Long non-coding RNA ANRIL is a potential indicator of disease progression and poor prognosis in acute myeloid leukemia

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Abstract. The present study explored the association of long non-coding RNA (lncRNA) antisense non-coding RNA in the INK4 locus (ANRIL) with the development of acute myeloid leukemia (AML) clinical features and prognosis of patients with AML. Bone marrow mononuclear cells (BMMCs) were obtained from 178 patients with *de novo* AML prior to initial therapy and from 30 healthy donors. The expression of lncRNA ANRIL in BMMCs was detected by reverse transcription-quantitative PCR. Complete remission (CR) was assessed after induction therapy. Event-free survival (EFS) and overall survival (OS) were evaluated during the follow-up. The levels of lncRNA ANRIL were increased in patients with AML compared with those in healthy donors and were capable of distinguishing patients with AML from healthy donors (area under the curve, 0.886; 95% CI, 0.820-0.952). Furthermore, lncRNA ANRIL was associated with an increased occurrence internal tandem duplications in the FMS-like tyrosine kinase 3, decreased occurrence inv(16) or t(16;6), intermediate-risk and poor-risk stratification while no association of lncRNA ANRIL was identified with French-American-British classification, cytogenetics, isolated biallelic CCAAT/enhancer-binding protein α mutation and nucleophosmin 1 mutation in patients with AML. Furthermore, lncRNA ANRIL was significantly associated with a lower CR rate. In addition, EFS and OS were shorter in patients with high expression of lncRNA ANRIL compared with those in patients with low expression of lncRNA ANRIL. Multivariate Cox regression analyses revealed that high expression of lncRNA ANRIL, poor-risk stratification and white blood cells (>$10.0x10^9$ cells/l) were independent prognostic factors for shorter EFS, while high expression of lncRNA ANRIL and poorer risk stratification were independent prognostic factors for shorter OS. The present results suggested that lncRNA ANRIL has clinical relevance as a biomarker for assisting diagnosis treatment decisions and prognosis prediction and the identification of potential drug target for AML.

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Introduction

Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous malignancy featured by abnormal clonal proliferation of immature myeloid precursors in the bone marrow, which can spread into the blood and other organs such as the spleen and liver (1,2). Patients with AML suffer from fatigue, recurrent infections and hemorrhage, as leukemic cells are dysfunctional (3). Current treatment protocols are restricted to intensive chemotherapy and the judicious use of allogeneic stem cell transplantation (1). However, only 20-30% of patients achieve long-term survival (4). This dismal prognosis is predominantly caused by the development of chemoresistance, unacceptable side effects of intensive chemotherapy and relapse (1). To improve the prognosis of patients with AML, efforts should be made to identify novel and sensitive biomarkers for recognizing patients with AML who are at risk of poor prognosis and for optimizing treatment strategies.

Long non-coding RNA (lncRNA) antisense non-coding RNA in the INK4 locus (ANRIL) is located in the chromosome 9p21 region and is identified in patients with familial melanoma with germline deletion in the INK4B-ARF-INK4A gene cluster (5,6). Several studies have reported that lncRNA ANRIL is essential for the pathogenesis of various cancers (7-13). For instance, lncRNA ANRIL enhances the growth, invasion and migration of cancer cells in laryngeal squamous cell and hepatocellular carcinoma (7,8). In AML, lncRNA ANRIL facilitates cell proliferation, migration and invasion, and represses cell apoptosis by modulating the expression of microRNA (miR)-34a, histone deacetylase 1 and ASPP2 (12). Furthermore, lncRNA ANRIL enhances malignant cell survival via a glucose metabolism pathway involving adiponectin receptor (AdipoR1)/AMP-activated protein kinase (AMPK)/sirtuin 1 (SIRT1) in AML (13). On this basis, it was hypothesized that lncRNA ANRIL may have a clinical implication in the prediction of risk, progression and prognosis of AML. However, to the best of our knowledge, no previous clinical study has reported on this topic. Therefore, the present study aimed to explore the association of lncRNA ANRIL with disease risk, clinical features and prognosis of AML.

Materials and methods

Participants. In the present prospective study, 178 patients with *de novo* AML who were admitted to the Central Hospital of Xiangtan (Xiangtan, China) were consecutively recruited between January 2016 and June 2019. All patients met the following criteria: i) Newly diagnosed with primary AML by morphology, immunophenotyping, cytogenetics or/and molecular genetic examinations, based on the World Health Organization Morphology, Immunology, Cytogenetics Molecular biology classification criteria (14); ii) age ≥18 years; iii) no history of hematopoietic or lymphoid tissue diseases prior to the diagnosis of AML; iv) no complication with other malignancies; and v) ability to be followed up regularly. However, patients were excluded if they had acute promyelocytic leukemia, secondary or relapsed AML, if they were infected with human immunodeficiency virus or if they were pregnant or lactating females. Furthermore, 30 healthy bone marrow donors were enrolled in the present study during the same period when they examined the eligibility for bone marrow transplantation. None of the healthy donors had a history of hematopoietic or lymphoid tissue malignancies and their health status was confirmed prior to bone marrow donation. The present study was approved by the Ethics Committee of the Central Hospital of Xiangtan (Xiangtan, China). All patients with AML and healthy donors voluntarily participated in the present study and signed the informed consent forms prior to enrollment.

Collection of bone marrow and clinical data prior to therapy. Bone marrow samples of enrolled patients were extracted prior to initial therapy, while bone marrow samples from healthy donors were collected when undergoing donation. Bone marrow mononuclear cells (BMMCs) were isolated from the collected samples by density-gradient centrifugation and were stored at -80˚C for subsequent detection of lncRNA ANRIL. The patients' baseline characteristics were documented after completion of diagnostic procedures, which comprised age, gender, French-American-Britain (FAB) classification (15), cytogenetics features, molecular genetic features, risk stratification [based on cytogenetics and molecular abnormalities according to the National Comprehensive Cancer Network AML Guidelines Version 2.2014 (16)] and white blood cell (WBC) count.

Detection of lncRNA ANRIL. The relative expression of lncRNA ANRIL in BMMCs was determined by reverse transcription-quantitative PCR (RT-qPCR). Total RNA from BMMCs was extracted using TRIzol™ reagent (Thermo Fisher Scientific, Inc.). Subsequently, complementary DNA (cDNA) synthesis was conducted with an iScript™ cDNA Synthesis kit (with redon primer; Bio-Rad Laboratories, Inc.) and qPCR was carried out with QTaq™ DNA Polymerase mix (Clontech Laboratories, Inc.) and Applied Biosystems 7900HT Fast Real-Time PCR system (Thermo Fisher Scientific, Inc.). The PCR amplification program was as follows: 95˚C for 30 sec, followed by 40 cycles of amplification (95˚C for 5 sec, 60˚C for 30 sec). GAPDH was applied as an internal reference for lncRNA ANRIL and the relative expression of lncRNA ANRIL was calculated by the $2^{-\Delta\Delta Cq}$ method (17). The primers used are listed in Table I.

Response assessment after induction therapy. Following standard induction therapy with anthracycline (daunorubicin, idarubicin or the anthracenedione mitoxantrone) for 3 days, followed by 7 days of cytarabine or therapies of comparable intensity, response assessment was commonly performed between days 21 and 28 after the start of induction therapy (18). Complete remission (CR) was evaluated according to the response criteria recommended by an international expert panel (on behalf of the European LeukemiaNet) (19).

Follow-up and survival assessment. Surveillance and follow-up were performed every 1-3 months for the first two years and every 3-6 months subsequently. The survival status of patients was recorded during follow-up until June 30, 2019. Event-free survival (EFS) was determined as the time from the date of initial therapy to the date of induction treatment failure, relapse from CR or death. Patients not known to have experienced any of these events at the last follow-up date were censored on the date of their last examination. Overall survival (OS) was determined as the time from the date of initial therapy to the date of death. Patients not known to have died at the last follow-up date were censored on the date they were last known to be alive.

Statistical analysis. Values are expressed as the mean ± standard deviation, median and interquartile range or n (%). The difference in expression of lncRNA ANRIL between patients with AML and healthy donors was determined by the Wilcoxon rank-sum test. For the analysis of correlation of lncRNA ANRIL with clinical features, patients were divided into an lncRNA ANRIL-high group and lncRNA ANRIL-low group according to the median value of lncRNA ANRIL relative expression of all patients with AML. Comparison of clinical features between the lncRNA ANRIL-high and -low groups was performed by χ^2, Fisher's exact and Wilcoxon rank-sum tests. A receiver operating characteristic (ROC) curve was used to evaluate the value of lncRNA ANRIL in differentiating patients with AML from healthy donors. Kaplan-Meier curves were plotted to display the EFS and OS, and the difference of EFS and OS between the lncRNA ANRIL-high and -low groups was determined by the log-rank test. Factors affecting EFS and OS were analyzed by univariate and multivariate Cox's proportional hazard regression models. SPSS version 22.0 (IBM, Corp.) was used for statistical analyses and figures were plotted using GraphPad Prism version 7.00 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of healthy donors and patients with AML. In healthy donors, the mean age was 52.2±22.5 years, and there were 16 (53.3%) females as well as 14 (46.6%) males. The median WBC count was 9.6 (7.6-12.5) $x10^9$ cells/l (Table II). In patients with AML, the mean age was 52.1±15.2 years). There were 66 (37.1%) females and 112 (62.9%) males. Regarding the FAB classification, 65 (36.5%), 48 (27.0%), 58 (32.6%) and 7 (3.9%) patients with AML were classified as FAB M2, M4, M5 and M6, respectively. In terms of cytogenetics, 95 (53.3%), 17 (9.6%), 16 (9.0%), 7 (3.9%), 7 (3.9%), 6 (3.4%), 5 (2.8%), 4 (2.2%), 3 (1.7%), 1 (0.6%), 1 (0.6%), 16 (9.0%) and

Table I. Primers used for quantitative PCR.

Item	Forward primer	Reverse primer
LncRNA ANRIL	TGCTCTATCCGCCAATCAGG	GGGCCTCAGTGGCACATACC
GAPDH	TGACCACAGTCCATGCCATCAC	GCCTGCTTCACCACCTTCTTGA

lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus.

14 (7.9%) patients with AML had normal karyotype (NK), complex karyotype (CK), inv(16) or t(16;16), t(8;21), -7 or 7q-, t(9;11), +8, t(9;22), 11q23, -5 or 5q-, t(6;9), others (undefined) and monosomal karyotype (MK), respectively. Regarding molecular genetics, 39 (21.9%), 16 (9.0) and 66 (37.1%) patients with AML exhibited internal tandem duplications in FMS-like tyrosine kinase 3 (FLT3-ITD), isolated biallelic CCAAT/enhancer-binding protein α (CEBPA) mutation and nucleophosmin 1 (NPMI) mutations, respectively. With respect to risk stratification, 53 (29.8%), 69 (38.8) and 56 (31.4%) patients with AML had better-, intermediate- and poor-risk stratification, respectively. In addition, the median WBC count was 17.7 (8.9-28.6) $x10^9$ cells/l [normal WBC range (4.0-10.0) $x10^9$/l] in patients with AML.

Association of lncRNA ANRIL with AML risk. The relative expression of lncRNA ANRIL was increased in patients with AML compared with that in healthy donors (P<0.001; Fig. 1A). ROC curve analysis revealed that lncRNA ANRIL was able to distinguish patients with AML from healthy donors [area under the curve (AUC), 0.886; 95% CI, 0.820-0.952], with a sensitivity of 84.8% and a specificity of 83.3% at the best cut-off point (where the value of sensitivity plus specificity was the largest) (Fig. 1B).

Association of lncRNA ANRIL with FAB classification and molecular genetics. lncRNA ANRIL was associated with elevated FLT3-ITD mutation (P=0.046; Fig. 2; Fig. 2C, while no association of lncRNA ANRIL with FAB classification (P=0.676; Fig. 2A), MK (P=0.578; Fig. 2B), isolated biallelic CEBPA mutation (P=0.600; Fig. 2D) or NPMI mutation (P=0.352; Fig. 2E) was observed in patients with AML.

Association of lncRNA ANRIL with cytogenetics. lncRNA ANRIL was associated with reduced occurrence inv(16) or t(16;16) cytogenetic type (P=0.009), while no association of lncRNA ANRIL with NK (P=0.881), CK (P=0.202), t(8;21) (P=1.000), -7 or 7q- (P=0.444), t(9;11) (P=1.000), +8 (P=0.368), t(9;22) (P=1.000), 11q23 (P=1.000), -5 or 5q- (P=1.000), t(6;9) (P=1.000) or others (undefined) (P=1.000) was found in patients with AML (Table III).

Association of lncRNA ANRIL with risk stratification. In the lncRNA ANRIL-low expression group, 41.6, 36.0 and 22.4% of cases had a better-, intermediate- and poor-risk stratification, respectively, while in the lncRNA ANRIL-high expression group, 18.0, 41.6 and 40.4% of cases had a better-, intermediate- and poor-risk stratification, respectively. Further comparison indicated that risk stratification

Table II. Baseline characteristics of healthy patients and patients with acute myeloid leukemia (n=178).

Item	Patients with AML, value	Healthy patients, value
Age (years)	52.1±15.2	52.2±22.5
Sex		
Female	66 (37.1)	16 (53.3
Male	112 (62.9)	14 (46.7)
FAB classification		
M2	65 (36.5)	-
M4	48 (27.0)	-
M5	58 (32.6)	-
M6	7 (3.9)	-
Cytogenetics		
NK	95 (53.3)	-
CK	17 (9.6)	-
inv(16) or t(16;16)	16 (9.0)	-
t(8;21)	7 (3.9)	-
-7 or 7q-	7 (3.9)	-
t(9;11)	6 (3.4)	-
+8	5 (2.8)	-
t(9;22)	4 (2.2)	-
11q23	3 (1.7)	-
-5 or 5q-	1 (0.6)	-
t(6;9)	1 (0.6)	-
Others (undefined)	16 (9.0)	-
MK	14 (7.9)	-
FLT3-ITD mutation	39 (21.9)	-
Isolated biallelic CEBPA mutation	16 (9.0)	-
NPMI mutation	66 (37.1)	-
Risk stratification		
Better-risk	53 (29.8)	-
Intermediate-risk	69 (38.8)	-
Poor-risk	56 (31.4)	-
WBC $(x10^9/l)_{\text{normal range (4.0-10.0)}}$	17.7 (8.9-28.6)	9.6 (7.6-12.5)

Values are expressed as the mean ± standard deviation, median (interquartile range) or n (%). Risk stratification was classified based on cytogenetics and molecular abnormalities according to the National Comprehensive Cancer Network AML Guidelines Version 2.2014 (16). AML, acute myeloid leukemia; FAB, French-American-Britain; NK, normal karyotype; CK, complex karyotype; MK, monosomal karyotype; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; CEBPA, CCAAT/enhancer-binding protein α; NPM1, nucleophosmin 1; WBC, white blood cells.

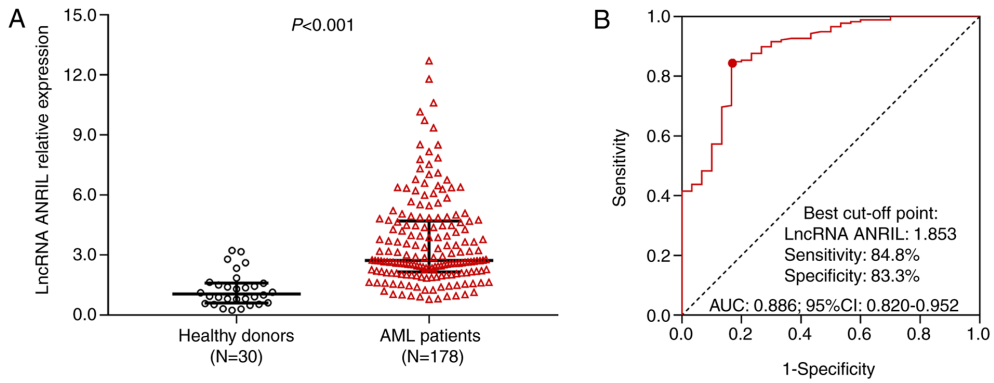

Figure 1. Value of lncRNA ANRIL in distinguishing patients with AML from healthy donors. (A) Comparison of the relative expression of lncRNA ANRIL between patients with AML and healthy donors. Comparison between patients with AML and healthy donors by the Wilcoxon rank-sum test. (B) ROC curve of lncRNA ANRIL for AML. The ability of lncRNA ANRIL to distinguish patients with AML from healthy donors was identified by ROC curve analysis and determination of the AUC with 95% CI. lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus; AML, acute myeloid leukemia; ROC, receiver operating characteristic; AUC, area under the ROC curve.

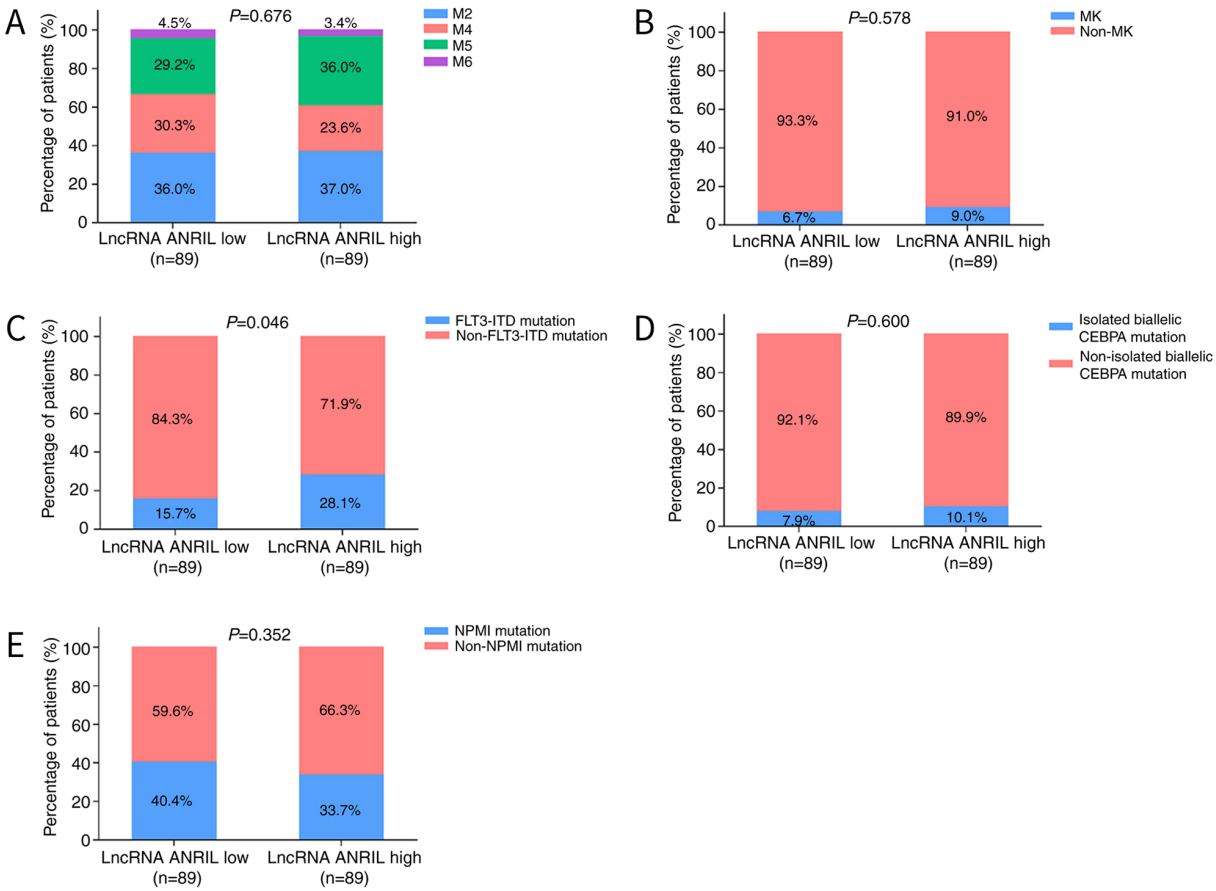

Figure 2. Differences in FAB classification, cytogenetics and molecular genetics between patients with acute myeloid leukemia with high and low expression of lncRNA ANRIL. Comparisons of (A) FAB classification, (B) MK, (C) FLT3-ITD mutation, (D) isolated biallelic CEBPA mutation and (E) NPMI mutation between patients with high and low expression of lncRNA ANRIL. Comparisons were performed with χ^2 tests. lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus; FAB, French-American-Britain; MK, monosomal karyotype; NK, normal karyotype; CK, complex karyotype; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; CEBPA, CCAAT/enhancer-binding protein α; NPM1, nucleophosmin 1.

was poorer in lncRNA ANRIL-high expression patients compared with that in lncRNA ANRIL-low expression patients (P<0.001; Fig. 3).

Predictive value of lncRNA ANRIL for treatment response. In the lncRNA ANRIL-low expression group, 84.3% of cases achieved CR, while 15.7% did not. In the lncRNA ANRIL-high expression group, 68.5% of cases achieved CR, while 31.5% did not. Further analysis suggested that the CR rate was lower in the lncRNA ANRIL-high expression group compared with that in the lncRNA ANRIL-low expression group (P=0.013; Fig. 4).

Table III. Comparison of cytogenetics between lncRNA ANRIL low group and lncRNA ANRIL high group.

Item	lncRNA ANRIL		P-value
	Low	High	
NK	48 (53.9)	47 (52.8)	0.881
CK	6 (6.7)	11 (12.4)	0.202
inv(16) or t(16;16)	13 (14.6)	3 (3.4)	0.009
t(8;21)	3 (3.4)	4 (4.5)	1.000
-7 or 7q-	2 (2.2)	5 (5.6)	0.444
t(9;11)	3 (3.4)	3 (3.4)	1.000
+8	1 (1.1)	4 (4.5)	0.368
t(9;22)	2 (2.2)	2 (2.2)	1.000
11q23	1 (1.1)	2 (2.2)	1.000
-5 or 5q-	1 (1.1)	0 (0.0)	1.000
t(6;9)	1 (1.1)	0 (0.0)	1.000
Others (undefined)	8 (9.0)	8 (9.0)	1.000

NK, normal karyotype; CK, complex karyotype; lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus.

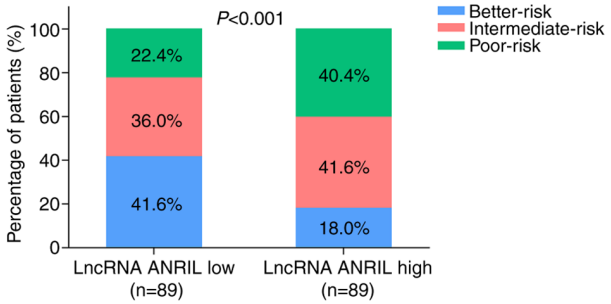

Figure 3. Risk stratification among patients with acute myeloid leukemia with high and low expression of lncRNA ANRIL. Risk stratification was classified based on cytogenetics and molecular abnormalities according to the National Comprehensive Cancer Network AML Guidelines Version 2.2014(16). Comparisons of better-, intermediate- and poor-risk stratification between patients with high and low expression of lncRNA ANRIL were conducted by a Wilcoxon rank-sum test. lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus.

Predictive value of lncRNA ANRIL for EFS and OS. The median EFS was shorter in the lncRNA ANRIL high-expression group than that in the lncRNA ANRIL-low expression group (P<0.001; Fig. 5A). The median OS was also lower in the lncRNA ANRIL-high expression group compared with that in the lncRNA ANRIL-low expression group (P=0.003; Fig. 5B).

Prognostic factors for EFS. Univariate Cox regression analysis indicated that high expression of lncRNA ANRIL [P<0.001, hazard ratio (HR)=1.548-3.300; 95% CI=2.260], poorer risk stratification (P<0.001, HR=1.859-3.124; 95% CI=2.410) and WBC >10.0x10^9 cells/l (P=0.005, HR=1.194-2.790; 95% CI=1.825) were predictors of unfavorable EFS in patients with AML (Table IV). Subsequent multivariate Cox regression

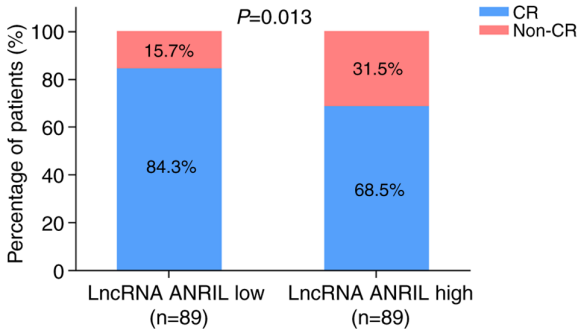

Figure 4. Influence of the expression of lncRNA ANRIL on CR of patients with acute myeloid leukemia. Comparisons of the proportions of CR and non-CR among patients with high and low expression of lncRNA ANRIL were performed using the χ^2 test. CR, complete remission; lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus.

analysis adjusted for lncRNA ANRIL high, age (>55 years), male, FAB classification, poorer risk stratification and WBC (>10.0x10^9 cells/l), which revealed that high expression of lncRNA ANRIL (P=0.002, HR=1.256-2.838; 95% CI=1.888), poorer risk stratification (P<0.001, HR=1.852-3.206; 95% CI=2.436) and WBC >10.0x10^9 cells/l (P<0.001, HR=1.470-3.629, 95% CI=2.309) were independent prognostic factors for poor EFS in patients with AML.

Prognostic factors for OS. Univariate Cox regression analysis revealed that high expression of lncRNA ANRIL (P=0.004, HR=1.311-4.061, 95% CI=2.308) and a poorer risk stratification (P<0.001, HR=1.984-4.446, 95% CI=2.970) were predictors of poor OS in patients with AML (Table V). Subsequent multivariate Cox regression analysis adjusted for lncRNA ANRIL high, age (>55 years), male, FAB classification, poorer risk stratification and WBC (>10.0x10^9 cells/l), which demonstrated that high expression of lncRNA ANRIL (P=0.047, HR=1.008-3.259, 95% CI=1.812) and poorer risk stratification (P<0.001, HR=2.884, 95% CI=1.885-4.413) were independent prognostic factors for shorter OS in patients with AML.

LncRNA ANRIL expression in relapsed/refractory patients with AML. The relative expression of lncRNA ANRIL was elevated in patients with relapsed AML compared with that in patients with *de novo* AML (P=0.044) and healthy donors (P<0.001; Fig. S1). Furthermore, the relative expression of lncRNA ANRIL was also higher in patients with refractory AML than that in patients with *de novo* AML (P=0.004) and healthy donors (P<0.001).

Discussion

The present study revealed that i) lncRNA ANRIL was elevated in patients with AML compared with its levels in healthy donors and was able to distinguish patients with AML from healthy donors; ii) lncRNA ANRIL was associated with increased FLT3-ITD mutation and poorer risk stratification in patients with AML patients; and iii) lncRNA ANRIL was associated with a lower CR rate after induction therapy and unfavorable survival in patients with AML.

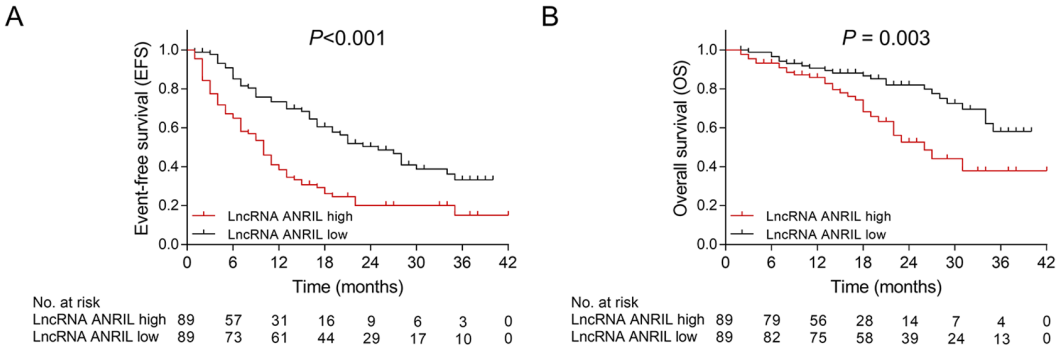

Figure 5. Differences in ESF and OS between patients with acute myeloid leukemia with high and low expression of lncRNA ANRIL. Comparisons of (A) EFS and (B) OS between patients with high and low expression of lncRNA ANRIL. Kaplan-Meier curves were drawn and log-rank tests were used to compare EFS and OS between patients with high and low expression of lncRNA ANRIL. lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus; EFS, event-free survival; OS, overall survival.

lncRNAs are abundant in the nucleus and cytoplasm, and have been established as important regulators of gene transcription, post-transcription processes, translation and epigenetic modification (20). Aberrant expression and dysfunction of lncRNAs are closely linked to tumorigenesis (21). Among the lncRNAs that have been identified thus far, lncRNA ANRIL has been reported to be involved in the development and progression of various cancer types (8,10,13,22,23). In triple-negative breast cancer, lncRNA ANRIL was reported to enhance cell proliferation and suppresses apoptosis via sponging miR-199a (22). In gastric cancer, lncRNA ANRIL knockdown was observed to inhibit cell viability, migration and invasion, and facilitate cell apoptosis by miR-99a-mediated downregulation of B-lymphoma Moloney murine leukemiavirus insertion region 1 (23). Regarding hematological malignancies, lncRNA ANRIL knockdown was indicated to suppress cell proliferation, migration and invasion, and facilitate cell apoptosis in AML (12). However, the clinical implications of lncRNA ANRIL in patients with AML have remained to be elucidated. In the present study, it was observed that lncRNA ANRIL was higher in patients with AML than in healthy donors, and it was able to differentiate patients with AML from healthy donors (AUC, 0.886; 95% CI, 0.820-0.952). Furthermore, lncRNA ANRIL was associated with increased FLT3-ITD mutation, reduced inv(16) or t(16;16) cytogenetic type, and poorer stratification in patients with AML. Several possible explanations have been proposed: i) lncRNA ANRIL may silence the tumor suppressor gene p15 (INK4B) by recruiting polycomb repressive complex 2, leading to the malignant growth of myeloid precursors (24). Thereby, lncRNA ANRIL is associated with a higher risk of AML; and ii) lncRNA ANRIL may repress the expression of AdipoR1 and modulate AMPK/SIRT1, which increases glucose uptake and malignant cell survival to accelerate AML progression (13). Thereby, lncRNA ANRIL is associated with increased FLT3-ITD mutation and poorer risk stratification in patients with AML.

lncRNA ANRIL is associated with dismal prognosis in multiple solid cancer types (10,11). *In vitro*, lncRNA ANRIL facilitates colorectal cancer cell chemoresistance through the regulation of ATP-binding cassette subfamily C member 1 by binding Let-7a (24). In the clinic, patients with head and neck squamous cell carcinoma (HNSCC) and high lncRNA ANRIL expression exhibit worse recurrence-free survival and OS compared with those of patients with HNSCC and low lncRNA ANRIL expression (10). In patients with esophageal squamous cell carcinoma, high expression of lncRNA ANRIL was determined to be associated with shorter disease-free survival (DFS) and OS, and to be an independent prognostic factor for DFS and OS according to the results of multivariate analyses (11). However, the prognostic value of lncRNA ANRIL in AML has not been investigated to date, to the best of our knowledge. Considering the present results that lncRNA ANRIL was associated with FLT3-ITD mutation, intermediate-risk and poor-risk stratification in patients with AML, it was hypothesized that lncRNA ANRIL may also be associated with poor prognosis in AML. Further analysis suggested that lncRNA ANRIL was associated with a lower CR rate after induction therapy, as well as with shorter EFS and OS in patients with AML. Furthermore, multivariate Cox regression analyses suggested that high expression of lncRNA ANRIL was an independent prognostic factor for shorter ESF and OS in patients with AML. The following are possible reasons: i) According to the present results, lncRNA ANRIL was positively associated with FLT3-ITD mutation; this reportedly leads to promotion of aberrant STAT5 activation and suppressed forkhead box O3 (a member of the pro-apoptotic regulator forkhead family of transcription factors), which in turn amplifies reactive oxygen species production, increases the frequency of double-strand DNA breaks and enhances AML cell survival and proliferation, resulting in genomic instability, advanced tumor stage and poor prognosis in patients with AML (25); ii) in the present study, lncRNA ANRIL was positively associated with intermediate/poor risk stratification, which was linked to poor prognosis in patients with AML; and iii) lncRNA ANRIL may induce the chemoresistance of tumor cells and increase their colony formation ability via positively regulating ATP-binding cassette subfamily C member 1, resulting in drug resistance in patients with AML (24). Thereby, patients with AML who exhibited high expression of lncRNA ANRIL had a lower CR rate and shorter EFS and OS compared with those of patients with AML who exhibited low expression of lncRNA ANRIL.

Several limitations of the present study should be noted. First, the follow-up duration of the present study was relatively short and should be extended to validate the present results

Table IV. Analysis of factors affecting EFS.

	Univariate Cox regression		Multivariate Cox regression	
Factor	P-value	HR (95%CI)	P-value	HR (95%CI)
LncRNA ANRIL high	<0.001	2.260 (1.548-3.300)	0.002	1.888 (1.256-2.838)
Age (>55 years)	0.856	0.966 (0.667-1.400)	0.694	1.080 (0.736-1.584)
Male sex	0.167	1.315 (0.892-1.938)	0.835	0.955 (0.622-1.467)
FAB classification				
M2	Reference	-	Reference	-
M4	0.275	0.767 (0.475-1.236)	0.329	0.781 (0.475-1.283)
M5	0.501	1.164 (0.748-1.812)	0.915	0.976 (0.623-1.529)
M6	0.737	0.852 (0.335-2.165)	0.974	1.016 (0.394-2.623)
Poorer risk stratification	<0.001	2.410 (1.859-3.124)	<0.001	2.436 (1.852-3.206)
WBC (>10.0x10^9/l)	0.005	1.825 (1.194-2.790)	<0.001	2.309 (1.470-3.629)

Factors affecting EFS were analyzed by univariate and multivariate logistic regression using Cox's proportional hazard model. For multivariate logistic regression, lncRNA ANRIL high, age (>55 years), male, FAB classification, poorer risk stratification and WBC (>10.0x10^9 cells/l) were adjusted. Risk stratification was classified based on cytogenetics and molecular abnormalities according to the National Comprehensive Cancer Network AML Guidelines Version 2.2014 (16). EFS, event-free survival; lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus; HR, hazard ratio; FAB, French-American-Britain; WBC, white blood cell.

Table V. Analysis of factors affecting OS.

	Univariate Cox's regression		Multivariate Cox's regression	
Factor	P-value	HR (95%CI)	P-value	HR (95%CI)
LncRNA ANRIL high	0.004	2.308 (1.311-4.061)	0.047	1.812 (1.008-3.259)
Age (>55 years)	0.682	0.891 (0.512-1.550)	0.917	0.970 (0.543-1.733)
Male sex	0.823	0.938 (0.535-1.645)	0.364	0.753 (0.409-1.388)
FAB classification				
M2	Reference	-	Reference	-
M4	0.929	1.032 (0.520-2.046)	0.612	0.830 (0.405-1.705)
M5	0.518	1.255 (0.630-2.499)	0.770	0.899 (0.442-1.831)
M6	0.871	0.885 (0.203-3.858)	0.845	0.862 (0.195-3.816)
Poorer risk stratification	<0.001	2.970 (1.984-4.446)	<0.001	2.884 (1.885-4.413)
WBC (>10.0x10^9/l)	0.705	0.897 (0.511-1.575)	0.840	1.063 (0.590-1.915)

Factors affecting OS were analyzed by univariate and multivariate logistic regression using Cox's proportional hazards model. For multivariate logistic regression, lncRNA ANRIL high, age (>55 years), male, FAB classification, poorer risk stratification and WBC (>10.0x10^9 cells/l) were adjusted. Risk stratification was classified based on cytogenetics and molecular abnormalities according to the National Comprehensive Cancer Network AML Guidelines Version 2.2014 (16). OS, overall survival; lncRNA, long non-coding RNA; ANRIL, antisense noncoding RNA in the INK4 locus; HR, hazard ratio; FAB, French-American-Britain; WBC, white blood cells.

in future studies. Furthermore, since it was difficult to enroll healthy donors for the present study due to the very limited healthy volunteers for bone marrow donation, the sample size of the healthy donor group was relatively small, which may reduce the statistical power. Hence, further studies with a larger sample size are required to validate the present results. Finally, only the expression level of lncRNA ANRIL at baseline was assessed, while changes in the expression levels of lncRNA ANRIL after treatment were not investigated; therefore, further investigation would be desirable in patients with AML.

To conclude, lncRNA ANRIL is associated with higher disease risk, exacerbated clinical features and poor prognosis of patients with AML, suggesting that lncRNA ANRIL may serve as a biomarker for facilitating treatment decisions and improving prognosis in patients with AML.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZT and ZL designed the study, KZ and YY performed the experiments, ZT and ZL analyzed the data and all authors wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of the Central Hospital of Xiangtan (Xiangtan, China). All AML patients and healthy donors voluntarily participated in the present study and provided written informed consent prior to enrollment.

Patient consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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