

The implication of circulating long non-coding RNA MALAT1 in diagnosis, disease surveillance and prognosis of acute ischemic stroke

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Abstract

Background We aimed to investigate predictive value of long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (lnc-MALAT1) for acute ischemic stroke (AIS) risk, and the association of lnc-MALAT1 expression with disease severity, inflammation as well as recurrence free survival (RFS) in AIS patients.

Methods 120 AIS patients and 120 controls were recruited. Venous blood samples from AIS patients (within 24 hours after symptoms onset) and controls (at entry to study) were collected to detect plasma lnc-MALAT1 expression by real-time quantitative polymerase chain reaction. For AIS patients, AIS severity was assessed by NIHSS score; plasma concentrations of inflammation factors (including C-reactive protein (CRP), tumor necrosis factor α (TNF- α), interleukin (IL)-6, IL-8, IL-10, IL-17 and IL-22) were measured; and RFS was calculated.

Results lnc-MALAT1 expression was decreased in AIS patients compared to controls, and it had a good predictive value for AIS risk (AUC=0.791, 95% CI: 0.735-0.846). For disease condition, lnc-MALAT1 expression negatively correlated with NIHSS score and pro-inflammatory factors expressions (including CRP, TNF- α , IL-6, IL-8 and IL-22), while it positively correlated with anti-inflammatory factor IL-10 expression. Besides, lnc-MALAT1 expression was elevated in AIS complicated with diabetes but numerically reduced in AIS complicated with hypertension. For prognosis, lnc-MALAT1 high expression numerically correlated with longer RFS, but without statistical significance.

Conclusion lnc-MALAT1 is downregulated and has a good predictive value of AIS risk, and its high expression correlates with decreased NIHSS score, reduced inflammation, as well as numerically better RFS in AIS patients.

Background

Acute ischemic stroke (AIS), a common disorder caused by embolic or thromboembolic occlusion in an artery supplying the brain, results in irreversible infarction of brain tissue and functional impairment, which brings economic and social burden for families.[1-3] Current treatments of AIS mainly focus on recovering cerebral circulation and lead to a great likelihood of independent living following AIS, while several restrictions exist in current therapy (such as narrow treatment time window and low recanalization rate), and the prognosis of AIS is still far from satisfactory.[4-6] Increasing studies disclose that early identification of AIS risk and prediction of prognosis may help improve treatment outcomes of AIS patients.[7, 8] Thus, searching for convincing biomarkers that monitoring AIS risk and predicting prognosis is urgently needed.

Long non-coding RNAs (lncRNAs) are transcripts with more than 200 nucleotides and rarely encode proteins, which play essential roles in regulation of protein-coding genes and signaling pathways related to development of diseases.[8] lncRNA metastasis-associated lung adenocarcinoma transcript 1 (lnc-MALAT1), located on human chromosome 11q13.1, is a lncRNA abundantly expressed and evolutionarily

conserved throughout a variety of mammalian species.[7] Accumulating data from basic and clinical studies have disclosed that lnc-MALAT1 could protect ischemia-induced brain microvascular endothelial cells (BMECs), implying that lnc-MALAT1 might regulate cerebrovascular pathologies in stroke.[9, 10] Meanwhile, lnc-MALAT1 has been reported to be downregulated and primarily plays an anti-inflammatory role in neurological diseases patients as well as cardiovascular and cerebrovascular diseases patients. [11, 12] Considering the participation of lnc-MALAT1 in cerebrovascular pathologies of stroke and its anti-inflammation effect on cardiovascular and cerebrovascular diseases, we hypothesized that lnc-MALAT1 might serve as a biomarker for disease risk and progression in AIS through affecting inflammation level, whereas related evidences were seldomly reported. Hence, we conducted this study to investigate the predictive value of lnc-MALAT1 for AIS risk, and the association of lnc-MALAT1 expression with disease severity, inflammation level as well as recurrence free survival (RFS) in AIS patients.

Methods

Participants

In total, 120 patients with AIS and 120 controls with high stroke risk factors were recruited from our hospital between July 2014 and June 2017. For the AIS patients, screening criteria were as follows: (i) primary diagnosis of AIS by clinical presentation, brain non-contrast computed tomography (CT) or diffusion-weighted magnetic resonance imaging, according to the "Guidelines for the Early Management of Patients with Acute Ischemic Stroke: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association (AHA/ASA)"[13]; (ii) age more than 18 years; (iii) admitted to our hospital within 24 hours after the symptoms onset; (iv) able to be regularly followed up. Following AIS patients were excluded: (i) had evidence of intracranial hemorrhage; (ii) treated with immunosuppressant within 1 month; (iii) suffered from infection (active or in the preceding 14 days of stroke); (iv) accompanied with hematological malignancies or solid tumors; (v) women who were pregnant or nursing. As for the controls, all of them had no history of stroke or malignancies and were presented with at least three stroke risk factors, such as hypertension, diabetes mellitus, hyperlipidemia, smoke and so on. The Institutional Review Board of our hospital approved the study protocol, and all participants or their guardians provided written informed consents.

Data collection and stroke severity assessment

At entry to the study, the baseline data of AIS patients was obtained through interview and medical records, which included age, gender, body mass index (BMI), smoke, hypertension, diabetes mellitus, hyperlipidemia, hypeluricemia and chronic kidney disease (CKD). Severity of AIS was assessed within the day of admission by use of the National Institutes of Health Stroke Scale (NIHSS) score. The NIHSS score aimed at assessing neurological impairment ranging from 0 to 42, a higher score indicated a more serious nerve damage, and the classification of severity was as follows: 0-1 point, normal or near normal; 2-4 points, mild stroke; 5-15 points, moderate stroke; 16-20 points, moderate-severe stroke; 21-42 points,

severe stroke. Besides, controls' basic characteristics including age, gender, BMI, smoke, hypertension, diabetes mellitus, hyperlipidemia, hypeluricemia and CKD were also documented on the enrollment.

Blood samples collection and determination

Venous blood samples were collected from AIS patients (within 24 hours after symptoms onset) and controls using ethylene diamine tetraacetic acid (EDTA) tubes, and subsequently centrifuged at 1600 g for 10 min (within 30 min) to acquire supernatant, then the supernatant was further centrifuged at 16000g for 10 min to obtain the plasma, which was scored at -80 °C for further analysis. Lnc-MALAT1 relative expression in the plasma of AIS patients and controls was determined by real-time quantitative polymerase chain reaction (RT-qPCR). AIS patients' CRP concentration in plasma was detected using Fully automatic POCT fluorescence immunoassay analyzer (GeteinBiotech, Nanjing, Jiangsu, China), and the plasma levels of inflammatory cytokines including tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), IL-8, IL-10, IL-17 and IL-22 were measured by human enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's recommendations.

RT-qPCR

Using QIAamp RNA Blood Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German), total RNA was extracted from plasm samples. Then, reverse transcription to cDNA was performed by PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara, Dalian, Liaoning, China), and qPCR was performed using TB Green™ Fast qPCR Mix (Takara, Dalian, Liaoning, China)). GAPDH was applied as the internal reference. Primers used in the RT-qPCR were as follows: Inc-MALAT1, forward (5'→3'): TCCTAAGGTCAAGAGAAGTGTCAG, reverse (5'→3'): GTGGCGATGTGGCAGAGAA; GAPDH, forward (5'→3'): TGACCACAGTCCATGCCATCAC, reverse (5'→3'): GCCTGCTTCACCACCTTCTTGA.

Follow up

All AIS patients received appropriate treatments as recommended by 2013 AIS Guidelines (AHA/ASA), and were followed up regularly or as clinically indicated. The last follow-up date was June 30, 2018, and the median follow-up duration was 25.0 months (range: 1.0-42.0 months). RFS was calculated from the date of hospital admission to the date of recurrence or death.

Statistical analysis

SPSS 24.0 statistical software (SPSS Inc, Chicago, IL, USA) was used for statistical data processing, and the GraphPad Prism 6.01 (GraphPad Software Inc., San Diego, CA, USA) was applied to the graphs plotting. Continuous variables were expressed as mean \pm standard deviation (SD) or median and interquartile range (IQR), and the categorical variables were expressed as number (percentage). Differences between groups were determined by Student's t test, Wilcoxon rank sum test or Chi-square test. Correlations between variables were analyzed by Spearman rank test. Diagnostic value of variable was assessed by receiver operating characteristic (ROC) curve analysis and the derived area under the

curve (AUC) as well as 95% confidence interval (CI). RFS profiles were illuminated by plotting the Kaplan-Meier (K-M) curve, and the difference of RFS between groups was determined by the log-rank test. Factors affecting RFS were analyzed by univariate and multivariate Cox's proportional hazards regression models. All tests were 2-sided, and P values <0.05 were considered statistically significant.

Results

Characteristics in AIS patients and controls

120 AIS patients and 120 controls were enrolled in this study (Table 1). The mean age of AIS patients and controls were 62.4 ± 12.3 years and 61.1 ± 11.0 years respectively. Meanwhile, there were 82 males as well as 38 females in AIS patients, and 87 males as well as 33 females in controls. Besides, mean BMI was 24.6 ± 2.8 kg/m² and 24.1 ± 2.8 kg/m² in AIS patients and controls respectively. No difference of age ($P = 0.363$), gender ($P = 0.479$), BMI ($P = 0.159$) between AIS patients and controls. Also, the percentage of smoke ($P = 0.796$), hypertension ($P = 0.421$), diabetes mellitus ($P = 0.182$), hyperlipidemia ($P = 0.296$), hypeluricemia ($P = 0.888$) as well as CKD ($P = 0.360$) was similar between two groups. The detailed information about other baseline characteristics were shown in Table 1.

Disease severity and inflammatory factors in AIS patients

In AIS patients, mean NIHSS score was 7.8 ± 3.5 and the median NIHSS score was 7.0 (5.0-10.0) (Table 2). As to inflammatory factors, mean values of CRP, TNF- α , IL-6, IL-8, IL-10, IL-17 and IL-22 were 35.3 ± 21.6 mg/L, 79.5 ± 56.1 pg/mL, 51.7 ± 29.9 pg/mL, 62.0 ± 35.1 pg/mL, 23.3 ± 12.6 pg/mL, 101.9 ± 49.1 pg/mL and 85.8 ± 64.9 pg/mL, and the median values of these factors were 29.0 (23.2-38.7) mg/L, 60.1 (44.9-94.6) pg/mL, 44.7 (38.1-54.3) pg/mL, 50.4 (40.2-76.4) pg/mL, 20.9 (14.1-30.9) pg/mL, 96.2 (65.3-122.7) pg/mL and 67.8 (48.5-96.9) pg/mL respectively.

Comparison of lnc-MALAT1 expression between AIS patients and controls

lnc-MALAT1 expression was decreased in AIS patients compared to controls ($P < 0.001$) (Figure 1A). And ROC curve showed that lnc-MALAT1 had a good predictive value for AIS risk (AUC=0.791, 95% CI: 0.735-0.846) (Figure 1B).

Correlation of lnc-MALAT1 expression with NIHSS score in AIS patients

Spearman rank test was used to determine the correlation of lnc-MALAT1 expression with NIHSS score, which showed that lnc-MALAT1 expression was negatively correlated with NIHSS score in AIS patients ($P < 0.001$, $r = -0.437$) (Figure 2).

Correlation of lnc-MALAT1 expression with inflammatory factors in AIS patients

lnc-MALAT1 expression was negatively associated with expressions of inflammatory factors including CRP ($P < 0.001$, $r = -0.354$) (Figure 3A), TNF- α ($P = 0.021$, $r = -0.211$) (Figure 3B), IL-6 ($P < 0.001$, $r = 0.328$)

(Figure 3C), IL-8 ($P = 0.037$, $r = -0.191$) (Figure 3D) and IL-22 ($P = 0.017$, $r = -0.218$) (Figure 3G), while it was positively correlated with expression of anti-inflammatory cytokine IL-10 ($P = 0.010$, $r = 0.235$) (Figure 3E) in AIS patients. Besides, no correlation of lnc-MALAT1 expression with IL-17 expression ($P = 0.250$, $r = -0.106$) (Figure 3F) was found.

Correlation of lnc-MALAT1 expression with complications in AIS patients

To investigate the correlation of lnc-MALAT1 expression with complications in AIS patients, all patients were categorized into different subgroups according to their complications (Figure 4). lnc-MALAT1 expression was elevated in diabetes compared to non-diabetes ($P = 0.017$) (Figure 4B), whereas it was numerically reduced in hypertension patients compared with non-hypertension patients ($P = 0.081$) (Figure 4A). Besides, no difference of lnc-MALAT1 expression was found between hyperlipidemia patients and non-hyperlipidemia patients ($P = 0.391$) (Figure 4C), hyperuricemia patients and non-hyperuricemia patients ($P = 0.657$) (Figure 4D), or CKD patients and non-CKD patients ($P = 0.264$) (Figure 4E). These data indicated that lnc-MALAT1 expression was positively correlated with diabetes occurrence in AIS patients.

Comparison of RFS between lnc-MALAT1 high expression patients and lnc-MALAT1 low expression patients

Patients were divided into lnc-MALAT1 high expression group and lnc-MALAT1 low expression group according to the median value of lnc-MALAT1 expression. RFS in AIS patients with lnc-MALAT1 high expression was numerically longer compared to AIS patients with lnc-MALAT1 low expression, but without statistical significance ($P = 0.053$) (Figure 5).

Analysis of factors affecting RFS in AIS patients

Univariate Cox's regression analysis displayed that lnc-MALAT1 high expression was numerically associated with better RFS in AIS patients (although no statistical significance) ($P = 0.063$), while CRP (≥ 29.0 vs. < 29.0 mg/L) was associated with worse RFS ($P = 0.006$) (Table 3). Moreover, multivariate Cox's regression analysis showed that lnc-MALAT1 high expression was not an independent predictive factor for RFS, whereas CRP (≥ 29.0 vs. < 29.0 mg/L) was an independent factor predicting reduced RFS ($P = 0.024$).

Discussion

Our results indicated that: (1) lnc-MALAT1 expression was decreased in AIS patients compared to controls, and it had a good predictive value of AIS risk; (2) lnc-MALAT1 high expression correlated with decreased NIHSS score as well as reduced inflammatory factors levels in AIS patients; (3) lnc-MALAT1 high expression numerically correlated with prolonged RFS in AIS patients, but without statistical significance.

LncRNAs are involved in a variety of biological processes (such as genes transcription, organizing RNA-protein complex, and management in protein activity).[14-17] As one of the frequently investigated lncRNAs, lnc-MALAT1 has various molecular functions (including alternative splicing, transcriptional regulation and post-transcriptional regulation), and also participates in multiple physiological functions (including neural development, skeletal myogenesis and vascular growth).[7] As to its role in neurological diseases or cerebrovascular diseases, previous studies demonstrate that lnc-MALAT1 has protective effects in these diseases via repressing proapoptotic or pro-inflammatory factors.[10, 11, 18, 19] For instance, a study displays that lnc-MALAT1 protects human brain vascular endothelial cells from OGD-induced apoptosis through activating phosphatidylinositol 3-kinase (PI3K).[19] In addition, lnc-MALAT1 expression is decreased in the spinal cords of mice with experimental autoimmune encephalomyelitis, and its knockdown raises levels of inflammatory cytokines (including IL-1 and IL-6).[11] Also, silencing of lnc-MALAT1 raises the levels of proapoptotic factor Bim and pro-inflammatory cytokines (including MCP-1 as well as E-selectin) in BMECs following oxygen-glucose deprivation (OGD), which is an *in vitro* mimic of ischemic stroke conditions.[10] Another study discloses that lnc-MALAT1 knockdown aggravates OGD-induced overexpression of pro-inflammatory cytokines including MCP-1 and IL-6 in mouse cerebral microvascular endothelial cells.[18] These studies reveal that lnc-MALAT1 has protective effects in neurological diseases or cerebrovascular diseases through decreasing inflammation level or inhibiting cell apoptosis.

Previous clinical trials mainly focus on the exploration of lnc-MALAT1 in cancer patients, which display that lnc-MALAT1 plays a tumor-promotive role in these cancer patients, while the information about the role of lnc-MALAT1 in neurological diseases patients or cerebrovascular diseases patients is still limited.[20-22] Just a few studies display that lnc-MALAT1 expression is reduced in central nervous system tissues from multiple sclerosis patients and carotid plaques from atherosclerosis patients.[11, 12] As for the correlation of lnc-MALAT1 with disease severity and inflammation in neurological diseases or cerebrovascular diseases patients, only a study shows that elevated lnc-MALAT1 expression is correlated with less advanced lesions in atherosclerosis patients.[12] Considering that lnc-MALAT1 might participate in the cerebrovascular pathologies of stroke according to previous studies, meanwhile, it presented anti-inflammatory effect in cardiovascular and cerebrovascular diseases, we hypothesized that lnc-MALAT1 expression might be related to the disease risk, progression or inflammation level of AIS, while related explorations in AIS are seldomly reported.[9-12, 23] In our study, we assessed the predictive value of lnc-MALAT1 for AIS risk, and we discovered that lnc-MALAT1 expression was lower in AIS patients compared to controls, and lnc-MALAT1 expression presented good diagnostic value for AIS, which might due to its protective effect in these diseases via repressing cell apoptosis of BMECs. Moreover, we investigated the correlation of lnc-MALAT1 expression with disease severity as well as inflammation in AIS patients, and we observed that lnc-MALAT1 high expression was associated with decreased NIHSS score, reduced levels of inflammatory factors (including CRP, TNF- α , IL-6, IL-8 and IL-22), meanwhile it reduced proportion of diabetes but numerically increased proportion of hypertension (without significant difference) in AIS patients. The possible reasons of these results might be as follows: (1) lnc-MALAT1 had protective influence on brain microvascular endothelial cells through inhibiting cell apoptosis, thereby

contributed to maintaining a healthy brain endothelium that was essential for normal cerebrovascular physiology, therefore the severity of AIS was attenuated and decreased NIHSS score was observed[7, 18]; (2) Inc-MALAT1 might decrease the expression of transcriptional factor nuclear factor-kappa B (NF-κB) that drove the transcription of a series of inflammatory factors, thus led to reduced inflammatory factors levels and alleviated inflammation in AIS patients[24]; (3) Inc-MALAT1 protected the brain microvascular endothelial cells against apoptosis, thereby facilitated microvascular permeability and helped restore cerebral vasoreactivity, thus it might attenuate hypertension in AIS patients[23] (4) Inc-MALAT1 was found to interact with transcription factor Foxo1 and SIRT1 transcription to induce poor glycemic control and insulin resistance, which might promote the occurrence of diabetes.[25, 26]

As to the prognostic value of Inc-MALAT1 in human diseases, it has been identified as a valuable biomarker for disease prognosis in several cancers, while limited clinical studies have been found in AIS, only a study displays that Inc-MALAT1 high expression is associated with prolonged major adverse cardiovascular and cerebrovascular events (MACCE)-free survival in atherosclerosis patients.[12] To our knowledge, there was still no evidence about the influence of Inc-MALAT1 on the prognosis in AIS patients. In our study, we observed that Inc-MALAT1 expression was numerically positively correlated with RFS in AIS patients, but without significant difference. These results might be due to: (1) Inc-MALAT1 reduced the production of inflammation cytokines and further decreased inflammation and attenuated disease progression, thus led to better RFS in AIS patients[10, 18]; (2) Inc-MALAT1 might facilitate sensitivity to treatment in AIS, thereby increased treatment efficacy and resulted in longer RFS in AIS patients.

Some limitations existed in our study: (1) sample size of 120 AIS patients was relatively small, thus the statistical power might be low; (2) the median follow-up duration (25.0 months (range: 1.0-42.0 months)) was relatively short, thus the correlation of Inc-MALAT1 expression with long-term prognosis was not investigated; (3) detailed mechanism of Inc-MALAT1 in AIS was still unclear, and further study was still needed.

Conclusions

In conclusion, Inc-MALAT1 is downregulated and has a good predictive value of AIS risk, and its high expression correlates with decreased NIHSS score, reduced inflammation, as well as numerically better RFS in AIS patients.

Abbreviations

Inc-MALAT1: long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; AIS: acute ischemic stroke; RFS: recurrence free survival; CRP: C-reactive protein; TNF-α: tumor necrosis factor α; lncRNAs: Long non-coding RNAs; BMECs: brain microvascular endothelial cells; CT: computed tomography; BMI: body mass index; CKD: chronic kidney disease; EDTA: ethylene diamine tetraacetic acid; RT-qPCR: real-time quantitative polymerase chain reaction

Declarations

Acknowledgments

Not applicable.

Ethics approval and written consent to participate

The Institutional Review Board of HanDan Central Hospital approved the study protocol, and all participants or their guardians provided written informed consents.

Authors contributions

HR designed and approved the entire study; HR prepared the draft; FW, BL and ZS collected and analyzed the data; DQ revised the manuscript; All authors have read and approved the final manuscript.

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None.

Availability of data and materials

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

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Characteristics of AIS patients and controls

Parameters	AIS patients (N=120)	Controls (N=120)	<i>P</i> value
Age, mean±SD, years	62.4±12.3	61.1±11.0	0.363
Gender (male/female)	82/38	87/33	0.479
BMI, mean±SD, kg/m ²	24.6±2.8	24.1±2.8	0.159
Smoke, No. (%)	58 (48.3)	60 (50.0)	0.796
Hypertension, No. (%)	108 (90.0)	104 (86.7)	0.421
Diabetes mellitus, No. (%)	26 (21.7)	18 (15.0)	0.182
Hyperlipidemia, No. (%)	55 (45.8)	47 (39.2)	0.296
Hypeluricemia, No. (%)	35 (29.2)	36 (30.0)	0.888
CKD, No. (%)	20 (16.7)	15 (12.5)	0.360

Differences were determined by Student's t test or Chi-square test. *P* value <0.05 was considered as significant. AIS: acute ischemic stroke; SD: standard deviation; BMI: body mass index; CKD: chronic kidney disease;

Table 2 Disease severity, CRP and inflammatory cytokines of AIS patients

Parameters	Mean	SD	Median	IQR
NIHSS score	7.8	3.5	7.0	5.0-10.0
CRP, mg/L	35.3	21.6	29.0	23.2-38.7
TNF- α , pg/mL	79.5	56.1	60.1	44.9-94.6
IL-6, pg/mL	51.7	29.9	44.7	38.1-54.3
IL-8, pg/mL	62.0	35.1	50.4	40.2-76.4
IL-10, pg/mL	23.3	12.6	20.9	14.1-30.9
IL-17, pg/mL	101.9	49.1	96.2	65.3-122.7
IL-22, pg/mL	85.8	64.9	67.8	48.5-96.9

SD: standard deviation; IQR: inter-quartile range; NIHSS: National Institutes of Health Stroke Scale; CRP: C-reactive protein; TNF- α : tumor necrosis factor α ; IL: interleukin.

Table 3 Factors affecting RFS by Cox's proportional hazards regression model analyses

Items	Univariate Cox's regression		Multivariate Cox's regression	
	<i>P</i> value	HR (95%CI)	<i>P</i> value	HR (95%CI)
Inc-MALAT1 (high vs. low)	0.062	0.402 (0.154-1.047)	0.200	0.482 (0.157-1.473)
Age (≥ 61 vs. < 61 years)	0.976	0.986 (0.406-2.397)	0.974	0.981 (0.307-3.137)
Gender (male/female)	0.853	1.095 (0.421-2.850)	0.965	0.974 (0.304-3.120)
BMI (≥ 24.2 vs. < 24.2 kg/m ²)	0.506	0.741 (0.307-1.791)	0.725	0.835 (0.305-2.282)
Smoke (yes vs. no)	0.456	0.712 (0.291-1.741)	0.509	0.726 (0.281-1.876)
Hypertension (yes vs. no)	0.836	1.167 (0.270-5.047)	0.704	1.371 (0.269-6.980)
Diabetes mellitus (yes vs. no)	0.193	0.378 (0.088-1.633)	0.526	0.585 (0.111-3.075)
Hyperlipidemia (yes vs. no)	0.853	0.920 (0.380-2.227)	0.978	1.015 (0.365-2.821)
Hypeluricemia (yes vs. no)	0.958	1.026 (0.394-2.673)	0.712	0.819 (0.283-2.370)
CKD (yes vs. no)	0.821	1.136 (0.377-3.418)	0.609	1.435 (0.360-5.728)
NIHSS score (≥ 7 vs. < 7)	0.428	0.701 (0.292-1.687)	0.229	0.557 (0.215-1.445)
CRP (≥ 29.0 vs. < 29.0 mg/L)	0.006	4.683 (1.563-14.028)	0.024	4.720 (1.224-18.197)
TNF- α (≥ 60.1 vs. < 60.1 pg/mL)	0.126	2.052 (0.818-5.151)	0.350	1.879 (0.501-7.041)
IL-6 (≥ 44.7 vs. < 44.7 pg/mL)	0.301	1.605 (0.655-3.931)	0.899	1.069 (0.381-2.996)
IL-8 (≥ 50.4 vs. < 50.4 pg/mL)	0.988	0.993 (0.413-2.387)	0.387	0.559 (0.149-2.088)
IL-10 (≥ 20.9 vs. < 20.9 pg/mL)	0.133	0.493 (0.196-1.239)	0.548	0.707 (0.227-2.195)
IL-17 (≥ 96.2 vs. < 96.2 pg/mL)	0.586	0.783 (0.324-1.889)	0.153	0.474 (0.170-1.319)
IL-22 (≥ 67.8 vs. < 67.8 pg/mL)	0.299	1.608 (0.656-3.942)	0.716	0.797 (0.235-2.705)

P value <0.05 was considered significant. RFS: recurrence free survival; HR: hazard ratio; CI: confidence interval; BMI: body mass index; CKD: chronic kidney disease; NIHSS: National Institutes of Health Stroke Scale; CRP: C-reactive protein; TNF- α : tumor necrosis factor α ; IL: interleukin.

Figures

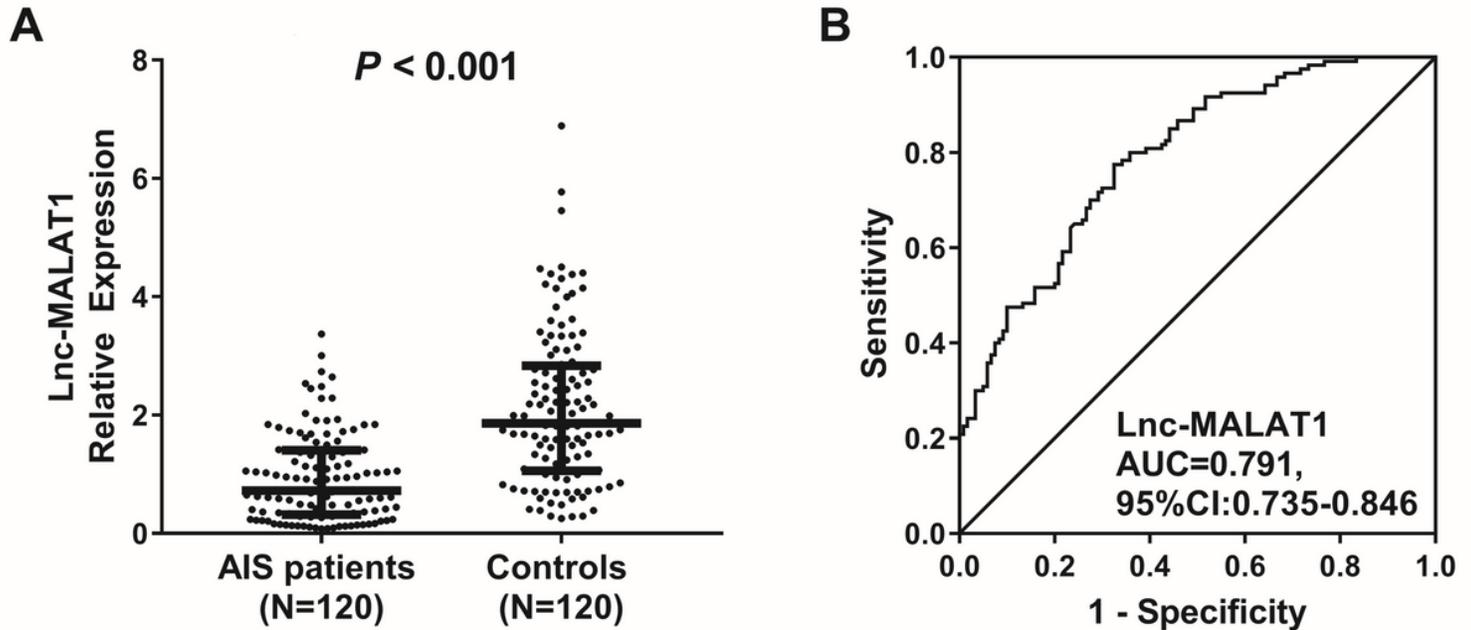


Figure 1

Lnc-MALAT1 expression in AIS patients and controls. Lnc-MALAT1 relative expression in AIS patients and controls (A). Diagnostic value of Lnc-MALAT1 for AIS assessed by ROC curve (B). Comparison between two groups was determined by Wilcoxon rank sum test. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; AIS, acute ischemic stroke; ROC curve, receiver operating characteristic curve. $P < 0.05$ was considered significant.

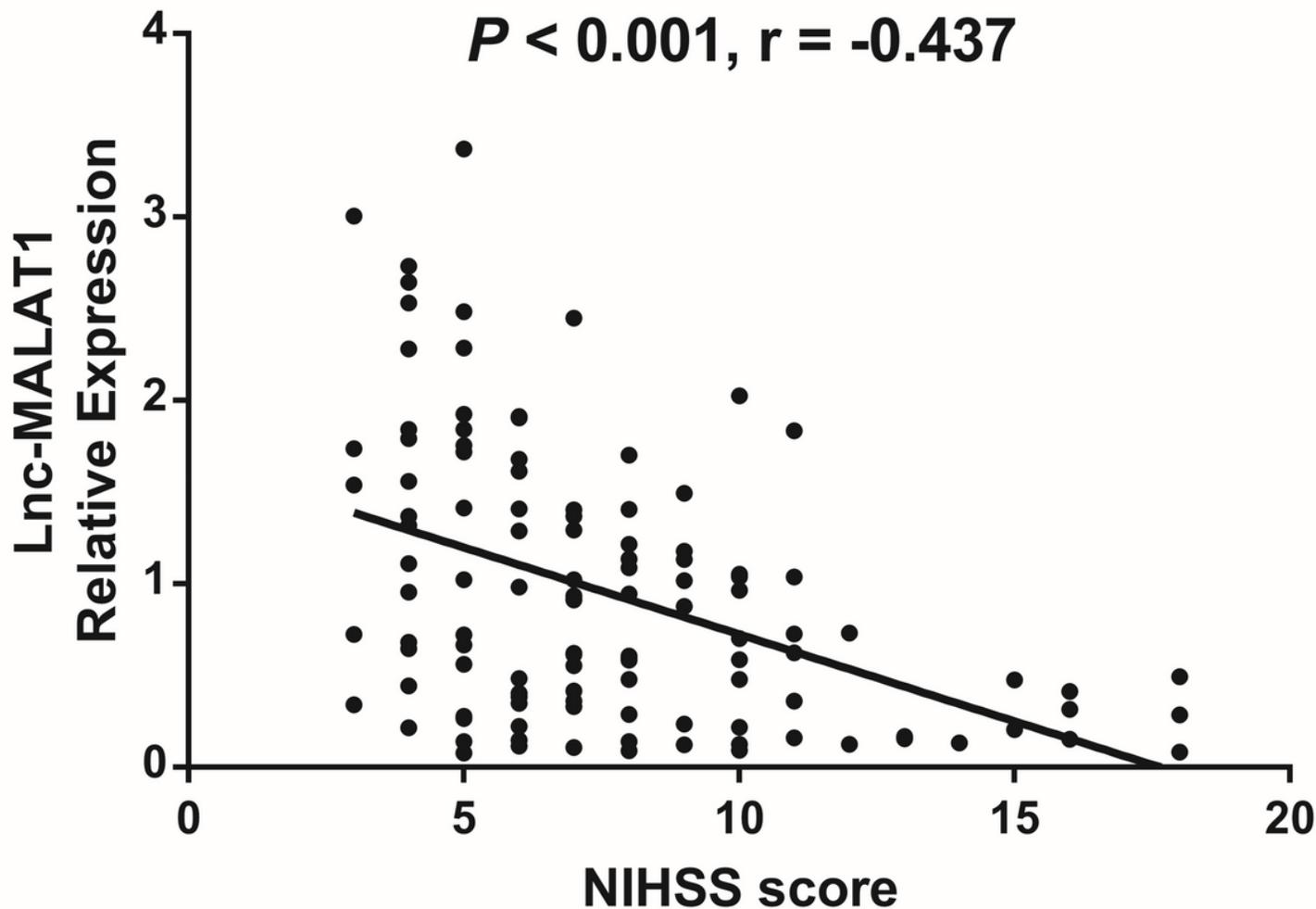


Figure 2

Correlation of Lnc-MALAT1 expression with NIHSS score. Lnc-MALAT1 high expression was correlated with decreased NIHSS score. Correlation of Lnc-MALAT1 expression with NIHSS score was determined by Spearman rank test. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; NIHSS score, National Institutes of Health Stroke Scale score. $P < 0.05$ was considered significant.

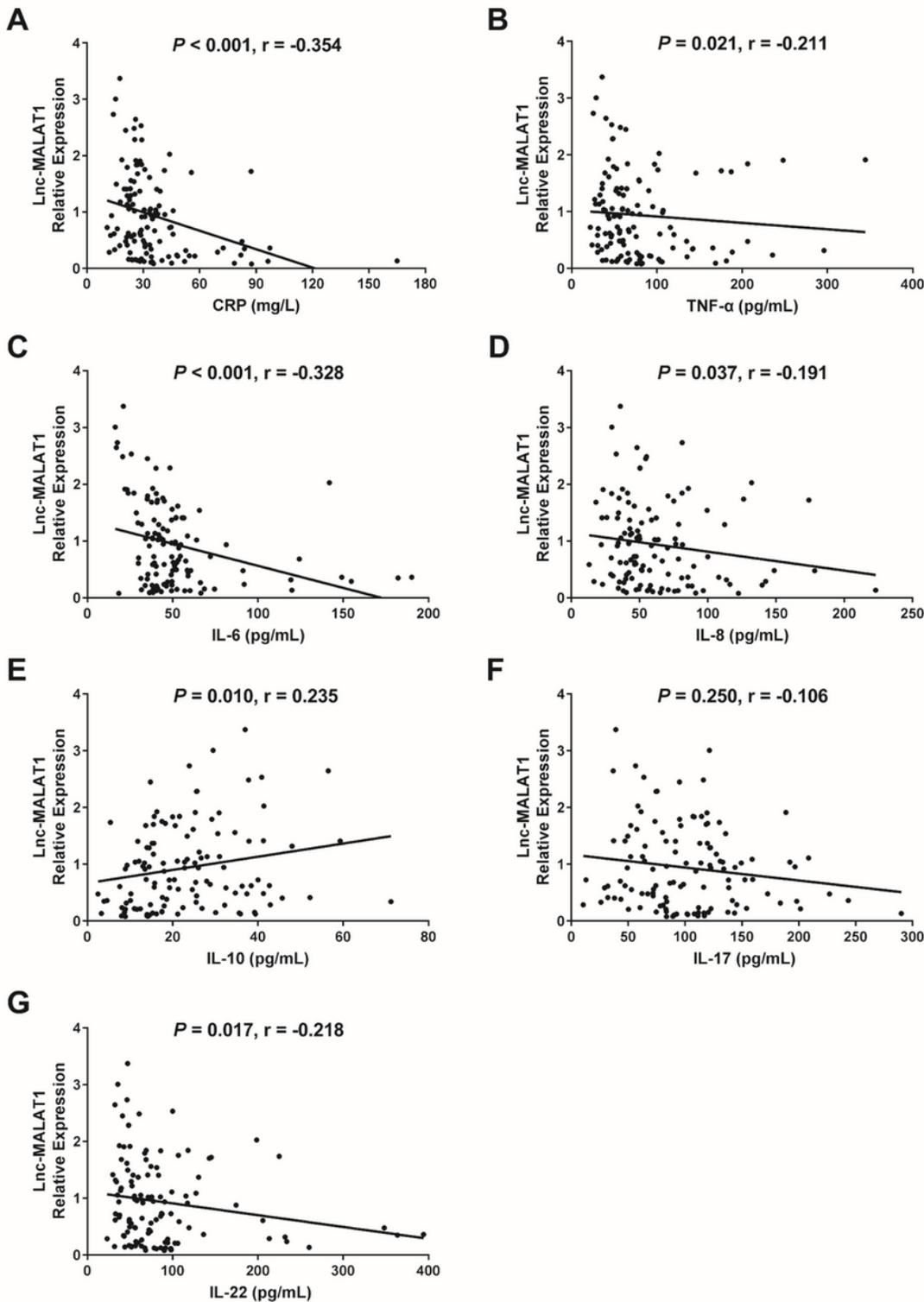


Figure 3

Correlation of Lnc-MALAT1 expression with inflammatory factors levels. Correlation of Lnc-MALAT1 expression with CRP (A), TNF- α (B), IL-6 (C), IL-8 (D), IL-10 (E), IL-17 (F), IL-22 (G) was performed. Correlation of Lnc-MALAT1 with inflammatory cytokines was determined by Spearman rank test. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; CRP, C-reactive

protein; TNF- α , tumor necrosis factor α ; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-17, interleukin-17; IL-22, interleukin-22. $P < 0.05$ was considered significant.

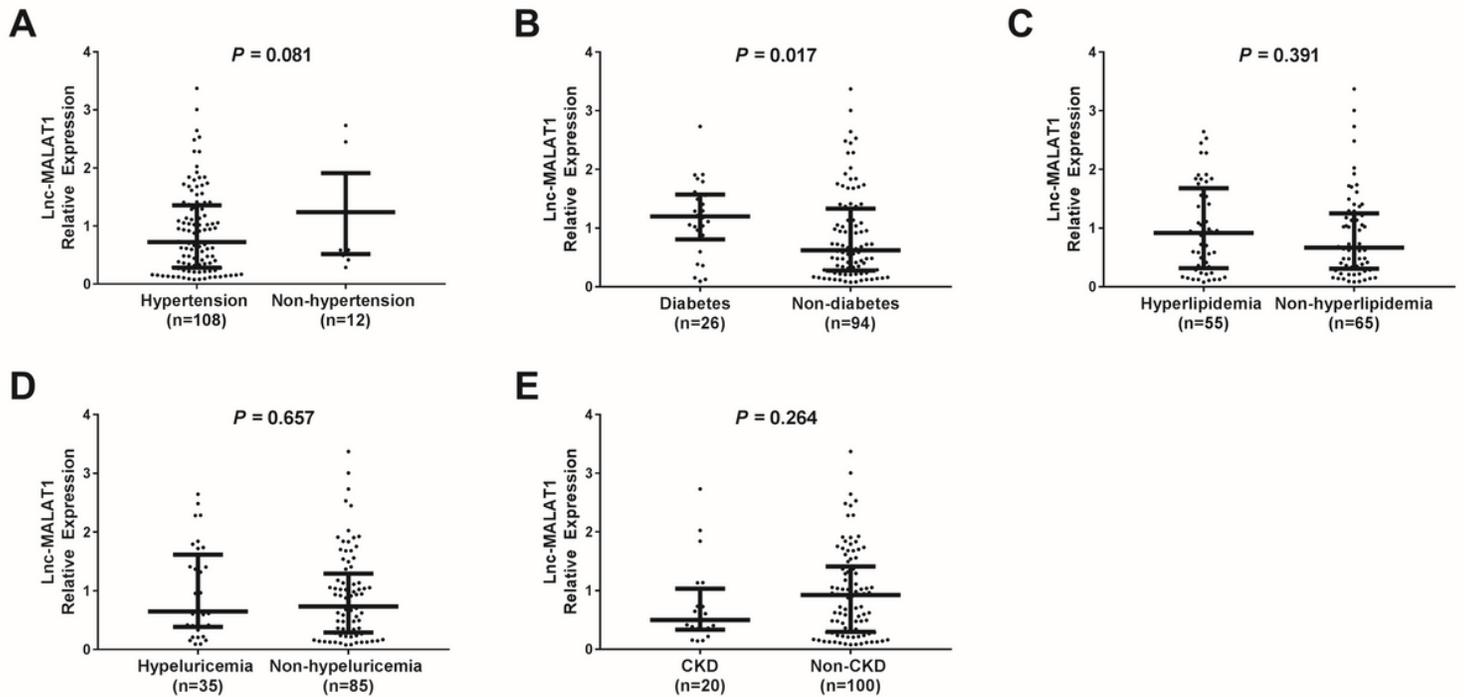


Figure 4

Lnc-MALAT1 expression in AIS patients with different complications. Lnc-MALAT1 in hypertension and non-hypertension patients (A), diabetes patients and non-diabetes patients (B), hyperlipidemia and non-hyperlipidemia patients (C), hypeluricemia and non-hypeluricemia patients (D) as well as CKD and non-CKD patients (E). Comparison between two groups was determined by Wilcoxon rank sum test. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; AIS, acute ischemic stroke; CKD, chronic kidney disease. $P < 0.05$ was considered significant.

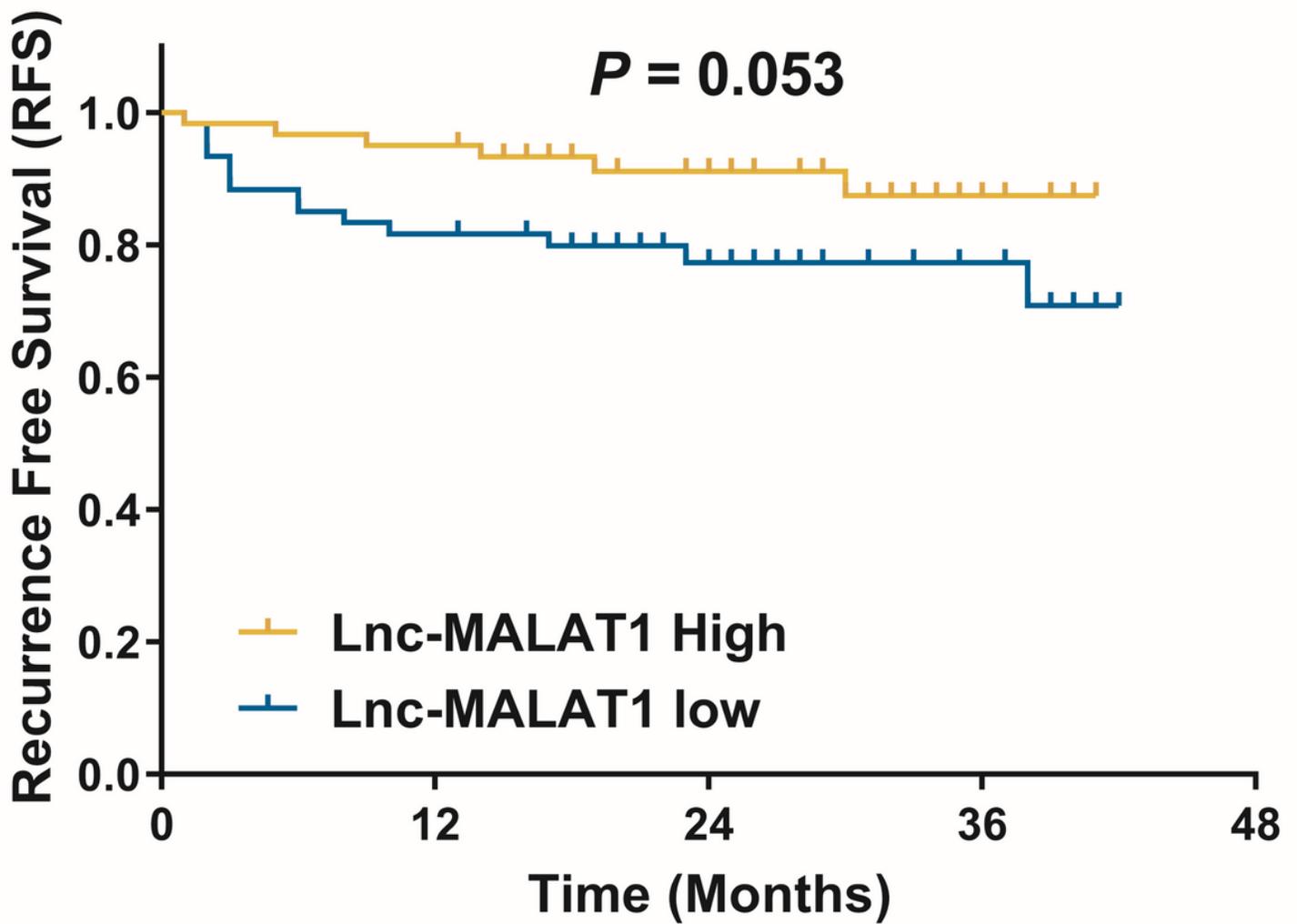


Figure 5

K-M curves analysis. K-M curves displayed the RFS in Lnc-MALAT1 high expression and Lnc-MALAT1 low expression patients. Comparison of RFS between two groups was determined by log-rank test. RFS, recurrence free survival; Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; K-M curves, Kaplan-Meier curves. $P < 0.05$ was considered significant.