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Early T-cell Precursor Acute Lymphoblastic Leukemia – A Characteristic Neoplasm Presenting the Phenotype of Common Hematopoietic Progenitors for both Myeloid and Lymphoid Lineages

Hiroko Tsunemine and Takayuki Takahashi

Abstract

Introduction: Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is a subtype of T-ALL and its clinical entity was established in recent years based on characteristic immunophenotyping and gene expression profiles. The cellular origin of ETP-ALL is supposed to be from common hematopoietic progenitors both for lymphoid and myeloid lineages because this leukemia phenotypically exhibits lymphoid, myeloid, and stem cell features. ETP-ALL comprises 5–15% of all T-ALL and is associated with a poor prognosis. The purpose of this chapter is to clarify the etiology, clinical picture, and therapeutic strategy of ETP-ALL showing two cases of this leukemia in our institution.

Cellular origin of ETP-ALL: The normal early T-cell precursors (ETPs) are considered to be a subset of early thymocytes which are originated from the bone marrow and subsequently reside in the thymus, retaining multilineage differentiation potential as the common lymphoid-myeloid hematopoietic progenitors. ETP-ALL is supposed to be a neoplastic counterpart of ETPs.

Immunophenotype and diagnosis of ETP-ALL: ETP-ALL is characterized by the lack of expression of T-lineage cell surface antigens (CD1a and CD8, weak or no expression of CD5) and expression of myeloid and hematopoietic stem cell markers such as CD13, CD33, CD34, and CD117. These characteristic immunophenotypic profiles have provided a scoring system or a criterion for the diagnosis of ETP-ALL, which distinguishes ETP-ALL from classical T-ALL.
Clinical pictures: Clinical features are not substantially different between ETP-ALL and classical T-ALL, although ETP-ALL is associated with a higher rate of relapse and induction failure and a significantly worse overall survival. Two cases of ETP-ALL in our institution, which exhibited unique clinical pictures, that is, marked intestinal involvement and lymphoma-like systemic lymphadenopathy, respectively, will be discussed later in this chapter.

Gene profiles: Whole-genome sequencing studies on ETP-ALL have demonstrated several recurrent mutations involving genes coding cytokines, RAS signaling mediators (NRAS, KRAS, FLT3, IL7R, JAK3, LAK1, SH2B3, and BRAF), epigenetic controllers (EZH2, EED, SUZ12, SETD2, EP300 and DNMT3A), and hematopoietic transcriptional regulators (GATA3, ETV6, RUNX1, IKZF1, and EP300). These mutational spectrums are similar to those of acute myeloid leukemia.

Therapeutic strategies: These gene profiles suggest that treatment of ETP-ALL may benefit from a new chemotherapeutic approach, which is directed to the myeloid or stem cell natures of this leukemia, such as high-dose cytarabine, or epigenetic or molecular targeting therapy. Allogeneic stem cell transplantation (allo-SCT) may be a promising option for the treatment of ETP-ALL.

Conclusion: More precise and extensive cellular and molecular investigations are required to establish definite cellular origin and genetic or epigenetic nature of ETP-ALL. Accumulation of ETP-ALL cases and larger clinical trials will establish an effective therapeutic strategy for this high risk leukemia.

Keywords: Early T-cell precursor acute lymphoblastic leukemia

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a clonal malignant disorder of immature T-cells that accounts for 10–15% of childhood and 25% of adult ALL patients [1]. Despite the relatively high morbidity and mortality of T-ALL when compared to B-cell ALL, the prognosis of T-ALL has dramatically improved following the advancement of chemotherapy, and its long-term survival has become as high as 85% in both pediatric and adult T-ALL patients [2, 3]. However, a refractory subset of pediatric T-ALL associated with a poor prognosis has remained. In 2009, a study performed at St. Jude Children’s Research Hospital identified a distinct subtype of pediatric T-ALL, which was designated as early T-cell precursor ALL (ETP-ALL) [4]. This new subtype of T-ALL was defined according to the characteristic gene expression profile and immunophenotypes of the leukemic cells and was found to be associated with a high rate of remission induction failure or relapse when the patients were treated with conventional chemotherapy [4].

The purpose of this chapter is to clarify the recent advances in the biology, genetics, clinical characteristics, and therapeutic strategy of ETP-ALL and discuss two cases experienced at our institution.
2. Cellular origin of ETP-ALL

Normal early T-cell precursors (ETPs) are a subset of thymocytes, which have newly immigrated from the bone marrow to the thymus, and they retain multilineage differentiation potential, suggesting their direct derivation from hematopoietic stem cells [5-7]. The initial stage of thymocyte development is characterized by the generation of cells that lack the expression of CD4 or CD8 antigen. Along with the differentiation of these double negative cells, a minimum of four distinct differentiation stages have been identified according to the differential expressions of CD44 and CD25, that is, DN1, DN2, DN3, and DN4 stages. The potential for myeloid, dendritic, and natural killer cell differentiation is retained at both the DN1 and early DN2 stages [6]. The ability to confer multilineage differentiation is lost at the DN3 stage, and provably, at the latter half of DN2 progression [8]. Therefore, it may be reasonable that the tumor-initiating cells in ETP-ALL could originate from DN1 and/or DN2 thymocytes (Figure 1). However, in recent years, a mouse model of T-ALL using a Sleeping-Beauty-based transposon system suggested that ETP-ALL may be derived from more mature T-cells [9]. Thus, the exact cellular origin of ETP-ALL remains to be elucidated.

3. Immunophenotyping and diagnosis of ETP-ALL

Immunophenotyping of ETP-ALL cells is characterized by the lack of CD1a and CD8 expressions, weak CD5 expression (< 75% positive blasts), and the expression of one or more of the following myeloid or stem cell antigens on at least 25% of the leukemic cells: CD117, CD34, HLA-DR, CD13, CD33, CD11b, and/or CD65 [4]. Subsequently, a study proposed a scoring system based on the expression of commonly available eleven markers: CD5, CD8, CD13, CD33, CD34, HLA-DR, CD2, smCD3, CD4, CD10, and CD56 (Figure 2A) [10]. The specificity and sensitivity of this scoring system were 100% and 94%, respectively, when applied to the
patients in the St. Jude cohort (Figure 2B) [10]. Recently, another study attempted to make a more simple diagnosis of ETP-ALL using the expression of CD5 and concluded that CD5-negative T-ALL could be diagnosed as ETP-ALL because CD5 negativity was associated with positive myeloid/stem cell antigens but not CD1a and CD8 expressions (Figure 3) [11]. Currently, precise immunophenotyping is the most important tool to make a diagnosis of ETP-ALL, and this analysis enables us to distinguish ETP-ALL from classical T-ALL.

### Figure 2.
A scoring system for immunophenotypical diagnosis of ETP-ALL. A: Scoring system based on the expression of 11 markers., B: Distribution of total score of 11-marker expression in ETP-ALL patients (right) and T-ALL (left) of the St Jude cohort. (Extract of Ref.10).

**4. Clinical characteristics**

Following the early reports from the St. Jude Children’s Research Hospital and the Associazione Italiana Ematologica Oncologica Pediatrica (AIEOP), comparative studies on the clinical characteristics between ETP-ALL and classical T-ALL were performed in six institutions: the Tokyo Children’s Cancer Study Group [10], the Shanghai Children’s Medical Center [12], the German Multicenter Study Group for adult ALL [13], Colombia University Medical Center [14], All India Institute of Medical Sciences [11], and the Medical Research Council UK-ALL 2003 trial [15] (Table 1). According to the results of these clinical studies, ETP-ALL was observed to comprise 5.5–16% of all T-ALL cases. The clinical characteristics were similar between ETP-ALL and classical T-ALL with regard to gender, hemoglobin concentration, and central nervous system involvement. However, ETP-ALL patients presented with a lower white blood cell (WBC) count [11, 12, 15], lower frequency of the mediastinal mass [13, 14], and higher age (10 years or older) [4, 11] at presentation when compared to those with classical T-ALL. Regarding the cytogenetic profile, Coustan-Smith et al. reported that ETP-ALL had
more 13q- and DNA copy number abnormalities than those in classical T-ALL [4]. Conversely, Allen et al. reported that the majority of patients with ETP-ALL exhibited a normal karyotype without recurrent cytogenetic abnormalities [14]. The monoclonal rearrangement of T-cell receptor genes was observed in 71% of the ETP-ALL cases, showing no significant difference between the two T-ALL subgroups [14].

Table 1. Comparative studies on the clinical characteristics between ETP-ALL and classical T-ALL.

Figure 3. A flowchart for the diagnosis of ETP-ALL based on CD5 expression (Extract of Ref.11).
As for the prognosis, ETP-ALL is associated with a higher rate of relapse and induction failure. ETP-ALL is additionally associated with a significantly worse overall survival with a 10-year event free survival (EFS) and overall survival (OS) rates of 22% and 19%, respectively, as compared with 69% EFS and 84% OS for all other subtypes of T-ALL, respectively, in the St. Jude cohort [4]. Similar results were obtained in the cohorts of four other institutions [4, 10-12]. More recently, however, two clinical studies showed no significant differences in the EFS and OS rates between the patients with ETP-ALL and classical T-ALL [14, 15]. Although the reason for this discrepancy is unclear, differences in the therapeutic protocol and patient cohort may have influenced the results of these clinical studies. However, an increased risk of relapse in the patients with ETP-ALL [4, 10-12, 15], especially children [4, 14], was a common result in all these previous studies. Thus, larger prospective studies are needed to determine the real prognosis of this T-ALL subtype.

5. Gene profiles

The expression levels of oncogenic transcription factor genes were examined to establish genetic profiles of ETP-ALL in the St Jude Children’s Research Hospital and AIEOP studies. Pediatric ETP-ALL had a higher expression of oncogenic transcription factors: LMO1, LMO2, LYL1, and ERG [4, 16]. LMO1 and LMO2 are binding partners with hematopoietic basic helix-loop-helix transcription factors, such as SCL/TAL1 or LYL1. These proteins interact together to form a transcription factor complex, and they are hypothesized to act through a common mechanism which leads to oncogenesis of T-ALL [17]. McCormack et al. demonstrated that LYL1 is critical for the oncogenic function of LMO2, including the upregulation of a stem cell-like gene signature, aberrant self-renewal of thymocytes, and subsequent generation of T-cell leukemia in LMO2-transgenic mice. Moreover, LMO2 and LYL1 are co-expressed in leukemic cells from the patients with ETP-ALL, and LYL1 is indispensable for the growth of ETP-ALL cell lines [18]. Whole-genome sequencing studies showed that ETP-ALL had a high frequency of activating mutations in the genes involved in cytokine receptor and RAS signaling (e.g., NRAS, KRAS, FLT3, IL-7R, JAK3, LAK1, SH2B3, and BRAF) and inactivating mutations in the genes encoding key transcription factors involved in hematopoietic development (e.g., GATA3, ETV6, RUNX1, IKZF1, and EP300) and involved in epigenetic gene control (e.g., EZH2, EED, SUZ12, SUZ12, and SETD2, and EP300 genes) [16]. The gene mutations which affect cytokine receptor regulation and/or RAS signaling pathway are observed in two-thirds of ETP-ALL cases but only in 19% of non-ETP T-ALL cases [16]. These mutational gene spectrums in ETP-ALL are similar to those in acute myeloid leukemia (AML), but not in T- or B-lineage ALLs. Furthermore, the global transcriptional profile of ETP-ALL is similar to that of normal hematopoietic stem cells, AML stem cells, and murine ETP. The activating mutations in the interleukin-7 receptor (IL-7R) gene were reported to be sufficient to generate ETP-ALL in mice, and this murine ETP-ALL model showed the blockage of thymocyte differentiation at the DN2 stage, at which the developmental potentials for both myeloid and T-cell lineages coexists [19]. These findings suggesting ETP-ALL is a neoplasm at the stage of less mature hematopoietic progenitor or stem cells may account for the capacity of ETP-ALL to exert myeloid differentiation.
Gene expression profiling was also investigated in adult ETP-ALL patients. Whole-exome sequencing in adult ETP-ALL cells demonstrated a distinct mutation spectrum from that of pediatric ETP-ALL, particularly in affecting genes involved in epigenetic regulation with higher frequencies of \textit{DNMT3A} and \textit{FAT3} mutations [20]. \textit{DNMT3A}, one of the genes for DNA-methyl-transferase, is a frequent mutational target in AML [21], whereas \textit{FAT3} mutations have been frequently reported in ovarian carcinomas but not AML [22]. The incidence of \textit{DNMT3A} mutations showed a clear age relationship [20]. Adult ETP-ALL patients also had mutations in the genes known to be involved in leukemogenesis, including \textit{ETV6}, \textit{NOTCH1}, \textit{JAK1}, and \textit{NF1}. In addition, more than 60\% of the adult patients with ETP-ALL harbored at least a single genetic lesion in \textit{DNMT3A}, \textit{FLT3}, or \textit{NOTCH1} [20]. Furthermore, adult ETP-ALL showed higher expression levels of \textit{BAALC}, \textit{IGFBP7}, \textit{WT-1}, and \textit{MN1} than those in classical T-ALL [4, 13, 18]. As described above, the high expression of \textit{BAALC} and \textit{ERG} were predictive for unfavorable outcomes in adult T-ALL [23, 24]. \textit{IGFBP7} is a stem cell-associated gene, which is functionally highly related to \textit{BAALC} and overexpressed in early T-ALL [25]. The \textit{WT-1} gene is commonly overexpressed in AML [26], and its overexpression is associated with a poor prognosis in thymic T-ALL patients [27]. The overexpression of the \textit{MN1} gene is additionally associated with a shorter survival in the patients with AML without karyotypic abnormalities [28, 29]. The \textit{FLT3} mutations which are frequently observed in AML were found in 37.5\% of the adult ETP-ALL but only in 1–3\% of the classical T-ALL patients (37.5\%) [13], although these \textit{FLT3} mutations in ETP-ALL more frequently generated tyrosine kinase domain (TKD) abnormalities rather than internal tandem duplication (ITD) mutations, which are frequently observed in AML. In relation to the above-mentioned observations, mice that received a transplant of \textit{FLT3}-ITD-transduced bone marrow cells developed myeloproliferative diseases, while those that received a transplant of \textit{FLT3}-TKD-transduced bone marrow cells developed lymphoid disorders [30]. Collectively, it may be reasonable to separate ETP-ALL from classical T-ALL due to the distinct genetic profiles between ETP-ALL and other T-ALL subtypes, and the characteristic gene profile of ETP-ALL may provide new therapeutic strategies for this leukemia.

6. Therapeutic strategies

Coustan-Smith et al. previously reported that the patients with ETP-ALL showed a poor initial response to standard intensive chemotherapies and unfavorable outcomes [4]. Subsequently, six clinical studies showed that ETP-ALL was associated with a very high risk for relapse, whereas two additional studies showed no significant differences in both the EFS and OS rates between the patients with ETP-ALL and classical T-ALL [14, 15]. In the TLLSGL99-15 study, three of four relapsed ETP-ALL patients were successfully treated with allogenic hematopoietic stem cell transplantation (allo-SCT), indicating that allo-SCT could be an effective therapeutic option for ETP-ALL [10]. Prior to this report, the Berlin-Frankfurt-Munster group showed that allo-SCT was superior to chemotherapy alone for high-risk childhood T-ALL [31]. The UKALL 2003 study, which showed better outcomes of ETP-ALL, suggested the beneficial effects of a more intensive chemotherapeutic regimen and the employment of dexamethasone.
and pegylated asparaginase [15]. High-dose cytarabine combined with epigenetic treatment may be a promising option for ETP-ALL according to the results of whole-genome sequencing, which showed that the mutational spectrum of ETP-ALL was similar to that of AML and that the transcriptional profile was similar to that of normal hematopoietic stem cells and granulocyte-macrophage progenitor cells [4], although these hypotheses need to be proven in future investigations. Additionally, other potential targets have been suggested according to the genetic alterations in the transcription factors. Stat4 phosphorylation was observed in IL-7R mutant-induced ETP-ALL cell lines, and consequently, ruxolitinib which is a selective JAK1 and JAK2 inhibitor, was shown to inhibit the proliferation of cells from the ETP-ALL cell lines and prolong the survival of mice xenotransplanted with the IL-7R mutated ETP-ALL cells [19]. Tyrosine kinase inhibitors may be effective when FLT3 mutations harboring ETP-ALL are molecularly targeted [13]. The antiapoptotic B-cell lymphoma-2 (BCL-2) protein is another attractive molecular target. BCL-2 is highly expressed in ETP-ALL and gradually decreases its expression along with the differentiation toward mature T-cells. ABT-199, an orally bioavailable BCL-2 specific inhibitor, was demonstrated to induce apoptosis of ETP-ALL cells from patients with this subtype of leukemia and dramatically reduced the tumor burden in the bone marrow, spleen, and peripheral blood in mice transplanted with ETP-ALL patient-derived xenografts [32, 33]. In addition, a WT-1 peptide cancer vaccine may be a therapeutic option for relapsed patients or those with minimal residual disease in WT-1-overexpressed ETP-ALL, because this approach has demonstrated objective clinical responses in other hematological neoplasms and solid tumors [34].

7. Case study

For a better understanding of ETP-ALL, we herein present two cases of ETP-ALL in our institution, which exhibited unique clinical pictures.

Case 1: A 24-year-old man developed epigastralgia and low-grade fever four months before the admission to our hospital. On gastrofiberscopy performed in a hospital, multiple non-ulcerative mucosal nodules were observed. A biopsy specimen from the nodule histologically showed diffuse infiltration of small lymphocytes, which were positive for CD3, CD7, CD8, and CD56 but negative for TIA-1, Epstein-Barr virus-encoded small RNAs-in situ hybridization (EBER-ISH), and a suspected pathological diagnosis was lymphomatoid gastroenteropathy. Three months later, the patient was admitted to our hospital due to the exacerbation of abdominal distress. On this admission, he presented with multiple ulcerative nodules in the gastric mucosa (Figure 4), marked wall thickening of the small intestine, hepatosplenomegaly (Figure 5) and multiple nodular lesions in the bilateral lungs. A histological examination of the biopsied gastric mucosal nodule showed dense infiltration with small immature lymphocytes (Figure 6). The WBC count elevated to 3.83×10⁹/L with 55% immature lymphocytes (Figure 7). Flow cytometry indicated that these cells were positive for cyCD3, CD7, CD8, CD13, and CD56 (partially), but negative for CD2, smCD3, CD34, TdT, B-cell antigens, and cytoplasmic myeloperoxidase (MPO). A multiplex PCR analysis for TCRγ chain and immunoglobulin heavy chain genes yielded negative results regarding the monoclonal gene rearrangements. A
cytogenetic examination of the bone marrow cells, including abundant leukemic cells, gave a normal karyotype of 46, XY. He was subsequently diagnosed with ETP-ALL according to these immunophenotypes of abnormal lymphocytes, which fulfilled the criteria of the TCCSG L99-15 study scoring system but not the St. Jude Criteria due to the CD8 positivity. Although the leukemia was resistant to CHOP (cyclophosphamide, adriamycin, vincristine, and prednisolone) and SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide) [35] regimens, a complete remission (CR) was obtained with the MEC regimen (mitoxantrone, etoposide, and cytarabine) followed by nelarabine. He underwent unrelated allogeneic bone marrow transplantation and is currently maintaining CR. Importantly, a marked intestinal involvement at presentation has not been reported in ETP-ALL.

Figure 4. Gastrofiberscopy of Case 1 on admission to our hospital. Multiple ulcerative nodules were visible on the gastric mucosa.

Case 2: An 83-year-old female who presented with generalized lymphadenopathy was referred to our hospital. She was tentatively diagnosed with peripheral T-cell lymphoma-unspecified according to the findings from a biopsy specimen from a cervical lymph node, which histologically showed diffuse infiltration of CD3-positive lymphocytes and a proliferation of Langerhans cells without dysplastic features. The lymphadenopathy disappeared after CHOP chemotherapy; however, blast cells (Figure 8A) appeared in the peripheral blood and rapidly increased in number without recurrence of the lymphadenopathy after the fourth round of CHOP chemotherapy. The blast cells expressed cyCD3, CD7, CD56, CD33, and CD34, but not CD2, smCD3, CD4, and CD8. PCR of the TCRγ chain gene
demonstrated a monoclonally rearranged faint band. These blast cells were negative for MPO staining; however, some of the cells were weakly positive for both α-naphthyl butyrate (Figure 8B) and naphthol AS-D chloroacetate esterase staining (Figure 8C), suggesting their ability to differentiate toward monocytes and granulocytes. A chromosomal analysis revealed an abnormal karyotype of 46, XX, t(12;20)(q13;q11.2) in seven of the 20 bone marrow cells analyzed. A final diagnosis of ETP-ALL was made according to these immunophenotypes, which fulfilled both the TCCSG L99-15 study scoring system and St. Jude criteria. Her leukemia was resistant to any chemotherapeutic protocols for lymphoma, ALL, and AML, and she ultimately died due to disease progression.

Figure 5. Contrast CT scanning of the abdomen (coronal image) in Case 1. Marked hepatosplenomegaly and wall thickening of the small intestine (arrows) were observed.

Figure 6. Histology of the biopsied gastric mucosal nodule in Case 1. Diffuse and dense infiltration of immature lymphocytes is shown (H-E staining, 100×).
In both cases, it was difficult to make a precise diagnosis with a histopathological strategy, and the immunophenotypic analysis was crucially important to determine the final diagnosis. Both cases are very interesting in terms of the phenotypic presentation reflecting an oncogenic development at the level of granulocyte-macrophage-T-cell progenitors in early normal hematopoiesis. In addition, morphologically, leukemic cells in these two cases had a slightly condensed chromatin network of the nucleus when compared with that of classical ALL blasts and these nuclei were irregular in shape.

8. Conclusion

More precise and extensive cellular and molecular investigations are required to establish the definite cellular origin and genetic or epigenetic nature of ETP-ALL. An accumulation of ETP-ALL cases and larger clinical trials will establish effective therapeutic strategies for this high-risk leukemia.
Author details

Hiroko Tsunemine and Takayuki Takahashi*

*Address all correspondence to: takahashi.takayuki@shinkohp.or.jp

Department of Hematology, Shinko Hospital, Kobe, Japan

The authors declare that there are no conflicts of interest with any individuals or companies.

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