High expression of microRNA-500 is associated with poor prognosis in patients with acute myeloid leukemia receiving allogeneic hematopoietic stem cell transplantation

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Abstract. MicroRNA-500 (miR-500) is a potential prognostic biomarker in a number of different types of cancer, such as prostate cancer and hepatocellular carcinoma. This study aimed to explore the clinical implications of miR-500 expression status in patients with acute myeloid leukemia (AML) that had received allogeneic hematopoietic stem cell transplantation (allo-HSCT). miR-500 expression status and clinical data were obtained from 74 patients with AML in the The Cancer Genome Atlas database receiving allo-HSCT. Patients with low expression level of miR-500 (miR-500$_{low}$) were significantly more likely to present with a French-American-British classification M2 subtype (P=0.003), and less likely to have the M5 subtype (P=0.040) compared with patients with high expression levels (miR-500$_{high}$). miR-500$_{low}$ patients were associated with low-risk AML (P=0.003) and core-binding factor subunit b-myosin heavy chain 11 translocation mutation (P=0.021). There was a significant difference in nucleophosmin 1 (P=0.009), NRAS proto-oncogene GTPase/KRAS proto-oncogene GTPase (P=0.047) and PHD finger protein 6 (P=0.040) expression levels between the two groups. miR-500$_{high}$ patients had a decreased overall survival (OS) time compared with the low expression group (P=0.035). Multivariate analysis revealed that miR-500 expression significantly affected OS time independent of other classical prognostic factors, such as age and common mutations. The analysis of survival curves further substantiated this result. The results obtained in the current study suggested that miR-500 may be a suitable prognostic marker for patients with AML receiving allo-HSCT.

Introduction

Acute myeloid leukemia (AML) is characterized by the infiltration of bone marrow and peripheral blood by proliferative, clonal, abnormally differentiated cells of the hematopoietic system (1).

A number of biomarkers are currently used for the diagnosis, prognosis evaluation and treatment strategy of patients with AML, including karyotype, molecular classifications and mutation analysis (2). Classically, specific recurrent chromosomal translocations, including inversion(16) [inv(16)], translocation(8;21) [t(8;21)] and t(15;17) indicate a positive prognosis, while chromosomal deletions (dels) including del(5q), -5 and -7 are associated with more severe disease phenotypes. Gene mutations in fms-related tyrosine kinase 3 (FLT3), DNA methyltransferase 3α (DNMT3A), KIT proto-oncogene, receptor tyrosine kinase (KIT), runt-related transcription factor 1 (RUNX1), isocitrate dehydrogenase (NADP(+))1 cytosolic and isocitrate dehydrogenase (NADP(+))2 mitochondrial indicate a poor outcome, while mutations in nucleophosmin 1 (NPM1) and CCAAT enhancer binding protein a are associated with a favorable prognosis (3,4). With advances in molecular biology, the understanding of the molecular causes for AML is gradually increasing. Therefore, an increased number prognostic factors and therapeutic targets, including epigenetic markers, have become topics of investigation.

MicroRNAs (miRNAs/miRs) are a class of short non-coding RNA molecules ~22 nucleotides in length which regulate gene expression at the post-transcriptional level (5). miRNAs bind to the 3'-untranslated region of specific target mRNAs, and may affect cell proliferation, differentiation, apoptosis and tumorigenesis (6). In the hematopoietic system, the expression of miRNAs and the related processing proteins is important for the maintenance of hematopoietic stem cells (7). Additionally, the dysregulation of miRNAs has been linked to the progression of hematopoietic malignancies, providing new avenues

for anticancer research. One of the first studies in this line of research investigated the effect of miR-15a and miR-16-1 in chronic lymphocytic leukemia (8).

miR-500 was first reported as a cancer-associated miRNA in hepatocellular carcinoma (9). Subsequent studies have suggested that miR-500 is involved in the development and progression of a number of solid tumors, including prostate (10) and gastric cancer (11). Although several prognostic factors have been identified for AML, there are only limited indicators for assessing the likely outcomes for patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). Thus, the aim of the present study was to investigate and analyze the clinical significance of miR-500 in patients with AML receiving allo-HSCT.

Patients and methods

Patients. The genomic and clinical data of 74 patients with AML were downloaded from The Cancer Genome Atlas database (TCGA; cancergenome.nih.gov). The diagnosis and classification of the patients were based on the World Health Organization and the French-America-British classification systems (12,13). The clinical data included age, sex and white blood cell (WBC) count. The mutation status of commonly mutated genes, as determined by next-generation sequencing, karyotypes and data regarding overall survival (OS) time were available.

All patients provided signed written informed consent in the original study (12), which was approved by the Human Research Ethics Committee of Washington University.

Statistical analysis. To explore the clinical relevance of the miR-500 expression level in patients with AML receiving allo-HSCT, the patients were divided into high and low miR-500 expression groups (miR-500$_{high}$ and miR-500$_{low}$, respectively) based on the median expression value, which was determined to be 8.766 (range, 0.545-72.375). Continuous data are presented as medians with ranges. The Mann-Whitney U and chi-square tests were used to evaluate continuous and categorical variables, respectively. A Cox proportional hazards model was performed to assess the effect of miR-500 expression on OS time. Survival curves were plotted using the Kaplan-Meier method to analyze the relationship between the miR-500 expression level and OS. The log-rank test was used to evaluate the differences between survival distributions. OS was defined as the time from the date of diagnosis until the date of mortality from any cause. The statistical analyses in the study were performed with SPSS (version 20; IBM Corporation, Armonk, NY, USA) and GraphPad Prism (version 6; GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic and biological characteristics associated with miR-500 expression level. Data regarding 74 patients with AML undergoing allo-HSCT, including 42 males and 32 females, were extracted from the TCGA database. The relevant biological and clinical characteristics of the patients are presented in Table I. There was no significant association between miR-500 expression level and age, sex, WBC count, bone marrow blasts,

peripheral blood blast proportion or karyotype. There were significantly more M2 subtype patients in the miR-500$_{low}$ group compared with the miR-500$_{high}$ group (P=0.003), while all M5 subtype patients were in the miR-500$_{high}$ group (P=0.040). The cytogenetic classification revealed that the miR-500$_{low}$ group was enriched in patients with inv(16)/core-binding factor subunit b (CBFB)-myosin heavy chain 11 (MYH11) (P=0.021). The miR-500$_{low}$ group was associated with a favorable molecular risk stratification, and all patients with a good molecular prognosis were in the miR-500$_{low}$ group (P=0.003). Among the common gene mutations with prognostic significance in AML with a frequency of at least 5%, significant differences were observed in the frequency of NPM1 (P=0.009), NRAS/KRAS (P=0.047) and PHD finger protein 6 (PHF6; P=0.040) mutations between the two groups. NPM1 mutation appeared more frequently in the miR-500$_{low}$ group, and the miR-500$_{high}$ group was associated with NRAS/KRAS and PHF6 mutations. However, the relapse rate and the sources of hematopoietic stem cells were not significantly associated with miR-500 level.

Univariate and multivariate analysis for OS. In order to investigate the prognostic significance of demographic and biological characteristics, survival analysis was performed based on the expression status of miR-500 (high vs. low), age (<60 vs. ≥60 years), WBC count (<30x10^9 vs. >30x10^9/l) and common gene mutations [FLT3, NPM1, DNMT3A, WT1 transcription factor, RUNX1, tumor protein (TP53), PHF6, lysine methyltransferase 2A (KMT2A) and NRAS/KRAS; mutated vs. wild type].

A Cox proportional hazards univariate analysis indicated that patients with high miR-500 expression had a shorter OS time compared with patients with low expression (P=0.035). Mutations in *PHF6*, *KMT2A*, *RUNX1* and *TP53* significantly affected the OS time compared with wild-type genes (all P<0.05; Table II). Multivariate Cox regression analyses were subsequently performed to determine the association of miR-500 expression level and classical clinical and molecular prognostic factors in AML (Table III). The results revealed miR-500 was independently associated with a poor prognosis, as determined by a decreased OS time (P=0.008), while classical biomarkers including mutations in the genes *FLT3*, *NPM1* and *DNMT3A* had no independent association with OS time.

Survival curves for the two miR-500 expression groups. Survival curves plotted with the Kaplan-Meier method revealed that the high expression of miR-500 was associated with a shorter OS time (P=0.033; Fig. 1).

Discussion

The present study revealed that high expression of miR-500 may be associated with a shorter OS time in patients with AML undergoing allo-HSCT compared with low expression. When considered with other known prognostic factors assessed by the multivariate analysis, miR-500 had an independent prognostic value.

A previous study suggested that determining the mutation status of NPM1 following allo-HSCT may predict the likelihood of relapse (14). While it was previously reported that FLT3 mutation status does not adversely impact OS time following allo-HSCT (15), another study revealed that

Table I. Comparison of the clinical and molecular characteristics of patients with AML stratified according to the expression of miR-500.

Characteristics	miR-500$_{low}$ (n=37)	miR-500$_{high}$ (n=37)	Statistics	P-value
Median age (range), years	49 (22-72)	53 (18-65)	U=654.0	0.741
Age group, n (%)			χ^2=1.096	0.295
<60 years	29 (78.4)	25 (67.6)		
≥60 years	8 (21.6)	12 (32.4)		
Sex, n (%)			χ^2=0.881	0.348
Male	19 (51.4)	23 (62.2)		
Female	18 (48.6)	14 (37.8)		
Median white blood cell count (range), x10^9/l	30.9 (0.6-202.7)	27.1 (0.8-233.8)	U=742.5	0.531
Median bone marrow blasts (range), %	67 (34-100)	75 (30-97)	U=592.0	0.317
Median peripheral blood blasts (range), %	48 (0-96)	45 (0-90)	U=791.6	0.166
French-American-British subtype, n (%)			χ^2=17.709	0.024
M0	5 (13.5)	4 (10.8)		0.722
M1	9 (24.3)	14 (37.8)		0.209
M2	15 (40.5)	4 (10.8)		0.003
M3	2 (5.4)	0 (0.0)		0.152
M4	5 (13.5)	9 (24.3)		0.235
M5	0 (0.0)	4 (10.8)		0.040
M6	0 (0.0)	1 (2.7)		0.314
M7	0 (0.0)	1 (2.7)		0.314
Karyotype, n (%)			χ^2=16.216	0.063
Normal	13 (35.1)	21 (56.8)		0.062
Complex	5 (13.5)	7 (18.9)		0.528
8 Trisomy	3 (8.1)	3 (8.1)		1.000
inv(16)/core-binding factor subunit β-myosin heavy chain 11	5 (13.5)	0 (0.0)		0.021
11q23/ lysine methyltransferase 2A	0 (0.0)	3 (8.1)		0.077
-7/7q-	2 (5.4)	1 (2.7)		0.556
t(15;17)/promyelocytic leukemia-retinoic acid receptor α	2 (5.4)	0 (0.0)		0.152
t(9;22)/BCR activator of RhoGEF and GTPase-ABL proto-oncogene 1, non-receptor tyrosine kinase	1 (2.7)	1 (2.7)		1.000
t(8;21)/runt-related transcription factor 1- RUNX1 translocation partner 1	1 (2.7)	0 (0.0)		0.314
Other/undefined subtypes	5 (13.5)	1 (2.7)		0.088
Molecular risk stratification, n (%)			χ^2=9.776	0.021
Good	8 (21.6)	0 (0.0)		0.003
Intermediate	18 (48.6)	23 (62.2)		0.242
Poor	11 (29.7)	13 (35.1)		0.619
Fms-related tyrosine kinase 3, n (%)			χ^2=1.909	0.167
Mutation	6 (16.2)	11 (29.7)		
Wild-type	31 (83.8)	26 (70.3)		
Nucleophosmin 1, n (%)			χ^2=6.852	0.009
Mutation	5 (13.5)	15 (40.5)		
Wild-type	32 (86.5)	22 (59.5)		
CCAAT enhancer binding protein α, n (%)			χ^2=5.345	0.069
Single mutation	4 (10.8)	1 (2.7)		
Double mutation	3 (8.1)	0 (0.0)		
Wild-type	30 (81.1)	36 (97.3)		

Table I. Continued.

Characteristics	miR-500$_{low}$ (n=37)	miR-500$_{high}$ (n=37)	Statistics	P-value
DNA methyltransferase 3α, n (%)			χ^2=0.294	0.588
Mutation	10 (27.0)	8 (21.6)		
Wild-type	27 (73.0)	29 (78.4)		
Isocitrate dehydrogenase (NADP(+)) 1, cytosolic, n (%)			χ=0.107	0.744
Mutation	5 (13.5)	6 (16.2)		
Wild-type	32 (86.5)	31 (83.8)		
Isocitrate dehydrogenase, n (%) [NADP(+)] 2, mitochondrial			χ^2=0.561	0.454
Mutation	3 (8.1)	5 (13.5)		
Wild-type	34 (91.9)	32 (86.5)		
WT1 transcription factor, n (%)			χ^2=0.126	0.722
Mutation	5 (13.5)	4 (10.8)		
Wild-type	32 (86.5)	33 (89.2)		
Runt-related transcription factor 1, n (%)			χ^2=2.242	0.134
Mutation	2 (5.4)	6 (16.2)		
Wild-type	35 (94.6)	31 (83.8)		
Lysine methyltransferase 2A, n (%)			χ^2=1.507	0.304
Mutation	1 (2.7)	3 (8.1)		
Wild-type	36 (97.3)	34 (91.9)		
NRAS/KRAS proto-oncogene GTPase, n (%)			χ^2=3.945	0.047
Mutation	6 (16.2)	1 (2.7)		
Wild-type	31 (83.8)	36 (97.3)		
Tet methylcytosine dioxygenase 2, n (%)			χ^2=1.057	0.304
Mutation	3 (8.1)	1 (2.7)		
Wild-type	34 (91.9)	36 (97.3)		
Tumor protein 53, n (%)			χ^2=1.057	0.304
Mutation	1 (2.7)	3 (8.1)		
Wild-type	36 (97.3)	34 (91.9)		
KIT proto-oncogene, receptor tyrosine kinase, n (%)			χ^2=1.057	0.304
Mutation	3 (8.1)	1 (2.7)		
Wild-type	34 (91.9)	36 (97.3)		
Protein tyrosine phosphatase non-receptor type 11, n (%)			χ^2=1.930	0.165
Mutation	1 (2.7)	4 (10.8)		
Wild-type	36 (97.3)	33 (89.2)		
PHD finger protein 6, n (%)				
Mutation	0 (0.0)	4 (10.8)	χ^2=4.229	0.040
Wild-type	37 (100.0)	33 (89.2)		
Relapse, n (%)			χ^2=0.063	0.802
Yes	26 (70.3)	25 (67.6)		
No	11 (29.7)	12 (32.4)		
Source of hematopoietic stem, n (%) cell transplantation			χ^2=4.900	0.086
Haplogeneic	2 (5.4)	0 (0.0)		
Sib allogeneic	12 (32.4)	20 (54.1)		
Matched unrelated donor	23 (62.2)	17 (45.9)		

U, Mann-Whitney U; t, translocation; sib, matched sibling.

Table II. Univariate analysis of OS.

Variables	OS Hazard ratio (95% confidence interval)	P-value
MicroRNA-500 (high vs. low)	0.563 (0.330-0.961)	0.035
Age (<60 vs. ≥ 60 years)	1.448 (0.807-2.599)	0.215
White blood cell count (<30 vs. ≥30×10⁹/l)	0.996 (0.585-1.694)	0.988
Fms-related tyrosine kinase 3 mutation	0.624 (0.333-1.170)	0.141
Nucleophosmin 1 mutation	1.099 (0.598-2.019)	0.761
DNA methyltransferase 3 mutation	0.757 (0.410-1.398)	0.374
WT1 transcription factor mutation	0.590 (0.276-1.262)	0.174
Runt-related transcription factor 1 mutation	0.431 (0.201-0.927)	0.031
Tumor protein 53 mutation	0.255 (0.087-0.750)	0.013
PHD finger protein 6 mutation	0.321 (0.114-0.906)	0.032
Lysine methyltransferase 2A mutation	0.309 (0.110-0.869)	0.026

OS, overall survival.

Table III. Multivariate analysis of OS.

Variable	OS Hazard ratio (95% confidence interval)	P-value
MicroRNA-500 (high vs. low)	0.440 (0.241-0.803)	0.008
Age (<60 vs. ≥60 years)	1.448 (0.777-2.697)	0.244
White blood cell count (<30 vs. ≥30x10⁹/l)	1.093 (0.615-1.943)	0.762
Fms-related tyrosine kinase 3 mutation	0.612 (0.288-1.302)	0.203
Nucleophosmin 1 mutation	2.315 (0.990-5.415)	0.053
DNA methyltransferase 3α mutation	0.643 (0.339-1.220)	0.177

OS, overall survival.

FLT3 and DNMT3A R882 mutations were associated with poor prognosis in patients with AML receiving allo-HSCT treatment (16). The results of the current study suggested that miR-500 had an adverse effect on OS time, while mutations in the genes *FLT3*, *NPM1* and *DNMT3A* were not independently associated with OS time in patents with AML receiving allo-HSCT. Thus, the results indicated that while allo-HSCT is an effective treatment strategy that may overcome the adverse effect of classical mutations, miR-500 is a more effective indicator of prognosis in patients with AML receiving allo-HSCT compared with the aforementioned mutations.

The subgroup of patients with AML with inv(16)/CBBB-MYH11, one of the most common cytogenetic aberrations in AML, tend to have an improved prognosis compared with patients without this aberration (17). This was also observed for patients with a low expression level of miR-500 in the present study. The lower expression of miR-500 was associated with a higher proportion of patients with inv(16), which may be associated with an improved prognosis. As presented in Table I, all the patients with a favorable molecular risk were in the miR-500$_{low}$ group in the current study, suggesting that miR-500 expression
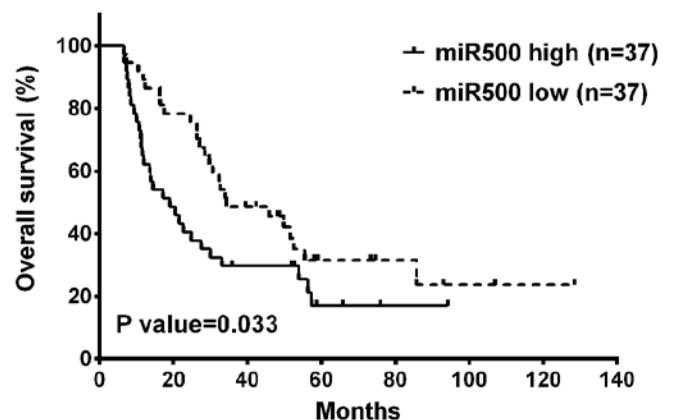

Figure 1. Kaplan-Meier curves for overall survival time. Patients in the high miR-500 expression group had a significantly decreased overall survival time compared with those in the low expression group.

adversely affects the prognosis of patients with AML receiving allo-HSCT. Mutations in the tumor suppressor gene PHF6 may be encountered in patients with AML (18), therefore a PHF6

mutation and high miR-500 expression may result in a poor prognosis. Although *NPM1* mutation is a favorable factor in AML, patients with high expression of miR-500 and *NPM1* mutation were associated with a poor prognosis in the present study.

miR-500 is located on the X chromosome. A previous study has demonstrated that miR-500 may be involved in cell proliferation and apoptosis (10). It may activate the nuclear factor (NF)-κb signaling pathway and suppress NF-κb negative regulatory genes. miR-500 may serve a role in tumorigenesis by sustaining NF-κb activation and disrupting ubiquitin deconjugation (11). Cai *et al* (10) demonstrated that miRNA-500 acts on the downstream target tissue factor pathway inhibitor, and may affect the outcome in human prostate cancer.

Due to the limited number of cases included in the present study, the results obtained have relatively low power. Further analysis with a larger cohort is warranted to validate the prognostic value of miR-500 in patients with AML receiving allo-HSCT. Furthermore, to the best of our knowledge, the miR-500 expression level in patients AML has not been previously investigated and the role of miR-500 in the development of hematological malignancies requires further experimentation *in vitro* and *in vivo*.

In conclusion, the results obtained in the current study indicated that miR-500 may be a potential prognostic biomarker for patients with AML undergoing allo-HSCT.

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Availability of data and materials

All the data in this study were derived from The Cancer Genome Atlas (TCGA) database.

Authors' contributions

XK and LF designed and refined the study, GZ wrote the manuscript, JS analyzed the data, and GZ, XY, XZ, JZ, SY, LZ, KH and JW performed the statistical analyses. XK and LF supervised the study throughout. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Human Research Ethics Committee of Washington University. All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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