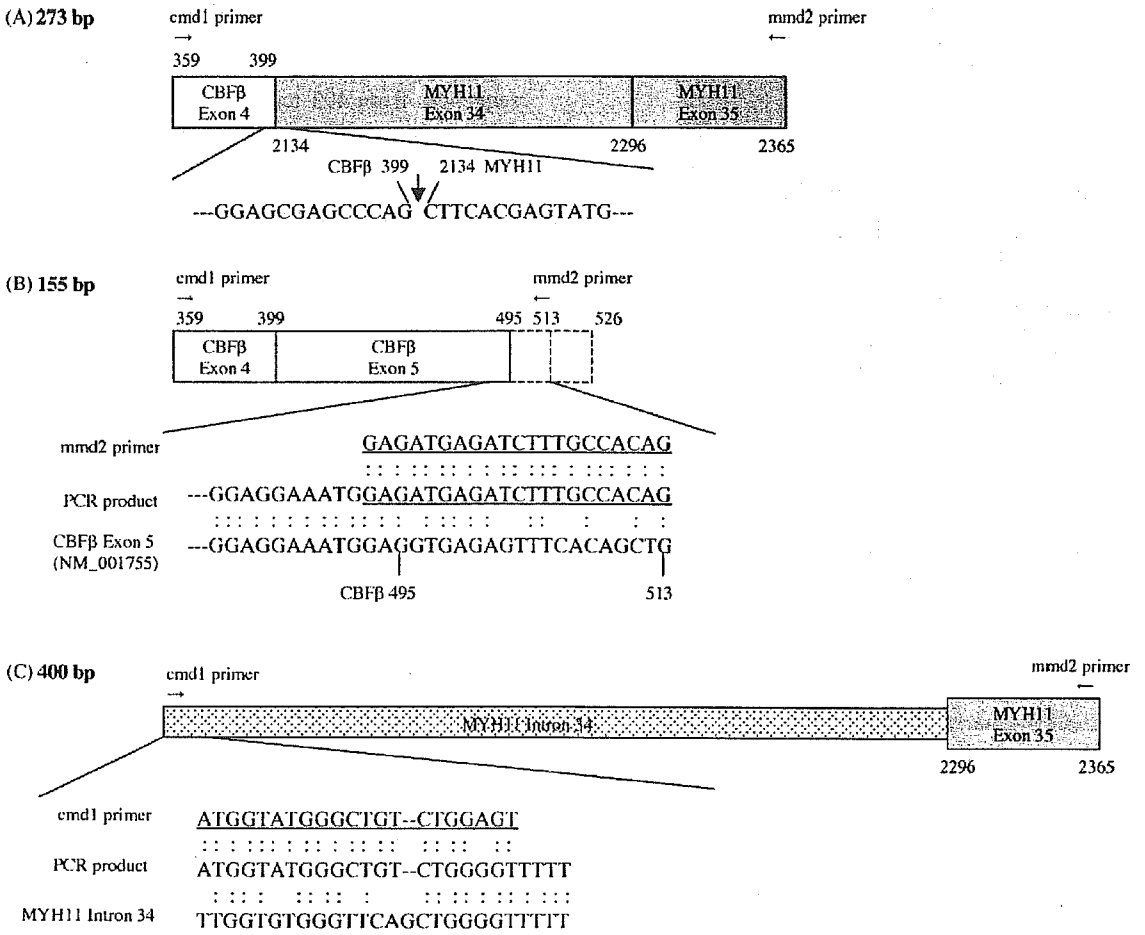

Fig. 5. Schematic illustration and partial nucleotide sequences of the PCR products shown in Fig. 4A. (A) The I type *CBFβ/MYH11* fusion transcript identified in lanes 3 and 4. The *CBFβ* exon 4 is fused to *MYH11* exons 34 and 35. The breakpoints are at nucleotide 399 of *CBFβ* and at nucleotide 2134 of *MYH11* genes and indicated by an arrow. (B) The PCR product of 155 bp detected in lane 2. The *CBFβ* exon 5 at nucleotide 495 is fused to 18 bp of the mmd2 primer. The sequences of the 3′ portion of the PCR product, the mmd2 primer, and another splicing variant of *CBFβ* exon 5 which extends to nucleotide 526, are compared. The sequence of mmd2 primer is underlined. Homologous sequences are indicated by colons. (C) The PCR product of 400 bp detected in lane 2. It is composed of *MYH11* intron 34 and exon 35. The sequences of the 5′ portion of the PCR product, cmd1 primer (underlined) and *MYH11* intron 34 are compared. Homologous sequences are indicated by colons.

topoisomerase II inhibitors (TIs), in particular anthracyclines, although it could be induced by radiotherapy and/or alkylating agents (AAs) alone [7,8]. In the present case, doxorubicin and etoposide were used for conventional chemotherapy and stem cell harvest, whereas cyclophosphamide was used for the conditioning regimen as well as previous therapy. These three drugs were the most frequently administered TIs and AAs in t-MDS/AML with inv(16) [8].

The cellular origin of t-MDS/AML following ASCT has been an important issue. Considering the very short latent period from ASCT to the development of t-MDS (6 months) and an association between TIs and inv(16), it is suspected that the stem cell damage occurred before ASCT in the present case. Moreover, Abruzzese et al. [3] demonstrated that cytogenetic abnormalities observed in t-MDS after ASCT were already present in the pretransplant stem cell harvest specimens from 9 of 12 patients. The percentages of abnormal cells were relatively high (11–46%), then these cytogenetic abnormalities, such as 5q−, −7, and +8, were easily detected by FISH. Lillington et al. [4] also showed that clonally abnormal cells were found before ASCT in all 20 t-MDS/AML patients screened using single locus-specific FISH probes. Nevertheless, we could not detect inv(16) or *CBFβ/MYH11* in the PBSC by RT-PCR as well as FISH. These findings indicate that the origin of t-MDS may be different from case to case. In contrast to our results, almost all karyotypes observed in the studies by Abruzzese et al. [3] and Lillington et al. [4] were unbalanced and complex abnormalities usually brought about by AAs, and there was no case having balanced translocation alone. Stem cells were harvested from bone marrow in all 20 patients [4] and in 8 of 9 patients with positive results [3], whereas only 1 of 2 patients whose stem cells were harvested from peripheral blood was positive [3]. It is unknown whether abnormal clones could be

detected more easily in bone marrow than PBSC by FISH, but these differences of stem cell sources and cytogenetic abnormalities might be associated with discrepant results.

On the other hand, several reports supported our results. Pedersen-Bjergaard et al. [1] suggested that some cases of t-MDS/AML could be directly initiated or triggered by transplantation procedure, because the use of TBI in the preparative regimen for ASCT has been reported to increase the risk of t-MDS/AML. Gilliland and Gibben [2] described that some patients who go on to develop t-MDS/AML have no detectable cytogenetic or interphase FISH abnormalities at the stem cell harvest. In addition, Weber et al. [5] screened whether chromosomal aberrations, including 5q−, −7, +8, and del(17)(p13), are already detectable in PBSC from 40 patients treated with ASCT. However, none of the stem cell preparations exhibited chromosomal abnormalities, indicating that chromosomal damage is a rare event in stem cell autografts. Consequently, the origin of t-MDS is varied, but at least a part of t-MDS/AML, including the present case, may be derived from transplantation procedure itself.

As shown in Table 1, a total of five cases with the I type *CBFβ/MYH11* fusion transcript has been reported [7,15,16]. The subtypes of the disease were not uniform, that is, only two AML cases were M4Eo. Morphologically, dysplastic changes of neutrophils were observed in all four cases examined and Auer rods of the blasts were detected in three cases. All patients achieved and maintained CR, confirming that patients with t-MDS/AML and inv(16) have favorable prognoses, even if the fusion transcript was I type [8]. As expected, three of five cases developed to MDS/AML following treatment for primary malignancies after a relatively short latent period. Cyclophosphamide, TIs and radiotherapy were commonly used as previous therapy. This finding is not contradictory to our speculation that the conditioning regimen consisting of TBI and cyclophosphamide mainly affected the onset of t-MDS in the present case. Thus, the I type *CBFβ/MYH11* fusion transcript may be preferentially, but not exclusively, associated with atypical phenotypes other than M4Eo, prior chemotherapy, dysgranulopoiesis, Auer rods and favorable prognoses.

The high percentage of therapy-related diseases in patients with I type *CBFβ/MYH11* fusion transcript indicates that breakpoints in the *CBFβ* and *MYH11* genes may vary between de novo AML and t-MDS/AML. Particularly, breakpoints of the *MYH11* gene may cluster within the intron 33 in t-MDS/AML. It has been shown that genomic breakpoints, leading to the formation of A type fusion transcript, are clustered within 370 bp of *MYH11* intron 32 [18], but those in t-MDS/AML with I type fusion transcript have never been studied. Recently, Mistry et al. [19] analyzed t(15;17) translocation breakpoints in acute promyelocytic leukemia that developed after exposure to mitoxantrone. The *PML* breakpoints were tightly clustered in an 8 bp region within intron 6, which was a common site of mitoxantrone-induced cleavage by topoisomerase II. Similar mechanisms might exist in t-MDS/AML with I type fusion transcript. Analyses

Table 1
Reported cases of myelodysplastic syndrome/acute myeloblastic leukemia with inv(16)(p13q22) and $CBF\beta^{399}/MYH11^{2134}$ (I type) fusion transcript

Age/sex	Diagnosis	Eosinophils in BM	Morphology of neutrophils and blasts	Karyotypes	Primary tumor	Previous therapy	Latent period (mo)	Treatment response/ duration (mo)	References
53/F	t-AML M4Eo	NA	NA	46,XY,inv(16)(p13q22)[13]/46,XX[23]	Breast cancer	CY, MIT, 5-FU, RT	11	CR/16+	Dissing et al. [7]
58/M	De novo AML M4Eo	6%, abnormal	Dysgranulopoiesis	Poor quality of metaphase, inv(16) confirmed by FISH[10]	None	None	None	CR/18+	van der Reijden et al. [15]
53/F	t-AML M2	5%, no abnormality	Hypogranulation, hyposegmentation, Auer rods, MPO+	46,XX,inv(16)(p13q22)[15]/46,XY[5]	Breast cancer	CY, EPI, 5-FU, RT	24	CR/6+	Grardel et al. [16]
36/M	De novo AML M2Eo	10%, dystrophic	Hypogranulation, Auer rods, MPO+	46,XY,inv(16)(p13q22)[9]/46,XY,+ 8,inv(16)(p13q22)[6]/46,XY[5]	None	None	None	CR/12+	Grardel et al. [16]
48/F	t-MDS RAEB-t	1.2%, no abnormality	Hypogranulation, pseudo-Pelger anomaly, Auer rods, MPO+	46,XX,inv(16)(p13q22)[5]/46,XX[15]	NHL	CY, DXR, VCR, PSL, ETP, Ara-C CDDP, TBI	12	CR/28+	Present case

M, male; F, female; t-AML, therapy-related acute myeloblastic leukemia; t-MDS, therapy-related myelodysplastic syndrome; NA, not available; MPO+, myeloperoxidase positive; NHL, non-Hodgkin's lymphoma; CY, cyclophosphamide; MIT, mitoxantrone; 5-FU, fluorouracil; RT, radiotherapy; EPI, epirubicin; DXR, doxorubicin; VCR, vincristine; PSL, prednisolone; ETP, etoposide; CDDP, cisplatin; Ara-C, cytosine arabinoside; TBI, total body irradiation; CR, complete remission; CR/+ indicates alive.

of genomic breakpoints in the *CBFβ* and *MYH11* genes are needed to clarify the molecular mechanism of t-MDS/AML.

Amplification of *CBFβ/MYH11* artifacts due to non-specific annealing is a persistent problem with false-positive results. Hackwell et al. [17] demonstrated the amplification of the *MYH11* gene comprising of intron 30 and exon 31 using CBFβ3 and MYH11 5M primers, instead of the *CBFβ/MYH11* fusion transcript. Their results were due to contamination with genomic DNA or unspliced primary RNA transcript and 7 bp sequence homology of CBFβ3 primer to intron 30. Amplification of *MYH11* intronic sequences in the present study could be explained similarly. Other PCR artifacts due to aspecific annealing of MYH11 M1 primer to another position of *MYH11* cDNA were also reported [20]. We have shown here that the nested RT-PCR method described by van der Reijden et al. [15] using cd–mm and cmd1–mmd2 primer sets could also amplify PCR artifacts such as *CBFβ* cDNA and *MYH11* genomic DNA, resulting in false-positive results. Therefore, even if these primers are used, it is essential to confirm the sequences of PCR products when the unexpected anomalous sized bands are detected.

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Casein kinase Iε down-regulates phospho-Akt via PTEN, following genotoxic stress-induced apoptosis in hematopoietic cells

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Abstract

Here, we show a functional role of casein kinase I (CKI) ε in hematopoietic cell survival through the modification of phosphatidylinositol 3-kinase (PI3K)/Akt signaling. Introduction of wild-type (WT)-CKIε into interleukin-3 (IL-3)-dependent 32D cells increased the sensitivity to genotoxic stresses, such as γ-irradiation, etoposide, and IL-3 deprivation, whereas kinase-negative (KN)-CKIε suppressed it. Contrary to KN-CKIε, WT-CKIε attenuated the IL-3-induced activation of Akt with the increase of PTEN activity. Similarly, the increase of Akt activation, as well as PTEN inactivation, was accompanied both by a decrease of CKIε expression induced by *all-trans* retinoic acid and by the addition of a specific inhibitor for CKIε in HL-60 cells. CKIε seems to activate PTEN by physical interaction. These results suggest that the CKIε-induced down-regulation of PI3K/Akt signaling through PTEN lead to amplified sensitivity to apoptosis. Thus, the suppression of CKIε in many human leukemia cell lines may play a role in the cell immortalization.

Keywords: Casein kinase Iε; Akt; PTEN; Cell survival; Leukomogenesis

Introduction

Members of the casein kinase I (CKI) family of monomeric serine/threonine kinases are highly conserved from yeast to human, and are ubiquitously expressed in different cell types (Gross and Anderson, 1998; Vielhaber and Virshup, 2001). In mammals, seven isoforms (α, β, γ1−3, δ, and ε) have been identified. By regulating the stability of potential substrates in vitro and transport-dependent cellular processes, CKI is likely to regulate DNA and RNA metabolism, cellular morphology, vesicular trafficking, DNA repair, and the activity of various transmembrane receptors. In addition, recent genetic analyses in diverse fields have demonstrated that CKIε plays an essential role in regulating several critical in vivo processes, such as circadian rhythm (Lowrey et al., 2000), and embryogenesis and morphogenesis via Wnt signaling in various species (Peters et al., 1999). In the CKI family, CKIδ and CKIε, which were first cloned while screening for a budding yeast mutant, *hrr25*, are known to have similar functions because of their structural similarity (Hoekstra et al., 1991; Fish et al., 1995).

Recently, we have reported the specific biological function of CKIε using recombinant CKIε cDNA-introduced murine myeloid progenitor 32D systems (Okamura et al., 2004). CKIε is down-regulated along with hematopoietic granulocytic differentiation. Then, it self-inhibits cytokine-induced granulocytic differentiation by stabilizing the suppressor of cytokine signaling 3 (SOCS3) and β-catenin. The 32D cells over-expressing CKIε grew as well as wild-type (WT) 32D cells in the standard culture condition. However, the 32D cells over-expressing CKIε seemed to be more apoptotic in the poor culture condition. Therefore, we speculated that CKIε might be involved in the regulatory mechanism of the cell survival. This study is focused on the sensitivity of various genotoxic stresses.

Phosphatidylinositol 3-kinase (PI3K)/Akt signaling, which can be activated by many growth factors and cytokines, was shown to be involved in a variety of cellular functions including inhibition of apoptosis (Nicholson and Anderson, 2002). The serine/threonine kinase Akt, a novel downstream target of this signaling, stimulates several pathways and

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synergistically promotes cell survival by phosphorylating suitable substrates such as Bad, caspase-9, IKKα, the Forkhead transcription factors, Mdm2, YAP, and the tumor suppressor TSC proteins (Nicholson and Anderson, 2002; McCormick, 2004). Recent findings indicate that Akt is constitutively activated and promotes cellular resistance to chemotherapy, ionizing radiation, and TRAIL in many tumors (Martelli et al., 2003; Pommier et al., 2004). Specifically, a branch of its pathways involving the kinase mTOR seems relevant for cancer cell survival (Bjornsti and Houghton, 2004). The activation of Akt is likely to be achieved by two main mechanisms (Pommier et al., 2004). First is due to direct *Akt* amplification. Second, Akt can be activated indirectly by PTEN inactivation.

Here, we showed the biological modulation of PTEN by CKIε for hematopoietic cell survival. CKIε increased the sensitivity to genotoxic stress-induced apoptosis by down-regulating PI3K/Akt signaling through stimulating PTEN.

Materials and methods

Cells and culture

The murine interleukin-3 (IL-3)-dependent myeloid progenitor cell line, 32D, was maintained in RPMI 1640 containing 10% fetal calf serum (FCS) (JRH Biosciences, Lenexa, KS, USA) and 15% WEHI-conditioned medium (WEHI-CM). Wild-type (WT)- or kinase-negative (KN)-CKIε cDNA (accession number: AB091043) fragment was introduced into 32D cells by the retroviral vector as described previously (Okamura et al., 2004). KN-CKIε interferes in the biological activity in a dominant-negative manner. As a control, 32D cells expressing only *Neo*-gene were employed. The human promyelocytic leukemia cell line, HL-60, was maintained in RPMI 1640 containing 10% FCS. Other human leukemia cell lines used in this study are the following; ML-1, THP-1, and U-937 derived from myeloid series, Mo7E from megakaryocytes, CCRF-CEM, HPB-ALL, HPB-MLT, Jurkat, MOLT-14, and MOLT-16 from T cell series, and Nalm-6 and Nalm-26 from B cell series. CD34-positive ($CD34^+$) hematopoietic cells were isolated from mobilized peripheral blood cells by Isolex50 (Baxter, Deerfield, IL, USA) (Matsuoka et al., 1997).

To investigate PI3K/Akt signaling, gene-infected 32D cells were starved in only RPMI 1640 for 60 min, and then stimulated with mouse recombinant IL-3 (Genzyme, Cambridge, MA, USA) in a medium containing 10% FCS. Similarly, starved HL-60 were stimulated with *all-trans* retinoic acid (ATRA) (Sigma Chemical Co. St. Louis, MO, USA) or cultured in the presence of CKIε-specific inhibitor, CKI-7 (Seikagaku Corp. Tokyo, Japan).

Antibodies

CKIε-specific polyclonal anti-sera were obtained after immunization of healthy rabbits with a peptide, IPASQT SVPFDHLGK, coupled to keyhole limpet hemocyanin by utilizing glutaraldehyde (Okamura et al., 2004). Antibodies to the total and serine 473-phosphorylated Akt (P-Akt), serine 380/threonine 382/383-phosphorylated PTEN (P-PTEN) and Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG were purchased from New England Biolabs (Beverly, MA, USA). An antibody to PTEN (FL-403) was kindly provided by Santa Cruz Biotech (Santa Cruz, CA, USA).

Apoptosis study

For induction of apoptosis, 1×10^5 cells/ml exponentially growing gene-infected 32D cells were irradiated at room temperature using a ^{60}Co γ-ray source with a dose of 7.0 Gy or treated with 0.2 μM etoposide (Sigma) in complete medium, and/or incubated without WEHI-CM as IL-3 deprivation. Apoptotic cells were assessed daily by flow cytometry (FACScan; Becton Dickinson San Jose, CA, USA) using fluorescence-conjugated anticoagulant (Annexin-V-FLUOS; Roche, Germany).

Immunoblot analysis

Immunoblot analysis was performed as described previously (Okamura et al., 2004). Briefly, cells were lysed in lysis buffer containing 50 mM Tris–HCl, pH 7.5, 50 mM NaCl, 2 mM EDTA, 1% Triton X-100, 10 mM sodium pyrophosphate, 1 mM sodium vanadate, 50 mM sodium fluoride, 1.0 μg/ml leupeptin, 1.0 μg/ml aprotinin, 1.0 μg/ml pestatin, and 1 mM phenylmethylsulfonyl fluoride. For immunoprecipitation, the total cell lysate was incubated with specific antibodies, followed by the addition of protein G-Sepharose (Amersham Pharmacia Biotech) on a rotating shaker for 4 h at 4 °C. The immunoprecipitates were washed three times with lysis buffer and three times with phosphate-buffered saline. The cell lysate was separated on 12–15% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) and transferred onto nitrocellulose membranes (Protran, Schleicher and Schuell, Germany). The membranes were probed with specific antibodies. Immune complexes were detected by chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL, USA).

RNA blot analysis

Total RNAs of human leukemia cell lines and $CD34^+$ cells were isolated by the acid guanidinium thiocyanate–phenol chloroform method (Nagata et al., 1996). RNA blots were hybridized with ^{32}P-labeled human CKIε-specific cDNA probe, which was prepared from the C-terminal-coding (nucleotide 920–1302) fragment.

Statistical analysis

Data are presented as the means±S.E.M of two or three clones of each gene-infected 32D cell line from three independent experiments. Student's *t*-test was applied in analyzing the results of the apoptosis study. $P<0.05$ was considered significant.

Results

CKIε increases the sensitivity of genotoxic stress-induced apoptosis

To elucidate the functional significance of CKIε in hematopoietic cell survival, apoptosis induced by several genotoxic stresses, such as γ-irradiation (7.0 Gy), etoposide (0.2 μM), and IL-3 deprivation in 32D cells expressing WT- or KN-CKIε-cDNA were examined. The Annexin V positive rate was measured as apoptotic cells by flow cytometry. Under normal culture conditions without any stress, there was no significant difference in cell growth or viability among the three cell lines as described previously (Okamura et al., 2004). However, WT-CKIε cells were significantly more sensitive to these apoptotic stresses than control cells (Table 1). In contrast, KN-CKIε cells were relatively less sensitive during the first few days after the treatments (Table 1). These results indicated that CKIε increased the sensitivity to genotoxic stress-induced apoptosis in hematopoietic cells.

CKIε down-regulates the phosphorylation of Akt via PTEN

In reference to this biological response, it was considered that CKIε might be involved in a cell survival signaling, PI3K/Akt. Because the pathway was one of the downstream targets of cytokine stimulation, the involvement of CKIε in IL-3-induced Akt activation of the 32D cell lines was investigated by immunoblot analysis. There were significant differences in the phosphorylation levels of Akt among the three cell lines (Fig. 1). By IL-3 stimulation, serine 473-phosphorylated Akt (P-Akt), an active form of Akt, was induced strongly within 20 min in all cell lines. In comparison to control cells, P-Akt decreased rapidly, and was barely detectable by 60 min in WT-CKIε cells. In contrast, a high level of P-Akt was sustained for 120 min in KN-CKIε cells.

Next, the activity of PTEN, a negative regulator of PI3K/Akt signaling, was evaluated after the IL-3 treatment. PTEN is known to be inactivated by phosphorylation of serine 380/threonine 382/383 in its regulatory domain (Vazquez et al., 2000; Miller et al., 2002). In the absence of IL-3, serine 380/threonine 382/383 was already phosphorylated to some extent in all cell lines (Fig. 1). In control cells, IL-3 increased the phosphorylation gradually in a time-dependent manner. On the other hand, it decreased in WT-CKIε cells, whereas it more clearly increased in KN-CKIε cells (Fig. 1). These results suggested that CKIε up-regulated the PTEN activity following the inhibition of IL-3-induced PI3K/Akt signaling.

Table 1
Apoptotic cells induced by genotoxic stresses (%)

Treatment	Cell	Day 1	Day 2	Day 3
γ-Irradiation (7.0 Gy)	Control	12.80±2.91	38.81±7.31	78.76±0.92
	WT-CKIε/32D	13.28±2.68	61.41±2.88*	89.31±1.46*
	KN-CKIε/32D	7.81±0.55*	38.30±5.11	79.47±2.29
Low-dose etoposide (0.2 μM)	Control	5.86±0.89	6.00±2.00	11.00±3.01
	WT-CKIε/32D	6.53±1.94	13.00±4.02*	27.50±6.59*
	KN-CKIε/32D	4.72±0.75**	5.50±2.25	10.75±2.95
IL-3 deprivation	Control	6.00±0.47	90<	ND
	WT-CKIε/32D	13.75±0.80*	90<	ND
	KN-CKIε/32D	5.47±0.46**	90<	ND

Data are presented as the means±S.E.M. of two or three clones of each gene-infected 32D cell line from three independent experiments.
ND=not determined.
* $P<0.01$ for comparison with control.
** $P<0.05$ for comparison with control.

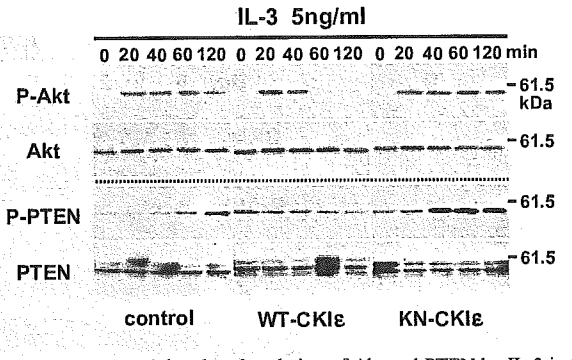

Fig. 1. CKIε suppressed the phosphorylation of Akt and PTEN by IL-3 in 32D cells. 1×10^5 cells/ml 32D transfectants were stimulated with 5 ng/ml IL-3 for the indicated periods (min). Total cell lysate was subjected to immunoblot for analysis of the expression levels of the total and serine 473-phosphorylated Akt (P-Akt) and the total and serine 380/threonine 382/383-phosphorylated PTEN (P-PTEN) using their specific antibodies. These data represent three independent experiments.

Physical interaction of CKIε with PTEN to down-regulate Akt signaling

Human promyelocytic leukemia HL-60 cells, which were differentiated into granulocytes by treatment with ATRA (Matsui et al., 1984), were also employed to assess the functional significance of CKIε in PI3K/Akt signaling. Immunoblot analysis was performed for the detection of CKIε and P-Akt or phosphorylated-PTEN (P-PTEN). The expression of CKIε was down-regulated along ATRA-induced granulocytic differentiation (Fig. 2A). On the contrary, the steady state of both P-Akt and P-PTEN gradually increased day by day (Fig. 2A).

To further confirm the functional interaction of CKIε with the Akt activation via PTEN, HL-60 cells were cultured in the presence of a specific inhibitor for CKIε, CKI-7. Both the P-Akt and P-PTEN were increased by CKI-7 in a dose-dependent manner, although the expression levels of CKIε were not affected (Fig. 2B).

We also examined the physical interaction of PTEN with CKIε in HL-60 cells treated with or without ATRA. As shown in Fig. 2C, PTEN was co-immunoprecipitated with CKIε-specific antibody. In addition, CKIε was co-immunoprecipitated with PTEN-specific antibody. These results suggested that CKIε physically interacted with PTEN to up-regulate its activity following the Akt inactivation.

The suppression of CKIε expression in several human leukemia cell lines

Finally, the CKIε expression of human leukemia cell lines was examined by RNA blot analysis. CKIε is strongly

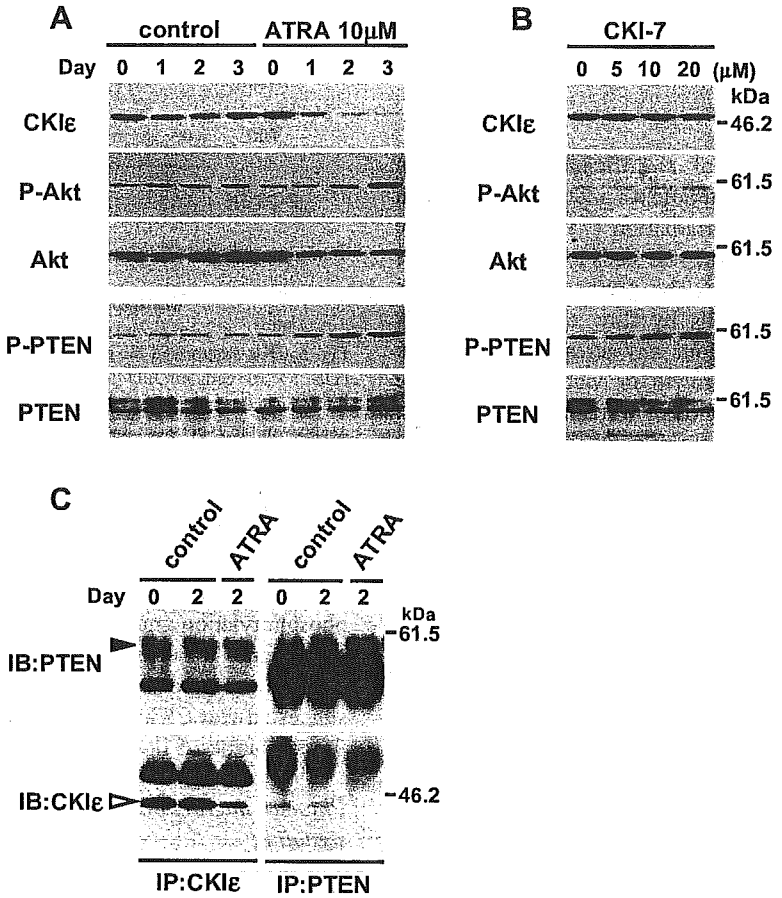

Fig. 2. (A) ATRA-induced down-regulation of CKIε activated Akt signaling in HL-60 cells. 1×10^5 cells/ml HL-60 cells were differentiated to granulocytes by the addition of 10 μM ATRA for the indicated days. Then, total cell lysate was subjected to immunoblot analysis using anti-CKIε, anti-P-Akt, anti-Akt, anti-P-PTEN, and anti-PTEN antibodies. (B) CKIε inhibitor activated Akt signaling with the phosphorylation of PTEN in a dose-dependent manner. HL-60 cells were cultured in the presence of a CKIε-specific inhibitor, CKI-7, for 24 h. Then, total cell lysate was subjected to immunoblot analysis as well. (C) Physical interaction of CKIε with PTEN. HL-60 cells were treated under conditions with or without 10 μM ATRA. After the indicated days, total cell lysate was immunoprecipitated with anti-CKIε (left panels) or anti-PTEN (right panels) antibody. Each blot was probed using anti-PTEN (upper panels) and anti-CKIε (lower panels) antibodies. Black and white arrowheads indicated the position of PTEN and CKIε, respectively. These data represent three independent experiments. IB=immunoblot; IP=immunoprecipitation.

expressed in immature normal hematopoietic cells, and down-regulated along granulocytic differentiation as described (Okamura et al., 2004). Thus, it is of interest how the CKIε expression is regulated in leukemia cells. As shown in Fig. 3, the expression level of CKIε mRNA was different in each human leukemia cell line. Compared to normal $CD34^+$ hematopoietic progenitors, it was suppressed in many cell lines, especially in ML-1, THP-1, Jurkat, and MOLT-16 cells, regardless of cellular origin. The suppression of CKIε might affect the biological characteristics of leukemia cells.

Discussion

Here, we show a new biological function of CKIε for hematopoietic cell survival. CKIε functionally up-regulated the PTEN activity by physical interaction following the inhibition of PI3K/Akt signaling. It seems compatible with the fact that CKIε increased the sensitivity to various genotoxic stress-induced apoptosis in 32D cells. However, the over-expression of recombinant CKIε could not change the IL-3 dependency of

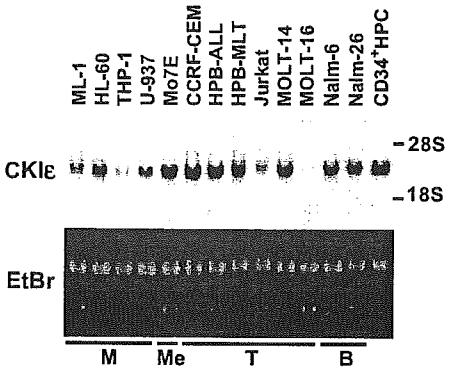

Fig. 3. Expression profile of CKIε in human leukemia cell lines. Total RNAs of human leukemia cell lines was subjected to RNA blot analysis using ^{32}P-labeled human CKIε-specific cDNA probes. Ethidium bromide (EtBr) staining indicated the amount of RNAs loaded in each lane. M=myeloid series; Me=megakaryocyte; T=T cell series; B=B cell series; HPC=human $CD34^+$ hematopoietic cells.

the 32D cell lines by itself. The cytokine deprivation finally led most cells to apoptosis without differences among the three cell lines 2 days after the treatment (Table 1). These results indicate that CKIε affects hematopoietic cell survival only due to exposure to a genotoxic stress. Therefore, the functional role of CKIε seems to be different from anti-oncogenes such as p53, loss of which cause cell immortalization by itself.

PTEN, known as a tumor suppressor, negatively regulates PI3K/Akt signaling by dephosphorylating a lipid second messenger, phosphatidylinositol-3,4,5-triphosphate, generated by activated PI3K (Cantley and Neel, 1999). The phosphatase activity of PTEN is conversely inhibited by the phosphorylation of serine 380/threonine 382/383 in its regulatory domain (Vazquez et al., 2000; Miller et al., 2002). However, it remains to be elucidated how PTEN is activated. Here, we showed that the phosphorylation of the negative regulatory sites of PTEN increased with the decreased expression of CKIε or in the presence of CKI-7. Thus, CKIε is thought to be involved in the phosphorylation of serine/threonine residue(s) other than the negative regulatory sites. Several possible recognition motifs for CKIε exist in the regulatory domain of PTEN including PDZ domain (Wu et al., 2000; Vazquez et al., 2000; Miller et al., 2002; Das et al., 2003). Thus, it is important to elucidate whether CKIε directly phosphorylates PTEN or indirectly activates it by interacting with an unidentified novel regulator for PTEN. Further analysis is necessary to determine the molecular mechanism of PTEN activation by CKIε.

In many leukemia cell lines, the CKIε expression was suppressed, which would contribute to obtaining the characteristics of leukemia cells through the modification of several intracellular signalings. The activation of PI3K/Akt signaling by the suppression of CKIε function would be beneficial to leukemia cell growth. We previously reported that CKIε inhibited signal transducers and activators of transcription 3 (STAT3) activation by stabilizing SOCS3 (Okamura et al., 2004). Recent analysis demonstrated that STAT3-deficient mice were resistant to skin tumor development (Chang et al., 1997). Constitutive activation of STAT3 had been found in various malignancies. Furthermore, somatic mutation of the *CKIε* gene was reported in mammary ductal carcinoma (Fuja et al., 2004). These findings arise a possibility that CKIε plays a role as a tumor suppressor to prevent tumorigenesis not only by the PTEN activation, but also by the STAT3 inactivation.

Moreover, therapy resistance of many malignancies appears to be controlled in part by constitutive activation of Akt due to the loss of PTEN function (Nicholson and Anderson, 2002; Martelli et al., 2003; McCormick, 2004; Pommier et al., 2004). In this study, we showed the CKIε-induced down-regulation of PI3K/Akt signaling through PTEN. Therefore, the suppression of CKIε seen in many leukemia cell lines might have contributed not only to leukomogenesis but also to the acquisition of resistance to anti-cancer therapy.

Conclusion

CKIε increases the sensitivity to genotoxic stress-induced apoptosis by down-regulating PI3K/Akt signaling through the physical interaction with PTEN in hematopoietic cells. The aberration of this CKIε-mediated suppression might be involved not only in leukomogenesis but also in the resistance to anti-cancer therapy of hematological malignancies.

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Non-myeloablative allogeneic haemopoietic stem-cell transplantation for treatment of metastatic invasive thymoma

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Thymoma is a rare epithelial neoplasm that is frequently associated with unique paraneoplastic syndromes, such as pure red-cell aplasia.[1] In small case series,[1–3] high-dose chemotherapy combined with autologous haemopoietic stem-cell transplantation (HCT) was shown to be effective for treatment of advanced invasive thymoma. However, the best salvage treatment to ensure durable remission has yet to be established.

Allogeneic HCT is a standard treatment for haematological malignant diseases that induce curative graft-versus-tumour effects. Non-myeloablative conditioning regimens based on fludarabine are effective in providing a sustained donor-cell engraftment with few toxic effects after allogeneic HCT.[4,5] Childs and colleagues[6] have also noted a graft-versus-tumour effect after allogeneic HCT for advanced renal-cell carcinoma.

Here, we describe a patient with metastatic invasive thymoma and pure red-cell aplasia who was treated successfully with non-myeloablative allogeneic haemopoietic stem cells from her HLA-identical brother. Despite thymoma relapse after previous surgery, radiotherapy, and high-dose chemotherapy followed by autologous HCT, the tumour regressed substantially and donor erythropoiesis was sustained at 6 months after HCT, suggesting that a graft-versus-thymoma effect was present.

In 1994, a 22-year-old Japanese woman was diagnosed with thymoma of mixed lymphoepithelial type by

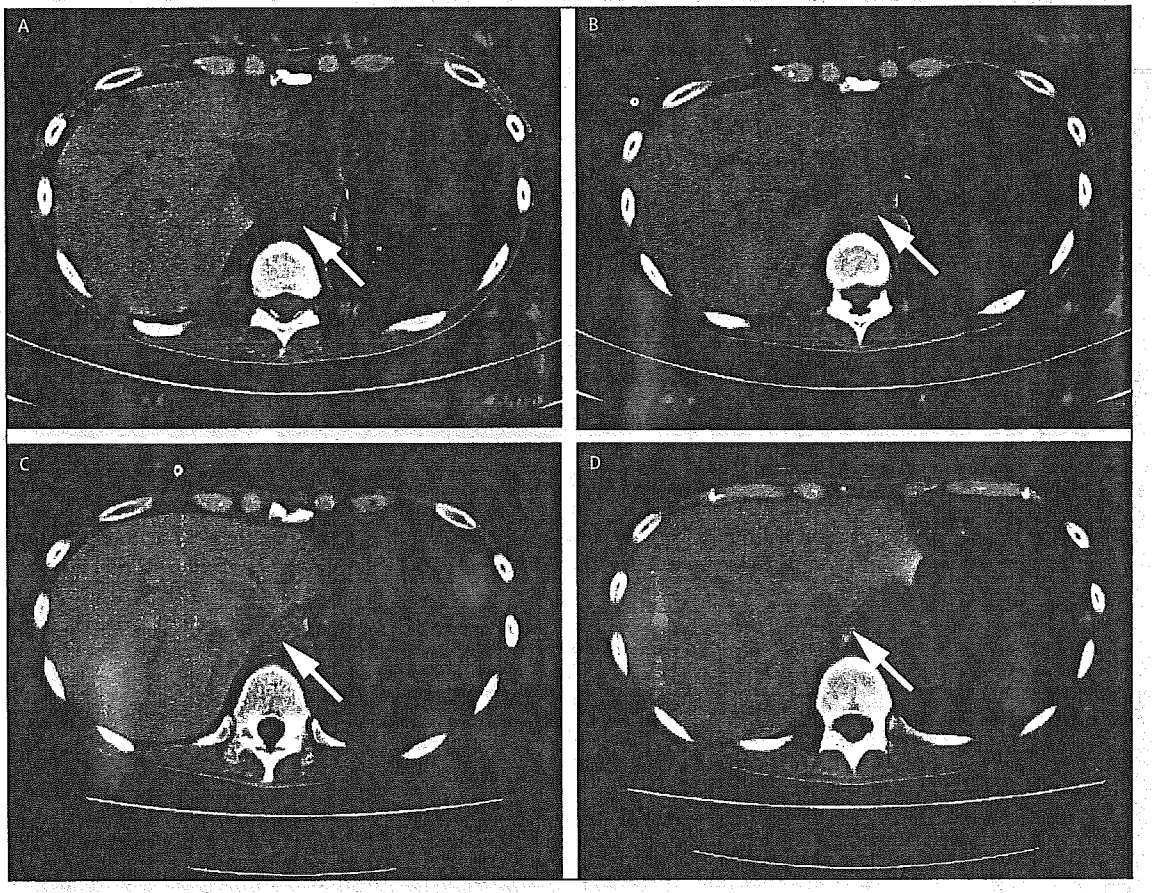

Figure 1: CT scans of retrocrural mass (arrows) before transplantation (A; 58×37 mm), and 12 days (B; 46×22 mm), 38 days (C; 33×13 mm), and 171 days (D; 21×9 mm) after transplantation

needle-aspiration cytology, and underwent extended thymectomy and local radiotherapy. She was diagnosed with invasive thymoma; she had infiltration of atypical large cells with polygonal nuclei and distinct nucleoli, and pale or clear abundant cytoplasm was seen in the fibrous capsules. After recurrence at several pleural sites in 1998, the patient received three cycles of chemotherapy consisting of doxorubicin, cisplatin, vincristine, and cyclophosphamide. After achieving complete remission, she underwent autologous HCT and high-dose chemotherapy with carboplatin, etoposide, and cyclophosphamide. Subsequently, the patient developed pure red-cell anaemia, which was treated successfully by combination immunosuppressive treatment with prednisolone and ciclosporin. 2 years after autologous HCT, the thymoma relapsed at several sites within the thoracic cavity, mediastinum, pleura, pericardium, and retrocrural space. The patient had no symptoms except anaemia-related fatigue. Because the patient refused further aggressive chemotherapy, she was given multiple cycles of local radiotherapy for several relapses within the thoracic cavity. Once the metastatic invasive thymoma started to progress, the patient became dependent on transfusion of red-blood cells despite continued immunosuppressive treatment. In June, 2004, the patient was referred to our transplantation team to participate in an institutionally-approved pilot protocol of non-myeloablative allogeneic HCT for metastatic solid tumours.

On admission, the red-blood cell count was 1890×10^9/L with a reticulocyte count of $3\cdot8\times10^9$/L. The bone marrow had a normal number of total nucleated cells, with extensive erythroid hypoplasia (myeloid/erythroid ratio of 70·3). A CT scan before transplantation showed an increase in the size of the retrocrural mass and multiple pleural metastases (figure 1). After obtaining written informed consent and approval of the institutional review board, the patient underwent non-myeloablative allogeneic HCT when she was 32 years old.

In view of the patient's history of previous autologous HCT and borderline renal insufficiency (serum creatinine 122 μmol/L), the patient received a conditioning regimen before transplantation of 20 mg/m² fludarabine per day for 5 days and 30 mg/kg cyclophosphamide per day for 2 days, which had been modified from the originally reported regimen.[6] After 4 days' administration of 400 μg/m² filgrastim subcutaneously to her HLA-identical, ABO major-minor mismatched brother, mobilised peripheral-blood stem cells were collected by leucopheresis. Unmanipulated peripheral-blood stem cells were infused on day 0; the grafts contained $9\cdot40\times10^8$ nucleated cells/kg, $8\cdot15\times10^6$ CD34-positive cells/kg, and $4\cdot64\times10^8$ CD3-positive cells/kg. 3 mg/kg ciclosporin per day, 10 mg/m² methotrexate on day 1, and 7 mg/m² methotrexate on days 3 and 6 were given intravenously for prophylaxis of graft-versus-host disease.

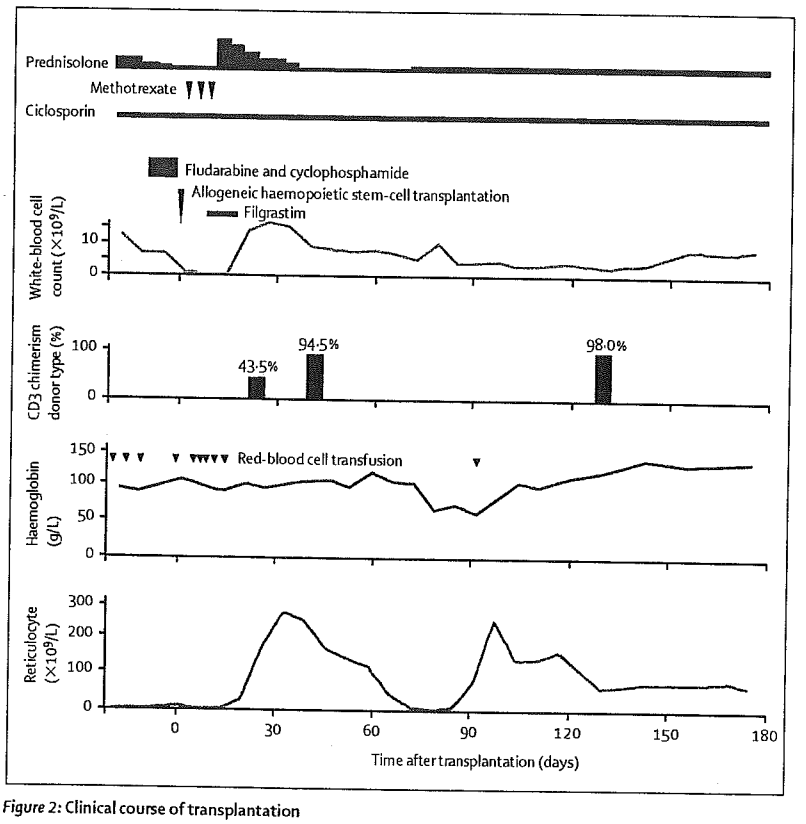

Figure 2: Clinical course of transplantation

Neutrophil engraftment occurred 15 days after transplantation. On day 9, the patient developed grade-III acute graft-versus-host disease (skin, stage 2; liver, stage 2; gut, stage 2), which was treated successfully with prednisolone. Donor chimerism in the CD3-positive T-cell fraction was 43·5% on day 28, 94·5% on day 42, and 98·0% on day 128 as assessed by fluorescence in-situ hybridisation (figure 2). Donor erythropoiesis began after a transient red-cell aplasia around day 80. CT scans showed slight regression of the retrocrural mass by day 12, with further regression of the residual retrocrural lesion and disappearance of the pleural lesions in accordance with the development of acute graft-versus-host disease and conversion to complete donor chimerism at days 38 and 171. At 6 months after transplantation, the patient had limited chronic graft-versus-host disease of the liver, with a 100% performance status, and remained independent on transfusions with sustained regression of the invasive thymoma.

Since Childs and colleagues[6] reported the effectiveness of allogeneic HCT for advanced renal-cell carcinoma, non-myeloablative allogeneic HCT has been used to treat other solid tumours, such as ovarian and breast cancer, with mixed results.[7] To our knowledge, allogeneic HCT has not been reported in invasive thymoma. Because the thymoma in this case relapsed after

chemoradiotherapy and autologous HCT, a poor prognosis with only a slight chance of cure was expected. However, the metastatic lesions regressed substantially after use of an investigational protocol of non-myeloablative allogeneic HCT.

We attribute the regression of thymoma in our case to the combined effects of the conditioning regimen and a graft-versus-tumour effect by donor T cells. The thymoma first started to regress early after transplantation, suggesting that fludarabine and cyclophosphamide have some antithymoma effects. Furthermore, the thymoma continued to regress in concordance with the development of acute graft-versus-host disease and subsequent conversion to complete donor T-cell chimerism. This delayed regression after non-myeloablative allogeneic HCT strongly supports the presence of a potent graft-versus-thymoma effect, which is consistent with previous reports of non-myeloablative allogeneic HCT for other solid tumours.[6,7]

Although the mechanisms of thymoma-associated pure red-cell anaemia remained unresolved, some cases document successful treatment of refractory acquired pure red-cell anaemia with allogeneic HCT.[8,9] In our case, donor erythropoiesis was sustained after a short period of red-cell aplasia around day 80, which is compatible with previous reports of delayed donor erythropoiesis in ABO-incompatible HCT after a similar non-myeloablative conditioning regimen.[10]

In conclusion, allogeneic HCT with non-myeloablative conditioning could be a promising treatment option with curative potential for patients with metastatic invasive thymoma that is unresponsive to conventional treatments. However, longer follow-up and more patients are needed to determine the effectiveness and safety of this new treatment method.

Conflict of interest

We declare no conflicts of interest.

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成人に対する臍帯血移植の治療成績と今後 — 3)成人T細胞白血病

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特集 成人における臍帯血移植

成人に対する臍帯血移植の治療成績と今後 3)成人T細胞白血病*

宇都宮 與**
米倉 健太郎**

Key Words: adult T-cell leukemia, allogeneic stem cell transplantation, cord blood transplantation, graft versus-ATL effect

はじめに

成人T細胞白血病(adult T-cell leukemia；ATL)はhuman T-lymphotropic virus type 1(HTLV-1)が原因ウイルスとして同定[1]され病態解明は進んだが，multidrug resistance(MDR)遺伝子やlung resistance-related protein(LRP)遺伝子が関与する抗がん剤耐性[2)3)]や免疫不全に伴う易感染性[4)]など種々の問題をかかえ，化学療法を施行しても予後はきわめて不良である[5)~7)]．化学療法の中でもっとも治療成績の良いJapan Clinical Oncology Group(JCOG)研究のLSG15プロトコールでも完全寛解率35.5%，治療開始からの生存期間の中央値が約13か月，2年生存率31.3%である[8)]．したがって，急性型やリンパ腫型のATLに対しては化学療法のみでは限界があると言わざるを得ない．

一方，造血幹細胞移植は近年造血器悪性腫瘍の再寛解例や予後不良と予測される例の寛解期に実施し，治癒をもたらす治療法としてもっとも効果を上げている．ATLにおいてはわれわれがretrospective studyではあるが，同種造血幹細胞移植10例を解析した報告以来，もっとも治癒が期待できる治療法として注目されている[9)10)]．また，臍帯血移植は1988年Gluckmanらにより小児重症Fanconi貧血の同胞間臍帯血移植の成功例の報告に始まった[11)]．日本においても血縁や非血縁でHLA一致のドナーが得られない症例や時間的に余裕のない症例に対して実施され，臍帯血バンクの整備と相まって急激にその症例数が増加しつつある．ATLにおける臍帯血移植はまとまった成績の報告はなく，その有効性は明らかではない．本稿では自験例を中心に報告し，今後の問題点についても言及する．

造血幹細胞移植

造血幹細胞移植には自家造血幹細胞移植と同種造血幹細胞移植がある．ATLにおける自家移植は再発と感染症が高率で患者の移植後の生存期間の延長は得られていない[12)13)]．一方，ATLに対する同種造血幹細胞移植は散発的に成功例が報告され[14)15)]，その後われわれの報告[9)]以来，急激に同種移植症例が増加しつつある．福島らはATL患者40例の骨髄破壊的同種移植をretrospectiveに解析し，3年時の全生存率は45.3%，無再発生存率33.8%であり，移植後40例中10例が再発したが，5例が再寛解したと報告している[16)]．さらに興味深いことに再寛解の得られた5例中3例は免疫抑制剤の中止のみで再寛解が得られており，graft versus-ATL(GV-ATL)効果を示唆していると考察している．これらの報告はほとんどが骨髄破壊的同種移植の成績であり，発症年齢の平均が55~60歳であるATLの多くは移植対象から外れてしまう．近

* Outcome and future direction of cord blood transplantation for ATL patients.

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年，高年齢の造血器悪性腫瘍患者に対して骨髄非破壊的同種造血幹細胞移植法が登場して低悪性度リンパ腫の再発例や急性および慢性白血病患者に有効性が示されている．ATLにおいても九州がんセンターの岡村 純先生を班長として50歳から70歳までの急性型とリンパ腫型の寛解例の患者を対象に骨髄非破壊的同種造血幹細胞移植の治療研究班が組織され，第1次プロトコール(第Ⅰ相試験)の成績が2003年の米国血液学会で発表された[17]．16例が本登録され，男性9例，女性7例で年齢の中央値が57歳(51～61歳)であった．病型は急性型11例，リンパ腫型5例，診断から移植までの期間の中央値は6か月(2～44か月)であった．移植時の寛解状態は完全寛解(complete remission；CR)3例，部分寛解(partial remission；PR)10例，非寛解(no remission；NR)3例であり，輸注CD34陽性細胞数は5.1×10^6/kg($1.6\sim8.0\times10^6$/kg)であった．ドナーの16例中8例は血清抗HTLV-1抗体陽性であった．前処置はfludarabine(FLU)/busulfan(BU)/antithymocyte globulin(ATG)を用いて行い，GVHD(graft versus host disease)予防はcyclosporin A(CsA)単独投与であった．主要評価項目は完全キメラの達成と早期移植関連死亡の2項目とし，治療関連の早期死亡がないこと，および完全キメラの達成をもって「成功」と定義した．16例全例に生着が得られた．16例のうち前処置施行中にATLが増悪し他の抗がん剤を追加した1例を除いた評価可能15例のうち14例に90日以内の完全キメラの達成が得られた．また，100日以内の早期死亡は1例のみであり，その結果13例が「成功」と判断された．移植時PRであった10例のうち8例にCR，1例にPRが得られ，NRの2例は一過性にATLが消失したものの30日以内に再燃した．移植後9例が再発した．再発後4例が寛解し，4例のうち2例が無病生存中である．2年生存率は33.3±12.2%であった．本研究において高年齢のATL患者においても安全に同種造血幹細胞移植が可能であることが示されたが，充分な生存率とはいえず，さらなる移植法の改良が必要である．

当院におけるATLに対する同種造血幹細胞移植は1998年6月から2004年9月までに18例(21回)であった(表1)．男性11例，女性7例，初回移植時の年齢の中央値は48歳(37～62歳)で，急性型15例，リンパ腫型2例，慢性型1例であった．初回移植は骨髄移植が5例，末梢血幹細胞移植10例，臍帯血移植3例で，化学療法開始から初回移植までの期間の中央値は6.4か月(3.1～27.0か月)であった．初回移植18例の寛解状態はCR 6例，PR 1例，NR 11例(stable disease；SD 4例，progressive disease；PD 7例)で，再移植の3例はCR 1例，SD 1例，PD 1例であった．初回造血幹細胞移植の内訳は血縁の骨髄破壊的移植が6例，骨髄非破壊的移植が6例，骨髄バンクを介した骨髄破壊的骨髄移植が3例，臍帯血バンクからの非血縁臍帯血移植が3例であった．血縁者間移植の12例中4例はHLA不一致移植であった．移植後の生存は18例中7例で，初回移植からの生存期間の中央値は9.0か月(1.4～78.7＋か月)であった(図1)．18例中11例が死亡しており，死因はATLの再発腫瘍死4例，GVHD 4例(急性3例，慢性1例)，血栓性微小血管障害2例，肺炎1例であった．当院での解析では非寛解例，HLA不一致の割合が高く，生存期間は決して満足すべき結果ではなかった．生存に関与する因子の検討を以下のように行った．移植時の寛解状態については寛解(CR, PR)での移植7例中5例(71.4%)が生存しているのに対し，NR(SD, PD)移植での生存は11例中2例(18.2%)であった(表2)．移植時期については化学療法開始後6か月以内に移植を行った例では9例中5例(55.6%)が生存し，6か月以降に移植を行った例での生存は9例中2例(22.2%)であった．化学療法開始より6か月以降に移植を行った例では移植後100日未満の早期死亡が4例(44.4%)と多かった(表2)．ATLの同種造血幹細胞移植においては年齢や全身状態(performance status；PS)のみでなく，移植前の寛解状態，HLAの一致度，化学療法開始からの期間などが重要な因子であることが示唆された．

HLA一致の血縁ドナーが存在しない場合，骨髄バンクを通してHLA一致の非血縁者間骨髄移植を行うことが推奨される．実際に骨髄バンクを通じた非血縁者間骨髄移植でも良好な生存率が得られている[18]．血縁・非血縁においてHLA一致のドナーが見つからない場合や非血縁者間移植では時間的に間に合わない場合において，血縁のHLA不一致移植を行うか，臍帯血移植を選

表1　同種造血幹細胞移植を施行したATL患者背景と移植成績

Case No.	Age/Sex	Clinical subtype	Disease status at SCT	Type of SCT	Stem cell source	Months from chemotherapy to SCT	Survival from SCT (months)	Outcome/ Causes of death
1-1	39/M	Acute	SD	NST(unrel)	CB	7.7	10.0	Engraftment failure
1-2	39/M	Acute	SD	NST(unrel)	CB	16.1	1.6	ATL
2-1	45/M	Acute	CR	CST(unrel)	CB	3.7	12.5+	Engraftment failure
2-2	45/M	Acute	CR	NST(unrel)	CB	4.9	11.3+	Alive
3-1	61/F	Acute	PD	NST(unrel)	CB	3.9	8.7	Rejection
3-2	61/F	Acute	PD	NST(rel*)	PB	7.5	4.7	ATL
4	62/M	Acute	PD	NST(rel)	PB	5.7	39.9+	Alive
5	52/M	Acute	PD	NST(rel)	PB	7.1	5.7	ATL
6	52/M	Acute	PD	NST(rel*)	PB	7.9	1.8	ATL
7	55/M	Lymphoma	PD	NST(rel)	PB	5.0	4.2	Acute GVHD
8	59/F	Acute	PD	NST(rel*)	PB	6.9	1.9	Acute GVHD
9	51/F	Acute	PD	NST(rel*)	PB	5.0	5.4	Acute GVHD
10	37/M	Lymphoma	CR	CST(rel)	PB	5.1	45.6+	Alive
11	47/F	Acute	SD	CST(rel)	PB	3.1	23+	Alive
12	40/F	Chronic	SD	CST(rel*)	PB	8.0	1.4	TMA
13	49/M	Acute	PR	CST(rel)	PB	5.9	3.6+	Alive
14	43/M	Acute	CR	CST(rel)	BM	7.1	78.7+	Alive
15	46/F	Acute	CR	CST(rel)	BM	7.7	2.3	TMA
16	39/F	Acute	CR	CST(unrel)	BM	15.4	13.1	Chronic GVHD(lung)
17	46/M	Acute	CR	CST(unrel)	BM	27.0	27.4+	Alive
18	47/M	Acute	SD	CST(unrel)	BM	5.5	9.4	Pneumonia

SCT：stem cell transplantation, CR：complete remission, PR：partial remission, SD：stable disease, PD：progressive disease, rel：related, unrel：unrelated, *：HLA-mismatched SCT, CB：cord blood, PB：peripheral blood, BM：bone marrow, GVHD：graft versus host disease, TMA：thrombotic microangiopathy

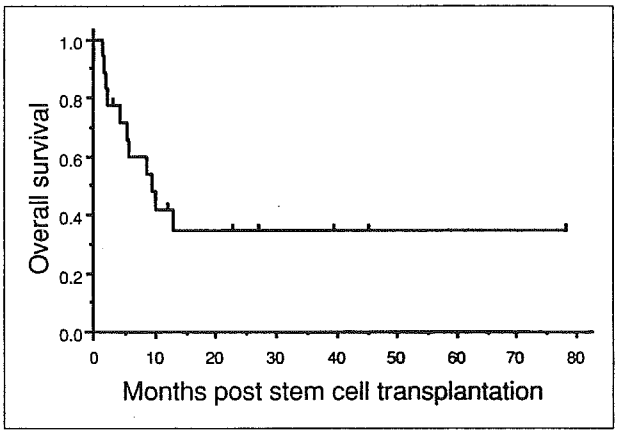

図1　同種造血幹細胞移植を施行したATL 18例の全生存率

択するかが問題である．本稿では数少ないATLに対する臍帯血移植の成績と適応や自験例，今後の方向性について述べる．

臍帯血移植の治療成績

臍帯血移植の造血器悪性腫瘍に対する有効性については欧米の報告[19)~23)]にひき続いてわが国でも東京大学医科学研究所のグループからきわめて良好な成績が報告されている[24)~26)]．彼らは，移植適応患者において血縁や非血縁でHLA一致ドナーが見つからない場合にはもっとも移植に適切な時期に臍帯血移植をするのがよいと述べている．臍帯血移植はHLA 2座不一致まで移植が可能であり，またすでに凍結保存されているので骨髄バンクドナーのようなコーディネートが不要であるため緊急の移植に適している．欠点としては成人の患者に対しては充分な細胞数が得られにくい，細胞数が少ないことも大きな要因となって生着までに長時間を要し，また生着不全も多いなどの特徴がある．生着不全を防ぐには，充分なCD34陽性細胞の輸注と全身放射線照射(total body irradiation；TBI)を併用することが重要であると考えられている．

当院においても造血器悪性腫瘍患者7例に臍帯血移植を実施している(表3)．男性5例，女性2例，初回移植時の年齢の中央値は43歳(21～61歳)であった．ATL 3例，慢性骨髄性白血病2例

表2 ATLに対する同種造血幹細胞移植成績

	No. of alive(%)	No. of death within 100 days from SCT	No. of death after 100 days from SCT	p value*
Disease status at SCT				
CR and PR(n=7)	5(71.4)	1	1	$p=0.03$
SD and PD(n=11)	2(18.2)	3	6	
Duration from chemotherapy to first SCT				
<6 months(n=9)	5(55.6)	0	4	$p=0.15$
≧6 months(n=9)	2(22.2)	4	3	
Stem cell sourse				
BMT(n=5)	2(40.0)	1	2	
PBSCT(n=10)	4(40.0)	3	3	
CBT(n=3)	1(33.3)	0	2	
HLA				
Match(n=11)	6(54.5)	1	4	
Mismatch(PB, BMT)(n=4)	0(0.0)	3	1	
Mismatch(CBT)(n=3)	1(33.3)	1	1	

SCT：stem cell transplantation, *：no. of alive cases versus no. of total death cases, CR：complete remission, PR：partial remission, SD：stable disease, PD：progressive disease, BMT：bone marrow transplantation, PBSCT：peripheral blood stem cell transplantation, CBT：cord blood transplantation, PB：peripheral blood

(2例とも第2慢性期)，急性骨髄性白血病1例(再発後のPR)，非ホジキンリンパ腫1例(CR導入困難PR)であった．臍帯血移植7例(10回)のHLA一致度は血清型で1座不一致が3回，2座不一致が6回，3座不一致が1回であった．10回の移植時の患者体重の中央値は53.9kg(48.0～67.0kg)であった．PSは0から2で，初回の移植法は骨髄破壊的移植が5例，骨髄非破壊的移植が2例であった．初回移植のTBIの併用は7例中2例のみであった．輸注有核細胞数の中央値は2.3×10^7/kg($1.8\sim9.0\times10^7$/kg)であった．移植結果としては7例中3例に生着不全，1例に生着後早期の拒絶がみられ，4例とも再移植を施行した．生着不全/拒絶4例中3例で2回目の臍帯血移植が実施され，そのうち1例のみに生着が得られている．生着不全/拒絶のみられた4例のうち3例はTBIが前処置に用いられておらず，このことが生着不全と関連しているものと考えられる．以後当院の臍帯血移植においてはTBIを前処置に併用することにしている．急性GVHDは評価可能であった5例中4例にみられ，Ⅱ度の3例は生存中で，Ⅳ度の1例はGVHDにより早期死亡している．広汎型の慢性GVHDが1例にみられた．現時点で7例中3例が無病生存中であり，初回移植からの全生存期間の中央値は8.3か月(2.4～28.6＋か月)であった．死亡4例の死因はATLの原病死2例，急性GVHD 1例，生着不全1例であった．

ATLに対する臍帯血移植については症例報告以外では，虎の門病院のreduced intensity stem cell transplantation(RIST)の2つの報告[27][28]がみられるのみである．前者の報告では，5例のATLに臍帯血RISTが施行されているがfeasible studyであり，詳細は記載されていない[27]．後者のATL 13例の報告では，患者の年齢の中央値は56歳，体重の中央値50kgで，輸注有核細胞数の中央値は2.69×10^7/kgであった．前処置にはFLU/melphalan(L-PAM)/TBIを用いている．臍帯血RIST後6か月の全生存率は29%，無病生存率は19%であり，非再発死亡が多いと述べている[28]．当院における臍帯血移植7例のうちATLに対する臍帯血移植は3例(5回)であった(表1, 3)．全例急性型で初回移植時の寛解状態はCR 1例，NR 2例であった．化学療法開始から初回移植までの期間はそれぞれ7.7か月，3.7か月，3.9か月であり，体重は54.0kg, 52.0kg, 50.0kgであった．5回の臍帯血移植におけるHLAの一致度は血清型で1座不一致が1回，2座不一致が4回であった．輸注有核細胞数の中央値は2.1×10^7/kg($1.8\sim9.0\times10^7$/kg)であった．2例が生着不全で，1例が生着後早期に拒絶された．NRの2例はともに再増

表3 臍帯血移植の患者背景と移植成績

Case	Age /Sex	Diagnosis	Disease status	PS	Conditioning	CST /NST	TNC ($\times 10^7$/kg)	Engraftment	GVHD		Survival from CBT (months)	Outcome/ Causes of death
									acute	chronic		
1-1	39/M	ATL	NR	1	FLU/ATG	NST	2.1	−	NE	NE	10.0	
1-2	39/M		NR	2	TBI/BU/FLU	NST	4.6	−	NE	NE	1.6	ATL
2-1	45/M	ATL	CR	0	TBI/BU/CY	CST	1.8	−	NE	NE	12.0+	
2-2	45/M		CR	1	FLU/L-PAM	NST	1.9	+	II	extensive	10.9+	Alive
3	61/F	ATL	NR	1	FLU/L-PAM	NST	9.0	+	0	NE	8.3	ATL
4	51/M	CML	2nd CP	0	TBI/BU/CY	CST	2.3	+	II	−	28.6+	Alive
5-1	43/M	CML	2nd CP	0	BU/CY	CST	2.4	−	NE	NE	3.0	
5-2	43/M		2nd CP	1	FLU/L-PAM/ATG	NST	2.1	−	NE	NE	1.4	Engraftment failure
6	39/M	AML	PR	1	BU/CY	CST	2.7	+	IV	NE	2.4	GVHD
7	21/F	NHL	PR	2	BU/CY	CST	2.3	+	II	−	24.7+	Alive

PS：performance status, CST：conventional stem cell transplantation, NST：nonmyeloablative stem cell transplantation, TNC：total nucleated cells, GVHD：graft versus host disease, CBT：cord blood transplantation, ATL：adult T-cell leukemia, CML：chronic myelocytic leukemia, NHL：non-Hodgkin's lymphoma, AML：acute myelocytic leukemia, NR：no remission, PR：partial remission, CR：complete remission, 2nd CP：second chronic phase, NE：not evaluable

悪し，腫瘍死したが，CRの1例は2回の臍帯血移植を要したものの再発なくCRを維持している．当院のATLでの臍帯血移植において生着不全や拒絶が多かったが，生着不全と生着後早期拒絶の2例はTBIを前処置に用いておらず，このことに関連があるものと考えられる．井関らは臍帯血移植にTBIを用いた前処置法により生着を含めてすばらしい成績を報告している[24)25)]．3例目では，TBIを用いて骨髄破壊的移植を行ったにもかかわらず，生着不全を起こしたが，輸注有核細胞数がやや少なかったものの生着不全の原因は明らかではない．今回はCRで移植した1例のみが，2回目の臍帯血移植後CRを維持しているが，少数例の解析であり，ATLに対する臍帯血移植の有効性を評価するには至らない．九州血液疾患治療グループ(K-HOT)の参加施設において，2004年9月までにATLに対して152例の同種移植が施行されている．その中で臍帯血移植が14例であった．詳細な解析はなされていないが，移植後の生存例は14例中3例とかなり厳しい成績である．血縁・非血縁の骨髄破壊的移植や血縁の骨髄非破壊的移植に比べ優れているとはいえないのが現状である．しかし，緊急を要する移植やドナーの見つからない患者にはもっとも期待されている治療法であるので，ATLにおける臍帯血移植の実態調査を施行し，ATLにおける臍帯血移植の位置づけをする必要があろう．

臍帯血移植の適応

われわれはK-HOTにおいてATLの同種造血幹細胞移植に対する適応基準についてアンケート調査を実施した．K-HOTの多くの施設の意見が一致した適応基準は，①年齢は骨髄破壊的移植では55歳以下，骨髄非破壊的移植では50/55歳から70歳まで，②臨床病型では急性型とリンパ腫型，③寛解状態はCR, PR, SD，④移植時期は化学療法開始から6か月以内，⑤前処置はcyclophosphamide(CY)/TBIもしくはBU/CY，⑥GVHDの予防はHLA一致の血縁者間移植ではCsAと短期methotrexate(sMTX)の併用，非血縁者間移植や血縁のHLA不一致移植ではtacrolimus(FK506)とsMTXの併用，⑦HTLV-1キャリアドナーの適格性については末梢血のサザンブロット検査で

HTLV-1プロウイルスのモノクローナルやオリゴクローナルな組込みがないことなどであった[29]．ATLに対する臍帯血移植については血縁・非血縁のHLA一致のドナーがいない場合や時間的に間に合わない場合に考慮するという施設が8割を占めたが，2割の施設ではまったく考慮しないとの結果であった[29]．臍帯血移植を考慮すると回答した施設においても積極的に臍帯血移植を実施しているわけではない．ATLに対する同種造血幹細胞の移植は有望であるもののprospective studyの報告はなく，骨髄破壊的同種造血幹細胞移植は現在JCOG study(第Ⅱ相臨床試験)として準備中の段階であり，ミニ移植においてのみ班研究(岡村班)として第Ⅰ相臨床試験が進行中である．ATLにおける臍帯血移植はretrospectiveなものや症例報告ですら少なく，有効性も明らかではない．当然ATLに対する臍帯血移植の適応も決まったものはなく，HLAの一致する血縁ドナーや非血縁ドナーの見つからない場合や非血縁においてはコーディネートが間に合わない場合などに限って，充分な移植経験を有する施設とATL診療に精通している施設が協力し臨床試験として実施されるべきである．

自験例

患者は44歳，男性．2003年5月頃より両腕，両膝に皮疹が出現する．近医で白血球増多20,600/μl(異常リンパ球64%)を指摘され2003年6月20日当科紹介受診した．一部浸潤を触れる全身性の紅斑および表在リンパ節の腫脹を認めた．末梢血中の異常リンパ球は特有の核変形がみられ，またモノクローナル抗体検査でT細胞形質(CD2, CD3, CD4, CD25陽性)を示し，血清抗HTLV-1抗体陽性でATLと診断した．皮膚生検では真皮内の血管周囲を主体とした異常リンパ球の浸潤がみられATLの皮膚病変と診断された．末梢血単核球のサザンブロット検査ではHTLV-1プロウイルスDNAのモノクローナルな組込みを確認した．下部消化管内視鏡検査で上行結腸と横行結腸を中心に多発性の発赤・びらんを認め，また病理組織検査により大腸粘膜内に多数の異常リンパ球(CD3, CD4陽性)の浸潤が確認され，急性型ATLと診断した．Modified LSG15を用いた化学療法2コースでCRが得られた．

若年者の急性型ATLであり同種移植の適応と判断したが，血縁者ではHLA一致のドナーが見つからなかった．骨髄バンクで非血縁者間骨髄移植を行うか臍帯血移植を実施するか慎重に検討し，家族を含めた充分なinformed consentのもとに臍帯血移植を選択した．BU/CY/TBIを前処置として2003年12月17日臍帯血移植を行った(輸注有核細胞数1.77×10^7/kg)．GVHDの予防はFK506とsMTXの併用で行った．生着は得られず2004年1月22日FLU/L-PAMを前処置(骨髄非破壊的前処置)として2回目の臍帯血移植を実施した(輸注有核細胞数1.85×10^7/kg)．GVHDの予防は1回目と同じFK506とsMTXの併用で行った．移植後25日目に好中球数＞500/μl，32日目に血小板数＞20,000/μlと増加し，また末梢血のY染色体のFISH検査で生着を確認した．移植後46日目に皮膚にstage 3の急性GVHD(gradeⅡ)がみられ，ステロイド治療を施行し改善した．免疫抑制剤中止後再び皮膚を中心にGVHDが再燃したが，ステロイドを含む免疫抑制療法で軽快し，移植後11か月でCRが持続している(図2)．

問題点および今後の可能性

ATLに対する臍帯血移植の効果と安全性の評価が必要である．どのようなタイプのATLに対して，いつ臍帯血移植を実施することが有効であるのかどうかを決定することは非常に重要なことである．免疫不全の強いATLに対して生着までに時間を要する臍帯血移植が妥当であるかどうか，GVHDが弱いとされる臍帯血移植で他の同種移植で確認されているGV-ATL効果[30]が得られるかどうか，原因ウイルスであるHTLV-1プロウイルスまで臍帯血移植で抑えることができるかどうかなど，解明すべき問題は多く残されている．しかし，GVHDの頻度やGV-ATL効果などが明らかにされていけば，ドナーが見つからない患者や緊急を要するATL患者の同種移植の重要な位置を占めるものと思われる．

おわりに

ATLに対する臍帯血移植の有効性や有用性の解明は今後の課題であるが，ドナーの負担がもっ

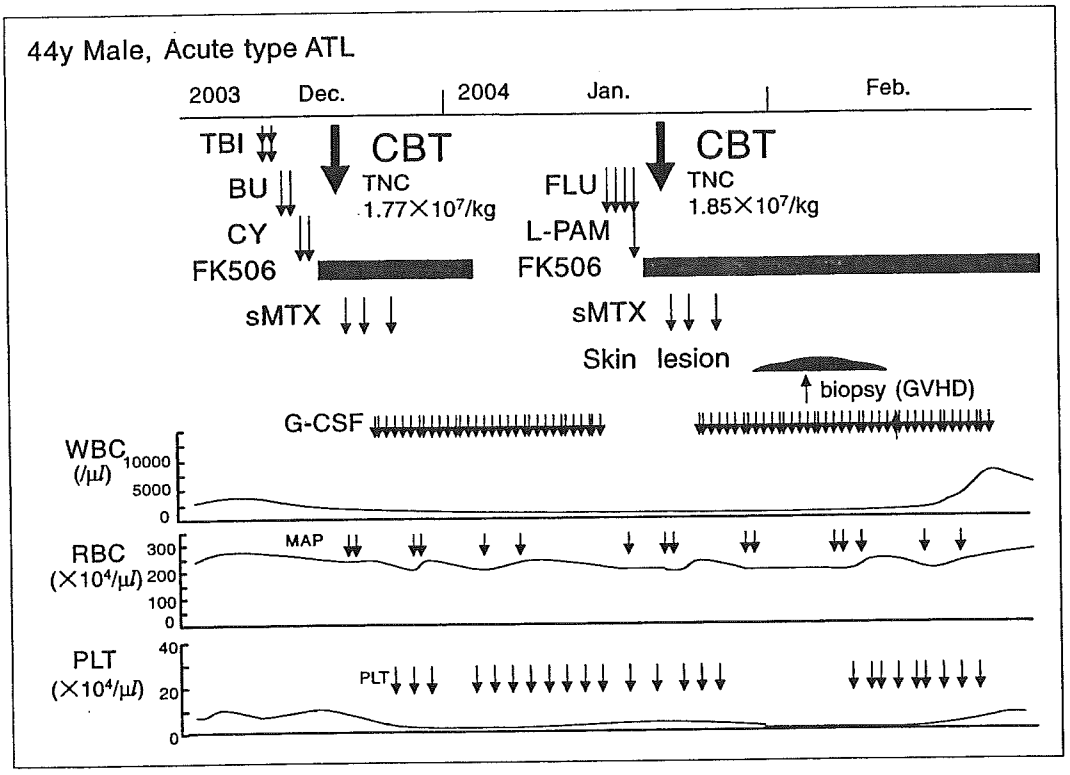

図2　臍帯血移植例の臨床経過

とも少なく，緊急移植にも対応可能である臍帯血移植がATLでも安全に実施できるようになることを願っている．

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