



# A novel *RARA–SNX15* fusion in *PML–RARA*-positive acute promyelocytic leukemia with $t(11;17;15)(q13;q21.2;q24.1)$

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## Abstract

Acute promyelocytic leukemia (APL) is characterized by a series of *retinoic acid receptor (RAR)* fusion genes that lead to the dysregulation of RAR signaling and onset of APL. *PML–RARA* is the most common fusion generated from  $t(15;17)(q24;q21)$ . In addition, the reciprocal fusion *RARA–PML* is present in over 80% of  $t(15;17)$  APL cases. The bcr3 types of *RARA–PML* and *RARA–PLZF* in particular are reciprocal fusions that contribute to leukemogenesis. Here, we report a variant APL case with  $t(11;17;15)(q13;q21.2;q24.1)$ . Massive parallel sequencing of patient RNA detected the novel fusion transcripts *RARA–SNX15* and *SNX15–LINC02255* along with the bcr3 type of *PML–RARA*. Genetic analysis revealed that *RARA–SNX15L* is an in-frame fusion due to intron retention caused by RNA mis-splicing. *RARA–SNX15L* consisted mainly of *SNX15* domains, including the Phox-homology domain, which has a critical role in protein–protein interactions among sorting nexins and with other partners. Co-immunoprecipitation analysis revealed that *RARA–SNX15L* is directly associated with *SNX15* and with itself. Further studies are needed to evaluate the biological significance of *RARA–SNX15L* in APL. In conclusion, this is the first report of APL with a complex chromosomal rearrangement involving *SNX15*.

**Keywords** Acute promyelocytic leukemia · Chromosomal translocation · Reciprocal fusion · *SNX15* · *LINC02255*

## Introduction

Acute promyelocytic leukemia (APL) is characterized by a series of *retinoic acid receptor (RAR)* fusion genes that lead to the dysregulation of RAR signaling and initiation of APL. *PML–RARA* is the most common fusion gene generated from the balanced chromosomal translocation  $t(15;17)(q24;q21)$  [1]. The reciprocal fusion *RARA–PML* is present in over 80% of  $t(15;17)$  APL cases [2]. While *PML–RARA* determines the disease phenotype, and is the therapeutic target of all-trans retinoic acid (ATRA) and arsenic trioxide,

the bcr3 type of *RARA–PML* was shown to significantly increase the penetrance of APL in *PML–RARA* transgenic mice, and induce a less mature morphology in the APL cells [3]. In the case of *PLZF–RARA*, the reciprocal fusion *RARA–PLZF* alone induced myeloproliferative hematopoiesis in *RARA–PLZF* transgenic mice, and was necessary to induce the full-blown APL phenotype in *PLZF–RARA* and *RARA–PLZF* double-transgenic mice [4]. Hence, some of the reciprocal fusions in APL can affect the disease phenotype. Over the past few decades, many APL variants caused by unique chromosomal translocations have been reported, [1, 5] including *NUMA1–RARA* in a case of variant APL with  $t(11;17)(q13;q21)$  [6]. Here, we report novel fusions, including *RARA–Sorting Nexin 15 (SNX15)* and *SNX15–LINC02255*, in a *PML–RARA*-positive APL case with  $t(11;17;15)(q13;q21.2;q24.1)$ .

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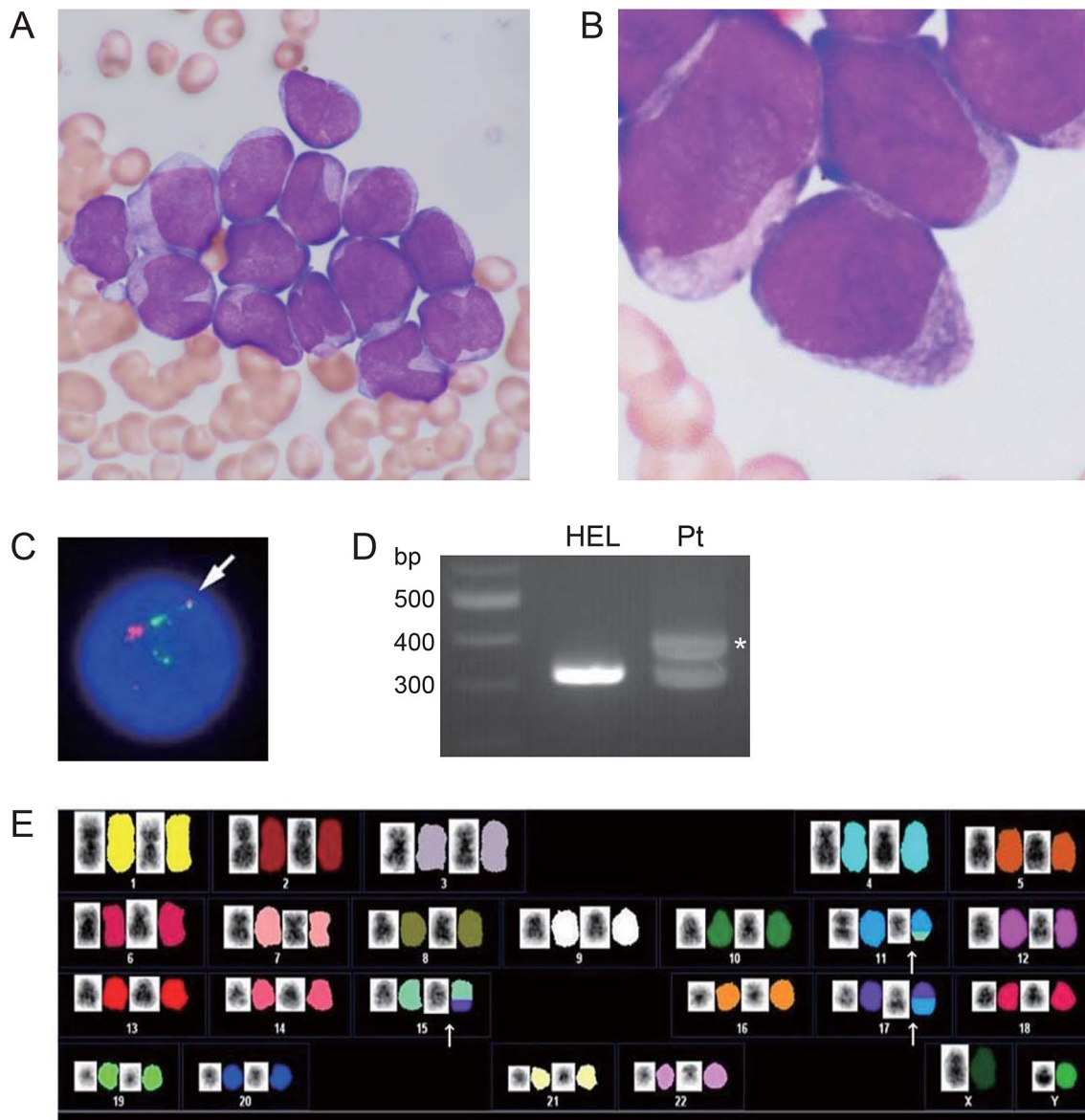
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## Case presentation

A 19-year-old man was admitted due to fever and bleeding. A full blood count showed a hemoglobin level of 12.6 g/dL, a platelet count of  $14 \times 10^9/L$ , and a white blood cell count of  $58.1 \times 10^9/L$ , in which 87% of the blasts had a monocytic morphology (Fig. 1A, B) and were positive for myeloperoxidase. The morphology of the blasts was compatible with acute myeloid leukemia M3 variant in

the French–American–British classification. Coagulopathy was present with an increased prothrombin time of 1.61 (international normalized ratio), an activated partial thromboplastin time of 30.2 s (normal, 26–39 s), decreased fibrinogen level of 122 mg/dL (normal, 200–400 mg/dL), and an increased level of fibrin/fibrinogen degradation products of 148.7 mg/L (normal, 0–5 mg/L). Flow cytometry analysis of the blasts revealed CD13+, CD33+, CD34+, CD117+, CD14–, and HLA-DR. Bone-marrow aspiration resulted in a dry tap, and bone-marrow biopsy



**Fig. 1** Morphology and cytogenetic analysis of the  $t(11;17;15)$  APL patient sample. Leukemic promyelocytes before treatment. **A** Monoblastic cell morphology. May-Giemsa staining. Original magnification  $\times 100$ . **B** Microgranular pattern in the cytoplasm. May-Giemsa staining. Original magnification  $\times 400$ . **C** Fluorescence in situ hybridization analysis. The arrow indicates a fusion signal between *PML*

and *RARA*. **D** *FLT3*-ITD genotyping [15]. HEL: germline control. Pt:  $t(11;17;15)$  APL sample. The asterisk indicates hemizygous *FLT3*-ITD detected by PCR of genomic DNA with primers *FLT3*\_11F (5'-GCAATTTAGGTATGAAAGCCAGC-3') and *FLT3*\_12R (5'-CTTTCAGCATTTTGACGGCAACC-3'). **E** Spectral karyotyping analysis showing  $t(11;17;15)$

showed hypercellular marrow with an excess of blasts. Computed tomography showed marked hepato-splenomegaly and tonsillar swelling. Reverse transcription quantitative polymerase chain reaction (PCR) analysis detected the chimeric *PML-RARA* fusion at  $1.1 \times 10^5/\mu\text{g}$  RNA. Fluorescence in situ hybridization analysis showed that the *PML-RARA* fusion signal was detected in 99% of the blasts (Fig. 1C). *FLT3* internal tandem duplication (*FLT3-ITD*) was present (Fig. 1D), and the *NPM1* mutation was not detected. Chromosomal analysis of the blasts revealed 46,XY,t(11;17;15)(q13;q21.2;q24.1), and spectral karyotyping analysis showed several chromosomal rearrangements among chromosomes 11, 15, and 17 (Fig. 1E). Taken together, the patient was diagnosed with the microgranular type of APL with a variant translocation, and was subsequently treated with systemic chemotherapy according to the Japan Adult Leukemia Study Group (JALSG) APL212 protocol (UMIN000008470). Administration of ATRA as induction therapy induced differentiation syndrome, but it was manageable using steroids. After a series of consolidation therapy courses according to the APL212 protocol, the patient achieved molecular complete remission.

To investigate the unknown fusion transcripts, we performed massive parallel sequencing of total RNA derived from the APL cells using the Illumina platform (Illumina, San Diego, CA). The RNA sequencing protocol was approved by the Institutional Review Board, and written informed consent was obtained from the patient. There were 56.5 million total reads, and the Q20 score was 98.52%. Chimeric fusions were analyzed by the TopHat-Fusion program (Center for Computational Biology, Johns Hopkins University, Baltimore, MD). In addition to the bcr3 type of *PML-RARA*, we found novel fusion transcripts, including *RARA-SNX15* and *SNX15-LINC02255* (Fig. 2A), which were confirmed by reverse transcription-PCR (Fig. 2B). To identify junction sequences between the related genes, the PCR products were cloned into the pGEM-T Easy Vector (Promega, Madison, WI), and analyzed by Sanger sequencing (Fig. 2C, D, and E). Unexpectedly, we found three splicing variants of *RARA-SNX15* (Fig. 2F). Although *RARA-SNX15S* and *RARA-SNX15M* were out-of-frame fusion transcripts, *RARA-SNX15L* was an in-frame fusion due to intron retention involving 68 base pairs. Colony PCR ( $n = 28$ ) revealed that *RARA-SNX15L*, *RARA-SNX15M*, and *RARA-SNX15S* comprised 17.9%, 57.1%, and 25.0% of the transcriptional variants, respectively. A schematic diagram of the *RARA-SNX15L* generated by the RNA mis-splicing form of *RARA-SNX15* is shown in Fig. 2G. The *RARA-SNX15L* consisted mainly of *SNX15* domains, including the Phox-homology (PX) domain, which is a pivotal structure shared by all sorting nexins that has a critical role in protein-protein interactions among sorting nexins and

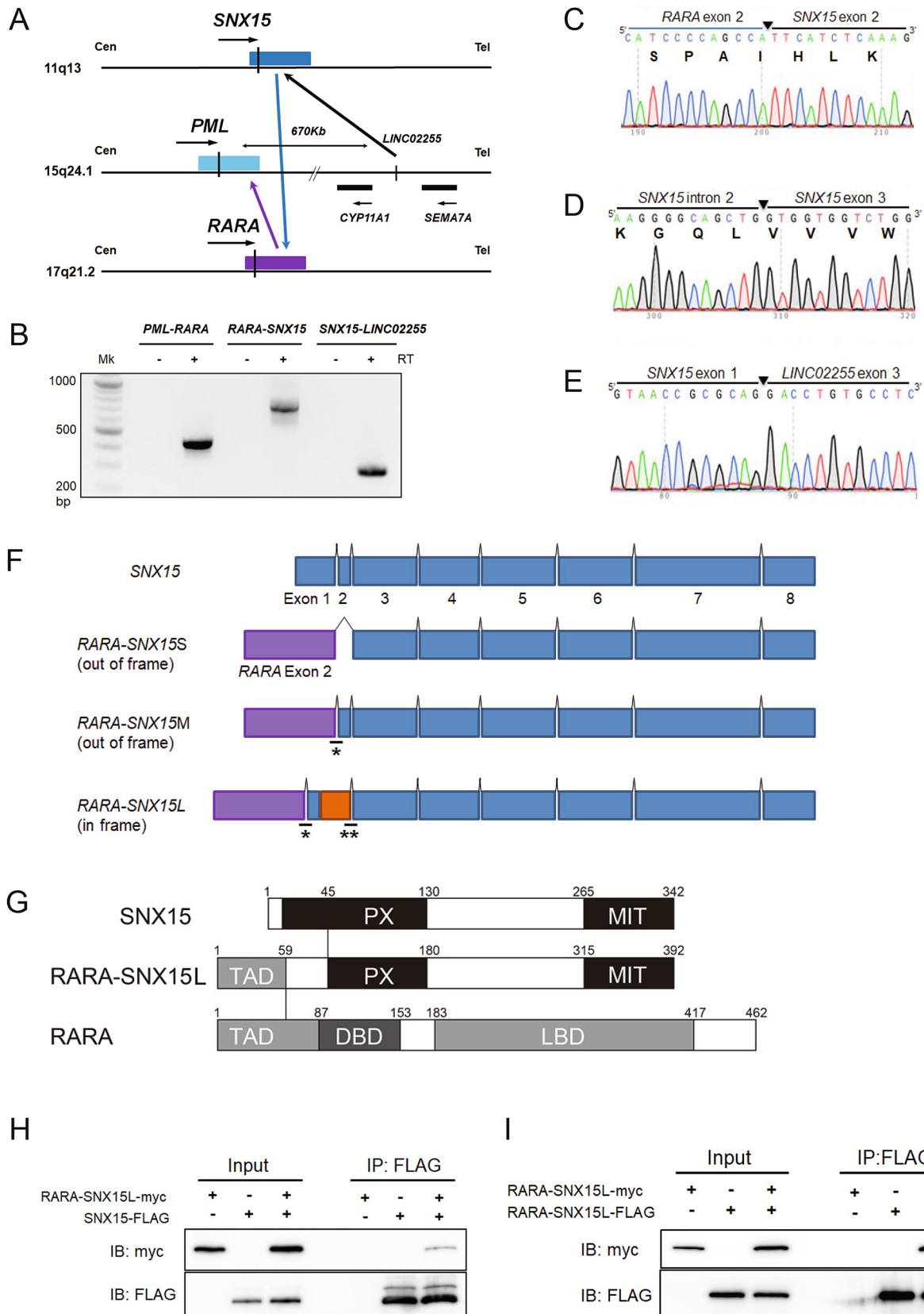
with other partners [7, 8]. To investigate the protein-protein interactions of *RARA-SNX15L*, we performed a co-immunoprecipitation analysis in HEK293T cells. As shown in Fig. 2H, I, *RARA-SNX15L* associated with *SNX15* and with itself.

## Discussion

In this report, we described the discovery of the novel fusions *RARA-SNX15* and *SNX15-LINC02255* in a *PML-RARA*-positive t(11;17;15)(q13;q21.2;q24.1) APL case. The current dogma is that *PML-RARA* primarily interferes with retinoic acid signaling, leading to a differentiation block. In addition, some gene mutations, such as *FLT3-ITD* [9, 10] or reciprocal fusions, influence the APL phenotype [3, 4]. In the present t(11;17;15) APL case, we found an in-frame fusion, *RARA-SNX15L*, generated by intron retention. Intron retention is primarily caused by RNA mis-splicing, which is a major mechanism of the pathogenesis of many inheritable diseases and cancers [11]. Of note, we did not detect any recurrent somatic mutations in the RNA-splicing machinery in this case.

The *SNX15* located on 11q13.1 is involved in intracellular trafficking, including the trafficking of platelet-derived growth factor receptor, insulin receptor (IR), and hepatocyte growth factor receptor [7]. *SNX15* associates with *SNX1*, *SNX2*, *SNX4*, and *SNX15* through the PX domain. *SNX15* not only directly regulates the surface localization of platelet-derived growth factor receptor, but it also impairs the post-translational processing of IR, resulting in negative regulation of IR signaling [7]. Although insulin is an important element for culturing cell lines in vitro [12], it also constitutively activates phosphatidylinositol-3 kinase-AKT-mammalian target of rapamycin signaling in acute myeloid leukemia cells [13, 14]. In Fig. 2H, I, we showed that *RARA-SNX15L* was directly associated with *SNX15* and *RARA-SNX15L* in HEK293T cells. This indicates that the novel fusion *RARA-SNX15L* might have some kind of biological interaction with wild-type *SNX15*. Further studies are needed to evaluate the biological significance of *RARA-SNX15L* in APL. The third fusion partner, *LINC02255* located on 15q24.1, is a long non-coding RNA, and its biological role has not yet been reported. However, *SNX15-LINC02255* was over-expressed when compared to *LINC02255* in normal bone marrow ( $n = 9$ ) and acute myeloid leukemia ( $n = 30$ ) samples.

In conclusion, this is the first report of APL with a complex chromosomal rearrangement involving *SNX15*.



**Fig. 2** Novel chimeric fusions *RARA-SNX15* and *SNX15-LINC02255* in t(11;17;15) APL. **A** Schematic diagram of the three-way translocation in t(11;17;15) APL. *Cen* centromere. *Tel* telomere. **B** Chimeric fusions detected by RT-PCR. The following primer pairs were used: *RARA-SNX15*, R18F (5'-TGGACAGCAGCTCCAGGACA-3') and *SNX15e5R* (5'-ATCAGAGGGGGTGGCAGGATGTGT A-3'); *SNX15-LINC02255*, *SNX15e1F* (5'-CCCCAAGGGCTACAC CGAGTACAAA-3') and *LINC2255R* (5'-ACCATCGTGGGCTTC CTCATTCTTG-3'). *RT* reverse transcription. **C** Sequence analysis of the *RARA-SNX15* transcript at the junction site. The junction of the *RARA* and *SNX15* transcripts is indicated by a bold arrowhead. The DNA and amino acid sequences spanning the junction are shown below. **D** Sequence analysis of the mis-spliced *RARA-SNX15* transcript between a part of intron 2 and exon 3 of *SNX15* that resulted in an in-frame *RARA-SNX15L* fusion protein. **E** Sequence analysis of the *SNX15-LINC02255* transcript at the junction site. **F** Schematic diagram of *SNX15* and the three variants of the *RARA-SNX15* transcript. The blue box indicates the *SNX15* exon. The violet box indicates the *RARA* exon. The orange box indicates the novel domain created by the mis-spliced sequence of *SNX15* intron 2. Single asterisks indicate the location of the sequence in (C). Double asterisks indicate the location of the sequence in (D). **G** Schematic diagram of *SNX15*, *RARA*, and the *RARA-SNX15L* fusion protein. The breakpoint is indicated by the black line. The domains of *RARA-SNX15L* and *RARA* are indicated as follows: Phox homology (PX) domain, microtubule interacting and trafficking (MIT) domain, transactivation domain (TAD), DNA-binding domain (DBD), and ligand-binding domain (LBD). The numbers indicate the position of the amino acid sequence. **H** *RARA-SNX15L* associated with *SNX15*. Co-immunoprecipitation in HEK293T cells between MYC-tagged and FLAG-tagged proteins as previously described [5]. *IP* immunoprecipitation. *IB* immunoblotting. **I** Identification of *RARA-SNX15L* self-association. Co-immunoprecipitation in HEK293T cells

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**Author contributions** YY designed the research; YY and HN performed the research; YY, HN, and AA analyzed the data; KH, SK, AI, MR, HK, and SI collected the clinical data; YY wrote the manuscript.

## Declarations

**Conflicts of interest** None of the authors have any relevant conflicts of interest to declare.

## References

1. Geoffroy MC, de The H. Classic and variants APLs, as viewed from a therapy response. *Cancers (Basel)*. 2020;12(4):967.
2. Walz C, Grimwade D, Saussele S, Lengfelder E, Haferlach C, Schnittger S, et al. Atypical mRNA fusions in PML-RARA

- positive, RARA-PML negative acute promyelocytic leukemia. *Genes Chromosomes Cancer*. 2010;49(5):471–9.
3. Pollock JL, Westervelt P, Kurichety AK, Pelicci PG, Grisolano JL, Ley TJ. A bcr-3 isoform of RARalpha-PML potentiates the development of PML-RARalpha-driven acute promyelocytic leukemia. *Proc Natl Acad Sci USA*. 1999;96(26):15103–8.
4. He LZ, Bhaumik M, Tribioli C, Rego EM, Ivins S, Zelent A, et al. Two critical hits for promyelocytic leukemia. *Mol Cell*. 2000;6(5):1131–41.
5. Yamamoto Y, Tsuzuki S, Tsuzuki M, Handa K, Inaguma Y, Emi N. BCOR as a novel fusion partner of retinoic acid receptor alpha in a t(X;17)(p11;q12) variant of acute promyelocytic leukemia. *Blood*. 2010;116(20):4274–83.
6. Wells RA, Catzavelos C, Kamel-Reid S. Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. *Nat Genet*. 1997;17(1):109–13.
7. Phillips SA, Barr VA, Haft DH, Taylor SI, Haft CR. Identification and characterization of SNX15, a novel sorting nexin involved in protein trafficking. *J Biol Chem*. 2001;276(7):5074–84.
8. Hanley SE, Cooper KF. Sorting nexins in protein homeostasis. *Cells*. 2020;10(1):17.
9. Madan V, Shyamsunder P, Han L, Mayakonda A, Nagata Y, Sundaresan J, et al. Comprehensive mutational analysis of primary and relapse acute promyelocytic leukemia. *Leukemia*. 2016;30(8):1672–81.
10. Callens C, Chevret S, Cayuela JM, Cassinat B, Raffoux E, de Botton S, et al. Prognostic implication of FLT3 and Ras gene mutations in patients with acute promyelocytic leukemia (APL): a retrospective study from the European APL Group. *Leukemia*. 2005;19(7):1153–60.
11. Scotti MM, Swanson MS. RNA mis-splicing in disease. *Nat Rev Genet*. 2016;17(1):19–32.
12. Sinclair J, McClain D, Taetle R. Effects of insulin and insulin-like growth factor I on growth of human leukemia cells in serum-free and protein-free medium. *Blood*. 1988;72(1):66–72.
13. Wahner Hendrickson AE, Haluska P, Schneider PA, Loegering DA, Peterson KL, Attar R, et al. Expression of insulin receptor isoform A and insulin-like growth factor-1 receptor in human acute myelogenous leukemia: effect of the dual-receptor inhibitor BMS-536924 in vitro. *Cancer Res*. 2009;69(19):7635–43.
14. Nepstad I, Hatfield KJ, Gronningsaeter IS, Aasebo E, Hernandez-Valladares M, Hagen KM, et al. Effects of insulin and pathway inhibitors on the PI3K-Akt-mTOR phosphorylation profile in acute myeloid leukemia cells. *Signal Transduct Target Ther*. 2019;4:20.
15. Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood*. 2001;97(8):2434–9.

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