PCR- Negative Atypical PML-RARA Rearrangement in Pediatric Acute Promyelocytic Leukemia

Dan-Ping Huang[1,#], Yi-Jun Chen[1,#], Jie-Si Luo[1], Shao-Qian Chen[2], Jing Cheng[2], Yu Li[1], Cong Liang[1], Li-Na Wang[1], Zhong Fan[1], Xiao-Li Zhang[1], Xue-Qun Luo[1], Li-Bin Huang[1,*] and Yan-Lai Tang[1,*]

[1]*Department of Pediatrics, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China*

[2]*Department of Clinical Laboratory, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China*

Abstract: Acute promyelocytic leukemia (APL) is a special type of acute myeloid leukemia (AML), accounting for about 5% to 10% of children with AML. At the genetic level, APL is featured by a unique chromosome translocation t(15;17) which results in the PML-RARA gene fusion. Most patients can be diagnosed by traditional karyotype analysis, Fluorescence-In-Situ Hybridization (FISH), or Reverse Transcription-Polymerase Chain Reaction (RT-PCR). We report the case of a child with acute promyelocytic leukemia (APL) who had characteristic chromosome translocation t(15;17) and rare PML-RARA gene mutation. This patient had an excellent response to chemotherapy, suggesting that this mutation will not affect the treatment and prognosis of APL.

Keywords: Acute promyelocytic leukemia, diagnosis, PML-RARA, FISH, t (15; 17).

INTRODUCTION

Acute promyelocytic leukemia (APL), which comprises 5%-10% of acute myeloid leukemia (AML) children, is typically characterized by the unique chromosome translocation t(15;17), resulting in the PML–RARA gene fusion [1, 2]. According to the different breakpoints in the PML gene, it can be divided into three different subtypes: long type(L-type, bcr1), variant type(V-type, bcr2) and short type (S-type, bcr3), and corresponding clinical characteristics are slightly different [3]. Patients with AML can be diagnosed as APL as long as t(15;17) (q22; q21) or PML/RARA gene is detected [4]. Most children with APL can be diagnosed by traditional karyotype analysis, Fluorescence In Situ Hybridization (FISH) or Reverse Transcription-Polymerase Chain Reaction (RT-PCR). However, some patients have rare breakpoints or cryptic translocations, and the use of one or two of these methods alone may cause missed diagnosis. We report the case of a child with acute promyelocytic leukemia (APL) who had characteristic chromosome translocation t(15;17) and rare PML-RARA gene mutation. The RT-PCR showed that common PML-RARA fusion genes (bcr1, bcr2, bcr3) were negative, but rare PML-RARA gene mutation was detected after sequencing and re-design of primers and probes.

*Address correspondence to these authors at the Department of Pediatrics, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China;
E-mail: huanglb3@mail.sysu.edu.cn, tangylai@mail.sysu.edu.cn

#These authors contributed equally to this manuscript.

CASE REPORT

A 13-year-old boy was admitted to our hospital due to "recurrent fever for 12 days and epistaxis for 6 days". His blood investigations in other hospital showed leukopenia and thrombocytopenia with a coagulation disorder. There was no significant improvement after anti-infection, platelet transfusion, and cryoprecipitate transfusion.

On physical examination, the patient's body temperature was 36 ° and had an acute face. There was a massive hemorrhage in the right bulbar conjunctiva and a small hemorrhage in the left bulbar conjunctiva. Hemostatic sponge tamponade was seen in the right nasal cavity without active bleeding and several bleeding spots were scattered in the oral mucosa. There was no generalized lymphadenopathy, hepatosplenomegaly or sternal tenderness. Pharynx hyperemia can be seen. The breath sounds of both lungs were coarse but without any rales. He has no arrhythmias or pathological murmurs. His testes are soft with a volume of about 20ml on each side.

Peripheral blood revealed: WBC 4.93x10^9/L, NEUT# 1.03x10^9/L，Hb 114g/L，PLT 26x10^9/L and examination of the coagulation function showed: Fib 1.8g/L, D-dimer 44.75mg/L, coagulation factor VIII 174.1%. No abnormality was found in the examination of liver and kidney function. The bone marrow smear of sternal puncture showed that bone marrow hyperplasia was active and the granulocytic system accounted for 83%, of which abnormal promyelocytic cells were dominant, accounting for 71.5%. The cell bodies of

ISSN: 1929-2260 / E-ISSN: 1929-2279/21

abnormal promyelocytic cells varied in size and were irregular in shape. The cytoplasm was rich, grayish-blue, full of thick purplish-red azurophilic granules and Auer bodies. There were 1-3 irregular, sunken, and folded nucleoli in each cell. POX was strongly positive. All of these were consistent with the myelogram of acute myeloid leukemia (M3a) (Figures **1**, **2**). The flow cytometry (FCM) showed that abnormal myeloid cells, accounting for 46.2% of nucleated cells, expressed CD13, CD33, MPO, CD64 and CD117, but did not express CD2, CD34 and CD56. Karyotype is 46, XY, t(15;17)(q22;q21) [4]/46, XY[11] (Figure **3**) and the FISH revealed 47% of PML/RARA fusion gene positive cells (Figure **4**). Qualitative screening of 31 leukemia fusion genes were negative, including PML/RARA fusion genes (L, S, V), PLZF/RARA, NPM/RARA, etc. WT1 gene expression score is 64.04%. No FLT3-ITD, C-kit/D816V, NPM1, CEBPA, and other gene mutations related to the prognosis of AML were detected. According to the chromosomal karyotype and FISH results, the PML-RARA fusion gene did exist in the child, but it could not be detected by RT-PCR, which may be due to the rearrangement of unconventional breakpoint regions. After sequencing and re-design of primers and probes, rare PML-RARA gene mutation was detected. Combined with all the test results, the patient was finally diagnosed as APL (medium-risk type), and then he received chemotherapy according to the SCCLG-APL clinical study. Twenty-three days after initial induction, bone marrow examination showed disease in remission. Three months later, after the consolidation treatment, a rare PML-RARA fusion gene turned negative detected by quantitative PCR. Maintenance chemotherapy was regularly administered subsequently and now the patient is in complete remission and doing well on follow-up. This study met the requirements of the Declaration of Helsinki. The patient and his guardians provided informed consent and signed the informed consent form. This research was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

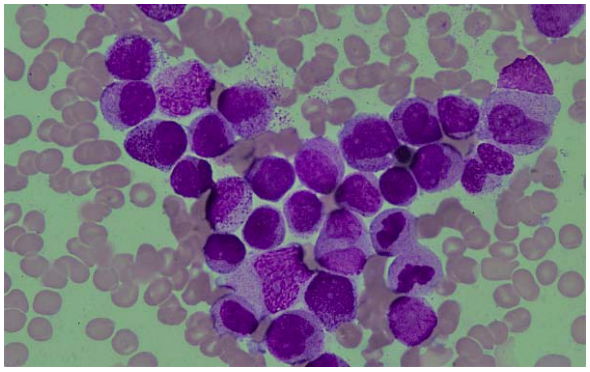

Figure 1: Bone marrow cell morphology.

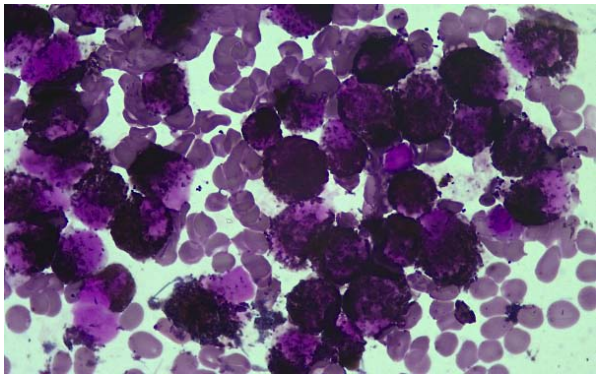

Figure 2: Peroxidase staining of bone marrow cells.

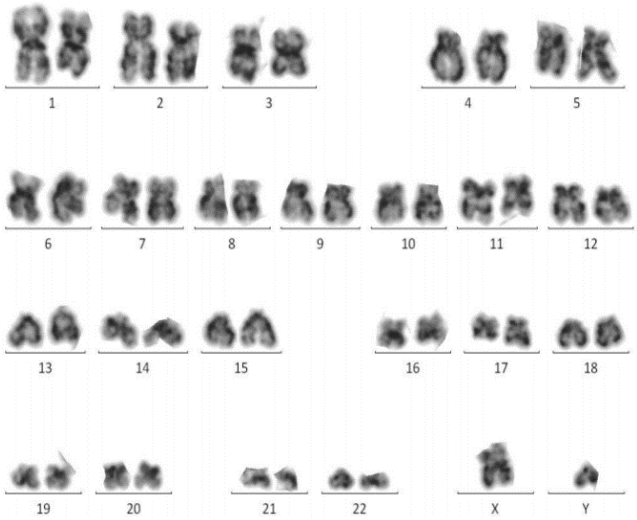

Figure 3: Chromosome karyotype of bone marrow cells.

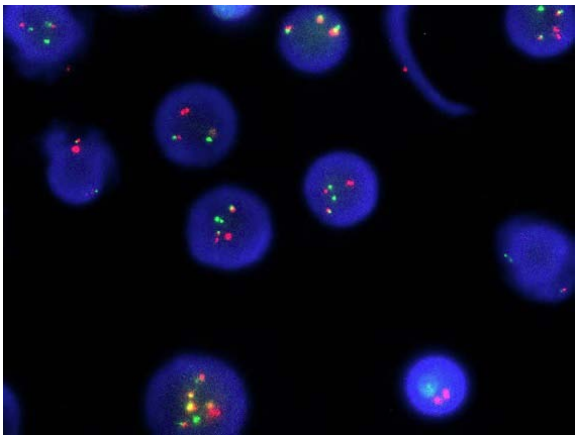

Figure 4: FISH showing.

DISCUSSION AND CONCLUSION

Acute promyelocytic leukemia (APL), a special type of acute myeloid leukemia (AML), is characterized by abnormal promyelocytosis in the bone marrow [1]. The t(15;17)(q24;q21) is the hallmark of APL, being present

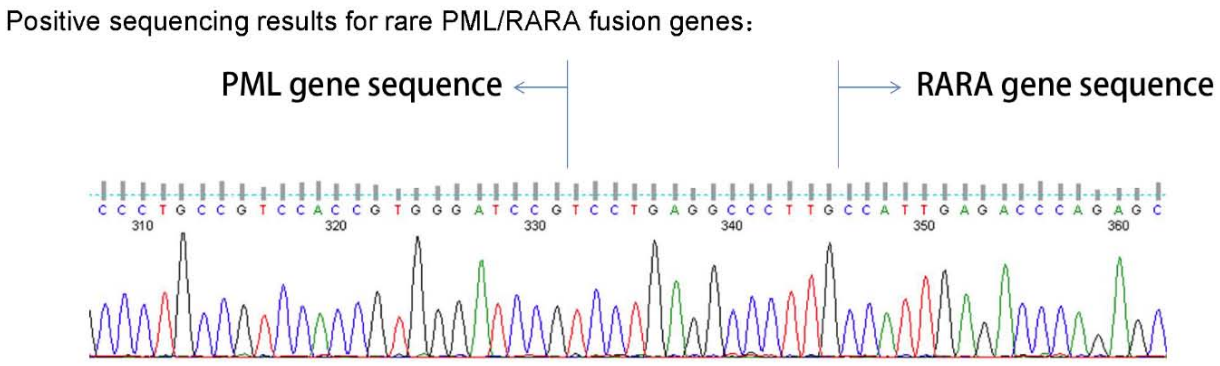

Figure 5: Partial sequencing map and breakpoint.

in about 90% of cases. During the production of the PML-RARA fusion gene, three different isomers are formed due to the different breakpoints of the PML gene [3]. They are long type (L-type, bcr1), variant type (V type, bcr2) and short type (S-type, bcr3) and most common types are L-type (40%- 60%) and S type (30% -50%). Different isomers encode different proteins, which block the differentiation and maturation of promyelocytes and eventually lead to APL. The early death rate remains high though the use of retinoic acid and arsenic has greatly improved the survival rate of patients with APL, so it is necessary to identify and diagnose APL early [5]. If M3 patients are misdiagnosed as non-M3-AML patients, the use of non-M3-AML chemotherapy will increase the mortality of the patients and the prognosis will be poor. At present, the most commonly used cytogenetic methods are karyotype analysis, FISH, and PCR. Each of these can diagnose APL if chromosome translocation t(15:17) or PML-RARA fusion gene is detected. Chinese guidelines recommend that eligible medical facilities use all three tests together, as the use of one or two tests alone may result in missed diagnosis [6]. Chromosome karyotype analysis can detect characteristic chromosome translocation t(15:17), but it is easy to cause false-negative results because of technical reasons or a small number of cells in the mitotic phase in the tested samples. At the same time, Karyotype analysis is also difficult to detect cryptic translocations caused by submicroscopic gene insertion or other complex translocation mechanisms [7-9]. FISH probes can cover different fusion sites, including PML-RARA fusion genes with rare breakpoints, but cannot specifically locate the fusion sites, which is not conducive to the continuous monitoring of minimal residual disease (MRD). FISH cannot detect the very small insertion because it is too small for the probe to hybridize; or the hybridized portion of the probe produces a fluorescence signal too faint for detection [10-12]. PCR can detect PML-RARA fusion gene in 99% of APL patients and detect cryptic translocations, and its high sensitivity and quantification make it the main method for regular monitoring of MRD level at present [13]. But it can only detect known mutations because primers need to be designed based on known PML-RARA fusion genes, and it often shows false-negative when detecting a small number of unknown fusion gene loci. All three detection methods are likely to be false-negative, so it is recommended to use three detection methods together to reduce the probability of missed diagnosis or misdiagnosis. Cases in which test results are inconsistent deserve further clinical and molecular analyses such as gene sequencing to provide definitive diagnoses.

In this case, Most test results supported APL, including bone marrow cell morphology and immunophenotype, classical chromosome translocation t(15;17)(q22;q21) detected by chromosomal karyotype, and the PML-RARA fusion gene in FISH. However, RT-PCR showed that conventional PML-RARA fusion genes (bcr1, bcr2, and bcr3) were negative. Considering the possibility of unconventional breakpoint rearrangement, we redesigned the primers after sequencing and finally confirmed the presence of a rare PML-RARA fusion gene in the child.

The PML-RARA fusion gene is both a pathogenic gene of APL and a target of ATRA and arsenic treatment. The 5-year Event-Free Survival (EFS) of APL children can be more than 90% by ATRA combined with arsenic induced therapy, making APL the highest cure rate leukemia at present [6]. Only a few APL children with negative PML-RARA fusion gene or other variant translocations are insensitive to retinoic acid and have a poor prognosis [3]. There are few reports on APL patients with rare PML-RARA fusion genes [14-19]. Most patients have a good prognosis after treatment with ATRA and arsenic, suggesting that although these patients have different gene breakpoint

regions, they can still form PML-RARA fusion gene eventually. However, it has also been reported that patients with the S-type of PML-RARA fusion gene are insensitive to retinoic acid and arsenic and have a poor prognosis [18]. The case report of the rare PML-RARA fusion gene is still rare, and there is no definite conclusion at present. Therefore, this kind of patient needs long-term follow-up and continuous monitoring of MRD. In our patient, the MRD of qRT-PCR monitoring remained negative 4 months after treatment, indicating complete remission and a good prognosis, the same as reported in most literature.

The formation mechanism of the PML-RARA fusion gene is complex and diverse, and there are still many rare translocations and partner genes that have not been discovered. The use of one or two detection methods alone may still cause misdiagnosis or missed diagnosis, so we propose the routine use of three methods as an initial diagnostic strategy for APL cases. We should strengthen our understanding of rare APL and carry out gene detection if necessary. Most patients with rare APL have a good prognosis, indicating that the new variant may not affect the prognosis of APL. However, due to the small number of existing patients, these findings should be confirmed or refuted through larger, randomized clinical trials with longer-term follow-up and continuous observation.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

FUNDING ACKNOWLEDGMENTS

This study was supported by the Guangdong Basic and Applied Basic Research Foundation [Grant No.2020A1515010282], the Science and Technology Planning Project of Guangdong Province, China [Grant No.2020B1010010003], the Youth Talent Promotion Project of Guangzhou Association for Science and Technology [Grant No.X20210201015] and the Grant Award from the Terry Fox Foundation, Canada.

REFERENCES

[1] Taga T, Tomizawa D, Takahashi H, Adachi S. Acute myeloid leukemia in children: Current status and future directions. Pediatr Int 2016; 58: 71-80. https://doi.org/10.1111/ped.12865

[2] Lo-Coco F, Hasan SK. Understanding the molecular pathogenesis of acute promyelocytic leukemia. Best Pract Res Clin Haematol 2014; 27: 3-9. https://doi.org/10.1016/j.beha.2014.04.006

[3] De Braekeleer E, Douet-Guilbert N, De Braekeleer M. RARA fusion genes in acute promyelocytic leukemia: a review. Expert Rev Hematol 2014; 7: 347-357. https://doi.org/10.1586/17474086.2014.903794

[4] Hui J. Examination and diagnosis of acute promyelocytic leukemia. Chinese Journal of Pediatric Hematology and Oncology 2019; 24: 116-118.

[5] Park JH, Qiao B, Panageas KS, Schymura MJ, Jurcic JG, Rosenblat TL, Altman JK, Douer D, Rowe JM, Tallman MS. The early death rate in acute promyelocytic leukemia remains high despite all-trans retinoic acid. Blood 2011; 118: 1248-1254. https://doi.org/10.1182/blood-2011-04-346437

[6] Specification for the Diagnosis and Treatment of Acute Promyelocytic Leukemia in Children (2018 Edition). Chinese Journal of Pediatrics 2019; 757-760.

[7] Lewis C, Patel V, Abhyankar S, Zhang D, Ketterling RP, McClure RF, Persons DL. Microgranular variant of acute promyelocytic leukemia with normal conventional cytogenetics, negative PML/RARA FISH and positive PML/RARA transcripts by RT-PCR. Cancer Genet 2011; 204: 522-523. https://doi.org/10.1016/j.cancergen.2011.09.001

[8] Schultz MJ, Blackburn PR, Cogbill CH, Pitel BA, Smadbeck JB, Johnson SH, Vasmatzis G, Rech KL, Sukov WR, Greipp PT, *et al*.. Characterization of a cryptic fusion by mate-pair sequencing in a case of acute promyelocytic leukemia with a normal karyotype and negative FISH studies. Leuk Lymphoma 2020; 61: 975-978. https://doi.org/10.1080/10428194.2019.1699081

[9] Avgerinou G, Katsibardi K , Filippidou M, Tzanoudaki M, Papadhimitriou SI, Kattamis A. Cytogenetically cryptic and fish negative PML/RARA rearrangement in acute promyelocytic leukemia detected by RT-PCR. Leuk Lymphoma 2020; 61: 3526-3528. https://doi.org/10.1080/10428194.2020.1808202

[10] Kim MJ, Cho SY, Kim M-H, Lee JJ, Kang SY, Cho EH, Huh J, Yoon H-J, Park TS, Lee W-I, *et al*. FISH-negative cryptic PML-RARA rearrangement detected by long-distance polymerase chain reaction and sequencing analyses: a case study and review of the literature. Cancer Genet Cytogenet 2010; 203: 278-283. https://doi.org/10.1016/j.cancergencyto.2010.08.026

[11] Blanco EM, Curry CV, Lu XY, Sarabia SF, Redell MS, Lopez-Terrada DH, Roy A. Cytogenetically cryptic and FISH-negative PML/RARA rearrangement in acute promyelocytic leukemia detected only by PCR: an exceedingly rare phenomenon. Cancer Genet 2014; 207: 48-49. https://doi.org/10.1016/j.cancergen.2014.01.001

[12] Campbell LJ, Oei P, Brookwell R, Shortt J, Eaddy N, Ng A, Chew E, Browett P. FISH detection of PML-RARA fusion in ins(15;17) acute promyelocytic leukaemia depends on probe size. Biomed Res Int 2013; 2013: 164501. https://doi.org/10.1155/2013/164501

[13] Gabert J, Beillard E, van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N, Barbany G, Cazzaniga G, Cayuela JM, Cavé H, *et al*. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe. Against Cancer program Leukemia 2003; 17: 2318-2357. https://doi.org/10.1038/sj.leu.2403135

[14] Jianwen X, Ying X, Yuxia G, Xianmin G. Two rare cases of PML/RARα fusion gene in children with acute promyelocytic leukemia. International Journal of Laboratory Medicine 2015; 36: 1636-1638.

[15] Iaccarino L, Divona M, Ottone T, Cicconi L, Lavorgna S, Ciardi C, Alfonso V, Travaglini S, Facchini L, Cimino G, *et al*. Identification and monitoring of atypical PML/RARA fusion transcripts in acute promyelocytic leukemia. Genes Chromosomes Cancer 2019; 58: 60-65. https://doi.org/10.1002/gcc.22708

[16] Hongxia S, Jing X, Guoliang H, Sanchun L, Zhiqiang Z. Acute promyelocytic leukemia of V. PML-RARA gene: A case report and review of the literature. Journal of Clinical Laboratory 2021; 39: 156-158.

[17] Cao Y, Yao L, Liu Y, Gu Q, Dong W, Wang Z, Wang F, Lin R, Xie X, Cen J, *et al.* An Atypical PML-RARA Rearrangement Resulting from Submicroscopic Insertion of the RARA Gene at the PML Locus with Novel Breakpoints within PML Exon 7b and RARA Exon 3. Acta Haematol 2019; 142. https://doi.org/10.1159/000498842

[18] Chengye W, Yulong L, Xiaoyan D, Lin Z, Baojun S. Acute promyelocytic leukemia with PML hidden breakpoint t(15; 17)(q22; q21): a case report and review of the literature. Chinese Journal of Hematology 2021; 42: 74-77.

[19] Ismail S, Ababneh N, Awidi A. Identification of atypical PML-RARA breakpoint in a patient with acute promyelocytic leukemia. Acta Haematol 2007; 118: 183-187. https://doi.org/10.1159/000109471

Received on 28-11-2021 Accepted on 12-12-2021 Published on 14-12-2021

https://doi.org/10.30683/1929-2279.2021.10.07