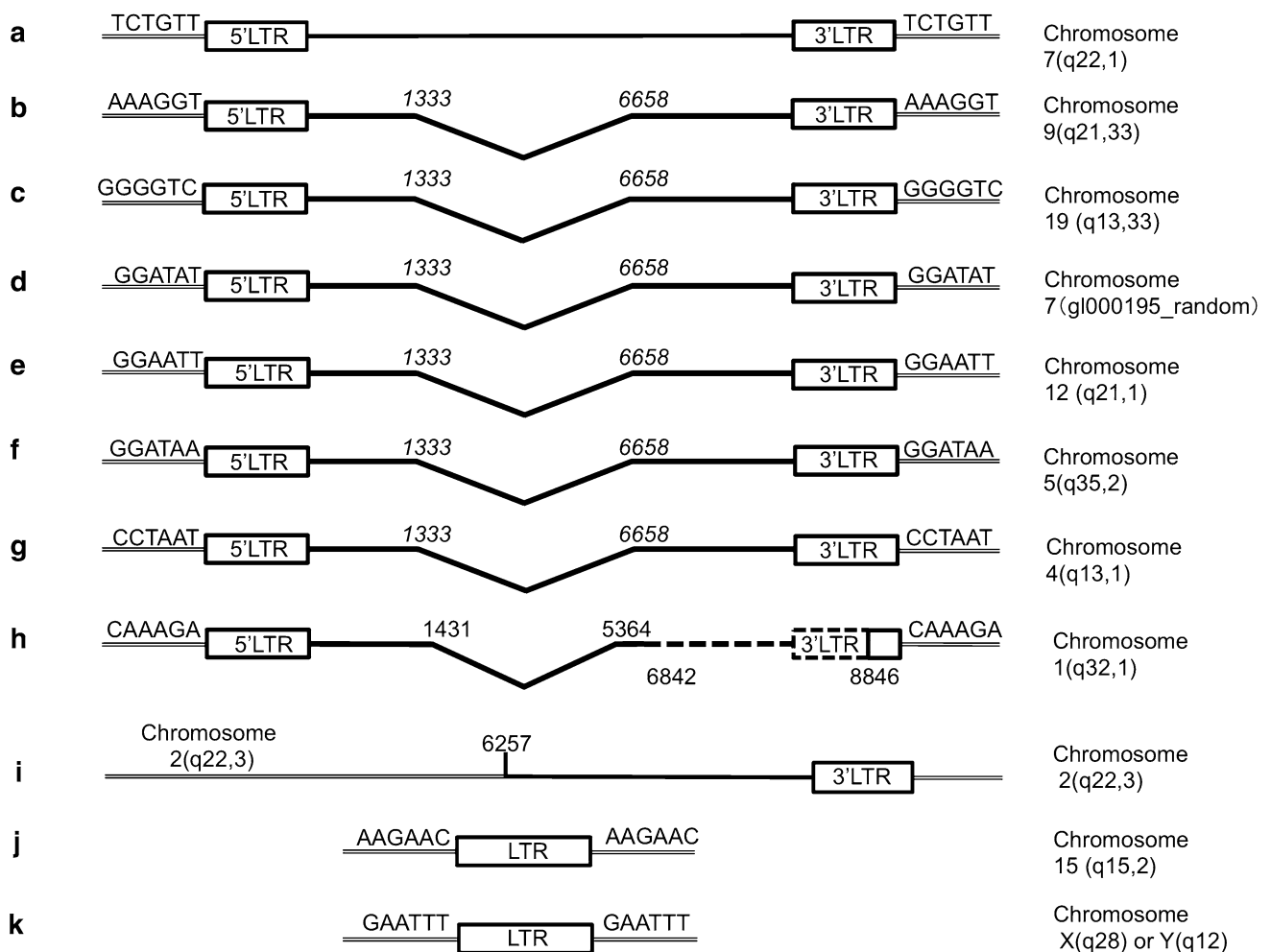


Fig. 2 Structure of human T-lymphotropic virus Type 1 (HTLV-1) provirus and their integration sites (chromosomes) in MT-2J cells. Positions of deletion or truncated provirus were indicated as HTLV-1

provirus base numbers. Six bases repeat sequences derived from host genome DNA were shown at the both end of provirus. Dotted line indicates unidentified internal sequence. LTR long terminal repeat

genome. Six bases repeat sequences derived from the host genome, which is characteristic of the integrated form of retrovirus [18], were found in both ends of each provirus, except a provirus (Fig. 2i).

Comparison of HTLV-1 provirus among MT-2J, MT-2A and MT-2B

To determine whether five types of different HTLV-1 proviral sequences in 11 different sites of the MT-2J genome were conserved in the other MT2 cell lines, two MT-2 cell lines (MT-2A and B), which have been independently passaged at two different laboratories, were tested for integration-site-specific PCR (Fig. 3a). Five (A, B, I, J and K) of 11 proviruses were detected in all cell lines; however, the others were not. D, E, F, and G were not detected in MT-2A, whereas, C, E, F, G, and H were not detected in MT-2B (Fig. 3b). Together, a complete provirus and three

of four types of defective provirus (except H) were consistently detected in 3 cell lines tested.

Detection of transcriptional products of HTLV-1 *Tax/Rex*, *HBZ* and a defective provirus in MT-2J, MT-2A and MT-2B

To determine whether RNA could be transcribed from proviruses in MT-2 cells, we performed RT-PCR to detect HTLV-1 *Tax/Rex* and *HBZ* RNA (Fig. 4a). In addition, we also tested whether the specific RNA for defective HTLV-1 provirus (with 5.3 kB deletion), which was most commonly found in MT-2J cells (Fig. 2 B, C, D, E, F, and G), was transcribed or not. As shown in Fig. 4, *Tax/Rex* and *HBZ* RNA was detected in all three cell lines. Transcriptional product for a specific defective provirus was detectable in all three MT-2 cell lines. RT-real time PCR revealed higher levels of *Tax/*

Fig. 3 Detection of human T-lymphotropic virus Type 1 (HTLV-1) provirus in MT-2A and MT-2B by the polymerase chain reaction (PCR) using primers, which are specific for each integrated provirus found in MT-2J cell line. **a** PCR products from MT-2J, MT-2A, and MT-2B cells were electrophoresed on 0.8 % agarose gel. **b** Summary of detection of HTLV-1 provirus (A–K) in MT-2J, MT-2A, and MT-2B cell lines

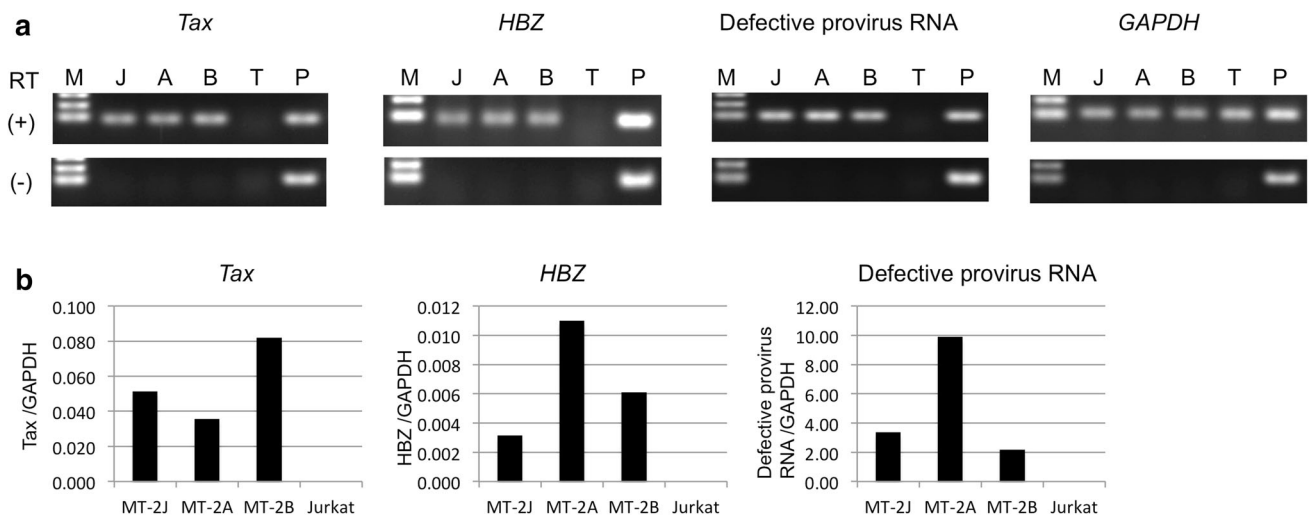
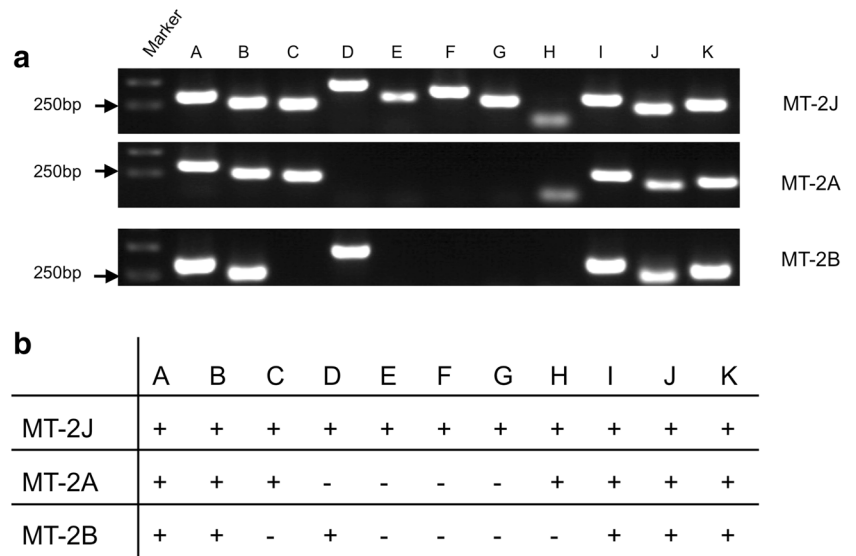


Fig. 4 Identification and quantitative measurement of transcriptional products of human T-lymphotropic virus Type 1 (HTLV-1) *Tax/Rex*, *HBZ* and a defective provirus using reverse transcription (RT)-polymerase chain reaction (PCR) and RT-real time PCR. **a** Identification of RNA transcribed from genes of HTLV-1 *Tax/Rex*, *HBZ*, a defective provirus and *GAPDH* in MT-2 cells (MT-2J, MT-2A, and MT-2B) with or without reverse transcription (RT+ and RT–,

respectively). PCR products were electrophoresed on 0.8 % agarose gel and visualized by ethidium bromide staining. M, 100 bp ladder marker; J, MT-2J; A, MT-2A; B, MT-2B; T, Jurkat cell; P, positive control (plasmid DNA). **b** Levels of RNA transcribed from genes of HTLV-1 *Tax/Rex*, *HBZ* and a defective provirus expressed as the ratio to that of *GAPDH* gene in MT-2J, MT-2A, and MT-2B cells

Rex RNA than *HBZ* RNA, and the level of transcriptional product for a specific defective provirus, was much higher than those of HTLV-1 *Tax/Rex* and *HBZ* RNA (Fig. 4b).

Discussion

In the current study, the genomic structure and site of integration of HTLV-1 provirus in MT-2J cells were analyzed in depth. The copy number of three different

regions of HTLV-1 provirus (5’LTR-*gag*, *gag*, and *pX* coding regions) in one cell (per 4 copies of albumin coding region) was assumed to be 11, 2, and 12, respectively, by real-time PCR. These data suggested that the majority of MT-2J cells harbor 10 or more HTLV-1 proviruses; and many of these proviruses are likely to have deficiencies or mutations in the *gag* region at least. This was also true in the case of HTLV-1 asymptomatic carriers, who were frequently shown to have HTLV-1 infected cells with defective provirus of the *gag* region [14].

Next, we attempted to identify the genomic structure of HTLV-1 provirus in MT-2J cells by IL-PCR and/or I-PCR. Eventually, five different types of HTLV-1 proviral sequences were detected in 11 sites of the MT-2J genome. No one method of IL-PCR nor I-PCR was sufficient to detect all of them [15, 16]. This may be due to the variation of DNA sequence of HTLV-1 proviral genome. The mutation or deficiencies of proviral sequences may be resistant to digestion by restriction enzymes used for IL-PCR or I-PCR. Alternatively, the large size of PCR products, which is defined by the location of sites for restriction enzyme in genome, may cause technical difficulty.

The five types of HTLV-1 proviral sequences were as follows; one complete proviral genome, two different types of proviruses with deletion of large internal viral sequence (5.3 and 3.9 kB), one provirus with large deletion (6.2 kB) from 5' LTR to position 6257, and a provirus with only LTR. The deficiencies found in these proviruses were likely to be produced after their integration into the host genome because six bases repeat sequences were found at the both ends of provirus except the case of provirus (I). This was true even in the case of the provirus containing LTR only. It was reported that a homologous recombination between two LTRs could delete the majority of the provirus resulting in the integration of a single LTR [19].

A provirus with identical deletion of large internal viral sequence (positions: 1333–6658, 5.3 kB) was found to be integrated into six different sites (chromosomes) of MT-2J genome. HTLV-1 provirus is known to have same 5 bases, CATCC at positions 1333–1337 and 6658–6662. Therefore, it is possible that this deficiency was the result of non-homologous recombination between these two sites. Similar non-homologous recombination was reported in human immunodeficiency virus type 1 genetic recombination [20]. The reason for that a provirus with identical deletion was integrated into multiple sites of genome of MT-2J cell was not clear.

Testing to determine whether the five different types of HTLV-1 proviral sequences in 11 different sites of MT-2J genome could be found in the other MT-2 cell lines (MT-2A and MT-2B) passaged in the different laboratories revealed conservation of a complete provirus and three of four types of defective provirus. Among the most common defective provirus with deletion of internal viral sequence (5.3 kB), the defective provirus integrated into chromosome 9 was found in all cell lines tested; however, the provirus with identical deletion of internal viral sequence (5.3 kB) in the other chromosomes was not always found in these cell lines. This observation suggested that at least this defective provirus integrated into chromosome 9 of the original MT-2 cell line and that integration into the other chromosomes occurred later during the long period of subculture.

Next, we tested to determine whether transcriptional product derived from provirus was detectable in MT-2 cell lines. As previously reported, *Tax/Rex* RNA and *HBZ* RNA were detectable among all three cell lines, although the level of *HBZ* RNA was low [21, 22]. The transcriptional product for a specific defective provirus with deletion of internal viral sequence (5.3 kB) was also detectable in all three MT-2 cell lines. The level of the transcriptional product derived from this defective provirus was much higher than those of *Tax/Rex* RNA and *HBZ* RNA. MT-2 cells have been reported to release two distinct types of virions [23]. The major “classic” type of particle contains the standard HTLV-I structural proteins. Approximately 5 % of particles are “light,” and contain 24S (3.4 kB) RNA transcript. DNA sequence of HTLV-1 defective provirus, which codes this 24S (3.4 kB) RNA transcript, was supposed to be same as that of defective provirus with 5.3 kB deletion in this study, when we compared their DNA sequence (data not shown) [24]. The 24S (3.4 kB) RNA transcript was reported to be translated into p28 [24]. It was not clear whether defective provirus with 5.3 kB deletion is functional in this study; however, p28 protein was suggested to play a role in the assembly of the light virions [23]. Takeuchi et al. also reported that 68 kD protein, which is a fused gene product of *env* and *tax* derived from a defective provirus, was detectable in their MT-2 cells [25]. Based on the DNA sequences clarified for the defective proviruses, only the complete provirus (A) can transcribe *Tax/Rex* and *HBZ* genes. Provirus (I) have the potential to transcribe *HBZ* genes; however, the other proviruses are not thought to transcribe any of these. Further study is necessary to clarify whether protein can be actually produced by the various defective proviruses found in MT-2 cells in the current study and what, if any, their functions are.

There are several limitations to the current study. The provirus integrated into MT-2 cells might not all be detected by the current methods employing IL-PCR and I-PCR. The number of copies of HTLV-1 *pX* region in MT-2J measured by the real-time PCR was assumed to be 12 per cell. A provirus (H), which has a deletion of at least 3.9 kB of internal sequence, was detected; however, the detailed internal DNA sequence between positions 6842 and 8846 could not be identified, possibly because the number of this provirus integrated into MT-2J was small. Further studies are necessary to identify greater detail of proviruses in MT-2 cells.

In conclusion, five different types of HTLV-1 proviral sequences were detected in 11 different sites of MT-2J genome. The provirus with identical deletion of large internal viral sequence (5.3 kB) was found to be integrated into the six different sites (chromosomes) of the MT-2J genome. A complete provirus and three of four types of defective provirus were consistently detected in the other MT-2 cell lines. Not only *Tax/Rex* RNA and *HBZ* RNA,

but also the transcriptional product for a specific defective provirus was detectable in all three MT-2 cell lines. The provirus with the deficiencies/mutations in the internal sequences is commonly found to be harbored by the lymphocytes in HTLV-1 carriers [13]. Because defective provirus is frequently detected in ATL cells, it may be related with the process of leukemogenesis [10]. In contrast, multiple integration of HTLV-1 provirus, which was seen in MT-2 cell line in this study, was hardly found in the peripheral blood lymphocytes in the HTLV-1 infected individuals [26]. Therefore, the HTLV-1 proviral polymorphism in the infected cells may be established in a different manner between in vitro culture system and in vivo infection; however, the data in the current study may be important when we think about their mechanism. In addition, because MT-2 cells were shown to have large variation of integrated HTLV-1 proviruses, it is important for researchers to exercise caution in their assessment and interpretation of results using them.

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Compliance with ethical standards

Conflict of interest We have no disclosure.

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