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SHORT COMMUNICATION

Methylation of DNA Repair Genes as a Prognostic Biomarker in AML of a TCGA-LAML Cohort

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SUMMARY

Background: **Dysregulation of DNA damage response and altered DNA methylation in acute myeloid leukemia (AML) have been reported, but the impact of methylation of DNA repair genes has not yet been researched. We aimed to predict the prognosis of non-APL AML patients based on the known CpG site methylation levels of DNA repair genes through The Cancer Genome Atlas AML project (TCGA-LAML).**
Methods: **We utilized TCGA-LAML cohort (174 non-APL AML) for the methylation data of 22 DNA repair genes.**
Results: **In univariate analysis among 174 non-APL AML patients of the TCGA-LAML cohort, the hypermethylation of *MLH1, RAD51,* and *ATM* showed superior overall survival (OS) than non-hypermethylated groups, while hypermethylation of *RAD23A, RAD23B, MLH1, MSH2, BRCA1, BRCA2, RAD50,* and *PARP1* was associated with poor OS. We demonstrated that CpG hypermethylation levels of DNA repair genes differed according to the AML cytogenetic risk groups. In multivariate analysis, hypermethylation of *MLH1* and *RAD51* showed better OS than non-hypermethylated patients, but hypermethylation of *MSH2* and *RAD50* showed worse OS than non-hypermethylated patients.**
Conclusion: **Methylation of 4 DNA repair genes, such as *MLH1, RAD51, MSH2,* and *RAD50,* have the potential to be independent risk factors in non-APL AML patients.**
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KEY WORDS

DNA repair gene, CpG methylation, acute myeloid leukemia, prognosis, biomarker

LIST OF ABBREVIATIONS

Allo-SCT - allogeneic stem cell transplantation
APL - acute promyelocytic leukemia
CBF - core binding factor
NK - normal karyotype

INTRODUCTION

With the rapid development of genetic testing methods, there are increasing reports that gene rearrangements, gene mutations, and epigenetic changes in various genes can affect the leukemic pathogenesis of AML [1,2]. Re-

current chromosomal aberrations and gene mutations are the most important factors in the diagnosis of and therapeutic decision for AML patients, but they can be eliminated only by killing the mutant cells. Histone modification and methylation are attractive therapeutic targets in that they have the potential to be restored to normal using targeting inhibitors. Therefore, research on methylation in AML continues.

Since cancer is basically caused by more than one genetic alteration, if DNA damage is not repaired normally, it can lead to apoptosis or proliferation of abnormal cells. Therefore, there have been reports on defects in DNA damage response in AML [3,4]. Leukemia with activated tyrosine kinases, such as BCR-ABL1 and FLT3/ITD, induces c-MYC expression and subsequently increases error-prone repair evidenced by the increased expression of DNA ligase IIIα and PARP1 [5]. DNA double strand break (DSB) response is reported to be abnormal in myeloblasts from therapy-related AML (t-AML) patients showing elevated, impaired, or delayed histone H2AX (γH2AX) induction measured by kinetics of γH2AX, a marker for DSB [6]. Distinct patterns of γH2AX were associated with *TP53* mutation and inferred chromothripsis [6]. Inherited mutations of DNA repair genes, such as Fanconi anemia genes, *TP53*, and *NBN* gene, predispose hematologic malignancies and polymorphisms in DNA repair genes, such as *RAD51* G135C and *XRCC3* Thr241met, are associated with increased risk of AML development [3,4]. The association between dysregulation of the DNA repair pathway and AML has been demonstrated, and epigenetic silencing is thought to be involved in DNA repair dysregulation in AML [7].

In this study, we intend to predict the prognosis of patients based on the known CpG site methylation levels of DNA repair genes in non-APL AML patients through The Cancer Genome Atlas AML project (TCGA-LAML) data. The purpose of this study was to compare the degree of methylation of 22 DNA repair genes between subgroups of AML, and to investigate their potential as prognostic factors.

MATERIALS AND METHODS

Materials and subjects

From the TCGA-LAML, the CpG site methylation data of DNA repair genes and clinical information across 174 de novo non-APL AML patients were downloaded using cBioProtal [8-10]. We analyzed 22 DNA repair genes of interest including 3 base excision repair pathway genes (*APEX1*, *POLB*, and *UNG*), 4 nucleotide excision repair pathway genes (*RAD23A*, *RAD23B*, *CCNH*, and *XPC*), 4 mismatch repair pathway genes (*MLH1*, *MLH3*, *MSH2*, and *PMS2*), 7 homologous recombination pathway genes (*BRCA1*, *BRCA2*, *RAD50*, *RAD 51*, *ATM*, *POLD3*, and *MRE11A*), and 4 non-homologous end joining pathway genes (*PARP1*, *LIG3*, *XRCC1*, and *FEN1*).

The beta value of methylation data (Illumina Infinium Human Methylation 450, TCGA Level 3 DNA methylation) was obtained. In the TCGA-LAML cohort, 408 DNA methylation probes of 22 DNA repair genes were analyzed (Supplemental data 1). To analyze methylation of DNA repair genes, the M values derived from the beta values [11] were used. M value is the log2 value of the intensities of methylated versus unmethylated probes [11].

The TCGA-LAML 174 non-APL AML patients included 22 in favorable risk group, 102 in intermediate risk group, and 50 in poor risk group according to cytogenetics and molecular abnormalities [12]. The characteristics of patients are described in the Table 1. The median follow-up period was 15.5 months (range: 0.0 - 118.1 months).

Statistics

Comparison of OS probability between hypermethylated AML and non-hypermethylated AML was analyzed by Kaplan-Meier log-rank test using MedCalc Statistical Software version 19.6 (MedCalc Software bvba, Ostend, Belgium). The cutoff point for hypermethylation of each DNA repair gene was set as the M value of each gene with statistically significant split. Cox proportional hazard regression for multivariate analysis used the cutoffs from the univariate analysis. The comparison analysis of the methylation of DNA repair genes between the AML patients were performed using the Kruskal-Wallis test along with post-hoc Mann-Whitney test for cytogenetic risk groups. p- values less than 0.05 was set as the level of statistical significance.

RESULTS

In the TCGA-LAML cohort, the hypermethylation of *MLH1* (cg10990993), *RAD51* (cg13422654), and *ATM* (cg14761454, cg25918541, cg27599391) genes had superior OS probability compared to the non-hypermethylated groups (Supplemental Figure 1A) by univariate log-rank survival analysis. However, hypermethylation of 8 DNA repair genes, such as *RAD23A* (cg22984992), *RAD23B* (cg13663912), *MLH1* (cg11291081, cg05670 953, cg18320188, cg21109167), *MSH2* (cg11311499), *BRCA1* (cg19442659, cg04582861, cg058152471, cg14 687474, cg19454999, cg24900425), *BRCA2* (cg272533 86), *RAD50* (cg14597804), and *PARP1* (cg24937136), was associated with a poor prognosis (Supplemental Figure 1B). The association of OS and methylation of *MLH1* (cg10990993 vs. cg11291081, cg05670953, cg1 8320188, and cg21109167) showed contradictory results according to the location of DNA methylation. Multivariate analysis demonstrated that hypermethylation of *MLH1* (cg10990993) or *RAD51* (cg13422654) was correlated with better OS than non-hypermethylated groups (Figure 1A, and Table 2). Two marker combined analysis demonstrated the prognostic impact of

Table 1. Demographic and laboratory characteristics of non-APL AML patients in The Cancer Genome Atlas AML cohort (TCGA-LAML).

Variable	TCGA-LAML (n = 174)	
	No. of Patients	%
Age, years		
Median (range)	58.5 (18 - 88)	
Gender (male:female)	96:78	55:45
WBC × 10^9/L		
Median (range)	19.2 (0.6 - 298.4)	
Hemoglobin, g/dL		
Median (range)	9.0 (6.0 - 14.0)	
Platelet × 10^9/L		
Median (range)	53 (8 - 351)	
Bone marrow blast, %		
Median (range)	72 (30 - 100)	
Non-APL AML cytogenetic risk group	174	
Favorable risk	22	13
CBF-AML	19	86
Normal karyotype*	3	14
Intermediate risk	102	59
t(9;11)	2	2
Normal karyotype†	79	77
Others‡	21	21
Adverse risk	50	29
KMT2A	5	10
Normal karyotype§	5	10
Others‖	16	32
Complex¶	24	48
Underwent Allo-SCT		
Yes	90	52
No	84	48

* Normal karyotype with mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} or normal karyotype with biallelic mutated *CEBPA*.
† Normal karyotype with wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}.
‡ Other karyotypes not included in favorable risk group or adverse risk group.
§ Normal karyotype with wild-type *NPM1* and *FLT3*-ITD^{high}.
‖ Other karyotypes with t(9;22), -5/-5q, -7/-7q, or monosomal karyotype.
¶ Complex karyotype was defined as the karyotype with 3 or more chromosomal abnormalities.

hypermethylation of 2 genes on better OS than non-hypermethylated groups (Figure 1A and Table 2). On the other hand, the hypermethylation of *MSH2* (cg1131 1499) or *RAD50* (cg14597804) showed worse OS than the non-hypermethylated groups (Figure 1B and Table 2). Two marker combined analysis showed worse OS in patients with hypermethylation of 2 genes (Figure 1B and Table 2). The age, stem cell transplant status, and cytogenetic risk group in AML patients were also confirmed to be independent risk factors from this analysis. In the TCGA cohort, the levels of methylation of DNA repair genes pertaining to significant different OS were analyzed for the AML cytogenetic risk groups. Most of the genes analyzed showed differential levels of methylation between AML cytogenetic risk subgroups (Supplemental Figure 2).

Methylation of the *ATM* gene (cg14761454) was higher in the favorable risk group than in the intermediate and the poor risk groups (Supplemental Figure 2A), while the methylation of *MLH1* (cg10990993) and *RAD51* (cg13422654) were not statistically different between AML risk groups.

Table 2. Multivariate analysis of the promoter hypermethylation of *MLH1* (cg10990993), *RAD51* (cg13422654), *MSH2* (cg11311499), and *RAD50* (cg14597804) in TCGA-LAML.

	Univariate		Multivariate (2 genes combined)	
Parameter	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Age *			**1.02 (1.00 - 1.03)**	**0.0196**
Stem cell transplant	**0.45 (0.31 - 0.65)**	**< 0.0001**	**0.36 (0.23 - 0.56)**	**< 0.0001**
Cytogenetic risk (reference favorable)				
Intermediate	**3.28 (2.04 - 5.27)**	**0.0001**	**3.20 (1.42 - 7.21)**	**0.0049**
Adverse	**4.92 (2.80 - 8.65)**		**5.89 (2.51 - 13.84)**	**< 0.0001**
Promoter hypermethylation				
MLH1	**0.42 (0.22 - 0.80)**	**0.0087**		
RAD51	**0.28 (0.15 - 0.55)**	**0.0002**		
MLH1 or RAD51			**0.77 (0.29 - 2.02)**	**0.5909**
MLH1 and RAD51			**0.32 (0.12 - 0.80)**	**0.0148**
MSH2	**1.52 (1.06 - 2.18)**	**0.0232**		
RAD50	**1.97 (1.03 - 3.79)**	**0.0414**		
MSH2 or RAD50			**1.95 (1.28 - 2.97)**	**0.0018**
MSH2 and RAD50			**3.86 (1.67 - 8.91)**	**0.0016**

*** Continuous variable.**

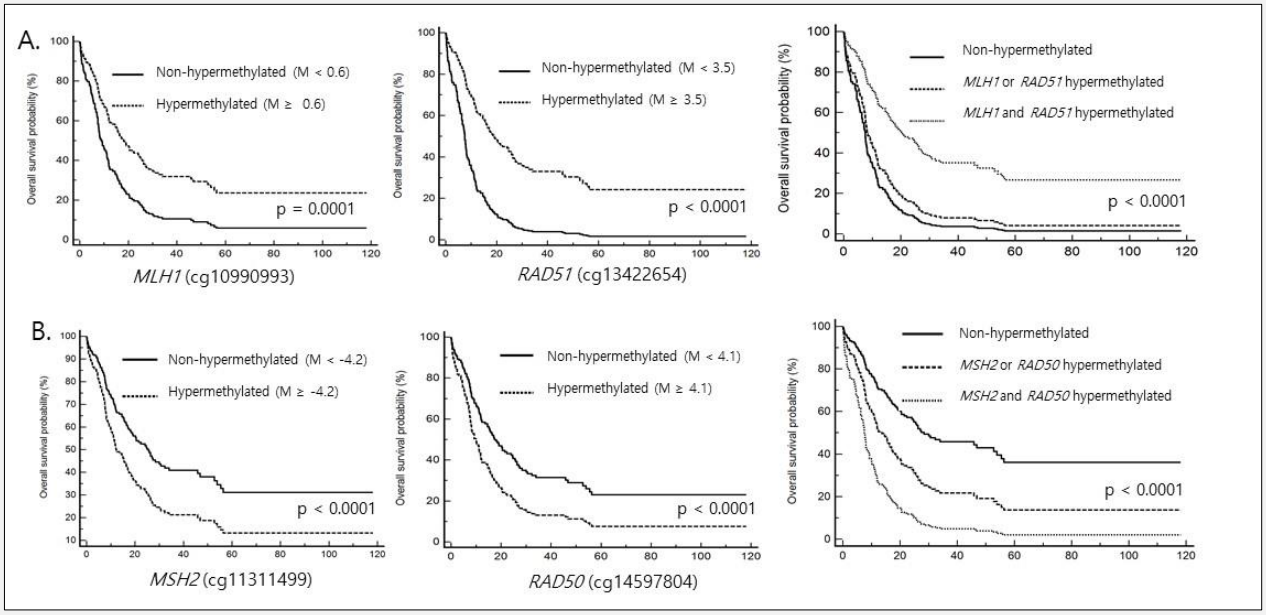

Figure 1. Promoter methylation status of DNA repair genes is associated with overall survival by Cox proportional hazard regression analysis in TCGA-LAML: (A) hypermethylation of *MLH1*, and *RAD51*, and the 2 markers combined showed better overall survival probability, and (B) hypermethylation of *MSH2* and *RAD50*, and the 2 markers combined showed worse overall survival probability.

Poor risk groups showed higher methylation levels in the following genes, such as *RAD23A* (cg22984992), *MLH1* (cg11291081), *MSH2* (cg11291081), *BRCA1* (19 442659), *BRCA2* (cg27253386), *PARP1* (cg24937136), than other risk groups (Supplemental Figure 2).

DISCUSSION

Through a study on genome wide DNA methylation profiling in AML, Figueroa et al. found 16 distinct methylation clusters in AML, and 3 clusters were correlated with the recurrent cytogenetic subgroup, such as inv(16)/t(16:16), t(8;21), and t(15;17) [13]. Among them, 5 clusters showed a unique pattern of methylation without any specific genetic or molecular characteristics, and biological differences were confirmed, suggesting the possibility of new AML subgroups [13]. The other report showed that methylation patterns are strongly associated with chromosomal abnormalities [8, 14]. It has been reported that a high methylation score among AML patients is associated with a poor prognosis [15,16]. AML with complex karyotype shows high levels of constitutive DNA damage and activation of check point signaling pathways, and may be a potential target for checkpoint therapy [17].

In our study, CpG methylation status of *ATM*, *RAD23A*, *RAD23B*, *MLH1*, *MSH2*, *BRCA1*, *BRCA2*, and *PARP1* were significantly different among non-APL AML cytogenetic risk subgroups (Supplemental figure 2). We confirmed that CpG hypermethylation of DNA repair genes resulted in poor prognosis (Supplemental figure 1B) but those genes showing differences according to the cytogenetic risk groups could not be independent risk factors through multivariate analysis. However, methylation of *MHL1*, *RAD50*, *MSH2*, and *RAD51* genes which were proven to have prognostic significance does not differ among the risk groups. Therefore, CpG methylation levels of those 4 genes have roles as independent factors.

The discovery of *MLH1* mutation or promoter methylation in 18 of 53 refractory or relapsed AML patients suggested that mismatch repair (MMR) deficiency was involved in the progression of AML [18]. Contrary to the assumption that promoter methylation of *MLH1* was associated with MLH1 deficiency, George Lenz et al. demonstrated that methylation of *MLH1* promoter was present in 4 out of 22 AML patients, but was not related to gene expression [15].

In a study on the MSH2 expression, abnormal expression of MSH2 protein (13 out of 42 AML patients) reported AML patients with abnormal MSH2 expression showed microsatellite instability (MSI) indicating that MMR deficiency from abnormal MSH2 expression could have a potential role in leukemogenesis [19]. Promoter hypermethylation of *MSH2* and *MLH1* was not common in AML (2 out of 44 de novo AML, 2 out of 7 secondary AML, and 1 out of 17 t-AML) while MSI was detected in 29.4% of AML (9 out of 44 de novo AML, 3 out of 7 secondary AML, and 8 out of 17 t-AML) [20].

Differences in results between this study and other researchers may be due to differences in study patient groups, probe regions, and test methods.

In conclusion, we found that methylation of DNA repair genes in non-APL AML patients differed according to the cytogenetic risk groups. The methylation of 4 genes show potential as prognostic factors, *MLH1* (cg109909 93), and *RAD51* (cg13422654) as favorable prognostic factors and *MSH2 (cg11311499)*, and *RAD50* (cg14597 804) as adverse prognostic factors. The usefulness as a prognostic factor in clinical practice should be elucidated through further investigation in the process of diagnosis and treatment in actual AML patients.

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Declaration of Interest:
The authors declare that they have no competing interests.

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