Letter to the Editor
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Serial Analysis and Comparison of Mutation Profiles in Decitabine-treated Myeloid Sarcoma and Subsequent Acute Myeloid Leukemia Using Next-Generation Sequencing

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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/m^2 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

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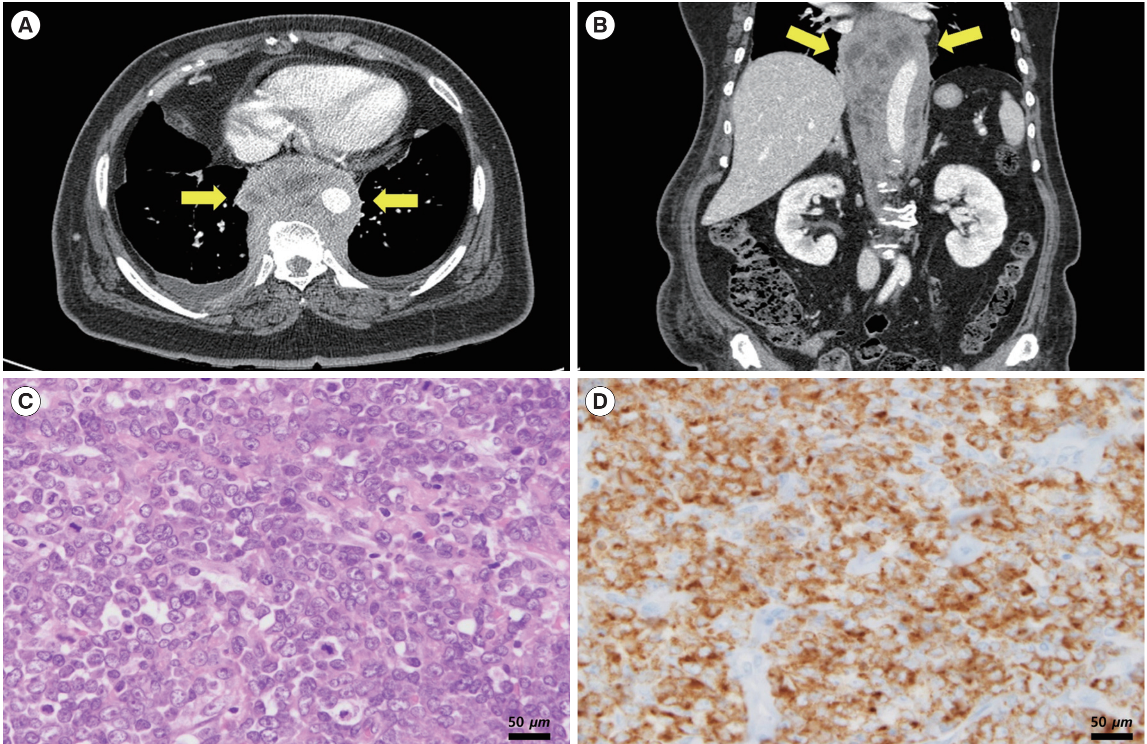

Fig. 1. Computed tomography and pathologic findings of the MS mass. (A, B) Computed tomography reveals the extent of soft tissue lesion surrounding the paraaortic and retrocrural spaces. (C) Pathological findings of the MS tissue (hematoxylin and eosin staining). (D) Immunostaining for myeloperoxidase (original magnification, ×200; scale bar, 50 μm).
Abbreviation: MS, myeloid sarcoma.

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

Diagnosis	Biopsy site	Interpretation	Immunophenotyping	Chromosome analysis	Genetic variants			
					KIT c.1328G>A (p.Cys443Tyr) (Tier1)	*ASXL1* c.2088_2089insGC (p.Leu697Alafs*7) (Tier1)	*TET2* c.4044+1G>A (Tier 1)	*DNMT3A* c.1903C>T (p.Arg635Trp) (Tier1)
Initial diagnosis (Isolated MS)	MS	MS	IHC stain: MPO$^+$, Pan-CK$^-$, CD34$^+$, CD117$^+$, CD56$^+$, CD43$^+$, TdT$^-$	Not analyzed	Detected (VAF 31%)	Detected (VAF 21%)	Not detected	Not detected
	BM	Normocellular BM without involvement of malignant cells	Not tested	46,XX[20]	Not detected	Not detected	Not detected	Detected (VAF 4%)
Isolated MS, relapse	MS	MS, recurrent	IHC stain: MPO$^+$, LCA$^-$, CD117$^+$, CD99$^-$	Not analyzed	Not detected	Detected (VAF 26%)	Detected (46%)	Not detected
AML	BM	AML with myelodysplasia-related change	FCM: MPO$^+$, CD13$^+$, CD19$^+$, CD33$^+$, CD34$^+$, CD56$^+$, CD117$^+$, HLA-DR$^+$	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]	Not detected	Detected (VAF 20%)	Detected (20%)	Detected (VAF 7%)

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; IHC, immunohistochemistry; MPO, myeloperoxidase; 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.

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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.

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