One novel ACOT7–NPHP4 fusion gene identified in one patient with acute lymphoblastic leukemia: a case report

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is a type of heterogeneous hematopoietic malignancy that accounts for approximately 20% of adult ALL. Although ALL complete remission (CR) rate has increased to 85–90% after induction chemotherapy, 40–50% of patients eventually relapsed. Therefore, it is necessary to improve the outcomes of ALL via accurate diagnosis and individualized treatments, which benefits in part from molecular biomarkers. Here, we identified a new fusion gene, Acyl-CoA Thioesterase 7–Nephrocystin 4 (ACOT7–NPHP4), in a 34-year-old patient with ALL. The fusion gene contributed to chemoresistance to doxorubicin and acted as a new molecular marker.

Case presentation: A 34-year-old male patient was diagnosed with ALL (common B cell) based on clinical manifestations and laboratory results. Although the patient received two cycles of the hyper-CVAD-L regimen as chemotherapy, the induction treatment failed. Because of the refusal of further treatments, the patient died of rapid progression of ALL one month later. Finally, a new fusion transcript, ACOT7–NPHP4, was detected in the patient’s lymphoblastic leukemia cells via RNA sequencing.

Conclusion: This is the first report of a patient with ALL carrying an ACOT7–NPHP4 fusion gene. These findings may help understand the impact of ACOT7–NPHP4 in clinical molecular monitoring and drug resistance to doxorubicin; furthermore, its leukemogenesis will be essential to explore in future.

Keywords: Case report, Acute lymphoblastic leukemia, ACOT7–NPHP4, Fusion gene, Molecular biomarker

Background

Acute lymphoblastic leukemia (ALL) is a subtype of hematological malignancies with an incidence of 1–1.5 per 100,000 people [1], which accounts for approximately 20% of adult ALL. In adult patients with ALL, the 5-year overall survival (OS) and event-free survival (EFS) are 30–40% and 30–45%, respectively [2, 3]. Currently, the complete remission (CR) rate in ALL is assessed mainly based on morphological criteria and immunophenotyping using flow cytometry [4]. Notably, molecular biomarkers such as BCR–ABL or TCF3–PBX1 are more sensitive biomarkers for minimal residual disease (MRD) than morphology and immunophenotyping [5]. For instance, BCR–ABL1 fusion-gene-guided diagnosis and targeted therapy with imatinib resulted in an improvement in 5-year disease-free survival (DFS) of 70% [6]; moreover, other fusion genes, including rearranged KMT2A, ETV6–RUNX1, DUX4-IGH, and TCF3–PBX1...
play important roles in the management of ALL. However, only 30–40% of patients with B-cell ALL and 10–20% of patients with T-cell ALL tested positive for chromosomal aberrations or chromosome translocations, which provided the basis for molecular marker-guided management [7].

With the development of next-generation sequencing (NGS), whole transcriptome sequencing (RNA-seq) has been widely used to identify the novel fusion genes [8]. At our center, patients with refractory/relapsed (R/R) ALL routinely undergo RNA-seq to identify new fusion genes as molecular biomarkers for monitoring and improving treatment. In the present case, one novel fusion gene, \textit{ACOT7–NPHP4}, was detected in one patient with ALL. This fusion gene was subsequently characterized, and its chemoresistance was assessed.

Case presentation

Clinical course

A 34-year-old male patient was referred to the Department of Hematology, Second Hospital of Dalian Medical University in China on January 16, 2020. Clinically, he manifested fatigue and progressive painless lumps on both sides of the neck for over one month. Physical examination showed lymphadenomegaly on both sides of the neck, with sizes of 1.4 \times 10^9/L, red blood cell count of 28 \times 10^12/L, hemoglobin (HB) level of 116.0 g/L, and platelet level of 188.0 \times 10^9/L. A bone marrow aspirate revealed hypercellularity, with 87.0% lymphoblasts expressing CD34+, CD117+, HLA-DR+, and CD38+ respectively. The 5' side of \textit{ACOT7} gene and exon 5 of the \textit{NPHP4} gene, was predicted in this patient.

The predicted breakage sites were located in exon 7 of the \textit{ACOT7} gene and exon 5 of the \textit{NPHP4} gene, respectively. The 5' side of \textit{ACOT7} joined into one part of 3' side of \textit{NPHP4}, yielding fusion transcript \textit{ACOT7–NPHP4}, then it was confirmed by Sanger sequencing using polymerase chain reaction (PCR) products amplified with sense primer (F) (5'–CGGGAAGTCTTGAGTTGATTC–3') and antisense primer (R) (5'–TGGCTTTAGCGTG–3'). Most of \textit{ACOT7} domains and \textit{NPHP4} domains remained in the fusion protein, as assessed based on an amino acid sequence analysis, suggesting that the novel fusion protein may have some aberrant function in lymphoblastic cells (Fig. 1c–e).
Fig. 1 Identification of ACOT7–NPHP4 in one patient with ALL. a May–Grunwald–Giemsa staining showed acute lymphoblastic leukemia blasts (87%) in a bone marrow (BM) smear with a magnification of 400. b Normal chromosomal karyotype with 46, XY. c The ACOT7–NPHP4 transcript contained ACOT7 exons 1–7 and NPHP4 exons 5–29. d PCR band (238 bp) and the fusion site by Sanger sequencing of ACOT7–NPHP4. e The domains of the wild-type ACOT7 and NPHP4, and the domains of the ACOT7–NPHP4 fusion proteins were shown. Breakpoints were indicated by the black dotted lines. HD1: HotDog ACOT-type 1; HD2: HotDog ACOT-type 2; SBBL: a domain named sufficient for basal bodies localization.
Drug resistance and monitoring of ACOT7–NPHP4

To investigate the chemoresistance to doxorubicin induced by ACOT7–NPHP4, NALM-6 cells (B-cell ALL cell line) were transiently transfected with plvx-ACOT7-NPHP4-Flag plasmid or the vector. The over-expression of ACOT7–NPHP4 was verified by western blotting using an antibody against Flag (Fig. 2a). The cell survival was inhibited by doxorubicin in ACOT7–NPHP4-expressing cells, with a half maximal inhibitory concentration (IC50) of 82.5 nM, as assessed using the cell counting kit-8 assay (mean ± SEM is displayed). **P < 0.01; ***P < 0.001; ****P < 0.0001. The expression of plvx-A-N-Flag in NALM-6 cells was tested via western blotting using an anti-Flag antibody. b The primary lymphoblastic leukemia cells from the patient were decreased after induction chemotherapy, as analyzed by flow cytometry in the bar chart on the left. The transcripts of ACOT7–NPHP4 were decreased after induction therapy, as assessed by quantitative real-time PCR in the bar chart on the right.

Fig. 2 Survival of NALM-6 cells and expression of the ACOT7–NPHP4. a The transfection of the plvx-ACOT7-NPHP4-Flag (plvx-A-N-Flag) or vector plasmids into NALM-6 cells showed that NALM-6 cells carrying ACOT7–NPHP4 (red line) presented a significant chemoresistance to doxorubicin after a 24-h exposure compared with NALM-6 cells carrying the vector plasmid (black line), as assessed using the cell counting kit-8 assay (mean ± SEM is displayed). **P < 0.01; ***P < 0.001; ****P < 0.0001. The expression of plvx-A-N-Flag in NALM-6 cells was tested via western blotting using an anti-Flag antibody. b The primary lymphoblastic leukemia cells from the patient were decreased after induction chemotherapy, as analyzed by flow cytometry in the bar chart on the left. The transcripts of ACOT7–NPHP4 were decreased after induction therapy, as assessed by quantitative real-time PCR in the bar chart on the right.

Discussion and conclusions

Fusion genes such as BCR–ABL, DUX4-IGH, or TCF3–PBX1, play critical roles in the diagnosis, targeted therapy, and prediction of prognosis in patients with leukemia. They also serve as molecular biomarkers and guide management in patients with ALL; for example, one retrospective investigation reported that 432 adult patients with Philadelphia chromosome-positive ALL in CR1 received allogeneic peripheral stem cell transplantation, among whom patients with detectable MRD of BCR–ABL before transplantation achieved 4-year
OS of 55% and DFS of 46%, in contrast, patients with undetectable MRD of BCR–ABL reached OS of 67% and DFS of 60%, indicating that the molecular marker BCR–ABL was helpful to achieve better outcomes [11]. The patient belonged to high-risk group based on World Health Organization (WHO) classification and guidelines of National Comprehensive Cancer Network (NCCN) for ALL [5, 12], he had markedly systemic lymph node infiltration with no chromosomal abnormalities, however, conventional chemotherapy hyper-CVAD was ineffective. At our center, 26 patients with R/R ALL were performed with RNA-seq to identify new fusion genes as molecular biomarkers, therefore, a novel fusion gene, ACOT7–NPHP4, was predicted through RNA-seq and subsequently was confirmed.

ACOT7–NPHP4 fusion gene detected in this patient comprised exons 1–7 of the ACOT7 gene and exons 5–29 of the NPHP4 gene. The HotDog folding structure presents in ACOT7, which is one of the main members of the ACOT family, is responsible for catalyzing the fatty acyl-CoA to free fatty acids and CoA-SH [13]. As reported elsewhere in 2019, high expression of wild-type (WT) ACOT7 in 156 patients with acute myelogenous leukemia often yielded a poor prognosis, even after treatment with allogeneic peripheral stem cell transplantation [14]. However, little is known about the correlation between ACOT7 and ALL, and our research reported that the breakage form of ACOT7 gene was fused with NPHP4 gene, with retaining functional domains. In addition, NPHP4 gene is closely associated with nephronophthisis type 4, Senior–Loken syndrome type 4 [15], and tumorigenesis. To clarify the function of ACOT7–NPHP4, NALM-6 cells transfected with transcript ACOT7–NPHP4 presented significant chemoresistance to doxorubicin, indicating that the new fusion gene may contribute to the failure to the induction therapy, to some extent. Moreover, ACOT7–NPHP4 transcripts may serve as a new molecular biomarker to monitor the MRD in ALL patients. Notably, mutations in multiple genes, such as ASXL1, PTPN11, RUNXI, or U2AF1, have been described by Klaus H. Metzeler et al. to be risk factors for resistance to chemotherapy in leukemia, and these mutated genes are usually persistent among patients exhibiting treatment failure [16]. In the present case, these mutated genes, including ASXL1, PTPN11, RUNXI, and U2AF1, but particularly ASXL1, were persistent, as detected by NGS, after induction therapy, suggesting that ASXL1 may partially contribute to chemoresistance, resulting in persistent ASXL1 expression. Furthermore, the combination of the ACOT7–NPHP4 fusion gene with ASXL1 may have enhanced the chemoresistance, thus may in part lead to treatment failure in this patient with ALL.

Collectively, our results showed that a novel fusion gene, ACOT7–NPHP4, was identified, which potentially served as a molecular biomarker and enhanced chemoresistance to doxorubicin. Furthermore, the leukemogenesis of ACOT7–NPHP4 in ALL should be further elucidated.

**Abbreviations**

ALL: Acute lymphoblastic leukemia; CR: Complete remission; ACOT7–NPHP4: Acyl-CoA Thioesterase 7–Nephrocystin 4; RNA-seq: RNA sequencing; OS: Overall survival; EFS: Event-free survival; MRD: Minimal residual disease; DFS: Disease-free survival; NGS: Next-generation sequencing; R/R: Refractory/relapsed; CT: Computed tomography; HB: Hemoglobin; IC50: Half maximal inhibitory concentration; WHO: The World Health Organization; NCCN: The National Comprehensive Cancer Network; PCR: Polymerase chain reaction; WT: Wild-type; AML: Acute myelogenous leukemia; BM: Bone marrow; HD1: HotDog-ACOT7-type 1; HD2: HotDog-ACOT7-type 2; SBR: A domain named sufficient for basal bodies localization; plvx-A-N-Flag: Plvx-ACOT7–NPHP4-Flag; CTX: Cyclophosphamide; VCR: Vincristine; Dex: Dexamethasone; Peg-ASNase: Peg-asparaginase.

**Supplementary Information**
The online version contains supplementary material available at https://doi.org/10.1186/s12920-022-01378-7.

**Additional file 1.** Fig. S1 Survival of NALM-6 cells inhibited by cyclophosphamide, vincristine, dexamethasone, or peg-asparaginase. The cell counting kit-8 assay showed that there was no significant difference in cell viability of 24-h inhibited by cyclophosphamide (CTX), vincristine (VCR), dexamethasone (Dex), or peg-asparaginase (Peg-ASNase) between plvx-A-N-Flag (red line) and vector plasmids (black line) into NALM-6 cells.

**Additional file 2.** Fig. S2 The original image of bone marrow smear. The original bone marrow smear was the field of view with a magnification of 100, and the red box was the field corresponding to Fig. 1a. The instrument of microscope was Olympus BX43, and the camera was Digital Camera. Smart V1050D. The image was taken at 300dpi resolution.

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**Author contributions**
XZ, ZK and JY collected and interpreted patient’s clinical information. DH, XHZ and YG performed karyotype and analyzed data. ZK and JY diagnosed and treated the patient. XZ and ZK drafted the manuscript. ZK, HW, WL and JY collected and interpreted patient’s clinical information. DH, XHZ and YG performed karyotype and analyzed data. ZK and JY diagnosed and treated the patient. XZ and ZK drafted the manuscript.

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