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BRIEF COMMUNICATION

Remission clone in acute myeloid leukemia shows growth advantage after chemotherapy but is distinct from leukemic clone

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In a previously published case study of acute myeloid leukemia, we tracked the dynamics of somatic mutations over 9 years. Interestingly, we observed a group of mutations that expanded during remission, which we named the "remission clone." To determine the nature of the remission clones, we performed flow cytometry-based cell sorting followed by ultradeep sequencing. The remission clone repeatedly expanded after chemotherapeutic cycles and was suppressed during relapse in the myeloid lineage (multipotent hematopoietic stem, progenitor, and myeloid cells). On the other hand, the remission clone was consistently observed in lymphoid lineages (B and T cells) regardless of the disease state. When transfected into the HEK-293 cell line, the *NR2C2*(A93V) mutant exhibited a growth advantage (all p values < 0.05). The results indicate that the remission clone seems to be another form of clonal hematopoiesis, but without a clear association with leukemia. As the remission clone is present in both myeloid and lymphoid lineages, it likely originates from ancestral hematopoietic cell lineages. More importantly, the remission clone is distinct from the leukemic clone; therefore, mutations expanded during remission require special interpretation when performing next-generation sequencing-based measurable residual disease assessment.

Acute myeloid leukemia (AML) is a genetically and morphologically heterogenous disease [1,2]. In The Cancer Genome Atlas project, the average number of mutations per patient was 13, and 23 significant gene mutations were frequently found with a complex interplay of genetic mutations at diagnosis [3]. Many studies have investigated the functional roles of these mutations [4–7]. For example, *DNMT3A*-R882H is one of the most common mutations. Several studies have

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suggested that it likely arises in the preleukemic hematopoietic stem cell compartment; leads to inactivation of apoptosis, induction of stem cell expansion, and inhibition of differentiation; and has been shown to have reduced DNA methylation activity [5,6,8].

We previously reported on clonal dynamics in a single AML patient over a 9-year period [9]. Interestingly, we also observed a clone, which we called the "remission clone," that expanded after chemotherapy treatments during remission, but was suppressed by the leukemic clone and exhibited distinct dynamics compared with the leukemic clone [9]. Mutations following a pattern similar to that of the remission clone also have been reported by Wong et al. [10], who described expansion of mutations unrelated to the initial AML after induction chemotherapy while mutations associated with the founding clone were cleared. To identify the distribution in cell lineages and dynamics of the remission clone, we performed amplicon sequencing of each cell fraction by sorting bulk cells in a single AML patient. In addition, we also examined the growth potential of mutations in the remission clone using a cell viability assay.

Methods

A 36-year-old male patient was diagnosed with normal karyotype AML, designated as M5b by the French–American–British classification. Intensive induction chemotherapy was administered (two cycles of 3 days of idarubicin 12 mg/m^2/day and 7 days of cytarabine 100 mg/m^2/day) with granulocyte colony-stimulating factor priming) to achieve the first complete remission (1CR) [11]. This was followed by two courses of consolidation therapy (cytarabine 3 g/m^2 every 12 hours on days 1, 3, and 5). After 23 months, the patient relapsed (first relapse: 1R), and the second CR (2CR) was achieved after re-induction therapy and maintained for 31 months. After the second relapse (2R), the patient achieved the third CR (3CR) after induction chemotherapy. After 19 months, the patient relapsed (third relapse: 3R), and a fourth CR (4CR) was achieved. The fourth relapse (4R) occurred 20 months after 4CR, and he received three courses of re-induction therapy. However, the patient failed to achieve CR and died of active AML 9 months after 4R (Supplementary Table E1, online only, available at www.exphem.org).

We selected samples taken at eight representative time points (diagnosis [Dx]; 1CR–4CR; and 1R, 2R, and 4R; the sample collected at 3R was excluded from the analysis because of an insufficient number of sorted cells) that appropriately cover the clinical event [9]. Mononuclear cells from all cryopreserved bone marrow samples were sorted into each cell fraction using a BD FACS ARIA III (BD bioscience, California, USA) cell sorter. The leukemic blasts in this patient were immunophenotypically CD34 negative at diagnosis and until the third relapse (Supplementary Table E2, online only, available at www.exphem.org). Therefore, multipotent hematopoietic stem cells (CD34+/CD38−), myeloid progenitor cells (CD34+/CD38+), leukemic/myeloid cells (CD33+), B cells (CD19+), and T cells (CD3+) were isolated. We performed whole-exome sequencing on this patient tracked over 9 years in the previous study [9]. Overall, 14 mutations were targeted for amplicon sequencing (*DNMT3A, NDC80, RBM5, GAS2L3, NR2C2, SF3B2, IDH1, DNAH9, SLC34A2, KIF17, C1orf158, U2AF2, OR2T12*, and *NPM1*), which represent the preleukemic, leukemic, and remission clones described in the previous study [9]. The 48 samples from eight time points (six fractions per each bone marrow sample collected [bulk cells, multipotent hematopoietic stem cells, myeloid progenitor cells, leukemic/myeloid cells, B cells, and T cells)] were sequenced using an Ion Proton sequencer with a 2358 × mean amplicon on-target coverage (Supplementary Table E3 and Figure E1, online only, available at www.exphem.org).

We chose the mutations, identified the remission clone, and conducted the cell growth study to examine the viability of the mutations. Each mutation of NDC80(L43S), NR2C2(A93V), GAS2L3(S487Y), and RBM5(N417K) was generated by site-directed mutagenesis. After DNA transfection into human embryonic kidney (HEK)-293 cells, the relative cell viability of each group was compared with that of the mock group in triplicate. Details of the treatment method, mutation calling, and experimental methods are described in the Supplementary Material (online only, available at www.exphem.org).

Results and discussion

Previously we identified clusters based on the pattern of clonal dynamics of this AML patient over 9 years [9]. The first cluster represents a preleukemic clone (*DNMT3A* mutation (*DNMT3A*^{mut}]). It was detected at all time points with a higher allelic burden than other clusters. The second clone is a remission clone and includes mutations in *NDC80, RBM5, GAS2L3, NR2C2*, and *SF3B2*. Unlike the other clusters, this remission clone expanded during remission after the chemotherapy treatments and collapsed during relapse, exhibiting a pattern opposite that of the leukemic clone. The third clone is a leukemic clone. The representative variants in the leukemic clone were *IDH1, DNAH9, SLC34A2, KIF17, C1orf158, U2AF2, OR2T12*, and *NPM1*. The leukemic clone exhibited mutation dynamics opposite those of the remission clone, and expanded at diagnosis and relapsed. Overall, 14 mutations were subjected to amplicon sequencing; these were representative subsets (preleukemic, leukemic, and remission clones) of mutation clusters defined in our previous study [9].

As shown in Figure 1 and Supplementary Figures E2 and E3 (online only, available at www.exphem.org), we observed that the dynamics in the mutant burden of bulk cells, multipotent hematopoietic stem cells, myeloid progenitor cells, and myeloid cells had a similar pattern. Thus, myeloid cells were selected in Figure 1 on behalf of the above group. The dynamics of *DNMT3A*^{mut}, the leukemic clone, and the remission clone in each cell fraction are illustrated in Figure 1.

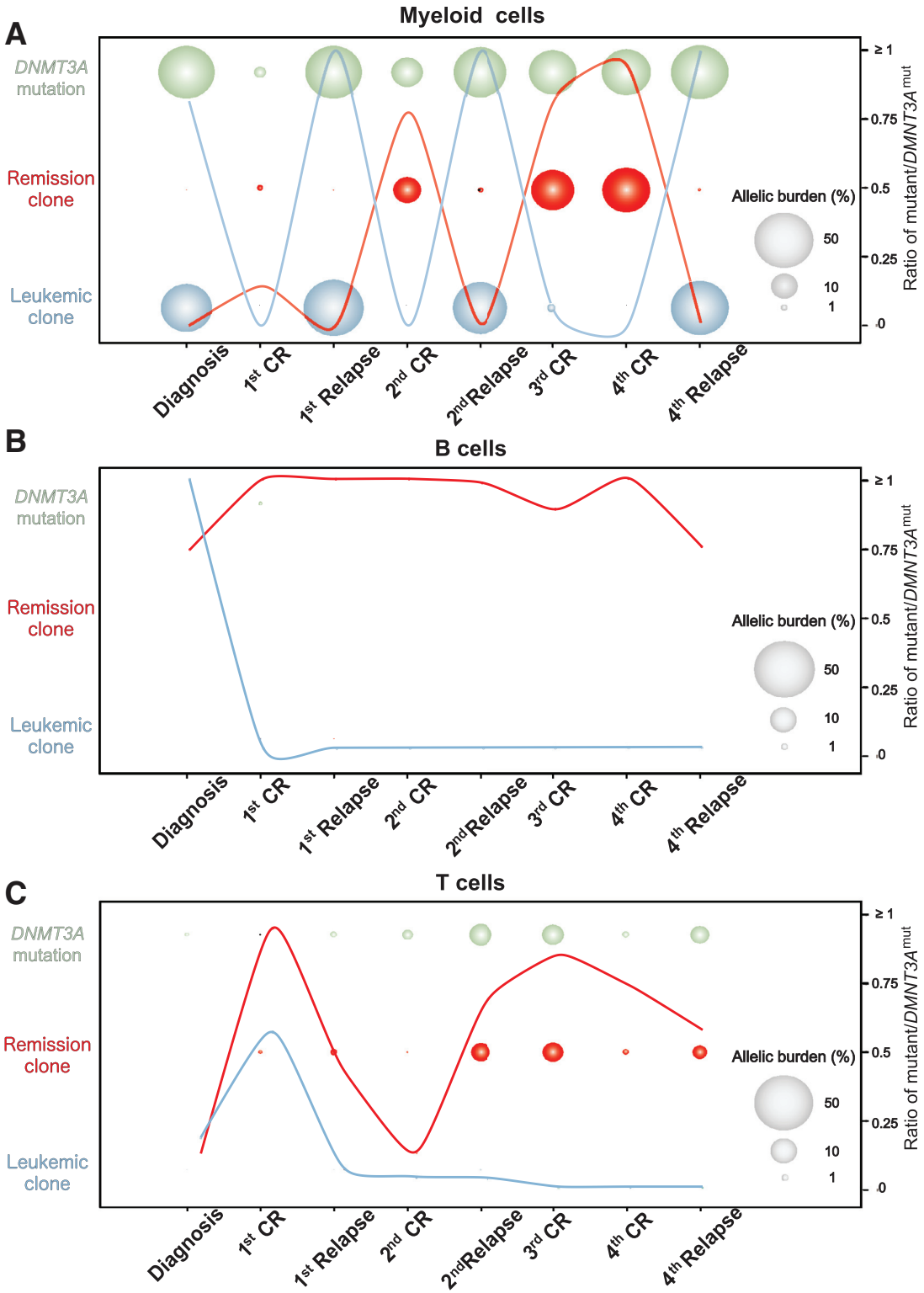

Figure 1. Clonal dynamics of somatic mutations in multiple cell lineages throughout the course of the disease. Bubble plot depicts allelic burdens of clonal mutations. Lines represent the ratios of the allelic burden of the remission clone (*blue line*) and the leukemic clone (*red line*) to that of the *DNMT3A* mutant (*DNMT3A*^{mut}) in each cell fraction during the clinical course. Bubble size represents the allelic burden of each clone. In our previous study on bulk cells, we identified somatic mutations using whole-exome sequencing and clustered identified mutations based on the pattern of clonal dynamics [9]. The first cluster represents a preleukemic clone (*DNMT3A* mutation [*DNMT3A*^{mut}]). The second clone is a remission clone (includes mutations in *NDC80, RBM5, GAS2L3, NR2C2*, and *SF3B2*). The third clone is a leukemic clone (includes mutations in *IDH1, DNAH9, SLC34A2, KIF17, C1orf158, U2AF2, OR2T12*, and *NPM1*). In myeloid cells **(A)**, the remission clone was observed

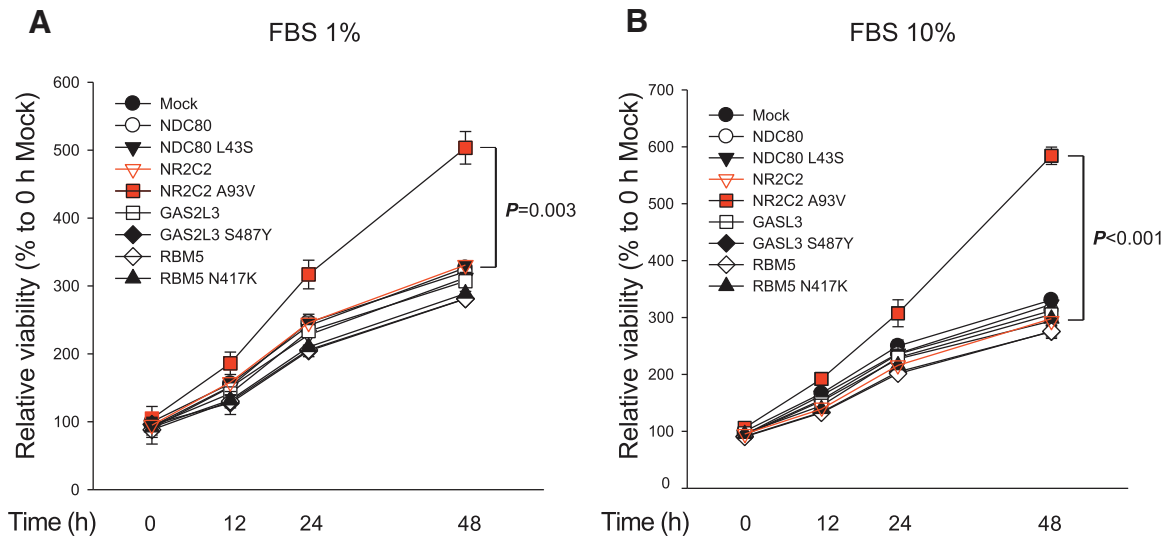

Figure 2. Growth potential of mutations in the remission clone determined using the cell viability assay. Each mutant of *NDC80*(L43S), *NR2C2* (A93V), *GAS2L3*(S487Y), and *RBM5*(N417K) was generated by site-directed mutagenesis. After DNA transfection into HEK-293 cells, the relative cell viability of each group was compared with that of the non-transfected group. After 48 hours of culture in 1% fetal bovine serum **(A)** and 10% fetal bovine serum **(B),** *NR2C2*(A93V) mutant-transfected HEK-293 cells (*red*) exhibited significantly increased viability (approximately 1.6-fold) compared with wild-type and other mutant-transfected cells (all p values < 0.05).

DNMT3A^{mut} was observed at all time points, except in the T-cell fraction of the CR1 sample. The allelic burden of *DNMT3A*^{mut} was significantly lower in the T-cell fractions than in other fractions (vs. myeloid, $p < 0.001$, vs. B cell, $p = 0.035$; Supplementary Figure E4, online only, available at www.exphem.org). *DNMT3A*^{mut} was observed in the nonmyeloid lineage with a low allelic burden, which is consistent with other studies in which *DNMT3A*^{mut} occurred in preleukemic hematopoietic stem cells as clonal hematopoiesis of indeterminate potential [12,13].

The remission clone was observed in myeloid cells at 1CR with very low allelic burden (0.12%), followed by further clonal expansion after chemotherapeutic cycles. The allelic burden of the remission clone in myeloid cells was increased to 6.11%–43.44%. However, the allelic burden was reduced to 0%–0.64% at the time of each leukemic relapse. Opposing ratios of remission clone/*DNMT3A*^{mut} and leukemic clone/*DNMT3A*^{mut} were observed in myeloid cells (Figure 1A). The remission clone expanded after chemotherapy in myeloid cell fractions, but decreased when the leukemic clone expanded. However, the dynamics of the remission clone in the lymphoid lineage were distinct from the pattern observed in the bulk cells [9]. Leukemic clones were rarely observed (maximum: 0.54%) in B- and T-cell fractions, but remission clones were continuously detected at an allelic burden ration similar to that of *DNMT3A*^{mut} (Figure 1B, C). These data suggest that the remission clone is not associated with active leukemic status. The remission clone was observed to expand after chemotherapy and collapsed at AML relapses in the myeloid cell fraction, whereas it was stable in the lymphoid lineage regardless of disease status. This characteristic of the remission clone differs from the general concepts of mutations in AML. Our results suggested that the remission clone has a growth advantage over normal hematopoietic cells. To test this hypothesis, we conducted a cell growth study to examine the viability of each mutant observed in the remission clone. Each mutant of *NDC80, NR2C2, GAS2L3*, and *RBM5* was generated by site-directed mutagenesis and transfected into HEK293 cells. After 48 hours of culture, the *NR2C2*(A93V) mutant-transfected HEK 293 cells showed significantly increased viability, approximately 1.6-fold compared with wild-type and other mutant-transfected cells (all p values < 0.05; Figure 2). Mutations in *NR2C2* were observed in other tumors at a frequency of 1%–3%, but *NR2C2*(A93V) has not been reported in AML [14]. Based on the results of the cell growth study, it is possible that *NR2C2*(A93V) expression contributes to the growth advantage of the remission clone. Investigation of this possibility will require long-term growth assays using hematopoietic cells. We propose that the other mutations

to expand further after chemotherapy cycles, and its allelic burdens were significantly reduced at every leukemic relapse. The remission clone/*DNMT3A*^{mut} and leukemic clone/*DNMT3A*^{mut} ratios exhibit opposite patterns. In the lymphoid lineage **(B, C),** leukemic clones were rarely observed (maximum = 0.54%) in the B-cell and T-cell fractions, but remission clones were continuously observed at almost the same allelic burden as *DNMT3A*^{mut} (blue lines in **B** and **C**).

identified in the remission clone are most likely passenger mutations.

Several recent studies have suggested that genetic mutations that remain after chemotherapy can be used to detect measurable residual disease (MRD) in AML [15–19]. However, our data and analyses revealed that MRD monitoring using mutations following a pattern to that of the remission clone may result in false MRD positives, and MRD assessment should be limited to mutations detected in leukemia that show significant reduction during remission. Wong and colleagues reported similar observations in 5 of 15 cases, which indicates that the presence of a remission clone may not be uncommon [10].

Overall, our study indicates recurrent expansion of the remission clone with lineage-specific dynamics in serial samples collected at multiple remissions and relapses. Whole-exome/genome sequencing of remission samples in a larger AML cohort is required to confirm the repertoire and clinical relevance of remission clones.

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Conflict of interest disclosure

The authors declare no competing financial interests.

Author contributions

JSA, THK, YKK, and HJK designed the study. JSA, YKK, SHJ, SYA, SYJ, DHY, JJL, SHC, JYL, and MGS collected samples and performed the experiments. SYC and YCC performed the cell growth study. THK, KY, SO, and ZZ analyzed the sequencing data. JSA, THK, YKK, ICK, and DDHK interpreted the data and performed statistical analyses. JSA, THK, YKK, HJK, and DDHK wrote the article.

References

1. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506:328–333.
2. Grimwade D, Ivey A, Huntly BJP. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. Blood. 2016;127:29–41.
3. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368:2059–2074.
4. Medinger M, Passweg JR. Acute myeloid leukaemia genomics. Br J Haematol. 2017;179:530–542.
5. Yang L, Rau R, Goodell MA. DNMT3A in haematological malignancies. Nat Rev Cancer. 2015;15:152–165.
6. Yan XJ, Xu J, Gu ZH, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011;43:309–315.
7. Naoe T, Kiyoi H. Gene mutations of acute myeloid leukemia in the genome era. Int J Hematol. 2013;97:165–174.
8. Bera R, Chiu MC, Huang YJ, Liang DC, Lee YS, Shih LY. Genetic and epigenetic perturbations by DNMT3A-R882 mutants impaired apoptosis through augmentation of PRDX2 in myeloid leukemia cells. Neoplasia. 2018;20:1106–1120.
9. Kim T, Yoshida K, Kim YK, et al. Clonal dynamics in a single AML case tracked for 9 years reveals the complexity of leukemia progression. Leukemia. 2015;30:295–302.
10. Wong TN, Miller CA, Klco JM, et al. Rapid expansion of preexisting nonleukemic hematopoietic clones frequently follows induction therapy for de novo AML. Blood. 2016;127:893–897.
11. Löwenberg B, van Putten W, Theobald M, et al. Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. N Engl J Med. 2003;349:743–752.
12. Buscarlet M, Provost S, Zada YF, et al. Lineage restriction analyses in CHIP indicate myeloid bias for TET2 and multipotent stem cell origin for DNMT3A. Blood. 2018;132:277–280.
13. Arends CM, Galan-Sousa J, Hoyer K, et al. Hematopoietic lineage distribution and evolutionary dynamics of clonal hematopoiesis. Leukemia. 2018;32:1908–1919.
14. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: Somatic cancer genetics at high-resolution. Nucleic Acids Res. 2017;45:D777–D783.
15. Morita K, Kantarjian HM, Wang F, et al. Clearance of somatic mutations at remission and the risk of relapse in acute myeloid leukemia. J Clin Oncol. 2018;36:1788–1797.
16. Klco JM, Miller CA, Griffith M, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. JAMA. 2015;314:811–822.
17. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med. 2018;378:1189–1199.
18. Christen F, Hoyer K, Yoshida K, et al. Genomic landscape and clonal evolution of acute myeloid leukemia with t(8;21): An international study on 331 patients. Blood. 2019;133:1140–1151.
19. Rothenberg-Thurley M, Amler S, Goerlich D, et al. Persistence of pre-leukemic clones during first remission and risk of relapse in acute myeloid leukemia. Leukemia. 2018;32:1598–1608.