Dear Editor,

Myeloid sarcoma (MS) is a rare myeloid neoplasm characterized by myeloblast proliferation outside the bone marrow (BM) [1]. The mutation profiles found in MS are generally consistent during paired BM analysis, whereas additional variants are detected in cases of MS following extramedullary relapse after AML development [2-5]. We report a comparison of sequencing data of BM and MS tissues in a patient with MS that relapsed in the form of AML after treatment with decitabine. The bone marrow specimens for this study were provided by the Chungbuk National University Hospital, a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare and Family Affairs. The samples derived from the National Biobank of Korea were obtained with informed consent, and consent for retrospective analysis of clinical and genetic information was waived under institutional review board-approved protocols. The study was approved by the institutional review board of the Chungbuk National University Hospital, Cheongju, Korea (CBNU-2018-05-006-001).

In April 2019, a 65-year-old woman presented to the Chungbuk National University Hospital with a large paraaortic mass lesion extending from the subcarina to the renal artery origin site (Fig. 1A, B). A biopsy revealed several malignant hematopoietic cells that were positive for CD34, CD117, and myeloperoxidase (Fig. 1C, D). No pathological findings were observed in her peripheral blood and BM cells, and karyotyping was normal. She was administered 20 mg/m² decitabine intravenously for five days every four weeks for isolated MS. She achieved partial response; however, multiple masses reappeared in the paraaortic area and pleural space after approximately 11 months. Tests of her peripheral blood and BM cells still yielded normal results. She attained a complete response following intensive chemotherapy with fludarabine, cytarabine, and idarubicin; however, the masses progressed again after six months, and BM examination revealed a 59.5% increase in myeloblasts. Flow cytometry findings revealed that the myeloblasts were positive for myeloperoxidase, CD13, CD33, and CD117. The result of chromosome analysis was complex karyotype: 45,X,-X,add(4)(q25),t(8;21)(q22;q22), del(9)(q13q22),add(22)(q11.2)[14]/45,idem,add(15)(p11.2)[3]/46,XX[3]. Repeated intensive chemotherapy provided only...
short-term improvement, and the patient died of disease exacerbation 24 months after her diagnosis.

We performed targeted next-generation sequencing of MS tissue, BM aspiration samples obtained at initial diagnosis, MS tissue obtained at the time of recurrence after decitabine treatment, and BM aspiration samples obtained following the increase in blasts in the BM, using MiSeqDx and NextSeq 550Dx platforms (Illumina, San Diego, CA, USA). The ASXL1 c.2088_2089insGC and KIT c.1328G>A variants were present in the MS sample obtained at initial diagnosis but not in the BM samples. The ASXL1 c.2088_2089insGC variant persisted in the recurrent MS tissue following decitabine treatment, whereas the KIT c.1328G>A variant disappeared. Instead, an additional TET2 c.4044+1G>A splice variant appeared. The KIT variant was not observed in BM cells obtained during leukemia; however, the ASXL1 c.2088_2089insGC and TET2 c.4044+1G>A splice variants were both detected. A DNMT3A c.1903C>T missense variant was observed in the BM at the time of diagnosis and after the onset of AML, but not in the MS sample (Table 1).

The mutation rates of genes related to the receptor tyrosine kinase (RTK)-RAS pathway, including NRAS, KRAS, and KIT, are higher in MS than in conventional AML [2, 6]. The researchers who discovered this suggested that the unique molecular pattern of MS, including alterations in the RTK-RAS pathway, may be involved in the mechanism underlying the migration of myeloid blasts to extramedullary organs. In the present case, the KIT c.1328G>A variant was found only in MS tissue, not in BM leukemia cells, which were eradicated by decitabine administration. When the patient relapsed after being administered decitabine, leukemic cells without KIT variants proliferated in both the BM and extramedullary tissues. We suggest that KIT variants may play a role in the extramedullary migration of leukemic cells; however, this is not considered essential for intramedullary leukemogenesis. The development of treatment resistance and disease progression may be due to mechanisms other than that involving the RTK-RAS pathway.

Molecular response predictors for hypomethylating agents such as decitabine have yet to be elucidated. Despite the limited treatment experience due to the very small number of patients, the findings of several studies have demonstrated that decitabine effectively treats MS [7, 8]. Although the prognostic role of TET2 variants remains controversial, some researchers...
Table 1. Pathological findings and genetic variants identified in MS and BM tissues at the time of diagnosis, relapsed MS tissue, and subsequent AML tissue.

| Genetic variants | BM | MS | Diagnosis (Isolated MS) | Interpretation | Initial diagnosis (Isolated MS) | Biopsy site | Immunophenotyping | chromosome analysis | Pathological findings |
|------------------|----|----|-------------------------|----------------|--------------------------------|-------------|-------------------|-------------------|-----------------------|
| KIT              |    |    |                         |                 |                                | MS          |                   |                   |                       |
| c.1326G>A        |    |    |                         |                 |                                | BM          |                   |                   |                       |
| (p.Cys443Tyr)    |    |    |                         |                 |                                |             |                   |                   |                       |
| ASXL1            |    |    |                         |                 |                                | MS          |                   |                   | Not detected          |
| c.2088_2089insGC |    |    |                         |                 |                                | MS          |                   |                   | Detected               |
| (p.Leu697Alafs*7) |    |    |                         |                 |                                | BM          |                   |                   | Not detected          |
| TET2             | c.4044+1G>A |    |                         |                 |                                | MS          |                   |                   | Not analyzed          |
| DNMT3A           |    |    |                         |                 |                                | MS          |                   |                   | Detected               |
| c.1903C>T        |    |    |                         |                 |                                | BM          |                   |                   | Detected               |
| (p.Arg635Trp)    |    |    |                         |                 |                                |             |                   |                   | Not detected          |

Reference sequences: NM_000222.2(KIT); NM_015338.5(ASXL1); NM_001127208.2(TET2); NM_022552.4(DNMT3A).

Abbreviations: MS, myeloid sarcoma; BM, bone marrow; AML, acute myeloid leukemia; RTK, receptor tyrosine kinase; iHC, immunohistochemistry; FCM, flow cytometry; VAF, variant allele frequency.

have reported that TET2 mutation status suggests responsiveness to hypomethylating agents [9, 10]. In the present case, despite the short response duration, decitabine caused a significant reduction in MS tumor size. The TET2 c.4044+1G>A splice variant newly appeared in MS tissue that recurred after decitabine treatment. The generation of an additional splice variant following decitabine administration possibly contributed to the development of decitabine resistance.

The current study provides information on the roles of the RTK-RAS pathway and particularly, KIT, in MS pathogenesis. The therapeutic effects of decitabine in MS and the development of resistance associated with TET2 variants were also described.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Park HS and Kwon JH conceived and designed the study and wrote the paper. Son SM performed bone marrow pathology and interpreted the findings.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

RESEARCH FUNDING

This study was supported by a research grant from Chungbuk National University in 2019 and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number 2020R1G1A1004157).

ORCID

Hee Sue Park https://orcid.org/0000-0002-8378-6066
Seung Myoung Son https://orcid.org/0000-0002-1646-4649
Jihyun Kwon https://orcid.org/0000-0001-8128-3310

REFERENCES

1. Avni B and Koren-Michowitz M. Myeloid sarcoma: current approach and therapeutic options. Ther Adv Hematol 2011;2:309-16.
2. Li Z, Stölzel F, Onel K, Sukhanova M, Mirza MK, Yap KL, et al. Next-generation sequencing reveals clinically actionable molecular markers in myeloid sarcoma. Leukemia 2015;29:2113-6.
3. Greenland NY, Van Ziffle JA, Liu YC, Qi Z, Prakash S, Wang L. Genomic analysis in myeloid sarcoma and comparison with paired acute myeloid leukemia. Hum Pathol 2021;108:76-83.
4. Kashofer K, Gornicec M, Lind K, Caraffini V, Schauer S, Beham-Schmid C, et al. Detection of prognostically relevant mutations and translocations in myeloid sarcoma by next generation sequencing. Leuk Lymphoma 2018;59:501-4.
5. Werstein B, Dunlap J, Cascio MJ, Ohgami RS, Fan G, Press R, et al. Molecular discordance between myeloid sarcomas and concurrent bone marrows occurs in actionable genes and is associated with worse overall survival. J Mol Diagn 2020;22:338-45.
6. Choi M, Jeon YK, Sun CH, Yun HS, Hong J, Shin DY, et al. RTK-RAS pathway mutation is enriched in myeloid sarcoma. Blood Cancer J 2018;8:43.
7. Gornicec M, Wöfler A, Stanzel S, Sill H, Zebisch A. Evidence for a role of decitabine in the treatment of myeloid sarcoma. Ann Hematol 2017;96:505-6.
8. Evers D, Bär BMAM, Gotthardt M, van der Velden WJFM. Activity of decitabine in pericardial myeloid sarcoma. Int J Hematol 2018;108:121-2.
9. Itzykson R, Kosmider O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Rauzy O, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 2011;25:1147-52.
10. Bejar R, Lord A, Stevenson K, Bar-Natan M, Pérez-Ladaga A, Zaneveld J, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 2014;124:2705-12.