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Case Report
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Novel ETV6-FOXO1 Fusion Transcript in Mixed Phenotype Acute Leukemia (T/Myeloid): Diagnosis and Treatment

Ka-Hei Murphy Sun[1]*, Yin-Ting Heylie Wong[2], Pui-Ha Natalie Chan[3], Ke Tian[3], Yuk-Lin Rabi Yung[3], Fung-Shan Kate Leung[1] and Heung-Ling Margaret Ng[3]

[1]Department of Pathology, Division of Hematopathology, Princess Margaret Hospital, Lai Chi Kok, Hong Kong

[2]Department of Pathology, Division of Chemical Pathology, United Christian Hospital, Kwun Tong, Hong Kong

[3]Department of Pathology, Division of Hematopathology, Prince of Wales Hospital, Shatin, Hong Kong

Introduction

Mixed Phenotype Acute Leukemia (MPAL) is a rare type of acute leukemia which accounts for only 2% - 5% of all acute leukemia [1]. The 2024 World Health Organisation (WHO) classification defines eight subtypes of MPAL: MPAL with BCR: ABL1 fusion, MPAL with KMT2A rearrangement, Acute leukaemia of ambiguous lineage with other defined genetic alterations, MPAL B/myeloid, MPAL T/myeloid, MPAL rare types, acute leukaemia of ambiguous lineage NOS and Acute undifferentiated leukaemia [2]. Different hematopoietic models were proposed to try to elucidate the genetic and cell origin of MPAL. Recent evidence suggests that founding lesions arise in primitive hematopoietic progenitors, rather than an accumulation of distinct genomic alterations which primes tumor cells for lineage promiscuity [3]. The prognosis of MPAL is worst among adult leukemias [4]. However, due to the rarity of cases and limited published studies, treatment guidelines and monitoring strategies are not established.

We report a case of a child who developed T/myeloid mixed phenotype leukaemia with translocation (12;13) (p12;q14). Subsequent RNA-seq study was performed to characterize the molecular basis of the disease. and identified a novel fusion transcript involving ETV6 (located at 12p13) and FOXO1 (located at 13q14), confirming the presence of an ETV6-FOXO1 fusion gene corresponding to the t(12;13) translocation observed in the karyotype. To our knowledge, this is the first reported case of an ETV6-FOXO1 fusion in pediatric MPAL, representing a novel finding with potential diagnostic and therapeutic implications.

Case Presentation

A 5-year-old female presented to our pediatric hematology department with a history of on-and-off fever and palpitations for two weeks. She had no significant past medical history, and her growth and development were normal. Physical examination showed pallor and bruising at left shin without palpable lymphadenopathy and organomegaly. Initial laboratory studies showed severe pancytopenia with a white blood cell count of 0.9 × 10^9/L, hemoglobin of 4.4 g/dL, and platelet count of 31 × 10^9/L. The Absolute Neutrophil Count (ANC) was 0, indicating severe neutropenia. Examination of the peripheral blood revealed hypochromic microcytic red cells with no obvious polychromasia and presence of rouleaux formation. There was marked neutropenia, with occasional blast cells (Figure 1) noted. Moderate thrombocytopenia was present with occasional giant platelets noted. The bone marrow aspirate was partially clotted with no marrow particles for assessment. However, blast cells were noted, predominantly medium to large-sized cells showing fine chromatin, inconspicuous nucleoli, high nuclear to cytoplasmic ratio, and basophilic cytoplasm. Occasional blasts showed small cytoplasmic vacuoles. No Auer rods were identified. Cytochemistry revealed that the blast cells were negative for both Sudan black and non-specific esterase. The bone marrow trephine biopsy (Figure 2) showed markedly hypercellular marrow with dense infiltration by blasts. These blasts were medium to large-sized cells showing slightly irregular nuclei, fine chromatin, and presence of nucleoli. Normal trilineage hematopoiesis was markedly reduced. Flow cytometry analysis demonstrated a blast population with a mixed phenotype profile. The blast cells co-expressed the myeloid-specific marker cytoplasmic myeloperoxidase (cMPO) and the T-lymphoid-specific marker cytoplasmic CD3 (cCD3). They were also positive for the myeloid markers CD13

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Correspondence:
Ka-Hei Murphy Sun, Department of Pathology, Division of Hematopathology, Princess Margaret Hospital, Lai Chi Kok, Hong Kong, E-mail: nancywilliammurphy@gmail.com

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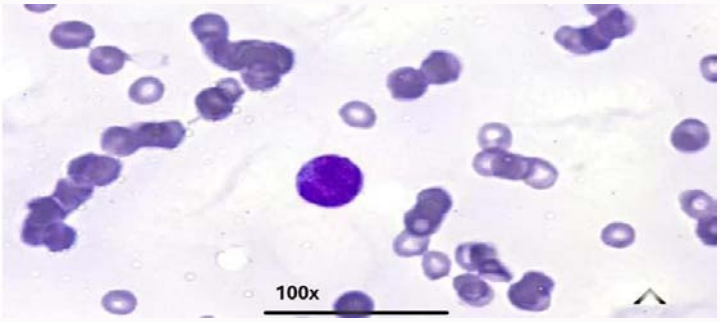

Figure 1: Bone marrow aspirate smears showed blast cells which are predominantly medium to large-sized cells showing fine chromatin, inconspicuous nucleoli, high nuclear to cytoplasmic ratio, and basophilic cytoplasm. May Grunwald Giemsa stain × 100.

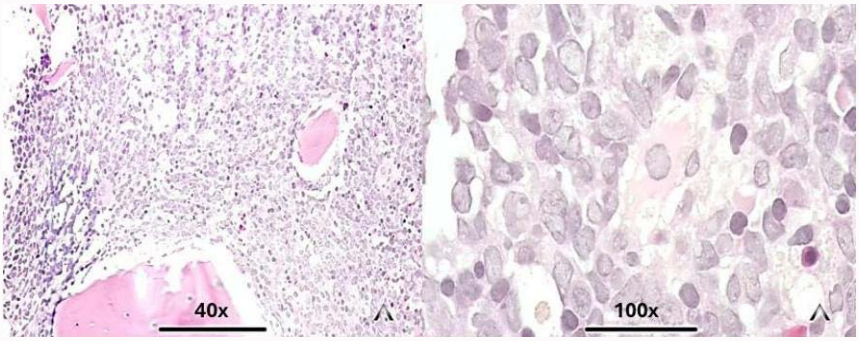

Figure 2: Bone marrow trephine biopsy showed markedly hypercellular marrow with dense infiltration by blasts. Normal trilineage hematopoiesis was markedly decreased.

and CD117, the T- lymphoid marker CD7, and the B-lymphoid marker cytoplasmic CD79a. Cytogenetics analysis (Figure 3) revealed complex translocation involving translocation between p12 region on chromosome 12 and q14 region on chromosome 13 with a derivative chromosome 15 from translocation between chromosome 1 and chromosome 15 as well as trisomy 22.

Methods

To characterize the molecular aberrations, we perform transcriptome sequencing using Novoseq 6000 system, which revealed a novel ETV6-FOXO1 fusion transcript. In detail, RNA sequencing reads were aligned to the human reference genome (hg38) using STAR aligner and subsequently, Arriba was used to detect fusion transcripts. Results were filtered to retain high-confidence fusion candidates defined as those supported by sufficient split or discordant reads, precise breakpoint definition, while excluding artefacts such as read through events. The ETV6 gene breakpoint occurred at chr12:11884587, located at the 5' end and the FOXO1 gene breakpoint occurred at chr13:40560860, located at the 3' end. This resulted in an in frame ETV6::FOXO1 fusion transcript containing exon 6 of ETV6 fused to exon 2 of FOXO1 and was predicted to generate a fusion protein ETV6/FOXO1 which contained Sterile Alpha Motif (SAM)/ Pointed domain of Tel/Yan protein (SAM_PNT- Tel_Yan) of ETV6 protein at the N terminal and part of the truncated Forkhead DNA binding domain and KIX-binding domain as well as transactivation domain.

Treatment and Outcome

The patient was treated with an intensive AML-oriented chemotherapy regimen, followed by ALL maintenance therapy. Induction chemotherapy was initiated with the ADE10+3+5 regimen (cytarabine, daunorubicin, and etoposide). The patient tolerated the treatment well despite expected cytopenia and achieved cytogenetic remission.

This was followed by a second course of chemotherapy following the ADE8+3+5 regimen (cytarabine, daunorubicin, and etoposide) which is followed by both cytogenetic and morphological remission. The patient continued to consolidation therapy with MACE (regimen amsacrine, cytarabine, and etoposide) followed by MidAC regimen (mitoxantrone and cytarabine). After completing consolidation, the patient was transitioned to maintenance chemotherapy according to the CCLG2008 intermediate-risk protocol, which included 6-mercaptopurine, dexamethasone, and methotrexate. At present, the patient remains in complete remission with normal blood counts and no evidence of disease recurrence. She has completed her treatment protocol and continues regular follow-up. No hematopoietic stem cell transplantation was required, and she has returned to normal activities with excellent performance status.

Discussion

Mixed phenotype acute leukaemia, T/myeloid is an aggressive cancer characterized by co-expression of T-cell and myeloid markers, poor prognosis, and somatic mutations typical of both T-ALL and AML [5,6]. Genetic aberrations have been identified in MPAL-T/M, with PICALM::MLLT10 rearrangements found in 10% - 15% of cases, sometimes with B-lineage differentiation, alongside other fusion genes such as NUP214, and BCL11A [7]. Recent molecular study identified ETV6-NCOA2 fusion gene acting as an oncogene, inducing T/myeloid leukemias by triggering a lymphoid gene expression program, arresting T-cell differentiation, and recruiting

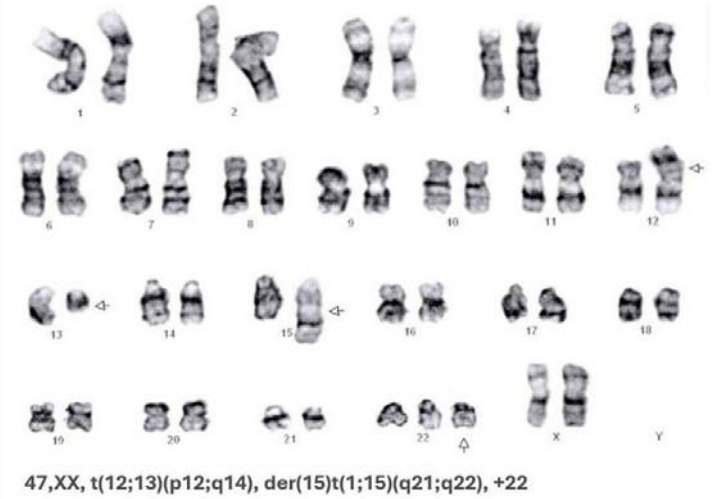

Figure 3: Karyotype analysis of bone marrow revealed a translocation between p12 region of chromosome 12 and q14 region of chromosome 13 and a derivative chromosome 15 from unbalanced translocation between chromosome 1 and chromosome 15 as well as trisomy 22.

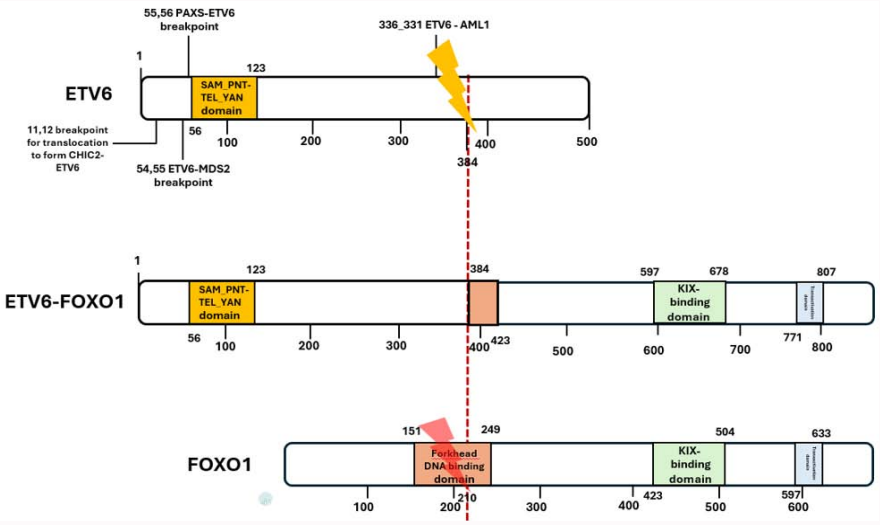

Figure 4: RNA sequencing revealed a novel in frame ETV6::FOXO1 fusion transcript containing exon 6 of ETV6 fused to exon 2 of FOXO1 and was predicted to generate a fusion protein ETV6/FOXO1 which contained Sterile alpha motif (SAM)/Pointed domain of Tel/Yan protein (SAM_PNT-Tel_Yan) of ETV6 protein at the N terminal and part of the truncated Forkhead DNA binding domain and KIX-binding domain as well as transactivation domain.

the histone acetyltransferase p300 to derepress ETV6 target genes, accompanied by NOTCH1-activating mutations [8]. Here, we report the identification of a novel fusion, ETV6-FOXO1, in MPAL-T/M, which may represent an additional driver of leukemogenesis and warrants further investigation into its role in disease pathogenesis.

ETV6 gene encodes codes for an ETS (E-Twenty-Six) family transcription factor which is a transcriptional repressor and is involved in maintenance of the vascular network, hematogenesis, embryogenesis and development of different tissues [9]. The protein encoded by ETV6 gene features two primary domains: the HLH (helix-loop-helix) domain and the ETS domain. The HLH domain is encoded by exons 3 and 4 and mediates protein-protein interactions, particularly homotypic oligomerization (self-association) and heterotypic interactions with other transcription factors such as members of the ETS family e.g. FLI1 [10]. The domain is also important for ETV6 to form dimers which is critical for its ability to participate in the formation of oncogenic fusion proteins in hematological malignancies [11,12]. A well-known example of such fusion is the ETV6::RUNX1 fusion, commonly found in B-cell acute lymphoblastic leukemia (B-ALL). This fusion typically retains the HLH domain of ETV6, which results in altered transcriptional regulation and a dominant- negative effect on the function of wild-type ETV6 [12]. Another example is the ETV6::PDGFRB fusion, where the HLH domain of ETV6 is fused to the PDGFRB tyrosine kinase domain. This fusion leads to constitutive dimerization and activation of PDGFRB signaling, driving the development of myeloproliferative neoplasms, particularly Chronic Eosinophilic Leukemia (CEL) [10].

Meanwhile, the ETS (E26 transformation specific) domain, encoded by exons 6 through 8, is a highly conserved C terminal DNA binding domain responsible for sequence-specific DNA binding, thereby regulating the transcription of target genes involved in hematopoiesis and cellular differentiation. In ETV6::RUNX1 fusion, the ETS domain is lost with retention of HLH domain with fusion with transcription activation domain of RUNX1, which functions

as a histone deacetylase dependent repressor, causing deregulation of the RUNX1 target genes [6]. Recent literature has also reported that ETV6-FGFR2 fusions in T/myeloid neoplasms result in aberrant FGFR2 tyrosine kinase activation, leading to an aggressive disease phenotype that may be refractory to conventional therapeutic approaches. In such cases, targeted therapies with FGFR inhibitors (such as pemigatinib or ponatinib) have been suggested as potential treatment options, with some reports of durable responses to such inhibitors in FGFR1-rearranged mixed-phenotype leukemias [10]. Other partners including ABL1, ABL2, JAK2, SYK, FRN, LYN, MN1, ARNT, EVI1, PAX5, MNX1, CHIC2 have also been reported in various other hematological neoplasms [10].

On the other hand, FOXO1 is one of the four members within the Forkhead box (FOX) family of transcription factors, which shares a conserved DNA-binding domain called the forkhead box or winged-helix domain. This domain enables FOXO1 to bind specific DNA sequences and modulate the transcription of genes involved in cell cycle regulation, apoptosis and differentiation. A molecular study showed that FOXO1 activation can promote CCND3 expression and formation of CCND3-CDK6 complex which could support cell cycle progression and inhibited apoptosis. FOXO1 depletion can reduce CCND3 expression and mTORC1signaling which is crucial for BCP-ALL proliferation and signaling. Interestingly, a small-molecule inhibitor AS1842856 effectively binds to FOXO1 and represses its transcriptional activity, showing anti-leukemia effects in primary and patient-derived BCP-ALL samples [13]. Meanwhile, FOXO transcription factors also play an essential role in maintaining LICs [14]. In CML, despite BCR-ABL activation of the PI3K-AKT pathway in the bulk leukemic population, FOXO3 remains active in the nucleus of LICs and promotes their self-renewal and persistence, even during tyrosine kinase inhibitor therapy [15]. Similarly, in AML models, LICs feature active FOXOs that support their stemness properties, with FOXO depletion leading to leukemic cell maturation and death.

To date, we are the first group to report a case of MPAL T/myeloid for ETV6-FOXO1 fusion gene. For ETV6, the breakpoint occurs at 5' splice junction of exon 6 and excludes the C-terminal ETS DNA-binding domain of ETV6, which is typically responsible for sequence-specific DNA binding. N-terminal PNT (Pointed) domain is retained which is critical for protein-protein interactions and oligomerization. For FOXO1, the breakpoint occurs at exon 2, retaining exon 2 and 3 of FOXO1 protein. The retained portion includes part of the FOXO1 DNA-binding domain (forkhead domain) and the transactivation domain, which likely preserves FOXO1's ability to bind DNA and regulate transcription. The resulting fusion protein combines the PNT domain of ETV6, which mediates oligomerization, with the DNA-binding domain and transactivation domain of FOXO1, enabling DNA binding and transcriptional regulation. Functionally, the PNT domain of ETV6 is likely to drive oligomerization of the fusion protein, while the FOXO1 DNA-binding domain directs this complex to specific FOXO1 target genes. This aberrant transcription factor is predicted to dysregulate FOXO1 target genes involved in critical cellular processes such as cell cycle regulation, apoptosis, and metabolism. This fusion is conceptually similar to other oncogenic ETV6 fusions, such as ETV6::RUNX1 fusion where the oligomerization domain of ETV6 is fused to the DNA-binding domain of RUNX1, creating an aberrant transcriptional regulator [10,16]. Similarly, the ETV6::FOXO1 fusion combines the oligomerizing capability of ETV6 with the transcriptional activity of FOXO1, likely driving oncogenesis through altered gene expression.

This novel fusion highlights a mechanism of pathogenic transcriptional dysregulation, contributing to leukemogenesis. The favorable outcome in this patient, despite the presence of a complex karyotype and a previously undescribed fusion gene, demonstrates that intensive chemotherapy protocols combining elements of AML and ALL treatment can be effective in pediatric MPAL T/myeloid with ETV6::FOXO1 fusion gene. Further studies are needed to elucidate the functional consequences of the ETV6::FOXO1 fusion and its role in leukemogenesis, which may lead to targeted therapeutic approaches in the future.

References

1. Khan M, Siddiqi R, Naqvi K. An update on classification, genetics, and clinical approach to mixed phenotype acute leukemia (MPAL). Ann Hematol. 2018;97(6):945-53.
2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: myeloid and Histiocytic/Dendritic neoplasms. Leukemia. 2022;36(7):1703-19.
3. Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature. 2018;562(7727):373-9.
4. Shi R, Munker R. Survival of patients with mixed phenotype acute leukemias: A large population-based study. Leuk Res. 2015;39(6):606-16.
5. Gerr H, Zimmermann M, Schrappe M, Dworzak M, Ludwig WD, Bradtke J, et al. Acute leukaemias of ambiguous lineage in children: characterization, prognosis and therapy recommendations. Br J Haematol. 2010;149(1):84-92.
6. Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JJK, Xu B, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature. 2018;562(7727):373-9.
7. WHO Classification of Tumours of Haematolymphoid Tumours. 5th ed. International Agency for Research on Cancer. 2022.
8. Fishman H, Madiwale S, Geron I, Bari V, Loocke WV, Kirschenbaum Y, et al. 2021. ETV6-NCOA2 fusion induces T/myeloid mixed-phenotype leukemia through transformation of nonthymic hematopoietic progenitor cells. Blood. 2021;139(3):399-412.
9. Biswas A, Rajesh Y, Mitra P, Mandal M. ETV6 gene aberrations in non-haematological malignancies: A review highlighting ETV6 associated fusion genes in solid tumors. Biochim Biophys Acta Rev Cancer. 2020;1874(1):188389.
10. De Braekeleer E, Douet-Guilbert N, Morel F, Le Bris MJ, Basinko A, De Braekeleer M. ETV6 fusion genes in hematological malignancies: a review. Leuk Res. 2012;36(8):945-61.
11. Wlodarska I, Mecucci C, Baens M, Marynen P, van den Berghe H. ETV6 gene rearrangements in hematopoietic malignant disorders. Leukaemia and lymphoma. 1996;23(3-4):287-95.
12. Monovich AC, Gurumurthy A, Ryan RJH. The diverse roles of ETV6 alterations in B-lymphoblastic leukaemia and other hematopoietic cancers. Adv Exp Med Biol. 2024:1459:291-320.
13. Wang F, Demir S, Gehringer F, Osswald CD, Seyfried F, Enzenmüller S. Tight regulation of FOXO1 is essential for maintenance of B cell precursors acute lymphoblastic leukemia. Blood. 2018;131(26):2929-42.
14. de Brachene AC, Demoulin JB. FOXO transcription factors in cancer development. Cell Mol Life Sci. 2006;73(6):1159-72.
15. Pellicano F, Scott MT, Helgason GV, Hopcroft LE, Allan EK, Aspinall-O'Dea M, et al. The antiproliferative activity of kinase inhibitors in chronic

myeloid leukemia cells is mediated by FOXO transcription factors. Stem Cells. 2014;32(9):2324-37.

16. Ito Y. Oncogenic potential of the RUNX gene family: 'overview'. Oncogene. 2004;23(24):4198-208.