CASE REPORT

Novel combined variants of \textit{WT1} and \textit{TET2} in a refractory and recurrent AML patient

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Abstract

Background: Somatic mutations in Wilms’ tumor 1 (\textit{WT1}) and tet methylcytosine dioxygenase 2 (\textit{TET2}) genes were separately perceived as contributors to hematopoietic disorders and usually thought to have a mutually exclusive effect in acute myeloid leukemia (AML). However, we found novel \textit{WT1} and \textit{TET2} variants persistently co-existed in a refractory and recurrent AML patient with t(9;11)(p21.3;q23.3); \textit{KMT2A-MLLT3}, and were only detectable genetic alteration in early recurrence. Hence, these two novel variants were further investigated in patient’s family, and the potential effect on disease progression was evaluated at follow-up.

Case presentation: A 27-year-old male was diagnosed with AML, having t(9;11)(p21.3;q23.3); \textit{KMT2A-MLLT3}, accompanied by \textit{WT1} (NM_024426.6:exon7:c.1109G>C:p.Arg370Pro) and \textit{TET2} (NM_001127208.3:exon11:c.5530G>A:p.Asp1844Asn) variants. After two cycles of induction chemotherapy, complete remission was achieved. A consolidation treatment was then completed. However, the evaluation of the bone marrow revealed that early recurrence, \textit{WT1}(p.Arg370Pro) and \textit{TET2}(p.Asp1844Asn) variants still detectable, instead of \textit{KMT2A-MLLT3}. Subsequently, these two variants were proved to be germline variants, which inherited from father and mother respectively. And the patient’s elder brother also carried \textit{TET2}(p.Asp1844Asn) variant. A sequential allogeneic HLA-matched sibling hematopoietic stem cell transplantation (allo-HSCT) was carried out, and the donor is the patient’s elder brother, the original two variants of patient were replaced by the donor-derived \textit{TET2}(p.Asp1844Asn) variant after allo-HSCT; the patient has remained in complete remission with regular follow-up.

Conclusions: In brief, it is firstly reported that \textit{WT1} p.Arg370Pro and \textit{TET2} p.Asp1844Asn variants co-existed in a refractory and recurrent AML patient by inheritance. These two variants of the patient were replaced with donor-derived \textit{TET2} p.Asp1844Asn after allo-HSCT, and the patient has remained in complete remission with regular follow-up.

Keywords: Acute myeloid leukemia, \textit{WT1}, \textit{TET2}, Germline variant, Next-generation sequencing, Prognosis, Case report

Background

Acute myeloid leukemia (AML) is a clonal hematopoietic disorder and is usually observed along with genetic alterations in hematopoietic stem cells [1]. Most of the molecular cytogenetic abnormalities involved in AML have been identified, and these may be potential biomarkers for differential diagnosis, risk stratification, and therapeutic response in AML patients [2, 3]. Nevertheless, genetic alterations with definite clinical significance are relatively rare [4], and the clinical significance of most of them is still unknown [5], especially for germline mutations [6].

In our hospital, a 27-year-old male was diagnosed with AML, having t(9;11)(p21.3;q23.3); \textit{KMT2A-MLLT3}, accompanied by Wilms’ tumor 1 (\textit{WT1}, NM_024426.6:exon7:c.1109G>C:p.Arg370Pro) and tet
methylcytosine dioxygenase 2 (TET2, NM_001127208.3:exon11:c.5530G>A:p.Asp1844Asn) variants were present. He had very abnormal WT1 mRNA expression. However, there are some noteworthy issues in this case, for instance: Why did WT1 and TET2 variants, which were previously reported to be mutually exclusive [7, 8], persistently co-exist in the AML patient? WT1 (p.Arg370Pro) and TET2 (p.Asp1844Asn) variants were only detectable genetic alterations in patients with early recurrence, instead of KMT2A-MLLT3, and whether co-exist of these two variants contribute to disease progression? To address these issues, blood and buccal mucosa cells samples of patient and his family were firstly collected, targeted multi-genes panel sequencing was performed to determine whether variation is hereditary or acquired. And after allo-HSCT, targeted multi-genes panel sequencing was performed again to investigate whether the variants were replaced, and regularly follow-up was performed.

**Case presentation**

A 27-year-old male was admitted to our hospital in Jan 2019 due to hypoleukocytosis lasting two weeks. His family history did not suggest an inherited susceptibility to cancer, as no first-degree relatives, including his parents, his brother, and his child, had cancer. The complete blood cell count (CBC) indicated a white blood cell (WBC) count of 0.77 × 10^9/L, platelet count of 129 × 10^9/L, and hemoglobin (Hb) concentration of 45 g/L. Bone marrow aspiration revealed monocyte hyperplasia with 93% monoblasts and 2.5% promonocytes (Fig. 1a). Moreover, non-specific esterase stain revealed intensely positive monoblasts, and the positive stain could be inhibited by sodium fluoride (data not shown). Immunophenotype analysis of the abnormal cells by flow cytometry revealed full expression of HLA-DR, CD38, CD33, CD11c, CD64 and CD56; partial expression of CD34, CD117, CD123, CD11b; and no expression of CD13, CD7, CD5, CD19, CD10, CD20, CD14, MPO, CD79a and cCD3 (Fig. 1b, c). Chromosome analysis of bone marrow cells indicated a karyotype of 46, XY, t(9;11)(p21.3;q23.3) (Fig. 1d). The KMT2A-MLLT3 fusion gene was identified using qRT-PCR. Targeted multi-genes panel sequencing revealed that WT1 (p.Arg370Pro VAF 50.11%) and TET2 (p.Asp1844Asn VAF 48.33%) variants were still present. Another IA regimen was then administered as the second induction chemotherapy from February 21, 2019.

At the end of first induction chemotherapy on February 20, 2019, the CBC revealed a WBC count of 0.64 × 10^9/L, Hb of 83 g/L, platelet count of 21 × 10^9/L. Bone marrow aspiration showed that primitive and immature cells made up 6% of the nucleated bone marrow cells. Flow cytometry detected 21% abnormal immunophenotype monocytes. The patient achieved partial remission. The CAG (Clarithromycin + Cytarabine + G-CSF) regimen was then administered as the second induction chemotherapy from February 21, 2019.

The evaluation of the bone marrow on March 21, 2019, showed that primitive and immature monocytes made up 0% of the bone marrow nucleated cells. Monocytes with abnormal immunophenotype were less than 0.01%. The KMT2A-MLLT3 fusion gene was not present. Complete remission was achieved. However, the WT1 (p.Arg370Pro VAF 50.11%) and TET2 (p.Asp1844Asn VAF 48.33%) variants were still present. Another IA regimen was then administered as consolidation chemotherapy from March 29, 2019.

The evaluation of the bone marrow on May 9, 2019 revealed that primitive and immature monocytes made up 7% of the nucleated bone marrow cells and CD34+CD117+CD33+ cells were 0.75% by flow cytometry. KMT2A-MLLT3 fusion remained negative. All these data suggested early recurrence. While WT1 (p.Arg370Pro) and TET2 (p.Asp1844Asn) variants were still positive, the VAF was 51.87% and 51.78%, respectively.

Owing to the fluctuation of the disease, the risk stratification was re-evaluated to high-risk. A HA regimen chemotherapy (homoharringtonine and intermediate-dose cytarabine) was administered from May 11, 2019. The second complete remission was achieved, with variants on WT1 (p.Arg370Pro) and TET2 (p.Asp1844Asn) genes still present, and the VAF was 48.35% and 49.88%, respectively (Table 1).

A sequential allogeneic HLA-matched sibling hematopoietic stem cell transplantation was carried out on July 9, 2019, and the donor is patient’s elder brother. After allo-HSCT, complete donor chimerism was received (Fig. 1e).
and donor-derived TET2 p.Asp1844Asn variant was also received. To date, the patient has remained in complete remission with regular follow-up.

Since the patient’s variants were persistent and does not change with treatment, we suspected the WT1 (p.Arg370Pro) and TET2 (p.Asp1844Asn) variants were germline variants. Then buccal mucosa cells (somatic control) were first obtained from the patient for targeted sequencing, and results revealed that shared variation sites of WT1 p.Arg370Pro (52.09%) and TET2 p.Asp1844Asn (37.26%) existed in buccal mucosa cells, indicating that WT1 p.Arg370Pro and TET2 p.Asp1844Asn may germline variants.

To further identify whether these two variants were heritable, DNA samples from the patient’s relatives were analyzed using the same target sequencing; WT1 p.Arg370Pro (VAF 44.75%) was detected in the patient’s father and TET2 p.Asp1844Asn (VAF 36.01%) was detected in his mother; the brother carried the TET2 p.Asp1844Asn variant (VAF 48.30%) (Fig. 2).

The above results thoroughly confirmed that germline variants WT1 p.Arg370Pro and TET2 p.Asp1844Asn co-existed in the patient by inheritance.

**Discussion and conclusions**

In this study, we first reported that WT1 p.Arg370Pro co-exists with TET2 p.Asp1844Asn variant in a refractory and recurrent AML patient. These two variants were derived from the patient’s father and mother respectively, and were only detectable genetic alteration in early recurrence. These two variants were replaced with donor-derived TET2 p.Asp1844Asn variant after allo-HSCT, and the patient has remained in complete remission with regular follow-up.

WT1 and TET2 mutations were mutually exclusive in AML patient, it is rare that WT1 and TET2 variants co-exist in an AML patient [8]. As the co-existing variants in this case were germline variants derived from the patient’s father and mother, respectively, this case serves as a reminder that mutually exclusive germline variants may co-exist in a patient due to inheritance. Recently, a growing number of germline mutations associated with tumor susceptibility have been reported; for instance, MITF (Microphthalmia-associated transcription factor) mutation impairs SUMOylation, which has an oncogenic function in the tumorigenesis of multiple tissues/melanocytes and kidney cells [9]. GATA2, RUNX1, DDX41,

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**Table 1** Molecular and cellular genetic alternation of patient at different disease stages

| Examination | Before allo-HSCT | After allo-HSCT |
|-------------|------------------|-----------------|
| Initial diagnosis | CR1 | Relapse | CR2 |
| 0 | 0 | 0.03 | 0.15 |
| Karyotype (Fusion) | t (9; 11) (p21.3;q23.3) | NA | – | NA |
| Variants (VAF %) | TET2 (52.59) | TET2 (52.59) |
| WT1 level (%) | 0 | 0 |

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**Fig. 2** Genetic change of WT1 and TET2 in patient's relatives. Variants status and VAF value of WT1/TET2 in the patient's relatives.
PAX5, CEBPA, and TP53 are associated with hematologic malignancies predisposition [10]. Identifying germline mutations is beneficial for therapy choice, donor selection for hematopoietic stem cell transplantation, evaluation of comorbidities, and surveillance strategies to improve the clinical outcomes [10].

Although it is unclear whether co-exist of these two variants closely associated with AML predisposition and recurrence, WT1 and TET2 genes are necessary for the normal development of blood cells based on previous findings. TET2 is important for normal myelopoiesis, and disruption of TET2 enzymatic activity favors myeloid tumorigenesis [11]. Slower proliferation and reduced clonogenicity have been observed in TET2 overexpressing cells in vitro compared to those transfected with the empty vector, which indicates the suppressor role of TET2 in AML. However, the inhibitory effects of TET2 on leukemic cell proliferation and colony formation could be abolished by WT1 knockdown [11]. Further studies revealed that TET2 suppresses leukemia cell proliferation and colony formation in a WT1-dependent manner [12]. All of the above conclusions are from in vitro and animal experiments; here, for the first time, TET2 and WT1 variants are reported to co-exist in an AML patient, with detectable alterations only in the complete remission and relapse stages. Interestingly, when these two variants were replaced with donor-derived TET2 p.Asp1844Asn variant, patient has been received complete remission during follow-up. It is necessary to explored whether co-exist of WT1 and TET2 variants have a worse effect on disease progression compared with existed alone.

Since the interaction of these two genes is essential for biological functions, we attempted to explain the pathogenesis at the molecular level. TET2 binds to the zinc finger domain (residue 323–449), but not the N-terminal region (residue 1–323) of WT1. In this patient, the missense mutation was in the zinc finger domain (residue 370) of WT1, which may disrupt the binding between WT1 and TET2. Moreover, WT1 binds to the CD domain of TET2, but not the N-terminal region (residue 1 to 1127) [11]. Unfortunately, in this case, the missense mutation was in the CD domain (residue 1844) of TET2, which may further disrupt the binding between WT1 and TET2. From a molecular point of view, this may be a reasonable explanation for the disease. In clinical practice, WT1 expression and disease progression are usually parallel to each other; WT1 expression is also used as the MRD marker of hematological neoplasms when other genetic alterations are absent [13]. However, in this case, abnormal up-regulation of WT1 was not observed at different stages, but the WT1 variant persisted. Similar to the WT1 mutation being related to poor prognosis [14], WT1 overexpression was also indicated to be associated with shorter OS [15]. However, the question remains which alteration could be a potential indicator of prognostic stratification for the patient; expression level or mutation status? It seems contradictory for the patient to have WT1 variant and no WT1 overexpression, and related reports in the literature are limited.

In this case, WT1 and TET2 variants were the only molecular alterations in the relapse stage. Then, this is only a phenomenon we observed, and the existing evidence is not enough to show that co-existing WT1 and TET2 variants were involved in the occurrence and progression of AML in the above manner. Previous studies have revealed that WT1 can bind TET2, acting as a guide for TET2 to specific genomic loci, leading to increased stem cell function and self-renewal with proliferation [16]. This seems a reasonable explanation for why the patient relapsed in a short time, but other possible reasons also need to be further explored. However, after allo-HSCT, two variants of patient were replaced with donor-derived TET2 p.Asp1844Asn, then patient has remained in complete remission with regular follow-up, we have to speculate whether there was the possibility of concurrent presence of WT1 gene mutation could contribute to second hit in the pathogenesis of AML. In brief, these two variants might play some role in the pathogenesis of refractory and recurrent AML, while a long term follow-up and regular monitoring by NGS is needed. At the same time, we just report a case and the result of the family pedigree study. More cases and related families, and further researches are necessary to prove our hypothesis.

In summary, we present the first report that WT1 and TET2 variants co-existed in a refractory and recurrent AML patient; these were identified as germline variants. After allo-HSCT, the original variants were replaced by the donor-derived TET2 variant; the patient has remained in complete remission with regular follow-up.

Abbreviations
AML: Acute myeloid leukemia; TET2: Tet methylcytosine dioxygenase 2; WT1: Wilms’ tumor 1; MRD: Minimal residual disease; VAF: Variant allele frequency; CBC: Complete blood cell count; WBC: White blood cell count; Hb: Hemoglobin; HSCT: Hematopoietic stem cell transplantation; qRT-PCR: Quantitative real-time PCR; OS: Overall survival.

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Authors’ contributions
QM/YXG wrote the manuscript. QM/XXL/GXW collected samples and performed data analysis. WLS designed this study, critically reviewed the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials
The patient’s DNA sequencing data generated during the current study are deposited in the NCBI Sequence Read Archive (SRA) repository under the accession number PRJNA729676. The datasets generated and/or analyzed during the current study are available in the Genbank repository (GRCh37/hg19, https://www.ncbi.nlm.nih.gov/genome/guide/human/) for NM_001127208.3 (https://www.ncbi.nlm.nih.gov/nuccore/NM_001127208.3) and NM_024426.6 (https://www.ncbi.nlm.nih.gov/nuccore/NM_024426.6/) and NM_001127208.3 (https://www.ncbi.nlm.nih.gov/nuccore/NM_001127208.3). The raw datasets of family members generated during the current study are not publicly available because it is possible that individual privacy could be compromised. It is possible to apply for permission to obtain access to the raw sequencing data through the corresponding author.

Declarations

Ethics approval and consent to participate
The present study was permitted by Ethics Committee of Xuanwu Hospital, Capital Medical University, and written informed consents were obtained from all of the participants.

Consent for publication
Written informed consent for publication of identifying images or other personal or clinical details was obtained from all of the participants. A copy of the written consent is available for review by the Editor of this journal.

Competing interests
The authors declare that they have no competing interests.

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