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Original Article

Mutation Analysis for *TP53* in Chronic-Type Adult T-Cell Leukemia/Lymphoma

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Adult T-cell leukemia/lymphoma (ATLL) is a T-cell neoplasm caused by human T-cell leukemia virus type I (HTLV-I). ATLL is classified into four clinical subtypes based on the clinical manifestation: acute, lymphoma, chronic and smoldering. Approximately half of chronic type ATLL cases progressed to the acute type. We previously demonstrated that genomic alterations related to the cell cycle de-regulation such as *CDKN2A* and immune escape such as *CD58* alteration can serve as predictive biomarkers for acute transformation of the chronic type. Although alteration of *TP53*, which is known to be a major regulator of cell cycle, has been identified in several types of cancers including acute type ATLL, no copy number alteration of *TP53* was found in the chronic type by array comparative genomic hybridization. In the present study, mutation of *TP53* was further analyzed by sequencing for these cases as well as HTLV-I carriers with oligo-clonality. However, no *TP53* mutation was identified. These results suggested that *TP53* mutation plays a role for the later stage of ATLL development. 〔*J Clin Exp Hematop 55(1) : 13-16, 2015*〕

Keywords: adult T-cell leukemia/lymphoma, acute transformation, *TP53* mutation

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is a T-cell neoplasm caused by human T-cell lymphotropic virus type I (HTLV-I).[1-3] Currently, approximately 10 to 20 million people are estimated to be infected by HTLV-I in the world, and high prevalence of the virus are recognized in south-western Japan, Caribbean islands and South America.[4,5] Over 90% of HTLV-I infected peoples remain asymptomatic carriers during one's life time, but approximately 2 to 5% of the carriers develop ATLL.[6]

ATLL has been classified into four types based on the clinical manifestation: smoldering, chronic, lymphoma and acute.[7] Among these subtypes, chronic and smoldering types are regarded as indolent ATLL because patients with these types have better prognosis than those with lymphoma and acute types. However, about half of patients with chronic type ATLL are reported to progress to the acute type and subsequently die.[8] Although combination therapy of interferon α and zidovudine are recommended for indolent ATLL patients who are symptomatic, chronic type ATLL patients with poor prognostic factors such as high level of lactate dehydrogenase are needed to be treated by intensive chemotherapy including stem-cell transplantation.[9]

We previously analyzed genomic alterations of chronic and acute types by using high-resolution array comparative hybridization (aCGH) and compared the molecular characteristics of both types.[10] The study revealed that cell cycle de-regulation and immune escape mechanism play critical roles in acute transformation of the chronic type. However, a few of the chronic type cases progressed to acute type ATLL without these alterations. Although *TP53* alteration, which causes de-regulation of cell cycle, has been frequently identified in several types of cancers, no copy number alteration of *TP53* were observed in our analyzed chronic type cases.[10] Several previous studies identified genomic mutation of *TP53* in ATLL cases[11-13] and other several types of cancers. Missense mutations of *TP53* rather than the copy number losses are more frequently observed in various cancers.[14] Although Tawara *et al*. found that two cases of chronic type ATLL possessed a *TP53* mutation and that such patients had a poor clinical outcome,[13] long-term follow-up duration was not described. Therefore, it has remained fully elucidated whether *TP53* mutation can work as predictive markers for

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acute transformation in chronic type ATLL. It should be also noted that mutation analysis for *TP53* has not been performed in HTLV-1 carriers to date.

In order to precisely reveal genomic alterations which are related to cell cycle de-regulation, we analyzed *TP53* mutation in our chronic type ATLL cases, whose copy number alterations had been analyzed by array CGH. HTLV-1 carriers who showed oligo- clonality were also investigated.

MATERIALS AND METHODS

Samples

Eight chronic type ATLL cases and eleven HTLV-1 asymptomatic carriers, for which adequate genomic DNA was available, were analyzed (Table 1). Seven of the eight cases with chronic type ATLL progressed to the acute type. Each DNA was extracted from CD4-positive cells in peripheral blood as described in previous reports.[10,15] Inverse polymerase chain reaction (PCR) analysis for HTLV-1 integration site showed oligoclonal proliferation of CD4-positive cells in five of the HTLV-1 carrier samples which were used in this study.[15] aCGH analysis revealed that five out of eight chronic type ATLL cases used in this study had genomic alterations of the genes which were related to cell cycle (Table 1).[10] This study was approved by the Institute Review Board of the Kurume University School of Medicine (Kurume, Japan) and was conducted with the basis of Declaration of Helsinki.

Mutation analysis for TP53

Mutation analysis was performed as previously described.[16] In brief, exons 4-8 of *TP53* were amplified from genomic DNA using PCR. PCR primers used in this study were detailed in the previous study.[17] The sequence results were evaluated by using International Agency for Research on Cancer (IARC) TP53 database (http://p53.iarc.fr/).[18]

RESULTS

Results for *TP53* mutation were summarized in Table 2. One-nucleotide substitution registered as a single nucleotide polymorphism (SNP) in the NCBI database (http://www.ncbi.nlm.nih.gov/gene/) was found in 17 cases (c.215C > G, SNP rs1042522). Except for this SNP, no mutation was identified in analyzed 19 cases. Even in the three chronic type cases which do not possess the copy number alteration of cell cycle related genes, no mutation of *TP53* was observed.

DISCUSSION

Our previous study revealed that 17 of the 35 (49%) acute type ATLL cases had genomic loss of *CDKN2A* located in 9p21.3, which was also found in 5 of the 27 (19%) chronic type ATLL cases.[10] The previous study also found that 15 of the 27 chronic type cases (56%) had copy number alterations related to cell cycle-related genes including *CDKN2A*.

Table 1. Characteristics of the analyzed samples

Case No.*	Sample type	Proliferation**	Cell cycle alteration***	*CDKN2A* loss	Acute transformation
Carrier-4	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-6	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-7	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-8	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-10	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-11	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-12	HTLV-1 carrier $CD4^+$	Oligoclonal	NA	NA	NA
Carrier-13	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-14	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-15	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
Carrier-16	HTLV-1 carrier $CD4^+$	Not determined	NA	NA	NA
C-1	Chronic type ATLL $CD4^+$	Monoclonal	+	−	+
C-3	Chronic type ATLL $CD4^+$	Monoclonal	+	−	+
C-6	Chronic type ATLL $CD4^+$	Monoclonal	+	+	+
C-10	Chronic type ATLL $CD4^+$	Monoclonal	+	+	+
C-12	Chronic type ATLL $CD4^+$	Monoclonal	+	−	+
C-21	Chronic type ATLL $CD4^+$	Monoclonal	−	−	−
C-23	Chronic type ATLL $CD4^+$	Monoclonal	−	−	+
C-24	Chronic type ATLL $CD4^+$	Monoclonal	−	−	+

NA, not available.

*: Representations in previous Ohshima *et al*. and Yoshida *et al*. papers were used.

**: These results were extracted from previous Ohshima *et al*. and Yoshida *et al*. results.

***: These results were extracted from previous Yoshida *et al*. result. "+" means presense of the genomic alterations related to cell cycle pathway.

Table 2. Results of *TP53* mutation analyses

Case No.*	Exon4	Exon5	Exon6	Exon7	Exon8
Carrier-4	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-6	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-7	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
Carrier-8	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-10	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-11	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
Carrier-12	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-13	Wt	Wt	Wt	Wt	Wt
Carrier-14	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
Carrier-15	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
Carrier-16	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
C-1	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-3	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
C-6	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-10	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-12	c.215C > G, SNP rs1042522 homozygous	Wt	Wt	Wt	Wt
C-21	Wt	Wt	Wt	Wt	Wt
C-23	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt
C-24	c.215C > G, SNP rs1042522	Wt	Wt	Wt	Wt

Wt, wild type.

*: Representations in previous Ohshima *et al*. and Yoshida *et al*. papers were used.

Because the chronic type ATLL cases with the alterations of cell cycle-related genes progressed to the acute type, presence of these alterations can serve as biomarkers predicting acute transformation in chronic type ATLL. However, no copy number alterations of *TP53* were found in our previous study. It is therefore surmised that *TP53* mutation rather than copy number alteration exert a predictive biomarker in the chronic type. Mutation analysis for *TP53* was therefore performed in this study, but no case with *TP53* mutation was found.

It is speculated that *TP53* mutation could not be identified in HTLV-1 carriers because of few *TP53* mutated cells. Under the current method, ultra-deep-next-generation sequencing might detect such *TP53* mutated cells in HTLV-1 carriers because the sensitivity of this method is reported to be approximately 0.3%.[19] Additionally, flow cytometrical analysis by using CD3, CD7 and CADM1 antibodies can purify the HTLV-1 infected clones which can progress to ATLL even in the carrier samples.[20] Combination of these techniques would help us more precisely evaluate *TP53* mutation in the carriers, and this analysis could reveal the role of *TP53* mutation in the pathophysiology of ATLL.

Tawara *et al*. found that 2 of the 13 chronic type ATLL cases had a *TP53* mutation and that *TP53* mutation and *CDKN2A* loss were mutually exclusive events in ATLL.[13] Our current analysis was also applied for the five chronic type cases, who progressed to the acute type without *CDKN2A* loss, but no *TP53* mutation was identified in these cases. This finding suggests that other alterations contribute to acute transformation of the chronic type besides *TP53* mutation. A previous report showed that *TP53* mutation was observed in 43% of acute type ATLL, while only 7% of the chronic type had the mutation.[12] Our current study also suggested that *TP53* mutation is a rare event in the chronic type. From these results, it is speculated that *TP53* mutation plays an important role especially in pathophysiology of the acute type ATLL and that the mutation immediately induces the acute type.

Monti *et al*. reported that copy number alterations of cell cycle-related genes including *CDKN2A* loss predict more precisely poor prognosis in diffuse large B-cell lymphoma than international prognostic index (IPI).[21] Although several clinical factors such as serum calcium levels, serum soluble interleukin-2 receptor levels, and performance status are reported to serve as IPI in ATLL,[22,23] these prognostic indexes do not include molecular pathological factors. Molecular characteristics of ATLL have been elucidated by several groups including us.[10,24,25] Development of prognostic and predictive factors based on these molecular aspects might be valuable to stratify treatments for incurable ATLL cases.

In summary, our previous study revealed that genomic alterations of cell cycle-related genes including *CDKN2A* can serve as predictive markers for acute transformation of the chronic type. However, our current study focusing on *TP53* indicates that copy number alteration and nucleotide mutation of *TP53* are not useful for the prediction of acute transformation in the chronic type.

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