The TCR $\gamma\delta$ Repertoire and Relative Gene Expression Characteristics of T-ALL Cases with Biclonal Malignant V δ 1 and V δ 2 T Cells

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Despite significant improvement in our understanding of T-cell acute lymphoblastic leukemia (T-ALL) biology and pathogenesis, many questions remain unanswered. In previous studies, we found a T-ALL case with two malignant T-cell clones with V δ 1D δ 2D δ 3J δ 1 and V δ 2D δ 3J δ 2 rearrangements. In this study, we further characterized T-ALL cases with two malignant clones containing V δ 1D δ 3J δ 1 and V δ 2D δ 1J δ 1 rearrangements using fine-tiling array comparative genomic hybridization, ligation-mediated polymerase chain reaction (LM-PCR), sequencing, and reverse transcription polymerase chain reaction (RT-PCR) analysis. We further analyzed the distribution and clonality of the T-cell receptor (TCR) V γ and V δ subfamily T cells in the two T-ALL cases by RT-PCR and GeneScan. Monoclonal V δ 1 and V δ 2 subfamilies were confirmed in both samples, the V δ 3 through V δ 7 subfamilies could not be detected in the T-ALL samples, whereas the oligoclonal V δ 8 subfamily could be identified. Based on the clinical finding that both of the T-ALL cases with two malignant T-cell clones had a poor outcome, we attempted to compare the expression pattern of genes related to T-cell activation and proliferation between cases with the malignant V δ 1 and V δ 2 T-cell clones and T-ALL cases with a mono-malignant V α T-cell clone. We selected two T-ALL cases with V α J α rearrangements and analyzed the expression level of *Notch1*, *TAL1*, and the *CARMA-BCL10-MALT-A20-NF- κ B* pathway genes by real-time PCR. *A20* had significantly higher expression in the biclonal compared with the monoclonal T-ALL group ($p=0.0354$), and there was a trend toward higher expression for the other genes in the biclonal group with the exception of *TAL1*, although the differences were not statistically significant. In conclusion, we identified two T-ALL cases with biclonal malignant T-cell clones and described the characteristics of the biclonal T-ALL subtype and its gene expression pattern. Thus, our findings may improve the understanding of biclonal T-ALL.

Introduction

T-CELL ACUTE lymphoblastic leukemia (T-ALL) is an aggressive malignancy that does not respond well to chemotherapy and is associated with a significant risk for disease relapse. The clinical outcome of T-ALL after chemotherapy, and allogeneic hematopoietic stem cell transplantation (allo-HSCT) is relatively different: even some cases with good outcome and long-term survival undergo disease relapse, and some *de novo* cases with poor prognosis, who have had unsuccessful treatment, die due to rapid disease progression and complications (Aifantis *et al.*, 2008; Kras-

zewska *et al.*, 2012; Van Vlierberghe *et al.*, 2013). This high failure rate is mainly due to an insufficient understanding of T-ALL biology, which hampers the identification of reliable prognosis factors that may enable appropriate therapy adjustment (Kraszewska *et al.*, 2012). Numerous genes were identified as predictors or related to the poor prognosis of T-ALL, for example, *MLLT10* was shown to predict poor prognosis in many T-ALL cases, and *TAL1* and *HOX11L2* rearrangements have been associated with trends toward good and poor outcomes, respectively, while *Notch1* mutations were present in all molecular-cytogenetic subgroups without restriction to a specific developmental stage (van Grotel *et al.*, 2006; van Grotel *et al.*, 2008; Kraszewska *et al.*,

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TABLE 1. LIST OF PRIMERS USED FOR LIGATION-MEDIATED POLYMERASE CHAIN REACTION

Name	Location	Sequence
V δ 1 (GSP1)	Chr14: 21633971–21633990	5'-GATTCAGGGGTGAGCCAGAC
V δ 1 (GSP2)	Chr14: 21634121–21634139	5'-GCTTCAGGCAGCACAACTC
V δ 2 (GSP1)	Chr14: 21961446–21961465	5'-GCCAAGCAAGACTCAGCAGC
V δ 2 (GSP2)	Chr14: 21961500–21961519	5'-TGGACTGTGCTCCTTACTC
J δ 1 (GSP1)	Chr14: 21989003–21989022	5'-GATGGAGGATGCCTTAACCT
J δ 1 (GSP2)	Chr14: 21988962–21988982	5'-GAGTTACTTACTTGGTTCCAC
J δ 2 (GSP1)	Chr14: 21996240–21996261	5'-CTATTGTCCTGGAGTTGGTGGC
J δ 2 (GSP2)	Chr14: 21996204–21996223	5'-ATACAGGCTCCTGAACCATT

2012; Lin *et al.*, 2012). Moreover, complex acquired genetic aberrations, including chromosomal translocations, gene rearrangements, and mutations resulting in the abnormal expression of oncogenes may be associated with advanced disease and resistance to treatment. For example, *CALM-AF10* has been associated with early relapse (Zheng *et al.*, 2011a; Huang *et al.*, 2012; Lin *et al.*, 2012; Zou *et al.*, 2013; Chen *et al.*, 2013b), whereas the molecular mechanism of the poor prognosis of T-ALL is far from clear.

T-ALL occurs through the proliferation of malignant T-cell clones, and these clones may be identified by their T-cell receptor (TCR) repertoire, which is specifically expressed on the T-cell surface. In general, T-ALL results from monoclonally expanded malignant T cells that alternatively express TCR $\alpha\beta$ or TCR $\gamma\delta$ (Chen *et al.*, 2013a). However, patients with T-cell leukemias and lymphomas carrying more than one malignant T-cell clone have been reported (Menin *et al.*, 2003). In some cases, mixed populations can be found, for example, a minor T-cell clone was found at diagnosis in a patient with acute myeloid leukemia (AML), and this minor T-cell subclone progressively increased and became dominant at relapse (Bellido *et al.*, 2000). In addition, secondary T-ALL following AML has been also reported (Liso *et al.*, 1998). Despite significant improvement in our understanding of T-ALL biology and pathogenesis, many questions remain unanswered, and many new ones arise (Kraszewska *et al.*, 2012). For example, little is known about the characteristics of T-ALL cases with two malignant T-cell clones. In this study, we characterized the distribution of the TCR $\gamma\delta$ repertoire and the expression of genes related to T-cell activation and response in two T-ALL cases with two malignant TCR V δ 1+ and V δ 2+ T-cell clones compared with two T-ALL cases with only one malignant TCR V α + T-cell clone.

Materials and Methods

Samples

Two newly diagnosed, untreated T-ALL cases that were identified as carrying two malignant V δ + T-cell clones were selected for this study: case 1 (C1): a 22-year-old male and case 2 (C2): a 4-year-old male. The clinical characteristics of these two cases were similar with high white blood cell counts (more than $100 \times 10^9/L$) and hepatosplenomegaly. The disease was more aggressive and had relatively poor prognosis. Two newly diagnosed, untreated T-ALL cases that were identified as carrying only one malignant V α + T-cell clone served as control: case 1: a 40-year-old male with a malignant T-ALL clone characterized as having a V α 26J α 16

rearrangement and case 2: a 20-year-old male with a V α 8-4J α 22 malignant T-ALL clone. Peripheral blood mononuclear cells (PBMCs) were collected from patients, and DNA and RNA extraction was performed according to the manufacturer's recommendations. All procedures were conducted according to the guidelines of the Medical Ethics Committee of the Health Bureau of Guangdong Province in China.

Fine-tiling array comparative genomic hybridization and ligation-mediated polymerase chain reaction

Fine-tiling array comparative genomic hybridization (FT-CGH) and ligation-mediated polymerase chain reaction (LM-PCR) were performed as previously described (Dittmann *et al.*, 2012; Chen *et al.*, 2013a). To achieve a high CGH resolution (<1 kb), which is necessary for subsequent *in vitro* DNA amplification, a custom designed, high-density fine-tiling long oligonucleotide array of 385,000 oligonucleotides 40–60 bp in length was prepared using the Maskless Array Synthesizer (MAS) technology (NimbleGen Systems, Reykjavik, Iceland). This array, covering 24 Mb of genomic regions, was selected using the Human Genome Browser hg18 assembly (University of California, Santa Cruz, CA). The array included *TRAD* from chromosome 14q11 (Chr14: 21,130–22,130 kb). Neighboring oligonucleotides with an average distance of 63 bp were grouped in 200, 400, and 1000 bp clusters. After normalization with reference DNA (HEK293 T-cell line), the mean fluorescence was analyzed using SignalMap software (NimbleGen Systems). Regions demonstrating DNA loss through FT-CGH were further analyzed by LM-PCR as previously described (Przybylski *et al.*, 2005; Przybylski *et al.*, 2010; Dittmann *et al.*, 2012). The primers used for the LM-PCR were listed in Table 1.

Reverse transcription polymerase chain reaction and GeneScan analysis

RNA extracted from PBMCs from patients with T-ALL was reverse transcribed into cDNA. Primers specific for the TCR V δ 1, V δ 2, and C δ 1 segments (Table 2) were used for PCR amplification. Reverse transcription-polymerase chain reaction (RT-PCR) and GeneScan analysis for the TCR $\gamma\delta$ subfamilies were performed as previously described (Geng *et al.*, 2012; Chen *et al.*, 2013a).

Real-time RT-PCR

To compare the expression level of V δ 1 and V δ 2 in the malignant T-cell clones, we used V δ 1-C δ 1 or V δ 2-C δ 1 primer pairs (Table 2) to amplify both gene segments by real-time

TABLE 2. LIST OF PRIMERS USED FOR REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION AND REAL-TIME PCR

Name	Sequence
Vδ1	5'-CGTCGCCTTAACCATTTTCAG
Vδ2	5'-ATGGACTGTGCTCCTTACTC
Cδ1	5'-AACAGCATTCGTAGCCCAAGCAC
Notch1-for	5'-GCGACAACGCCTACCTCT
Notch1-rev	5'-CTGCTGGCACAGTCATCC
TAL1-for	5'-GGATGCCTTCCCTATGTTC
TAL1-rev	5'-AAGATACGCCGCACAACATTT
MALT1-for	5'-TCTTGGCTGGACAGTTTGTA
MALT1-rev	5'-GCTCTCTGGGATGTCGCAA
MALT1-V1-for	5'-AAGCCCTATTCCTCACTACCAG
MALT1-V1-rev	5'-CACTCCACTGCCTCATCTGTTC
CARMA1-for	5'-TTGTGGGAGAAATGTGGAGTGT
CARMA1-rev	5'-TGCCCTTGGTATGTAGAATG
BCL10-for	5'-CCCGCTCCGCCTCCTCTCCTT
BCL10-rev	5'-GGCGCTTCTTCCGGGTCCG
A20-for	5'-CTGGGACCATGGCACAACCTC
A20-rev	5'-CGGAAGGTTCCATGGGATTC
NF-κB-for	5'-CCACAAGACAGAAGCTGAAG
NF-κB-rev	5'-AGATACTATCTGTAAGTGAACC
β 2m-for	5'-TACACTGAATTCACCCCCAC
β 2m-rev	5'-GCGGCATCTTCAAACCTC

PCR. PCR was performed as previously described (Geng *et al.*, 2012; Chen *et al.*, 2013a). In addition, to compare the gene expression characteristics of T-ALL cases with Vδ1 and Vδ2 T-cell clones and T-ALL cases with a Vα T-cell clone, the *Notch1*, *TAL1*, *MALT1*, *CARMA1*, *BCL10*, *A20*, and *NF-κB* mRNA expression levels were detected by real-time RT-PCR using specific primers (Table 2). The relative amount of the genes of interest and the β2M reference gene was measured in two independent assays. Specific amplification of the PCR products was confirmed by melting curve analysis. The data are presented as the relative expression of the genes of interest compared with the internal control gene as determined by the $2^{(-\Delta CT)}$ method (Zha *et al.*, 2012; Shi *et al.*, 2013).

Results and Discussion

The malignant T-cell clones in T-ALL

The combination of FT-CGH and LM-PCR analysis is a novel approach for characterizing novel chromosomal translocations at the molecular level (Przybylski *et al.*, 2005; Przybylski *et al.*, 2010; Dittmann *et al.*, 2012). For lymphocytic malignancies, it is also an accurate technique for identifying malignant T-cell and B-cell clones. In previous studies, we could clearly identify malignant T-cell clones based on analysis of breakpoints at the TCR locus using this technique (Chen *et al.*, 2013a). Moreover, this technique has allowed for the identification of more than one malignant T-cell clone, and we found a case with two malignant T-ALL clones with Vδ1Dδ2Dδ3Jδ1 and Vδ2Dδ3Jδ2 rearrangements (Zheng *et al.*, 2011b). According to the cancer clonal theory, leukemia develops from a single cell due to malignant transformation (Kraszewska *et al.*, 2012). Therefore, every T-ALL clone has a unique TCR gene rearrangement, and T-ALL clones express either TCR αβ or TCR γδ (Chen *et al.*, 2013a). However, few reports have described the characteristics of T-ALL cases

containing two malignant clones. In this study, we characterized a T-ALL case with two malignant clones. FT-CGH analysis of the TCR αδ locus (chr14: 21,130–22,130) in the T-ALL sample revealed breakpoints at the 216,300-kb (TCR Vδ1), 219,600-kb (TCR Vδ2), and 219,900-kb (TCR Jδ1) loci (Fig. 1). LM-PCR using nested forward primers specific for the TCR Vδ1 or Vδ2 locus revealed two rearrangements: TCR Vδ1 to Jδ1 and TCR Vδ2 to Jδ1. Direct sequencing of these LM-PCR products demonstrated the following details for the Vδ1Dδ3Jδ1 and Vδ2Dδ3Jδ1 rearrangements (Fig. 1). To verify that these were functional TCR rearrangements, we used RT-PCR to detect the rearrangements with specific primers, and clear PCR products were detected (Fig. 2). This result may indicate that multiple malignant T-cell clones exist in some T-ALL cases, and these can be identified using new techniques such as FT-CGH and LM-PCR. Differences in TCR rearrangements in diagnostic samples may indicate the divergent subclonal evolution of an original preleukemic clone (Zuna *et al.*, 2003). Umino *et al.* (2011) demonstrated that the genomic profiles of PB from 13 patients with acute adult T-cell leukemia/lymphoma (ATLL) frequently differed from those of lymph node (LN) samples using oligo-array CGH analysis, indicating that multiple subclones in the LNs originate from a common clone and a selected subclone from the LN subclones appears in the PB in many ATLL cases. Moreover, a recent finding showed that more than one-third of late T-ALL recurrences are a second T-ALL incidence that demonstrates different TCR rearrangements and patterns of genomic aberrations (Szczepanski *et al.*, 2011). Although none of the above studies provided direct data demonstrating the identification of two malignant T-cell clones in the same sample at the same time, these findings at least partially support our findings. In this study, two malignant T-cell clones could be identified in the same sample at diagnosis, and this may be related to use of the FT-CGH and LM-PCR techniques, which can display chromosomal breakpoints and directly identify T-cell clones.

Of 14 T-ALL cases (data not shown), we found only 2 cases containing 2 malignant clones. Interestingly, all of the cases expressed Vδ1 and Vδ2 rearrangements. The Vδ2 subfamily is most frequently used in healthy individuals, while the Vδ subfamily members preferentially expressed in T-ALL clones include Vδ1 and Vδ2 and this is similar to our finding (Langerak *et al.*, 1999; Kode *et al.*, 2004). In addition, it may be well understood that the most frequent Vδ T-cell clones may be attacked during T-cell differentiation. We then analyzed the expression level of the Vδ1 and Vδ2 T-cell clones and found that Vδ2 was highly expressed in both T-ALL cases, while the expression of Vδ1 was low, particularly in case 1 (Fig. 2). Thus, this finding may indicate that the Vδ2+T-cell clone is the main malignant T-cell clone in T-ALL patients with two malignant clones, and the Vδ1+T-cell clone may be a minor subclone. However, confirmation by FACS using specific Vδ subfamily antibodies, which can directly show the percentage of malignant T-cell clones, is needed. Thus, these malignant T-cell clones may at least serve as biomarkers for the detection of minimal residual disease (MRD) (Kode *et al.*, 2004; Toubai *et al.*, 2005).

The TCR γδ repertoire in biclonal malignant T-ALL

We further analyzed the distribution and clonality of the TCR Vγ and Vδ subfamily T cells in two patients with T-ALL

FIG. 1. Identification of two malignant T-ALL clones by FT-CGH, ligation-mediated polymerase chain reaction (LM-PCR), and sequencing in a T-ALL case (C1). V δ 1D δ 3J δ 1 and V δ 2D δ 1J δ 1 rearrangements were confirmed. **(A)** FT-CGH analysis results for the TCR $\alpha\delta$ locus in chromosome 14 with arrows indicating breakpoints. **(B, D)** Comparison of the GenBank sequences within the V δ 1, D δ 3, and J δ 1 and V δ 2, D δ 1, and J δ 1 segments. **(C, E)** Sequence of the V δ 1D δ 3J δ 1 and V δ 2D δ 1J δ 1 splice junction from a purified LM-PCR product. The sequences within the ellipses indicate the CDR3 segment, including the D δ 3 or D δ 1 and N regions. T-ALL, T-cell acute lymphoblastic leukemia; FT-CGH, fine-tiling array comparative genomic hybridization. Color images available online at www.liebertpub.com/dna

with two malignant T-cell clones by RT-PCR and GeneScan. Monoclonal V δ 1 and V δ 2 subfamilies were confirmed in both the T-ALL samples, and V δ 3 through V δ 7 could not be detected in the T-ALL samples, whereas the oligoclonal V δ 8 subfamily could be identified in both samples (Fig. 3). These results were further supported by the identification of two

malignant V δ 1 and V δ 2 T-cell clones in the T-ALL cases with two malignant clones. However, the oligoclonal expanded V δ 8 T cells were thought of as reactive T-cell clones, which respond to leukemia-associated antigen (Cabillic *et al.*, 2010; Yin *et al.*, 2011), and unlike the malignant T-cell clone, the percentage of this clone was low; thus, the number of T cells

FIG. 2. The expression of the V δ 1 and V δ 2 clones in two patients with T-ALL. **(A)** The expression level of the V δ 1 and V δ 2 clones in T-ALL case 1; **(B)** the expression level of the V δ 1 and V δ 2 clones in T-ALL case 2; **(C)** amplification of V δ 1-J δ 1 and V δ 2-J δ 1 rearrangement segments in T-ALL case 1 by RT-PCR; (1) DNA ladder, (2) V δ 1-J δ 1 PCR product, (3) V δ 2-J δ 1 PCR product. RT-PCR, reverse transcription polymerase chain reaction.

FIG. 3. The distribution and clonality of the TCR V γ and V δ subfamily T cells in two patients with T-ALL. **(A)** T-ALL case 1, **(B)** T-ALL case 2. The monoclonal V δ 1 and V δ 2 subfamilies are found in both T-ALL samples, and V δ 3 through V δ 7 could not be detected in the T-ALL samples. The oligoclone V δ 8 was found in both samples. TCR, T-cell receptor.

containing the same breakpoint in the TCR locus could not be detected by FT-CGH. Therefore, a combination of the FT-CGH and GeneScan techniques may provide an accurate representation of the different properties, clonality, and distribution of TCR subfamilies. This protocol may not only provide biomarkers for MRD, but also facilitate the design of tailored and targeted therapies and immunotherapy for T-ALL subsets (Cabillic *et al.*, 2010; Yin *et al.*, 2011; Wang *et al.*, 2012).

The relative gene expression pattern characteristics of T-ALL cases with biclonal malignant V δ 1 and V δ 2 T-cell clones

It has been noted that most T-ALL patients display pathological gene expression even in the absence of chromosome aberrations. Ferrando *et al.* were the first to report different gene expression patterns in T-ALL using microarray analysis (Ferrando *et al.*, 2002; Kraszewska *et al.*, 2012). Whereas different gene expression patterns may be linked to different prognoses for T-ALL, it is notable that T-ALL patients with *Notch1* mutations have different outcomes depending on the therapeutic protocol applied (Kraszewska *et al.*, 2012).

Based on the clinical finding that both the T-ALL cases with bi-malignant T-cell clones had poor outcome, we attempted to compare the expression pattern of genes related

to T-cell activation and proliferation between the cases with bi-malignant V δ 1 and V δ 2 T-cell clones and T-ALL cases with a mono-malignant V α T-cell clone. We selected two T-ALL cases with V α 26J α 16 or V α 8-4J α 22 rearrangements that were identified by FT-CGH and LM-PCR (data not shown) as control. We analyzed the expression level of *Notch1*, *TAL1*, and the *CARMA-BCL10-MALT-A20-NF- κ B* pathway genes. The expression level of these genes in biclonal T-ALL was higher than that in monoclonal T-ALL with the exception of the *TAL1* gene (Fig. 4), and only *A20* had a significant difference ($p=0.0354$), which may be due to the limited number of samples, although the gene expression tendencies appeared to be unequivocal.

Notch1 signaling is crucial for T-cell differentiation and proliferation, and the mutational activation of *Notch1* is an important factor in T-ALL pathogenesis (Koch and Radtke, 2011; Zou *et al.*, 2013). Translocation and mutations in *Notch1* may alter its function and result in overexpression and independent activation, and ~60% of T-ALL cases show an increased *Notch1* activity (Asnafi *et al.*, 2009; Erbilgin *et al.*, 2010; Koch and Radtke, 2011). The effect of *Notch1* activation on the T-ALL outcome is controversial. In this study, a high *Notch1* expression level was found in the biclonal T-ALL group, and the significance of its differential expression in biclonal and monoclonal T-ALL requires further investigation. T-cell acute lymphoblastic leukemia 1 (*TAL1*), also

FIG. 4. The relative expression level of the *CARMA1* (A), *BCL10* (B), *MALT1* (C), *MALT1-V1* (D), *A20* (E), *NF- κ B* (F), *Notch1* (G), and *TAL1* (H) genes in T-ALL cases with bi-malignant V δ 1 and V δ 2 T-cell clones (biclonal T-ALL) and the T-ALL cases with a mono-malignant V α T-cell clone (monoclonal T-ALL).

known as stem cell leukemia (SCL), plays an important role in the differentiation of hematopoietic stem cells. Aberrant *TAL1* transcription often occurs in T-ALL (Patel *et al.*, 2013). Dysregulation of the *TAL1* activity has been associated with T-cell leukemogenesis and initiates T-ALL in mouse models (Tremblay *et al.*, 2010; Li *et al.*, 2012b). Interestingly, a low *TAL1* expression level was found in the biclonal T-ALL group, and this result may support the finding that high *TAL1* expression is associated with a trend toward good outcome in other T-ALL cases (van Grotel *et al.*, 2008). Because we did not find *TAL1* translocations in the four T-ALL cases in both groups (data not shown) using previously published techniques (Bash *et al.*, 1993; Li *et al.*, 2012b; Patel *et al.*, 2013), the differential *TAL1* expression pattern requires further investigation.

A20 (also called TNFAIP3) is a zinc finger protein that negatively regulates the expression of proinflammatory genes by downregulating NF- κ B activation in response to various stimuli (Beyaert *et al.*, 2000; Coornaert *et al.*, 2008; Zhang *et al.*, 2012). The novel data demonstrating the significant contribution of A20 inactivation resulting in the constitutive activation of the NF- κ B pathway is considered to be associated with cancer pathogenesis; A20 deficiency has also been found in the Sezary syndrome, which is a cutaneous T-cell lymphoma, particularly those characterized by constitutive NF- κ B activation (Honma *et al.*, 2009; Braun *et al.*, 2012). Our previous findings demonstrated that the A20 expression level was significantly decreased in T-ALL (Ma *et al.*, 2012). Interestingly, the expression level of the A20 gene was significantly upregulated in the biclonal T-ALL group compared with the monoclonal T-ALL group (Fig. 4). A20 is regulated by the CARMA1 (caspase-recruitment domain [CARD]-containing membrane-associated guanylate kinase protein 1, also called CARD11), B-cell lymphoma 10 (BCL10), and mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1 (paracaspase MALT1) (CBM) upstream signaling pathway complex, which bridges TCR signaling with the canonical I κ B kinase/NF- κ B pathway (Ruland *et al.*, 2003; Coornaert *et al.*, 2008; Li *et al.*, 2012a). We further analyzed the expression levels of the CBM genes, the *CARMA1-BCL10-MALT1-NF- κ B* expression pattern was high in biclonal T-ALL patients compared with those with monoclonal T-ALL, and this may be one of the gene expression patterns that distinguish both the T-ALL subsets. Few studies have focused on the expression characteristics of the CBM complex in T-cell malignancies. It has been reported that BCL10 is commonly downregulated in peripheral T-cell lymphomas, but has no significant correlation with progression-free survival and overall survival (Rossi *et al.*, 2012). The high expression level of these genes suggests that T-ALL cells have more active proliferation. However, this result is in contrast with the finding that MALT1 mediated the rapid proteolytic cleavage and inactivation of A20 after TCR stimulation (Coornaert *et al.*, 2008; Duwel *et al.*, 2009). Because *MALT1* has two transcripts (*MALT1-V1* and *V2*), we detected not only the total *MALT1* expression level, but also the *MALT1-V1* expression level (Shi *et al.*, 2013), and also, the latter is expressed higher in the biclonal T-ALL group (Fig. 4). Therefore, whether A20 is linked to negative feedback regulation of NF- κ B activation in different T-ALL subtypes remains an open question (Beyaert *et al.*, 2000; Coornaert *et al.*, 2008).

Conclusions

Recent intensive research into the molecular biology of T-ALL revealed significant heterogeneity (Kraszewska *et al.*, 2012). We identified two cases with T-ALL carrying biclonal malignant T-cell clones, and characterized the TCR $\gamma\delta$ repertoire and *CARMA1*, *BCL10*, *MALT1*, *A20*, *NF- κ B*, *Notch1*, and *TAL1* gene expression patterns, which are related to the T-cell activation and proliferation of T-ALL cases with biclonal malignant V δ 1 and V δ 2 T cells. However, the current conclusion was based on the limited two T-ALL cases with biclonal malignant T cells; further data are needed to support the finding by a large cohort analysis. Overall, to our knowledge, this is the first description of such a T-ALL subtype and its gene expression pattern, and this study may improve our understanding of biclonal T-ALL.

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Authors' Contributions

Y.Q.L. and G.K.P. contributed to the concept development and study design. H.T.Z. performed FT-CGH and LM-PCR analysis, X.W. and Y.M. performed the real-time PCR, S.H.C. performed the RT-PCR and GeneScan, L.J.Y. prepared PBMCs and DNA, X.L.W. and B.L. prepared RNA and cDNA, T.Z.Y., and B.X. were responsible for clinical diagnoses and performed clinical data acquisition. Y.Q.L., H.T.Z., and X.W. coordinated the study and helped draft the manuscript, and S.M.H. and T.Z.Y. helped edit the manuscript. All authors read and approved the final manuscript.

Disclosure Statement

The authors declare that they have no competing interests.

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