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TP53 Mutation Status in Myelodysplastic Neoplasm and Acute Myeloid Leukemia: Impact of Reclassification Based on the 5th WHO and International Consensus Classification Criteria: A Korean Multicenter Study

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Background: *TP53* mutations are associated with poor prognosis in myelodysplastic neoplasm (MDS) and AML. The updated 5th WHO classification and International Consensus Classification (ICC) categorize *TP53*-mutated MDS and AML as unique entities. We conducted a multicenter study in Korea to investigate the characteristics of *TP53*-mutated MDS and AML, focusing on diagnostic aspects based on updated classifications.

Methods: This study included patients aged ≥18 yrs who were diagnosed as having MDS (N=1,244) or AML (N=2,115) at six institutions. The results of bone marrow examination, cytogenetic studies, and targeted next-generation sequencing, including *TP53*, were collected and analyzed.

Results: *TP53* mutations were detected in 9.3% and 9.2% of patients with MDS and AML, respectively. Missense mutation was the most common, with hotspot codons R248/R273/G245/Y220/R175/C238 accounting for 25.4% of *TP53* mutations. Ten percent of patients had multiple *TP53* mutations, and 78.4% had a complex karyotype. The median variant allele frequency (VAF) of *TP53* mutations was 41.5%, with a notable difference according to the presence of a complex karyotype. According to the 5th WHO classification and ICC, the multi-hit *TP53* mutation criteria were met in 58.6% and 75% of MDS patients, respectively, and the primary determinants were a *TP53* VAF >50% for the 5th WHO classification and the presence of a complex karyotype for the ICC.

Conclusions: Collectively, we elucidated the molecular genetic characteristics of patients with *TP53*-mutated MDS and AML, highlighting key factors in applying *TP53* mutation-related criteria in updated classifications, which will aid in establishing diagnostic strategies.

Key Words: Acute myeloid leukemia, *TP53* mutation, Myelodysplastic syndromes, International Consensus Classification, World Health Organization

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INTRODUCTION

The *TP53* gene located on chromosome 17p13.1 encodes the tumor suppressor protein p53, which consists of 393 amino acids [1]. Wild-type p53 primarily acts as a stress-activated transcription factor that regulates the transcription of various target genes, thereby playing pivotal roles in numerous vital biological processes and mediating tumor suppression [2]. In contrast, mutated p53 promotes tumor-cell invasion, migration, and development through loss of function, a dominant negative effect, or potential gain of function [3]. Notably, the biological potency of different *TP53* mutations is variable, with recurrent alleles exhibiting potent oncogenic effects [3].

TP53 is the most commonly mutated gene in human cancer; it is mutated or deleted in approximately 50% of all cancers, and mutant p53 is strongly associated with advanced stages and poor prognosis [4]. Particularly in myelodysplastic neoplasm (MDS) and AML, *TP53* mutations lead to a dismal prognosis, with a median overall survival of 5–10 months [5]. Therefore, the updated 5th WHO classification of hematolymphoid tumors and International Consensus Classification (ICC) of myeloid neoplasms and acute leukemias have categorized *TP53*-mutated MDS and AML as unique entities [6, 7]. Notably, in MDS, multi-hit *TP53* mutations are considered clinically significant; however, the criteria for defining multi-hit *TP53* mutations differ between the 5th WHO classification and ICC.

Recently, various novel therapeutic strategies have been tested in patients with *TP53*-mutated MDS or AML [4, 5, 8], making accurate classification of this patient group increasingly important. Emerging data on the impact of cytogenetic abnormalities, *TP53* mutation burden, and the immunobiology of *TP53*-mutated MDS and AML are highlighting the complexity of these diseases [5]. We conducted a multicenter study in Korea to investigate the characteristics of *TP53* mutations in patients with MDS or AML, with a focus on their diagnostic aspects, based on the 5th WHO classification and ICC.

MATERIALS AND METHODS

Study population

This study included patients aged ≥18 yrs diagnosed as having MDS or AML according to the 2016 WHO classification for myeloid neoplasms [9] between January 2017 and December 2022 at six institutions. The diagnostic criteria and definitions of multiple-hit *TP53* mutations according to the 5th WHO classification and ICC are summarized in Supplemental Data Table S1 [6, 7]. The study was approved by the Institutional Review Board of each institution, and the requirement for informed consent was waived because this study involved a retrospective analysis using anonymized data.

Data collection

Patient age, sex, and various laboratory data, including the results of bone marrow examination, chromosome analysis, *TP53* fluorescence *in-situ* hybridization (FISH), and targeted next-generation sequencing (including *TP53* and ICC-defined myelodysplasia-related (MR) genes *ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *STAG2*, *SRSF2*, *RUNX1*, *U2AF1*, and *ZRSR2* [7]), were collected and analyzed in 1,244 patients with MDS and 2,115 patients with AML. For 459 patients, MDS or AML subtype information was not available. The frequency, spectrum, and variant allele frequency (VAF) of *TP53* mutations were analyzed. 17p loss was defined as whole chromosome 17 loss, whole 17p loss, or partial 17p loss, including *TP53*, based on the chromosome analysis and FISH results.

Statistical analysis

The primary study objective was to evaluate the *TP53* mutation status in Korean patients with MDS or AML. In subgroup analysis, categorical variables were compared using Pearson's chi-squared test or Fisher's exact test, as appropriate, and continuous variables were compared using a paired *t*-test or Wilcoxon's signed-rank test, as appropriate. All statistical analyses were performed using IBM SPSS Statistics, version 27 (IBM, Armonk, NY, USA). $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Patient characteristics

TP53 mutations were detected in 9.3% (N = 116) of patients with MDS and 9.2% (N = 194) of patients with AML, respectively ($P = 0.883$) (Table 1). Patients with MDS or AML harboring *TP53* mutations were older than those without mutations ($P < 0.001$). Although information regarding prior therapy was available for only some patients, *TP53* mutations were detected in 29.0% and 36.8% of patients with therapy-related MDS and AML, respectively. The frequency of MDS with excess blasts (MDS-EB) was higher in patients with *TP53* mutations than in those without (56.9% vs. 36.4%, $P < 0.001$). Notably, one-third of patients with MDS with del(5q) harbored *TP53* mutations. Two-thirds of patients with AML with *TP53* mutations were diagnosed as hav-

Table 1. Characteristics of patients according to their *TP53* mutation status

Characteristics	MDS			AML		
	Non-mutated *TP53* (N = 1,128)	Mutated *TP53* (N = 116)	*P*	Non-mutated *TP53* (N = 1,921)	Mutated *TP53* (N = 194)	*P*
Age, yrs, median (range)	61 (18–98)	65 (27–92)	< 0.001	60 (18–94)	65 (22–90)	< 0.001
Sex, N (%)						
Male	709 (62.9%)	71 (61.2%)	0.727	1059 (55.1%)	113 (58.2%)	0.405
Female	419 (37.1%)	45 (38.8%)		862 (44.9%)	81 (41.8%)	
Therapy-related, N (%)	22 (2.0%)	9 (7.8%)	0.001	43 (2.2%)	25 (12.9%)	< 0.001
MDS subtype*, N (%)						
MDS-Non-EB	717 (63.6%)	50 (43.1%)	< 0.001			
MDS-del(5q)	10 (0.9%)	5 (4.3%)				
MDS-RS	59 (5.2%)	7 (6.0%)				
MDS-SLD	97 (8.6%)	3 (2.6%)				
MDS-MLD	320 (28.4%)	23 (19.8%)				
MDS-U	41 (3.6%)	6 (5.2%)				
MDS[†]	190 (16.8%)	6 (5.2%)				
MDS-EB1	232 (20.6%)	37 (31.9%)				
MDS-EB2	179 (15.9%)	29 (25%)				
AML subtype*, N (%)						
AML-recurrent genetic abnormalities				864 (45.0%)	9 (4.6%)	< 0.001
AML-*RUNX1*::*RUNX1T1*				149 (7.8%)	1 (0.5%)	
AML-*CBFB*::*MYH11*				101 (5.3%)	0	
AML-*PML*::*RARA*				168 (8.7%)	1 (0.5%)	
AML-*MLLT3*::*KMT2A*				28 (1.5%)	3 (1.5%)	
AML-*DEK*::*NUP214*				9 (0.5%)	0	
AML-*GATA2*, *MECOM*				14 (0.7%)	3 (1.5%)	
AML-*BCR*::*ABL1*				16 (0.8%)	0	
AML-mutated *NPM1*				252 (13.1%)	1 (0.5%)	
AML-biallelic *CEBPA* mutations				79 (4.1%)	0	
AML-mutated *RUNX1*				48 (2.5%)	0	
AML-MRC				363 (18.9%)	129 (66.5%)	
AML-NOS				447 (23.3%)	40 (20.6%)	
AML[‡]				247 (12.9%)	16 (8.2%)	
17p loss, N (%)	28/1100 (2.5%)	32/115 (27.8%)	< 0.001	60/1871 (3.2%)	75/188 (39.9%)	< 0.001
Cytogenetics, N (%)						
NK	549 (52.4%)	9 (8.0%)	< 0.001	730 (39.7%)	15 (8.0%)	< 0.001
CK	115 (11.0%)	87 (77.0%)		259 (14.1%)	149 (79.3%)	
Others[§]	384 (36.6%)	17 (15.0%)		849 (46.2%)	24 (12.8%)	

*MDS and AML subtypes according to the 2016 WHO classification.
[†]MDS subtype information was not provided, but there was no increase in blasts.
[‡]AML subtype information was not provided.
[§]These include cytogenetic abnormalities that are neither normal nor complex karyotype (defined as having three or more abnormalities).
Abbreviations: MDS, myelodysplastic neoplasm; EB, excess blasts; RS, ring sideroblasts; SLD, single-lineage dysplasia; MLD, multilineage dysplasia; U, unclassifiable; MRC, myelodysplasia-related changes; NOS, not otherwise specified; NK, normal karyotype; CK, complex karyotype.

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ing AML with MR changes (AML-MRC), which was significantly higher than the rate in patients with AML without *TP53* mutations (66.5% vs. 18.9%, $P<0.001$). Notably, *TP53* mutations were observed in only 1% (9/873) of patients with AML with recurrent genetic abnormalities, whereas the frequency of *TP53* mutations in patients with AML, not otherwise specified (AML-NOS) was relatively high (8.2%, 40/487). A complex karyotype was observed at substantially higher frequencies in patients with *TP53* mutations in both MDS and AML (77% and 79.3%, respectively) than in those without *TP53* mutations (11.0% and 14.1%, respectively; all $P<0.001$).

Characteristics of p53 mutations in MDS and AML

We identified 346 *TP53* mutations in total in 310 patients, 188 of which were unique (Fig. 1A and Supplemental Data Table S2). Further, 89.3% of the p53 mutations were located within the DNA-binding domain (codons 94–292) [10]. Missense mutations were predominant, accounting for 76.9% of all mutations, followed by frameshift (7.8%), canonical splice-site (7.5%), and nonsense (6.9%) mutations. Within the DNA-binding domain, missense mutations were predominant (82.2%), whereas outside the DNA-binding domain, frameshift, nonsense, and splice-site mutations were more common (67.6%). The distribution of mutation types did not significantly differ between MDS and AML ($P=0.163$) (Fig. 1B). Interestingly, C>T was the most frequent substitution, accounting for 38.2% of all mutations, with those occurring at CpG sites constituting 18.2%. The most commonly mutated codon was R248 (7.5%), followed by R273 (4.3%), G245 (4%), Y220 (3.8%), R175 (2.9%), C238 (2.9%), V272 (2.6%), G244 (2%), M237 (2%), I195 (1.7%), C176 (1.7%), and K132 (1.7%). R248Q (5.8%) was the most common *TP53* mutation, followed by R175H (2.9%), Y220C (2.9%), V272M (2.6%), R273H (2.6%), C238Y (2.3%), G245D (2%), and M237I (1.7%).

The median VAF of *TP53* mutations was 41.5% (interquartile range, 20.5%–69.4%), with no significant difference between MDS and AML ($P=0.958$) (Fig. 1C). The VAF did not differ according to the *TP53* mutation type ($P=0.085$). The VAF of *TP53* mutations was higher in patients with 17p loss and a complex karyotype than in those without these characteristics (48% vs. 39%, $P=0.022$; 46% vs. 24%, $P<0.001$, respectively), with a particularly significant difference observed for the presence of a complex karyotype. Notably, when the VAF of *TP53* mutations exceeded 50%, 17p loss, and a complex karyotype were observed at high rates of 43% and 90%, respectively (Fig. 1D). Among patients with a single *TP53* mutation, 74.9% (209/279) had a complex karyotype, of whom 45.5% (95/209) had concurrent 17p loss (Fig. 1E). Only 1.4% (4/279) of patients had 17p loss without a complex karyotype.

Reclassification of *TP53*–mutated MDS and AML according to the 5th WHO classification and ICC criteria

First, we reclassified the patients with *TP53* mutations into MDS (MDS-WHO) according to the 5th WHO classification and into MDS (MDS-ICC), MDS/AML, and AML according to the ICC and compared their molecular and cytogenetic characteristics (Table 2). 17p loss was identified in 39.9% of patients with AML, which was significantly higher than the 27.8% observed in MDS-WHO ($P=0.033$). According to the ICC, the frequency of 17p loss was significantly higher in patients with MDS/AML than in those with MDS-ICC (50% vs. 20.7%, $P=0.003$), but there was no difference between MDS/AML and AML ($P=0.393$). Among all patients with *TP53* mutations, 90% (279/310) had a single mutation, and 78.4% (236/301) had a complex karyotype, with no significant differences among disease categories. Mutations in MR genes were found in 19.4% (60/310) of these patients, with *ASXL1* and *RUNX1* mutations being the most frequent, and no differences among disease categories were observed.

Next, each *TP53* mutation-related multi-hit criterion based on the 5th WHO classification, and ICC was applied to the patients with MDS, MDS/AML, and AML (Table 3 and Supplemental Data Fig. S1). According to the 5th WHO classification, 58.6% of these patients were diagnosed as having MDS with biallelic *TP53* inactivation (MDS-TP53m-WHO), mostly based on the criterion of *TP53* mutations with VAF >50%. In contrast, the ICC classified 75.0% (66/68) of these cases as MDS with mutated *TP53* (MDS-TP53m-ICC), 96.4% (27/28) as MDS/AML with mutated *TP53* (MDS/AML-TP53m), and 89.7% (174/194) as AML with mutated *TP53* (AML-TP53m). Notably, among patients with <10% blasts, ~20% more patients were diagnosed as having MDS-TP53m-ICC than as having MDS-TP53m-WHO (75% vs. 55.7%, $P=0.007$) because the complex karyotype-related multi-hit *TP53* criterion is included only in the ICC. Among patients with 10%–20% blasts, 67.9% of cases were classified as MDS-TP53m-WHO based on multi-hit *TP53* criteria, whereas 96.4% were classified as MDS/AML-TP53m based on the criterion of any *TP53* mutation with a VAF >10%. AML-TP53m is a unique category in the ICC, whereas in the 5th WHO classification, as in the case of the 2016 WHO classification [9], a significant number of patients in this category are expected to be classified as having AML, MR because of a complex karyotype. Fig. 2 presents a mutation heat map depicting the karyotypes, 17p loss,

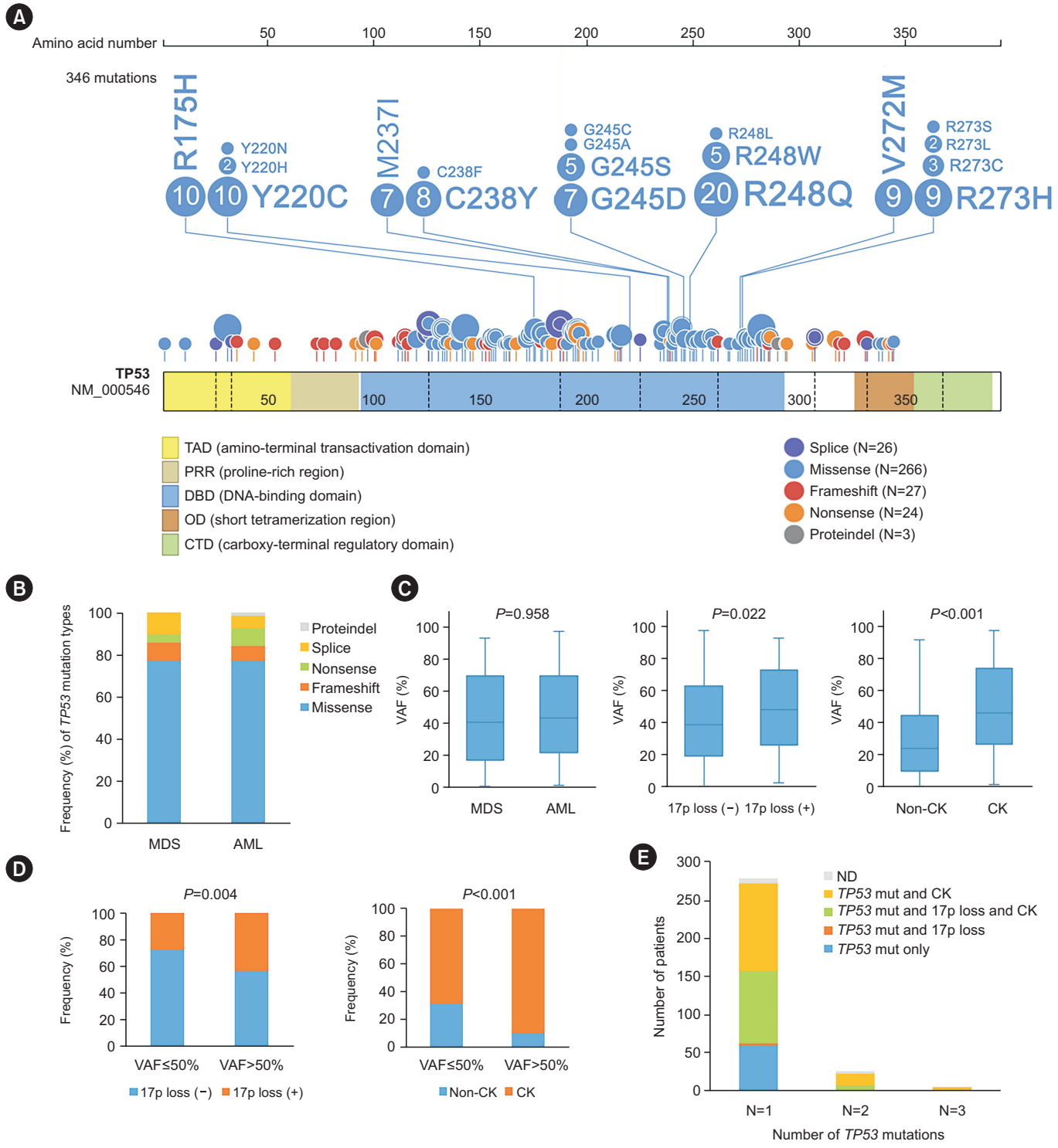

Fig. 1. Characteristics of *TP53* mutations in patients with MDS or AML. (A) Spectrum of *TP53* mutations (numbers in circular discs are the numbers of *TP53* mutations identified). (B) Types of *TP53* mutations. (C) Distribution of the *TP53* mutation VAF according to disease categories, presence of 17p loss, and a CK. (D) Proportions of 17p loss and a CK based on a 50% VAF threshold. (E) Status of 17p loss and CK according to the number of *TP53* mutations.

Abbreviations: MDS, myelodysplastic neoplasm; VAF, variant allele frequency; CK, complex karyotype; ND, not determined.

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Table 2. Molecular and cytogenetic characteristics of patients with *TP53* mutations according to the 5th WHO classification and ICC

Characteristics	WHO	ICC	ICC	WHO/ICC	*P* (B vs. C)	*P* (A vs. D)
	A: MDS (blasts < 20%) (N = 116)	B: MDS (blasts < 10%) (N = 88)	C: MDS/AML (10% ≤ blasts < 20%) (N = 28)	D: AML (blasts ≥ 20%) (N = 194)		
TP53 mutation, N (%)						
Single	107/116 (92.2%)	81/88 (92.0%)	26/28 (92.9%)	172/194 (88.7%)	1.000	0.309
Multiple (≥2)	9/116 (7.8%)	7/88 (8.0%)	2/28 (7.1%)	22/194 (11.3%)		
Median VAF of *TP53* mutation, % (IQR)	40.4 (16.9–69.6)	38.3 (14.4–68.6)	43.2 (28.8–76.8)	43.1 (21.9–69.8)	0.297	0.958
17p loss, N (%)	32/115 (27.8%)	18/87 (20.7%)	14/28 (50.0%)	75/188 (39.9%)	0.003	0.033
Chromosome study	30/113 (26.5%)	17/85 (20.0%)	13/28 (46.4%)	72/188 (38.3%)	0.006	0.037
TP53 FISH	20/79 (25.3%)	12/63 (19.0%)	8/16 (50.0%)	21/50 (42.0%)	0.011	0.047
Cytogenetics, N (%)						
NK	9/113 (8.0%)	6/85 (7.1%)	3/28 (10.7%)	15/188 (8.0%)	0.396	0.854
CK	87/113 (77.0%)	64/85 (75.3%)	23/28 (82.1%)	149/188 (79.3%)		
Others*	17/113 (15.0%)	15/85 (17.6%)	2/28 (7.1%)	24/188 (12.8%)		
Any MR gene mutations, N (%)	23/116 (19.8%)	19/88 (21.6%)	4/28 (14.3%)	37/194 (19.1%)	0.398	0.871
ASXL1	8/116 (6.9%)	5/88 (5.7%)	3/28 (10.7%)	11/194 (5.7%)		
BCOR	0/116 (0%)	0/88 (0%)	0/28 (0%)	5/194 (2.6%)		
EZH2	4/116 (3.4%)	4/88 (4.5%)	0/28 (0%)	6/194 (3.1%)		
SF3B1	3/116 (2.6%)	3/88 (3.4%)	0/28 (0%)	1/194 (0.5%)		
SRSF2	1/116 (0.9%)	0/88 (0%)	1/28 (3.6%)	4/194 (2.1%)		
STAG2	2/116 (1.7%)	1/88 (1.1%)	1/28 (3.6%)	6/194 (3.1%)		
U2AF1	6/116 (5.2%)	6/88 (6.8%)	0/28 (0%)	2/194 (1.0%)		
ZRSR2	2/116 (1.7%)	2/88 (2.3%)	0/28 (0%)	3/194 (1.5%)		
RUNX1	4/116 (3.4%)	2/88 (2.3%)	2/28 (7.1%)	11/194 (5.7%)		

*These include cytogenetic abnormalities that are neither normal nor complex karyotypes (defined as having three or more abnormalities).
Abbreviations: MDS, myelodysplastic neoplasm; ICC, International Consensus Classification; IQR, interquartile range; FISH, fluorescence *in-situ* hybridization; NK, normal karyotype; CK, complex karyotype; MR, myelodysplasia-related

multi-hit *TP53* mutations, and the presence of mutations in MR genes in patients with MDS or AML with *TP53* mutations.

DISCUSSION

To our knowledge, this study was the first large-scale investigation in Korea to identify the prevalence and characteristics of *TP53* mutations in MDS and AML and to evaluate the impact of *TP53* mutation-related reclassification according to the 5th WHO classification and ICC.

The frequency of *TP53* mutations varies significantly among cancers, with >90% in ovarian cancer and <15% in AML [11], suggesting that *TP53* mutations may exhibit tissue-specific acquisition. *TP53* mutations have been reported in 8%–10% of patients with MDS or AML [12–14]. In line herewith, we identified *TP53* mutations in 9.2% of patients. Reportedly, 73%–80% of *TP53* mutations are missense mutations, 86% of which are clustered in the DNA-binding domain [11, 12, 14, 15]. Specifically, missense mutations predominate in the DNA-binding domain, whereas nonsense and truncating mutations are more frequent outside this region, a pattern also observed in our study. Among patients with MDS or AML with *TP53* mutations, the presence of multiple *TP53* mutations has been reported at a frequency of 18%–30% [12, 15]. We found a slightly lower frequency of 10%. In terms of the *TP53* mutation pattern, most hotspot residues include methylated CpG sites, which are five times more likely to undergo spontaneous deamination leading to C>T mutation than unmethylated cytosines [11]. Approximately 25% of single-base substitutions are reported to be C>T transitions at CpG sites. In our study, while C>T substitutions

Table 3. Application of multiple-hit *TP53* mutation criteria in patients with *TP53* mutations according to the 5th WHO classification and ICC

Multi-hit *TP53* mutation	5th WHO	ICC	ICC	5th WHO/ICC	*P*	*P*
	A: MDS (blasts < 20%) (N = 116)	B: MDS (blasts < 10%) (N = 88)	C: MDS/AML (10% ≤ blasts < 20%) (N = 28)	D: AML (blasts ≥ 20%) (N = 194)	(B vs. C)	(A vs. D)
Criteria for multi-hit *TP53* mutation						
1. 5th WHO: two or more *TP53* mutations	9/116 (7.8%)	7/88 (8.0%)	2/28 (7.1%)	22/194 (11.3%)	1.000	0.309
2. 5th WHO: one *TP53* mutation with 17p loss	32/115 (27.8%)	18/87 (20.7%)	14/28 (50.0%)	75/188 (39.9%)	0.003	0.033
3. 5th WHO/ICC: one *TP53* mutation with VAF > 50%	48/116 (41.4%)	36/88 (40.9%)	12/28 (42.9%)	83/194 (42.8%)	0.855	0.809
4. ICC: two or more *TP53* mutations (each with a VAF > 10%)	8/116 (6.9%)	6/88 (6.8%)	2/28 (7.1%)	17/194 (8.8%)	1.000	0.559
5. ICC: one *TP53* mutation (with a VAF > 10%) with 17p loss	31/115 (27.0%)	18/87 (20.7%)	13/28 (46.4%)	71/188 (37.8%)	0.008	0.053
6. ICC: one *TP53* mutation (with a VAF > 10%) with CK	85/113 (75.2%)	63/85 (74.1%)	22/28 (78.6%)	143/188 (76.1%)	0.636	0.869
Multi-hit *TP53* mutation, N (%) according to the 5th WHO 2022 classification*	**68/116 (58.6%)**	49/88 (55.7%)	19/28 (67.9%)	134/194 (69.1%)	0.255	0.062
Multi-hit *TP53* mutation, N (%) according to the ICC†	89/116 (76.7%)	**66/88 (75.0%)**	23/28 (82.1%)	156/194 (80.4%)	0.436	0.44
Multi-hit *TP53* mutation (excluding CK), N (%) according to the ICC‡	66/116 (56.9%)	48/88 (54.5%)	18/28 (64.3%)	127/194 (65.5%)	0.365	0.132
Any *TP53* mutation with VAF > 10%	107/116 (92.2%)	80/88 (90.9%)	**27/28 (96.4%)**	**174/194 (89.7%)**	0.685	0.455

*Corresponding to criteria 1, 2, or 3 for multi-hit *TP53* mutations.
†Corresponding to criteria 3, 4, 5, or 6 for multi-hit *TP53* mutations.
‡Corresponding to criteria 3, 4, or 5 for multi-hit *TP53* mutations.
Bold indicates cases that meet the multi-hit criteria for *TP53*-mutated MDS, MDS/AML, and AML according to the 5th WHO classification and ICC.
Abbreviations: MDS, myelodysplastic neoplasm; ICC, International Consensus Classification; VAF, variant allele frequency; CK, complex karyotype.

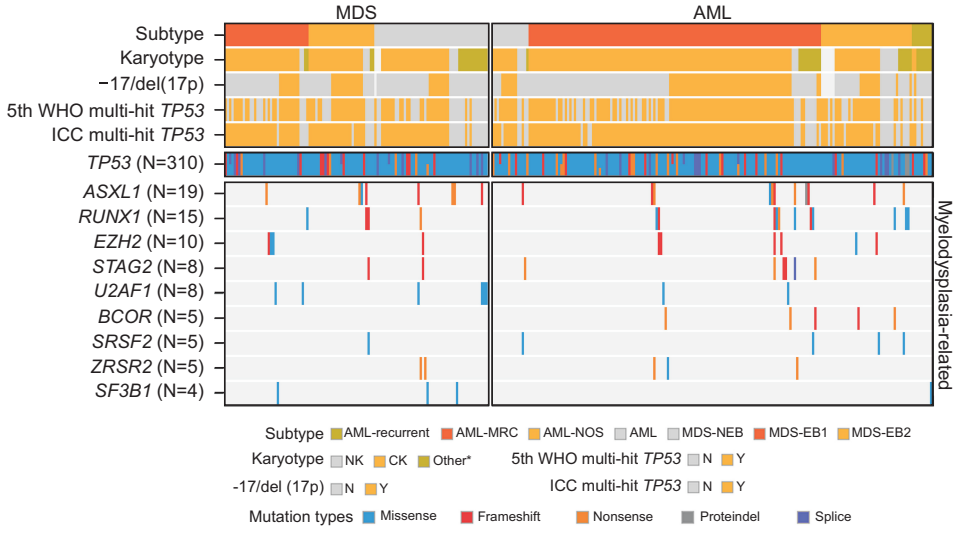

Fig. 2. Mutation heatmap of patients with MDS and AML with *TP53* mutations.
*These include cytogenetic abnormalities that are neither normal nor complex karyotypes (defined as having three or more abnormalities).
Abbreviations: MDS, myelodysplastic neoplasm; ICC, International Consensus Classification; AML-recurrent, AML with recurrent genetic abnormalities; AML-MRC, AML with myelodysplasia-related changes; AML-NOS, AML, not otherwise specified; MDS-NEB, MDS with non-excess blasts; MDS with EB1, MDS with excess blasts 1; MDS with EB2, MDS with excess blasts 2; NK, normal karyotype; CK, complex karyotype; N, no; Y, yes.

were the most common, the frequency at CpG sites was slightly lower (18.2%) than that in the previous study [11].

Hotspot *TP53* mutations are located in residues involved in DNA contact or the DNA-binding domain structure [14]. R273C/H, R248Q/W, and R282W are classified as contact mutations, which render p53 unsuitable for DNA binding. In contrast,

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R175H, Y220C, and G245S are classified as structural mutations characterized by reduced thermodynamic stability and an unfolded structure compared to wild-type p53. In metastatic cancer, hotspot codons for *TP53* mutations account for 25% of all *TP53* mutations, with the most frequently mutated codons being R273, R248, R175, R213, G245, and R282 [16]. In our study, six hotspot codons, R248, R273, G245, Y220, R175, and C238, accounted for 25.4% of all *TP53* mutations. This pattern slightly differs from the hotspot mutations observed in solid tumors. Notably, R248Q was the most common mutation in both MDS and AML, a finding consistent with findings in a previous study on AML [17]. Y220C, which is associated with targeted therapy [18, 19], was the second most common mutation, with a frequency of 2.9%, indicating an enrichment in hematologic malignancies compared to solid tumors. The targeted therapy drug PC14586 converts the Y220C-mutated p53 conformation into the wild-type conformation and induces the activation of p53 transcriptional pathways. Clinical trials of PC14586 in solid tumors and AML/MDS with the Y220C mutation are ongoing [18, 19].

The median VAF was ~40% in both MDS and AML, with ~40% of patients with *TP53* mutations exhibiting a VAF of ≥50%. The VAF was higher in patients with 17p loss than in those without and notably differed depending on the presence of a complex karyotype. Furthermore, most patients with a *TP53* VAF >50% had a complex karyotype, suggesting that a VAF >50% may be a predictor of a complex karyotype and indicates the potential co-occurrence of loss of heterozygosity in complex karyotypes. Similarly, Sallman, *et al*. [20] found a strong correlation between a complex karyotype and the VAF; patients with a complex karyotype had a significantly higher median VAF than those with a non-complex karyotype based on a cutoff of VAF >40%.

In addition to acquiring a *TP53* mutation in one allele, most tumors lose the second allele through deletion or copy-neutral loss of heterozygosity [21]. Lie, *et al*. [21] demonstrated that ~50% of AML patients with a *TP53* mutation also had 17p deletion, and conversely, approximately half of the patients with 17p deletion had a *TP53* mutation. We observed 17p loss in 41.2% (89/216) of patients with ≥10% blasts (including both MDS/AML and AML), which was twice the frequency in those with <10% blasts (MDS-ICC), indicating an association with disease severity. However, we found no difference between patients with MDS/AML and those with AML.

The criteria used for multi-hit *TP53* mutation vary slightly among studies, including differences in the VAF threshold [12, 22, 23]. Grob, *et al*. [12], in a study of patients with AML or MDS-EB who had *TP53* mutations, defined biallelic *TP53* mutation status using criteria similar to those of the 5th WHO classification. Their study showed that 76% of patients had biallelic *TP53* mutations, with a median *TP53* VAF of 47%. In our study, 69% (153/222) of patients with MDS/AML or AML met the WHO criteria for multi-hit *TP53* mutations, which is slightly lower than the frequency in the previous study. Notably, Grob *et al*. observed that patients with *TP53* mutations accompanied by a complex karyotype had a significantly worse prognosis than those with *TP53* mutations alone [12]. They found no other molecular genetic factors that affected prognosis in patients with AML or MDS-EB with *TP53* mutations. Similarly, Weinberg, *et al*. [23] found that 83% of patients with a complex karyotype had accompanying *TP53* mutations and that this co-occurrence was associated with aggressive disease. Additionally, they showed that 64% of patients with MDS and 83% of patients with AML with *TP53* mutations had multi-hit *TP53* mutations, which were defined as a single *TP53* mutation with a VAF >60% or a single *TP53* mutation with 17p loss.

Based on these previous studies, the 5th WHO classification and ICC have proposed different criteria for multi-hit *TP53* mutations [6, 7]. The most significant difference is that the ICC considers only *TP53* mutations with a VAF >10% as meaningful and includes a complex karyotype as a criterion for multi-hit mutation. Additionally, the ICC applies the multi-hit criteria only when blasts are <10%. Both classifications consider a single *TP53* mutation with a VAF >50% as indicative of loss of heterozygosity, as identifying loss of heterozygosity is difficult in actual clinical settings. In our study, among the patients who met the 5th WHO multiple-hit criteria, the majority (60%–70%) fulfilled this criterion of having a VAF >50%. Conversely, according to the ICC criteria, patients with a VAF >50% frequently overlapped with those having a complex karyotype, whereas among patients with a VAF <50%, 27% were classified as having multi-hit *TP53* mutations solely based on the presence of a complex karyotype. Therefore, the inclusion of a complex karyotype in the ICC multi-hit criteria has significant implications. As mentioned above, the ICC classification considers the prognostic implications of a *TP53* mutation being accompanied by a complex karyotype [12, 23]. While the proportion of multi-hit *TP53* mutations did not significantly differ among disease categories, the discrepancy in multi-hit *TP53* mutations between the two classifications remains 20%–30%. Further research based on clinical data is required to determine the optimal application of these criteria.

This study had some limitations. As we did not perform survival analysis, we could not verify the prognostic significance of

each multi-hit *TP53* mutation criterion. Nonetheless, numerous studies have demonstrated the association between *TP53* mutations in MDS and AML and poor survival [12, 23–28]. We anticipate that future prospective studies based on these diagnostic criteria will provide new insights. Furthermore, we assumed that all *TP53* mutations detected were of somatic origin and we did not assess whether they were of germline origin.

In conclusion, we identified the molecular genetic characteristics of patients with MDS or AML with *TP53* mutations, as well as the key factors that significantly impact the application of *TP53* mutation-related criteria according to the updated classifications. We believe that our findings provide valuable evidence to support the establishment of diagnostic strategies.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0351

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AUTHOR CONTRIBUTIONS

Kim HY analyzed the data and drafted the manuscript. Shin S edited the manuscript. Lee JM and Kim IS conceptualized and supervised the study and reviewed the manuscript. Kim B, Kim HJ, Choi YJ, Bae B, Kim Y, Kim H, Lee JS, Chang YH, Kim HY, Lee JY, Yu S, Kim MY, Cho YU, Jang S, and Kim MS provided laboratory information and participated in manuscript review.

CONFLICTS OF INTEREST

None declared.

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