LETTER TO THE EDITOR

Open Access

Recurrent SETD2 mutation in NPM1-mutated acute myeloid leukemia

Jiewen Sun1, Wenjuan Yu2,3* and Xiang Zhang2,3*

Abstract

SETD2 is the only methyltransferase for H3K36me3, and our previous study has firstly demonstrated that it functioned as one tumor suppressor in hematopoiesis. Consistent with it, SETD2 mutation, which led to its loss of function, was identified in AML. However, the distribution and function of SETD2 mutation in AML remained largely unknown. Herein, we integrated SETD2-mutated AML cases from our center and literature reports, and found that NPM1 mutation was the most common concomitant genetic alteration with SETD2 mutation in AML, with its frequency even higher than MLL rearrangement and AML1-ETO. Though this result indicated the cooperation of SETD2 and NPM1 mutations in leukemogenesis, our functional study showed that SETD2 was required for the proliferation of NPM1-mutated AML cell line OCI-AML3, but not MLL-rearranged AML cell line THP-1, via maintaining its direct target NPM1 expression, which was just opposite to its role of tumor suppressor. Therefore, we speculated that SETD2 possibly had two different faces in distinct subtypes and stages of AML.

Keywords: SETD2 mutation, NPM1 mutation, Acute myeloid leukemia

To the editor

SETD2 has been demonstrated as one tumor suppressor in hematopoiesis [1], and SETD2 mutation affected AML, in which its distribution remained not fully understood [2]. Herein, we analyzed the SETD2 mutation in NPM1-mutated AML.

One 36-year-old woman was committed due to abdominal pain and fever for 7 and 3 days, respectively. PB test showed WBC: 52.4 × 10^9/L, Hb: 98 g/L, PLT: 48 × 10^9/L, circulated blast: 80%. BM examination exhibited 67.5% myeloblasts with the immunophenotype of CD11b-CD13dim + CD14-CD15dim + CD33 + CD34partial + CD35-CD38dim + CD45 + CD64-CD65dim + CD71 + CD117 + CD123dim + HLA-DR-. Though karyotype was normal and CBF or MLL rearrangements were negative, NPM1, SETD2, NRAS and ETV6 mutations were identified.

Therefore, AML with mutated NPM1 was diagnosed. After receiving the operation for co-existed acute appendicitis, she accepted IA regimen as induction therapy, and CR1 was achieved. Subsequently, she received three cycle of medium-dose cytarabine regimen. However, AML relapsed at the 3 months after cessation of chemotherapy, and 72% myeloblasts re-emerged in BM. Due to the early recurrence, she accepted HAA and CLAG regimen successively, and achieved CR2. However, the leukemic clones were not eradicated reflected by persistent above mutations. Therefore, allogeneic semicompatible HSCT was immediately conducted. As follow-up, CR was still maintained at the 15 months after HSCT (Fig. 1a).

In this patient, SETD2R2109X was identified, and it was also found in other malignancies from COSMIC database (Fig. 1b), so SETD2R2109X was one driver in cancer. However, SETD2 deficiency was not sufficient to generate AML, so additional hits were required [1, 3]. Therefore, we reviewed AML studies involving SETD2 mutation [2, 4–9], and found that NPM1 mutation

© The Author(s). 2020. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
rather than MLL rearrangement or AML1-ETO was the most common co-existent genetic alteration of SETD2 mutation in AML (Fig. 1c-d). To establish their association, we displayed subgroup analysis in above studies, then submitted it Pearson’s chi-square test, and calculated OR. Strikingly, SETD2 and NPM1 mutations were the concomitant mutation in AML (P = 0.031; OR = 3.28) (Fig. 1e-f). To address whether SETD2 mutation mediated drug resistance in AML, we analyzed their therapeutic response to standard chemotherapy. Among 22 SETD2-mutated AML patients, the data were available in 11 patients, while CR, PR, and NR was 72.7%, 9.09%, and 18.2%, respectively. Notably, the CR was comparable to it in total AML. Interestingly, all with NPM1-mutated AML achieved CR, and two with MLL-rearranged AML exhibited NR. Therefore, SETD2 mutation was possibly not one determinant in drug sensitivity for AML. Furthermore, we analyzed the OS between SETD2-mutated and wild-type groups with cBioPortal database [10, 11], but no significance between two groups was found (Additional file 1: Figure S1). Regrettably, the data about EFS were not available.

Loss of SETD2 function accelerated the progression of MLL-rearranged or AML1-ETO-positive AML, but whether it was the same in NPM1-mutated AML remained unknown. Herein, we displayed shRNA-mediated SETD2 knockdown, which simulated its loss of function caused by SETD2 frame-shift or nonsense mutation, in NPM1-mutated AML cell line OCI-AML3 and MLL-rearranged AML cell line THP-1. Interestingly, SETD2 knockdown impaired the proliferation of OCI-AML3 but not THP-1 cells (Fig. 2a-d). Furthermore, the proliferative defect of OCI-AML3 was caused by increased cell apoptosis (Fig. 2e) and cell cycle arrested at G1/G0 phase (Fig. 2f). It has been reported that the viability of OCI-AML3 relied on the function of NPM1 mutation [12], while NPM1 expression was regulated by the transcriptional activation mark, H3K36me3, which indicated by ChIP-Seq in the HSPCs of Mll-af9-positive leukemia (Fig. 2g) [13]. Consistently, we demonstrated that NPM1 and its direct targets MEIS, HOXA9 were significantly down-regulated in SETD2 knockdown OCI-AML3 cells (Fig. 2h-i). Therefore, our results indicated that SETD2 knockdown-mediated OCI-AML3 proliferation inhibition was possibly attributed to NPM1 down-regulation.

The detailed role of SETD2 mutation in NPM1-mutated AML remained mysterious. Theoretically, SETD2 and NPM1 mutations probably cooperated in leukemogenesis. However, our results showed that SETD2 was required for the maintenance of OCI-AML3. To our knowledge, two possibilities existed: firstly, SETD2 mutation played different roles in the initiation and maintenance of NPM1-mutated AML; secondly,
additional genetic alteration influenced SETD2 function in NPM1-mutated AML. Therefore, further investigations were needed in the future.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s40364-020-00243-y.

**Fig. 2** SETD2 was required for the maintenance of NPM1-mutated AML cell line OCI-AML3. a and b The proliferation (a) and SETD2 expression (b) of scramble and SETD2 knockdown OCI-AML3 cells. c and d The proliferation (c) and SETD2 expression (d) of scramble and SETD2 knockdown THP-1 cells. e Annexin-V staining for detecting cell apoptosis in OCI-AML3 cells. f PI staining for cell cycle analysis in OCI-AML3 cells. g NPM1 has been demonstrated as one direct target of H3K36me3 in the literature report. h and i The expression of NPM1 (h) and its direct downstream targets, MEIS and HOXA9 (i), was analyzed in scramble and SETD2 knockdown OCI-AML3 cells. ***, P < 0.001; **, P < 0.01; *, P < 0.05; T test was used for each graph.

**Abbreviations**
AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BM: Bone marrow; ChIP-Seq: Chromatin immunoprecipitation-sequencing; CLAG: Cladribine, cytarabine plus granulocyte colony-stimulating factor; CR: Complete remission; EFS: Event-free survival duration; H3K36me3: Tri-methylated histone 3 lysine 36; HAA: Homoharringtonine, aclacinomycin, plus cytarabine regimen; HB: Hemoglobin; HSCT: Hematopoietic stem cell transplantation; HSPCs: Hematopoietic stem progenitor cells; IA: Idarubicin plus cytarabine regimen; MDS: Myelodysplastic syndrome; NR: No response; OR: Odds ratio; OS: Overall survival duration; PB: Peripheral blood; PLT: Platelet; PR: Partial remission; WBC: White blood cell

**Additional file 1:** Figure S1. The OS of SETD2 wild type and mutated AML patients from the summary of TCGA, TARGET, and OHSU studies.
Acknowledgements
We would also like to thank all members of Zhejiang Key Laboratory of Diagnosis and Treatment for Hematologic Malignancies in diagnostic supports.

Authors’ contributions
X.Z. designed the experiments. W.-J. Y. collected and integrated clinical materials. J.-W. S. displayed the experiments. X. Z. integrated and analyzed all the data. X. Z. wrote the manuscript. J.-W. S. and W.-J. Y. revised the manuscript. The authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (81800199, 81670124).

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
This study was approved by the ethical review committees of the First Affiliated Hospital to Zhejiang University School of Medicine.

Consent for publication
Written informed consent was obtained from this patient.

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

Author details
1Center Laboratory, Affiliated Secondary Hospital, Zhejiang Chinese Medical University, Zhejiang, Hangzhou, China. 2Department of Hematology, The First Affiliated Hospital, Zhejiang University School of Medicine, #79 Qingchun Rd, Zhejiang 310003, Hangzhou, China. 3Key Laboratory of Hematologic Malignancies, Diagnosis and Treatment, Zhejiang, Zhejiang, Hangzhou, China.

Received: 4 September 2020 Accepted: 29 October 2020
Published online: 11 November 2020

References
1. Zhang YL, Sun JW, Xie YY, Zhou Y, Liu P, Song JC, et al. Setd2 deficiency impairs hematopoietic stem cell self-renewal and causes malignant transformation. Cell Res. 2018;28(4):476–90.
2. Zhu X, He F, Zeng H, Ling S, Chen A, Wang Y, et al. Identification of functional cooperative mutations of SETD2 in human acute leukemia. Nat Genet. 2014;46(3):287–93.
3. Zhou Y, Yan X, Feng X, Bu J, Dong Y, Lin P, et al. Setd2 regulates quiescence and differentiation of adult hematopoietic stem cells by restricting RNA polymerase II elongation. Haematologica. 2018;103(7):1110–23.
4. Faber ZJ, Chen X, Gedman AL, Boggs K, Cheng J, Ma J, et al. The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet. 2016;48(12):1551–6.
5. Masetti R, Castelli I, Astolfi A, Bertuccio SN, Indio V, Togni M, et al. Genomic complexity and dynamics of clonal evolution in childhood acute myeloid leukemia studied with whole-exome sequencing. Oncotarget. 2016;7(35):56746–57.
6. Shin SY, Lee ST, Kim HJ, Cho EH, Kim JW, Park S, et al. Mutation profiling of 19 candidate genes in acute myeloid leukemia suggests significance of DNMT3A mutations. Oncotarget. 2016;7(34):54825–37.
7. Cancer Genome Atlas Research N, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
8. Bolouri K, Farrar JE, Tchich T Jr, Ris SE, Lim EL, Alonzo TA, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med. 2018;24(1):103–12.
9. Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, Savage SL, et al. Functional genomic landscape of acute myeloid leukaemia. Nature. 2018;562(7728):526–31.
10. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4.
11. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.
12. Balusu R, Fiskus W, Rao R, Chong DG, Nalluri S, Mudunuru U, et al. Targeting levels or oligomerization of nucleophosmin 1 induces differentiation and loss of survival of human AML cells with mutant NPM1. Blood. 2011;118(11):3096–106.
13. Bu J, Chen A, Yan X, He F, Dong Y, Zhou Y, et al. SETD2-mediated crosstalk between H3K36me3 and H3K9me2 in MLL-rearranged leukemia. Leukemia. 2018;32(4):850–9.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.