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Long noncoding RNA NEAT1/microRNA-125a axis predicts increased major adverse cardiac and cerebrovascular event risk independently in patients with unprotected left main coronary artery disease underwent coronary artery bypass grafting

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Abstract

Background: The study aimed to investigate the long noncoding RNA nuclear-enriched abundant transcript 1 (lnc-NEAT1) and microRNA-125a (miR-125a) expressions, and further explore the role of lnc-NEAT1/miR-125a axis in predicting major adverse cardiac and cerebrovascular event (MACCE) risk in patients with unprotected left main coronary artery disease (ULMCAD) underwent coronary artery bypass grafting (CABG).

Methods: A total of 280 patients with ULMCAD underwent CABG were consecutively enrolled in our prospective study, and their plasma samples were collected before CABG for the detection of lnc-NEAT1 and miR-125a expressions by reverse transcription quantitative polymerase chain reaction. Lnc-NEAT1/miR-125a axis was calculated via dividing lnc-NEAT1 by miR-125a. After CABG, regular follow-up was continued until MACCE occurrence or 36 months.

Results: Lnc-NEAT1 expression, miR-125a expression, and lnc-NEAT1/miR-125a axis were 0.998 (IQR: 0.440-1.720, range: 0.116-5.771), 0.997 (IQR: 0.461-1.650, range: 0.055-3.621), and 1.018 (IQR: 0.384-2.782, range: 0.041-52.832), respectively. And lnc-NEAT1 was negatively associated with miR-125a. The 1-, 2-, and 3-year MACCE occurrence was 19 (6.8%), 29 (10.4%), and 38 (13.6%), respectively. Lnc-NEAT1/miR-125a axis (χ^2 = 11.207, P = .001) and lnc-NEAT1 expression (χ^2 = 5.345, P = .021) positively associated with accumulating MACCE occurrence, while miR-125a expression (χ^2 = 5.869, P = .015) negatively correlated with accumulating MACCE occurrence. Notably, lnc-NEAT1/miR-125a axis presented numerically better predictive value compared with lnc-NEAT1 or miR-125a alone for MACCE risk. Furthermore, lnc-NEAT1/miR-125a axis high, elderly age, increased BMI, diabetes, previous stroke, LVEF, and higher disease extent (all P < .05) were independent predictive factors for increased accumulating MACCE occurrence.

Conclusion: Lnc-NEAT1/miR-125a axis, as a combined index, presents potential value to be a prognostic biomarker for MACCE risk in ULMCAD management.

1 | INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality and responsible for plenty of healthcare burden globally.[1] Unprotected left main coronary artery disease (ULMCAD) is a severe cardiovascular disease, where there is a ≥50% stenosis in left main coronary artery without protective bypass grafts to the left anterior descending/left circumflex coronary arteries.[2] Coronary revascularization for ULMCAD has undergone considerable advancement in the last few decades, and coronary artery bypass grafting (CABG), as one of revascularization strategy, has been considered as the first line management for patients with ULMCAD.[3-5] However, patients with ULMCAD still suffer unfavorable prognosis due to several safety composite endpoints of major adverse cardiac and cerebrovascular events (MACCE).[5-7] Hence, it is necessary to discover novel biomarkers, which can facilitate the evaluation of MACCE risk earlier and then improve disease management of ULMCAD.

Long noncoding RNA nuclear-enriched abundant transcript 1 (lnc-NEAT1) is exhibited to function as a structural component of paraspeckles, which regulates multiple gene expressions by nuclear retention.[8] Existing researches report that lnc-NEAT1 participates in various cellular and biological processes in cardiac and cerebrovascular diseases, such as atherosclerosis, myocardial infarction, and stroke.[9-13] For example, one functional experiment in cardiomyocyte injury suggests that lnc-NEAT1 is upregulated in the ischemia/reperfusion myocardium, and its knockdown decreases the trend of hypoxia/reoxygenation-induced cardiomyocyte apoptosis.[12] Furthermore, based on the miRanda database and the previous studies reported, lnc-NEAT1 serves as the sponge of microRNA-125a (miRNA-125a), and miR-125a is involved in the glycolipid metabolism and inflammatory responses of cardiac and cerebrovascular diseases, including heart failure, myocardial infarction, and acute ischemic stroke.[9,14-18] According to these previous evidence and the data that lnc-NEAT1 was upregulated while miR-125a was downregulated in MACCE-occurred ULMCAD patients compared to non–MACCE-occurred ULMCAD patients in our preliminary findings, and meanwhile, considering the targeted interaction between lnc-MALAT1 and miR-125a, we hypothesized that lnc-NEAT1/miR-125a axis might be correlated with the prognosis in ULMCAD patients underwent CABG, which was not ever explored before. Therefore, we performed the present study to investigate the lnc-NEAT1 and miR-125a relative expressions in patients with ULMCAD underwent CABG and further explore the role of lnc-NEAT1/miR-125a axis in predicting MACCE risk in these patients.

2 | MATERIALS AND METHODS

2.1 | Study design

The objective of our study was to investigate the lnc-NEAT1 and miR-125a expressions, and further explore the role of lnc-NEAT1/miR-125a axis in predicting MACCE risk in patients with ULMCAD underwent CABG. We prospectively enrolled 280 eligible patients with ULMCAD who underwent CABG, and their plasma samples were collected before CABG for the detection of lnc-NEAT1 and miR-125a expressions by reverse transcription quantitative polymerase chain reaction (RT-qPCR). After CABG, all patients were followed up until MACCE occurrence or 36 months.

2.2 | Patients

A total of 280 eligible patients with ULMCAD who underwent CABG in our hospital between January 2014 and July 2016 were consecutively enrolled in this prospective study. The inclusion criteria were as follows: (a) confirmed as ULMCAD according to the coronary angiography (left main artery luminal narrowing of more than 50% without patent bypass grafts to its branches[2]); (b) age between 18 and 80 years old; (c) willingness to receive CABG and had no contraindication for CABG; and (d) no history of CABG, aortic surgery, or cardiogenic shock. Besides, the patients who had concomitant valvular or ST-segment elevation myocardial infarction (MI) within 1 week were excluded. This study was approved by the Institutional Review Board of Zibo Central Hospital, and all patients signed the informed consents before enrollment.

2.3 | Data and sample collection

For the patients, clinical characteristics were recorded after they were enrolled, which included demographic characteristics, CAD risk factors, clinical presentation, left ventricular ejection fraction (LVEF) level, and angiographic information. Peripheral blood samples (5 mL) of patients were collected on the day before CABG, and then, the blood samples were centrifuged at 2000 g for 20 minutes under 4°C. Subsequently, plasma samples were isolated from the blood samples and stored at −80°C until detection.

2.4 | Reverse transcription quantitative polymerase chain reaction

Reverse transcription quantitative polymerase chain reaction was used to detect the relative expressions of lnc-NEAT1 and miR-125a in plasma. Total RNA (200 ng) was extracted from plasma using TRIzol™ Reagent (Thermo Fisher Scientific) and then reversely transcribed using iScript™ cDNA Synthesis Kit (Bio-Rad).[19,20] Following that, qPCR was performed using KOD SYBR® qPCR Mix (Toyobo) to quantify expressions of lnc-NEAT1 and miR-125a. And the expression levels of lnc-NEAT1 (with GADPH as an internal reference[21,22]) and miR-125a (with U6 as an internal reference [20,23,24]) were calculated using $2^{-\triangle\triangle Ct}$ method. Primers were listed as follows:

FIGURE 1 Study flow. ULMCAD, unprotected left main coronary artery disease; CABG, coronary artery bypass graft; lnc-NEAT1, long noncoding RNA nuclear-enriched abundant transcript 1; miR-125a, microRNA-125a; and MACCE, major adverse cardiac and cerebrovascular event

lnc-NEAT1 forward primer: TGTCCCTCGGCTATGTCAGA, reverse primer: GAGGGGACGTGTTTCCTGAG; miR-125a forward primer: ACACTCCAGCTGGGTCCCTGAGACCCTTTAAC, reverse primer: TGTCGTGGAGTCGGCAATTC; GAPDH forward primer: TGAC CACAGTCCATGCCATCAC, reverse primer: GCCTGCTTCACCAC CTTCTTGA; U6 forward primer: CTCGCTTCGGCAGCACATATACTA; reverse primer: ACGAATTTGCGTGTCATCCTTGC.

2.5 | Follow-up

After CABG, patients were followed up every 3 ~ 6 months by clinic visits or telephone calls, during which, MACCE occurrence status was recorded in the follow-up documents. The follow-up was continued for all patients until MACCE occurrence or 36 months. Reference to a pervious study,[25] MACCE was defined as a composite of death, myocardial infarction, stroke, or repeat revascularization. The accumulating MACCE occurrence was calculated from the date of CABG to the date of MACCE occurrence. For the patients who lost follow-up, they were censored on the date of last visit.

2.6 | Statistical analysis

Correlation between lnc-NEAT1 relative expression and miR-125a relative expression was determined by Spearman's rank correlation test. Accumulating MACCE occurrence was displayed by Kaplan-Meier curve, and the difference of accumulating MACCE occurrence between two groups was determined by log-rank test. Factors predicting MACCE occurrence were analyzed by univariate and forward stepwise multivariate Cox's proportional hazard regression model. Statistical analyses were performed using SPSS software (version 22.0; IBM). Figures were plotted using GraphPad Prism software (version 7.00; GraphPad Software). All tests were two-side, and *P* value <.05 was considered as significant.

3 | RESULTS

3.1 | Study flow

Totally 301 patients with ULMCAD who were about to receive CABG treatment were screened, and among them, 21 patients were excluded (including 16 patients who did not meet the inclusion criteria or met the exclusions, 5 patients who disagreed to sign informed consents) (Figure 1). The remaining 280 ULMCAD patients were eligible, and then their blood samples were collected for determination of lnc-NEAT1 as well as miR-125a relative expressions before CABG. Regular follow-up was continued for all patients until MACCE occurrence or 36 months after CABG, and 242 (86.4%) patients completed the follow-up, and 38 patients (13.6%) lost follow-up (including 23 patients (8.2%) could not be
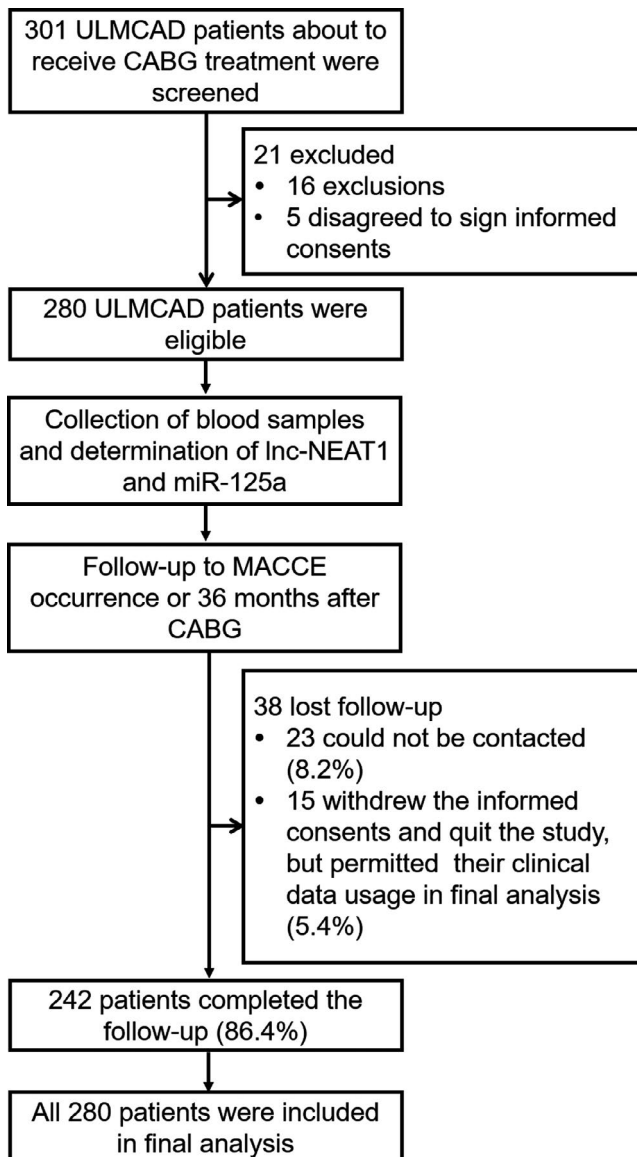
contacted and 15 patients (5.4%) withdrew the informed consents and quit the study, but permitted their clinical data usage in final analysis. For the 38 patients who lost follow-up, their MACCE data were censored on the date of last visit. Finally, 280 patients were included in the final analysis.

3.2 | Baseline characteristics of ULMCAD patients underwent CABG

The mean age of all patients with ULMCAD was 64.6 ± 7.7 years (Table 1). The number of male and female patients were 220 (78.6%) and 60 (21.4%), respectively. There were 70 (25.0%) patients with previous myocardial infarction, 18 (6.4%) patients with previous stroke, 11 (3.9%) patients with previous heart failure, and 33 (11.8%) patients with previous percutaneous coronary intervention (PCI). As for clinical presentation, 140 (50.0%) patients were with unstable angina, and another 140 (50.0%)

TABLE 1 Baseline characteristics

Items	ULMCAD patients (N = 280)
Age (years), mean ± SD	64.6 ± 7.7
Gender (male/female), No.	220/60
BMI (kg/m^2), mean ± SD	25.1 ± 2.9
Hypertension, No. (%)	178 (63.6)
Hyperlipidemia, No. (%)	155 (55.4)
Diabetes, No. (%)	85 (30.4)
Current Smoker, No. (%)	78 (27.9)
Chronic lung disease, No. (%)	12 (4.3)
Chronic renal failure, No. (%)	11 (3.9)
Family history of CAD, No. (%)	50 (17.9)
Previous myocardial infarction, No. (%)	70 (25.0)
Previous stroke, No. (%)	18 (6.4)
Previous heart failure, No. (%)	11 (3.9)
Previous PCI, No. (%)	33 (11.8)
Clinical presentation, No. (%)	
Unstable angina	140 (50.0)
Stable angina	140 (50.0)
LVEF < 50%, No. (%)	54 (19.3)
Disease extent, No. (%)	
Left main only	7 (2.5)
Left main + 1 vessel disease	19 (6.8)
Left main + 2 vessel disease	57 (20.3)
Left main + 3 vessel disease	197 (70.4)
Distal bifurcation involvement, No. (%)	192 (68.6)
Right CAD involvement, No. (%)	215 (76.8)

Abbreviations: BMI, body mass indexes; CAD, coronary artery disease; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; SD, standard deviation; ULMCAD, unprotected left main coronary artery disease.

patients were with stable angina. Furthermore, the number of patients with LVEF <50% was 54 (19.3%). Regarding the disease extent, patients with left main only, left main + 1 vessel disease, left main + 2 vessel disease, and left main + 3 vessel disease were 7 (2.5%), 19 (6.8%), 57 (20.3%), and 197 (70.4%), respectively. More detailed baseline information of patients with ULMCAD was listed in Table 1.

3.3 | The correlation between lnc-NEAT1 and miR-125a in ULMCAD patients underwent CABG

In patients with ULMCAD, the lnc-NEAT1 relative expression was 0.998 (inter-quartile range (IQR): 0.440-1.720) (range: 0.116-5.771) (Figure 2A). The miR-125a relative expression was 0.997 (IQR: 0.461-1.650) (range: 0.055-3.621) (Figure 2B). Lnc-NEAT1/miR-125a axis was 1.018 (IQR: 0.384-2.782) (range: 0.041-52.832) (Figure 2C). As for the correlation of lnc-NEAT1 with miR-125a, lnc-NEAT1 relative expression was negatively associated with miR-125a relative expression ($P < .001$, $r = −0.331$) (Figure 2D).
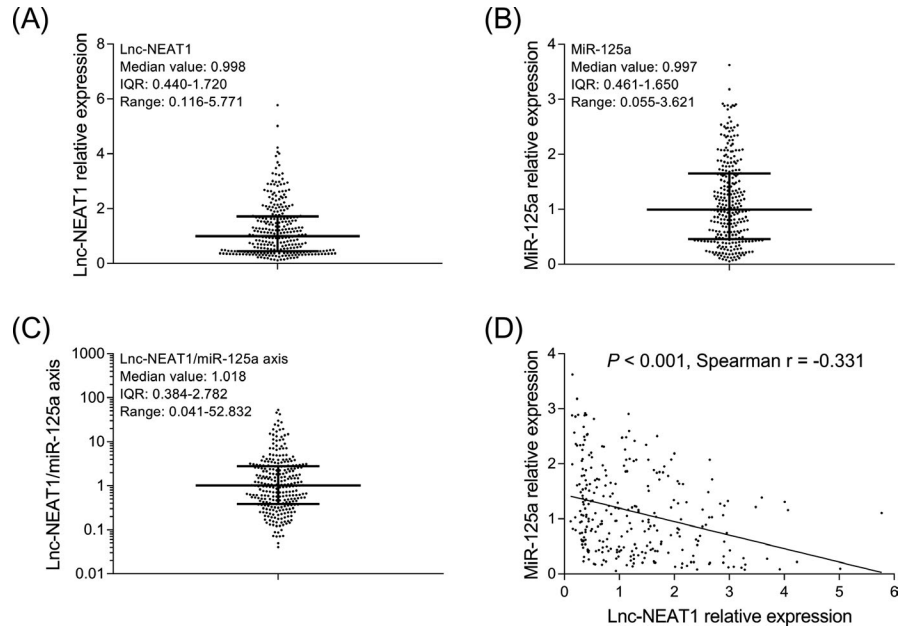
3.4 | The MACCE occurrence in ULMCAD patients underwent CABG

The 1-year, 2-year, and 3-year MACCE occurrence was 19 (6.8%), 29 (10.4%), and 38 (13.6%), respectively, in patients with ULMCAD underwent CABG (Figure 3A). Furthermore, accumulating MACCE occurrence was calculated and shown by Kaplan-Meier curve in Figure 3B.

3.5 | Correlation of lnc-NEAT1, miR-125a, and lnc-NEAT1/miR-125a axis with accumulating MACCE occurrence in ULMCAD patients underwent CABG

According to the median level of lnc-NEAT1 relative expression, miR-125a relative expression or lnc-NEAT1/miR-125a axis, all patients with ULMCAD were divided into patients with high level of each index and those with low level of each index. Accumulating MACCE occurrence was increased in patients with lnc-NEAT1 high expression compared with those with lnc-NEAT1 low expression ($\chi^2 = 5.345$, $P = .021$) (Figure 4A). Furthermore, accumulating MACCE occurrence rate was decreased in patients with miR-125a high expression compared with those with miR-125a low expression ($\chi^2 = 5.869$, $P = .015$) (Figure 4B). Notably, accumulating MACCE occurrence was greatly increased in patients with lnc-NEAT1/miR-125a axis high compared with those with lnc-NEAT1/miR-125a axis low ($\chi^2 = 11.207$, $P = .001$) (Figure 4C). In addition, lnc-NEAT1/miR-125a axis presented numerically increased χ^2 value and decreased P value compared with lnc-NEAT1 or miR-125a alone in K-M curves, suggesting the numerically better predictive value of lnc-NEAT1/miR-125a axis for MACCE risk in patients with ULMCAD underwent CABG.

FIGURE 2 Lnc-NEAT1, miR-125a, and lnc-NEAT1/miR-125a in ULMCAD. Lnc-NEAT1 relative expression in plasma of ULMCAD patients (A). MiR-125a relative expression in plasma of ULMCAD patients (B). Lnc-NEAT1/miR-125a axis in plasma of ULMCAD patients (C). The correlation of lnc-NEAT1 with miR-125a in ULMCAD patients (D). ULMCAD, unprotected left main coronary artery disease; lnc-NEAT1, long noncoding RNA nuclear-enriched abundant transcript 1; miR-125a, microRNA-125a; and IQR, inter-quartile range

3.6 | Univariate Cox's regression analyses of factors predicting accumulating MACCE occurrence in ULMCAD patients underwent CABG

Univariate Cox's regression model indicated that lnc-NEAT1 high expression (HR = 2.193, P = .024), lnc-NEAT1/miR-125a axis high (HR = 3.326, P = .002), BMI (>25.0 kg/m^2) (HR = 3.515, P = .002), hyperlipidemia (HR = 2.036, P = .042), diabetes (HR = 4.248, P < .001), previous stroke (HR = 6.428, P < .001), LVEF <50% (HR = 3.128, P = .001), and higher disease extent (HR = 2.126, P = .032) were associated with increased accumulating MACCE occurrence; however, miR-125a high expression (HR = 0.440, P = .019) was correlated with decreased accumulating MACCE occurrence in patients with ULMCAD underwent CABG (Table 2).

3.7 | Multivariate Cox's regression analyses of factors predicting accumulating MACCE occurrence in ULMCAD patients underwent CABG

Forward stepwise multivariate Cox's regression model exhibited that lnc-NEAT1/miR-125a axis high (HR = 2.583, P = .016), age (>60 years) (HR = 3.521, P = .003), BMI (>25.0 kg/m^2) (HR = 6.575, P < .001), diabetes (HR = 4.973, P < .001), previous stroke (HR = 10.969, P < .001), LVEF (HR = 4.955, P < .001), and higher disease extent (HR = 3.283, P = .001) independently predicted increased accumulating MACCE occurrence in patients with ULMCAD underwent CABG (Table 3).

4 | DISCUSSION

In the present study, we found that (a) lnc-NEAT1 expression was negatively associated with miR-125a expression in ULMCAD patients underwent CABG. (b) Lnc-NEAT1/miR-125a axis presented

a numerically better predictive value for MACCE risk compared with lnc-NEAT1 or miR-125a alone in ULMCAD patients underwent CABG. (c) lnc-NEAT1/miR-125a axis high predicted increased accumulating MACCE occurrence independently in patients with ULMCAD underwent CABG.

Lnc-NEAT1, as an essential regulator for paraspeckle formation, is implicated in several physiologic and pathophysiologic processes of cardiac and cerebrovascular diseases.[8-13] For example, one research discloses that lnc-NEAT1 is upregulated in ischemia/reperfusion myocardium, and lnc-NEAT1 knockdown exerts a protective effect against hypoxia/reoxygenation-induced cardiomyocyte apoptosis via mediating apoptotic protein of Bcl-2.[12] Another study reveals that lnc-NEAT1 is a critical regulator of vascular smooth muscle cell (VSMC) phenotype, which is associated with the progression of intimal lesions in vascular-related diseases.[26] In addition, one clinical study indicates that lnc-NEAT1 is elevated in patients with acute ischemic stroke compared with healthy controls, and it presents a good predictive value for increased disease risk with area under the curve of 0.804.[9] As to miR-125a, it serves as the target of lnc-NEAT1, and previous evidence demonstrates that miR-125a is involved in bidirectional control mechanism of immunity and inflammation in diseases.[17,27,28] For example, miR-125a regulates lipid uptake and decreases the secretion of several inflammatory cytokines in oxidized low-density lipoprotein (LDL)-induced macrophages, which associates with the development of hyperlipidemia and atherosclerosis.[17,27] However, some other previous studies indicate that higher level of miR-125a reduces autophagy, but increase the expression of pro-inflammatory factors via downregulating PI3K/Akt/mTOR signaling pathway.[28,29] In addition, miR-125a is exhibited to regulate urocortin-1 protection against the ischemia-reperfusion injury of acute MI, and one clinical study reports that miR-125a presents good ability in differentiating ischemic stroke patients from healthy controls.[30,31] Meanwhile, considering the complex calculation method of using Cox's predicting combing lnc-NEAT1 and miR-125a, which was too
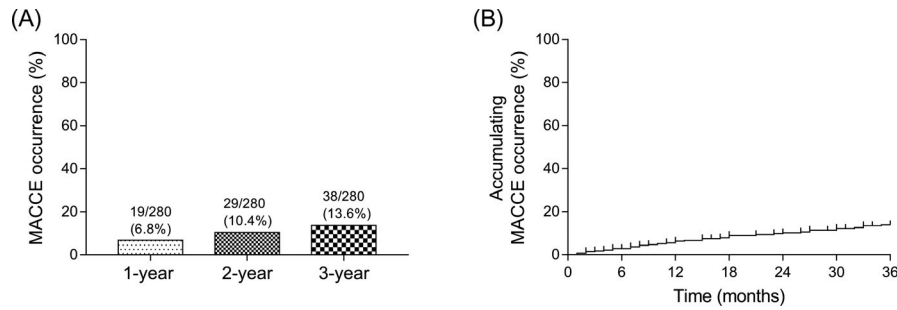
FIGURE 3 MACCE occurrence. The 1-year, 2-year, and 3-year MACCE occurrence in ULMCAD patients underwent CABG (A). The accumulating MACCE occurrence in ULMCAD patients underwent CABG (B). MACCE, major adverse cardiac and cerebrovascular event; ULMCAD, unprotected left main coronary artery disease; and CABG, coronary artery bypass graft
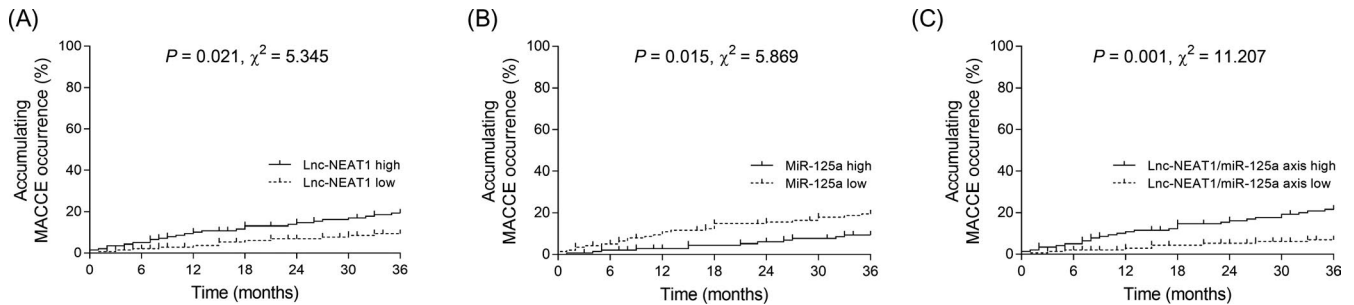

FIGURE 4 Correlation of lnc-NEAT1/miR-125a, lnc-NEAT1, and miR-125a with accumulating MACCE occurrence. Comparison of accumulating MACCE occurrence between ULMCAD patients with lnc-NEAT1 high expression and those with lnc-NEAT1 low expression in ULMCAD patients underwent CABG (A). Comparison of accumulating MACCE occurrence between ULMCAD patients with miR-125a high expression and those with miR-125a low expression in ULMCAD patients underwent CABG (B). Comparison of accumulating MACCE occurrence between ULMCAD patients with lnc-NEAT1/miR-125a axis high and those with lnc-NEAT1/miR-125a axis low in ULMCAD patients underwent CABG (C). MACCE, major adverse cardiac and cerebrovascular event; ULMCAD, unprotected left main coronary artery disease; CABG, coronary artery bypass graft; lnc-NEAT1, long noncoding RNA nuclear-enriched abundant transcript 1; and miR-125a, microRNA-125a

complex for application in the clinical practice, we chose lnc-NEAT1/miR-125a axis via dividing lnc-NEAT1 expression by miR-125a for the investigation. According to these prior studies and the preliminary findings that lnc-NEAT1 was upregulated while miR-125a was downregulated in MACCE-occurred ULMCAD patients compared to non–MACCE-occurred ULMCAD patients, we speculated that lnc-NEAT1/miR-125a axis might be associated with increased occurrence of MACCE in ULMCAD patients underwent CABG; therefore, we performed the present study to explore the association of lnc-NEAT1/miR-125a axis with the MACCE occurrence in ULMCAD patients underwent CABG. Initially, we detected the relative expressions of lnc-NEAT1 and miR-125a in plasma of ULMCAD patients and found that there existed negative association between lnc-NEAT1 and miR-125a in ULMCAD patients. These data suggested that miR-125a might be targeted by lnc-NEAT1 in ULMCAD patients, which was consistent with the previous studies.[9,14]

Existing previous studies have revealed the role of lnc-NEAT1 or miR-125a in the management of cardiac and cerebrovascular diseases in clinical setting.[9,32,33] For example, lnc-NEAT1 expression is associated with increased myocardial inflammation, decreased LVEF, higher level of low-density lipoprotein (LDL) cholesterol, and reduced level high-density lipoprotein cholesterol in post-myocardial infarction patients, and its high expression correlates with worse recurrence-free

survival in patients with acute ischemic stroke.[9,10] Besides, miR-125a is correlated with decreased pro-inflammatory cytokine level and increased anti-inflammatory cytokine level in patients with atherosclerosis, and its decreased level predicts favorable prognosis in acute ischemic stroke.[17,32] Taken together, we speculated that lnc-NEAT1/miR-125a axis might be of predictive significance for MACCE occurrence in patients with ULMCAD underwent CABG. In this present study, we observed that lnc-NEAT1/miR-125a axis and lnc-NEAT1 expression were positively associated with accumulating MACCE occurrence, while miR-125a expression was negatively correlated with accumulating MACCE occurrence in ULMCAD patients underwent CABG. Notably, the combined index of lnc-NEAT1/miR-125a axis presented numerically better value in predicting MACCE risk compared with the single index of lnc-NEAT1 or miR-125a expression in ULMCAD patients underwent CABG. We further performed the univariate Cox's proportional hazard regression analysis and observed that the value of lnc-NEAT1/miR-125a high axis in predicting MACCE occurrence was comparable to high BMI, LVEF <50%, but was better compared to advancing age, hypertension, diabetes, etc We further performed the and forward stepwise multivariate Cox's proportional hazard regression and found that lnc-NEAT1/miR-125a axis high is one independent predictive factor for increased MACCE occurrence in patients with ULMCAD underwent CABG, which could improve

TABLE 2 Univariate Cox's proportional hazard regression model analyses of factors predicting accumulating MACCE occurrence

| Items | Univariate Cox's regression model | | 95%CI | |
	P value	HR	Lower	Higher
Lnc-NEAT1 high expression	.024	2.193	1.107	4.346
MiR-125a high expression	.019	0.440	0.222	0.872
Lnc-NEAT1/miR-125a axis high	.002	3.326	1.575	7.027
Age (>60 years)	.118	1.779	0.864	3.664
Male	.235	0.654	0.324	1.319
BMI (>25.0 kg/m^2)	.002	3.515	1.611	7.672
Hypertension	.123	1.764	0.857	3.633
Hyperlipidemia	.042	2.036	1.027	4.037
Diabetes	.001	4.248	2.214	8.151
Current Smoker	.867	0.940	0.457	1.936
Chronic lung disease	.581	1.493	0.359	6.202
Chronic renal failure	.397	0.047	0.000	56.328
Family history of CAD	.885	1.062	0.468	2.412
Previous myocardial infarction	.144	1.649	0.843	3.223
Previous stroke	<.001	6.428	2.934	14.081
Previous heart failure	.096	2.722	0.836	8.858
Previous PCI	.196	1.718	0.757	3.902
Clinical presentation (stable angina)	.607	1.183	0.624	2.242
LVEF < 50%	.001	3.128	1.615	6.057
Higher disease extent	.032	2.126	1.069	4.228
Distal bifurcation involvement	.647	1.178	0.584	2.375
Right CAD involvement	.474	1.349	0.594	3.064

Abbreviations: BMI, body mass indexes; CAD, coronary artery disease; CI, confidence interval;HR, hazard ratio; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events; PCI, percutaneous coronary intervention.

predictive accuracy combing with conventional predictive factors (such as diabetes, hyperlipidemia) in MACCE management. The possible reasons might be listed as follows: (a) lnc-NEAT1 high expression might increase cardiomyocyte apoptosis via increasing production of intracellular reactive oxygen species level and activating transcriptional activity of NF-κB signaling, which strengthened the hypoxia/reoxygenation-induced cardiomyocyte injury in patients with ULMCAD, contributing to increased MACCE occurrence. (b) The inhibition of miR-125a might aggravate glycolipid metabolism dysfunction via elevating level of blood glucose and accumulation of lipid droplets, which led to the exacerbation of glycolipid metabolism disorder and diabetes severity, and meanwhile, hyperlipidemia and diabetes were found to be predictive factors for increased MACCE occurrence; therefore, miR-125a might be negatively correlated with MACCE occurrence in

TABLE 3 Forward stepwise multivariate Cox's proportional hazard regression model analyses of factors predicting accumulating MACCE occurrence

| Items | Forward stepwise multivariate Cox's regression model | | 95%CI | |
	P value	HR	Lower	Higher
Lnc-NEAT1/miR-125a axis high	.016	2.583	1.191	5.599
Age (>60 years)	.003	3.521	1.532	8.091
BMI (>25.0 kg/m^2)	<.001	6.575	2.850	15.168
Diabetes	<.001	4.973	2.440	10.137
Previous stroke	<.001	10.969	4.404	27.320
LVEF < 50%	<.001	4.955	2.322	10.571
Higher disease extent	.001	3.283	1.630	6.611

Note: All the factors in baseline were included in multivariate Cox's regression model.

Abbreviations: BMI, body mass indexes; CI, confidence interval;HR, hazard ratio; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events.

ULMCAD patients underwent CABG via interaction with hyperlipidemia and diabetes.[18] (c) Lnc-NEAT1 high expression might enhance VSMC proliferation via regulating phenotypic switching of VSMCs, and meanwhile, suppression of miR-125a presented similar effect on VSMCs proliferation via targeting ETS-1 and regulating VSMCs phenotypic transition; therefore, the combined effect of lnc-NEAT1 and miR-125a could lead to the aggravated intima hyperplasia as well as vessels stenosis compared with single effect of lnc-NEAT1 or miR-125a, contributing to the increased MACCE occurrence.[15,26]

There existed some limitations in the present study. (a) The underlying mechanism of lnc-NEAT1/miR-125a axis in regulating cardiomyocytes, VSMCs, vascular epithelioid cells, neurons, etc needed further cellular experiments to explore, so as to clarify its application in MACCE. (b) The present study was a single-centered study with a small sample size; therefore, a study with more sample from multiple regions was needed to validate the results in the future. (c) We only included the patients with ULMCAD who underwent CABG, whether the predictive role of lnc-NEAT1/miR-125a in MACCE occurrence was suitable for the patients with ULMCAD who underwent other treatments, such as medical therapy, PCI, needed further exploration. (d) GAPDH and U6 might not perform stable when the combination of lnc-NEAT1 and miR-125a was measured as axis.

In conclusion, lnc-NEAT1/miR-125a axis independently predicts increased MACCE risk in patients with ULMCAD underwent CABG, which suggests that lnc-NEAT1/miR-125a axis, as a combined index, presents potential value to be a prognostic biomarker in ULMCAD management.

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REFERENCES

1. Thuijs D, Kappetein AP, Serruys PW, et al. Percutaneous coronary intervention versus coronary artery bypass grafting in patients with three-vessel or left main coronary artery disease: 10-year follow-up of the multicentre randomised controlled SYNTAX trial. *Lancet*. 2019;394(10206):1325-1334.

2. Deb S, Wijeysundera HC, Ko DT, Tsubota H, Hill S, Fremes SE. Coronary artery bypass graft surgery vs percutaneous interventions in coronary revascularization: a systematic review. *JAMA*. 2013;310(19):2086-2095.

3. Head SJ, Milojevic M, Daemen J, et al. Mortality after coronary artery bypass grafting versus percutaneous coronary intervention with stenting for coronary artery disease: a pooled analysis of individual patient data. *Lancet*. 2018;391(10124):939-948.

4. Rahouma M, Abouarab A, Di Franco A, et al. Percutaneous coronary intervention versus coronary bypass surgery for unprotected left main disease: a meta-analysis of randomized controlled trials. *Ann Cardiothorac Surg*. 2018;7(4):454-462.

5. Khan MR, Kayani WT, Ahmad W, et al. Effect of increasing age on percutaneous coronary intervention vs coronary artery bypass grafting in older adults with unprotected left main coronary artery disease: A meta-analysis and meta-regression. *Clin Cardiol*. 2019;42(11):1071-1078.

6. Cavalcante R, Sotomi Y, Lee CW, et al. Outcomes after percutaneous coronary intervention or bypass surgery in patients with unprotected left main disease. *J Am Coll Cardiol*. 2016;68(10):999-1009.

7. Slim M, Meddeb MA, Neffati E, et al. A comparative study of bypass versus percutaneous intervention for left main disease. *Tunis Med*. 2019;97(4):533-540.

8. Clemson CM, Hutchinson JN, Sara SA, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell*. 2009;33(6):717-726.

9. Li P, Duan S, Fu A. Long noncoding RNA NEAT1 correlates with higher disease risk, worse disease condition, decreased miR-124 and miR-125a and predicts poor recurrence-free survival of acute ischemic stroke. *J Clin Lab Anal*. 2019;e23056.

10. Gast M, Rauch BH, Haghikia A, et al. Long noncoding RNA NEAT1 modulates immune cell functions and is suppressed in early onset myocardial infarction patients. *Cardiovasc Res*. 2019;115(13):1886-1906.

11. Du XJ, Wei J, Tian D, et al. NEAT1 promotes myocardial ischemia-reperfusion injury via activating the MAPK signaling pathway. *J Cell Physiol*. 2019;234(10):18773-18780.

12. Wu HJ, Tang GM, Shao PY, et al. Long non-coding RNA NEAT1 modulates hypoxia/reoxygenation-induced cardiomyocyte injury via targeting microRNA-520a. *Exp Ther Med*. 2019;18(3):2199-2206.

13. Ren L, Chen S, Liu W, Hou P, Sun W, Yan H. Downregulation of long non-coding RNA nuclear enriched abundant transcript 1 promotes cell proliferation and inhibits cell apoptosis by targeting miR-193a in myocardial ischemia/reperfusion injury. *BMC Cardiovasc Disord*. 2019;19(1):192.

14. Yan H, Liang H, Liu L, Chen D, Zhang Q. Long noncoding RNA NEAT1 sponges miR125a5p to suppress cardiomyocyte apoptosis via BCL2L12. *Mol Med Rep*. 2019;19(5):4468-4474.

15. Gareri C, Iaconetti C, Sorrentino S, Covello C, De Rosa S, Indolfi C. miR-125a-5p modulates phenotypic switch of vascular smooth muscle cells by targeting ETS-1. *J Mol Biol*. 2017;429(12):1817-1828.

16. Wong LL, Armugam A, Sepramaniam S, et al. Circulating microRNAs in heart failure with reduced and preserved left ventricular ejection fraction. *Eur J Heart Fail*. 2015;17(4):393-404.

17. Chen T, Huang Z, Wang L, et al. MicroRNA-125a-5p partly regulates the inflammatory response, lipid uptake, and ORP9 expression in oxLDL-stimulated monocyte/macrophages. *Cardiovasc Res*. 2009;83(1):131-139.

18. Xu L, Li Y, Yin L, et al. miR-125a-5p ameliorates hepatic glycolipid metabolism disorder in type 2 diabetes mellitus through targeting of STAT3. *Theranostics*. 2018;8(20):5593-5609.

19. Seeley JJ, Baker RG, Mohamed G, et al. Induction of innate immune memory via microRNA targeting of chromatin remodelling factors. *Nature*. 2018;559(7712):114-119.

20. Gorbea C, Mosbruger T, Cazalla D. A viral Sm-class RNA base-pairs with mRNAs and recruits microRNAs to inhibit apoptosis. *Nature*. 2017;550(7675):275-279.

21. Hua JT, Ahmed M, Guo H, et al. Risk SNP-mediated promoter-enhancer switching drives prostate cancer through lncRNA PCAT19. *Cell*. 2018;174(3):564-575.e18.

22. Cho SW, Xu J, Sun R, et al. Promoter of lncRNA gene PVT1 Is a tumor-suppressor DNA boundary element. *Cell*. 2018;173(6):1398-1412.e22.

23. Kleaveland B, Shi CY, Stefano J, Bartel DP. A network of noncoding regulatory RNAs acts in the mammalian brain. *Cell*. 2018;174(2):350-362.e17.

24. Elbarbary RA, Miyoshi K, Myers JR, et al. Tudor-SN-mediated endonucleolytic decay of human cell microRNAs promotes G1/S phase transition. *Science*. 2017;356(6340):859-862.

25. Kang SH, Ahn JM, Lee CH, et al. Differential event rates and independent predictors of long-term major cardiovascular events and death in 5795 patients with unprotected left main coronary artery disease treated with stents, bypass surgery, or medication. *Circ Cardiovasc Interv*. 2017;10(7):e004988.

26. Ahmed ASI, Dong K, Liu J, et al. Long noncoding RNA NEAT1 (nuclear paraspeckle assembly transcript 1) is critical for phenotypic switching of vascular smooth muscle cells. *Proc Natl Acad Sci U S A*. 2018;115(37):E8660-E8667.

27. Rink C, Khanna S. MicroRNA in ischemic stroke etiology and pathology. *Physiol Genomics*. 2011;43(10):521-528.

28. Hsu AC, Dua K, Starkey MR, et al. MicroRNA-125a and -b inhibit A20 and MAVS to promote inflammation and impair antiviral response in COPD. *JCI Insight*. 2017;2(7):e90443.

29. Chen D, Huang X, Lu S, et al. miRNA-125a modulates autophagy of thyroiditis through PI3K/Akt/mTOR signaling pathway. *Exp Ther Med*. 2019;17(4):2465-2472.

30. Diaz I, Calderon-Sanchez E, Toro RD, et al. miR-125a, miR-139 and miR-324 contribute to Urocortin protection against myocardial ischemia-reperfusion injury. *Sci Rep*. 2017;7(1):8898.

31. Tiedt S, Prestel M, Malik R, et al. RNA-Seq Identifies Circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as Potential Biomarkers for Acute Ischemic Stroke. *Circ Res*. 2017;121(8):970-980.

32. Zhaolin Z, Jiaojiao C, Peng W, et al. OxLDL induces vascular endothelial cell pyroptosis through miR-125a-5p/TET2 pathway. *J Cell Physiol*. 2019;234(5):7475-7491.

33. Wang SM, Liu GQ, Xian HB, Si JL, Qi SX, Yu YP. LncRNA NEAT1 alleviates sepsis-induced myocardial injury by regulating the TLR2/NF-kappaB signaling pathway. *Eur Rev Med Pharmacol Sci*. 2019;23(11):4898-4907.