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 (Inc-NEAT1) and microRNA-125a (miR-125a) expressions, and further explore the role of Inc-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 Inc-NEAT1 and miR-125a expressions by reverse transcription quantitative polymerase chain reaction. Lnc-NEAT1/miR-125a axis was calculated via dividing Inc-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 Inc-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 ($\chi^2 = 11.207, P = .001$) and Inc-NEAT1 expression ($\chi^2 = 5.345, P = .021$) positively associated with accumulating MACCE occurrence, while miR-125a expression ($\chi^2 = 5.869, P = .015$) negatively correlated with accumulating MACCE occurrence. Notably, Inc-NEAT1/miR-125a axis presented numerically better predictive value compared with Inc-NEAT1 or miR-125a alone for MACCE risk. Furthermore, Inc-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 ULM CAD management.
1 INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality and responsible for plenty of healthcare burden globally. 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. 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. However, patients with ULMCAD still suffer unfavorable prognosis due to several safety composite endpoints of major adverse cardiac and cerebrovascular events (MACCE). 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. 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. 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. Furthermore, based on the miRanda database and the previous studies reported, Inc-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. According to these previous evidence and the data that Inc-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 Inc-MALAT1 and miR-125a, we hypothesized that Inc-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 Inc-NEAT1 and miR-125a relative expressions in patients with ULMCAD underwent CABG and further explore the role of Inc-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 Inc-NEAT1 and miR-125a expressions, and further explore the role of Inc-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 Inc-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); (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 Inc-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). Following that, qPCR was performed using KOD SYBR® qPCR Mix (Toyobo) to quantify expressions of Inc-NEAT1 and miR-125a. And the expression levels of Inc-NEAT1 (with GADPH as an internal reference) and miR-125a (with U6 as an internal reference) were calculated using 2^{-\Delta\Delta C_T} method. Primers were listed as follows:

- Inc-NEAT1 forward primer: TGTCCCTCGGCTATGTCAGA, reverse primer: GAGGGGACGTGGTTCCTGAG; miR-125a forward primer: ACACTCCAGCTGGTCCTTGAGGACCCCTTAA, reverse primer: TGTCGGGTAGTCGGCAATTT; GAPDH forward primer: TGACACATCCATGCGCATAC, reverse primer: GCCTGCTTCACCACCTTGA; U6 forward primer: CTCGCTTCGGCAGCACATATACTA; reverse primer: ACGAATTTCGGTGTCATCCTTG.

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, 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 Inc-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 Inc-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%) 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.

#### 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/m²), 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.

### 3.3 The correlation between Inc-NEAT1 and miR-125a in ULMCAD patients underwent CABG

In patients with ULMCAD, the Inc-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). Inc-NEAT1/miR-125a axis was 1.018 (IQR: 0.384-2.782) (range: 0.041-52.832) (Figure 2C). As for the correlation of Inc-NEAT1 with miR-125a, Inc-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 Inc-NEAT1, miR-125a, and Inc-NEAT1/miR-125a axis with accumulating MACCE occurrence in ULMCAD patients underwent CABG

According to the median level of Inc-NEAT1 relative expression, miR-125a relative expression or Inc-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 Inc-NEAT1 high expression compared with those with Inc-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 Inc-NEAT1/miR-125a axis high compared with those with Inc-NEAT1/miR-125a axis low ($\chi^2 = 11.207, P = .001$) (Figure 4C). In addition, Inc-NEAT1/miR-125a axis presented numerically increased $\chi^2$ value and decreased P value compared with Inc-NEAT1 or miR-125a alone in K-M curves, suggesting the numerically better predictive value of Inc-NEAT1/miR-125a axis for MACCE risk in patients with ULMCAD underwent CABG.
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 = 3.515, P = .002), BMI (>25.0 kg/m²) (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/m²) (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 0.804.7 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
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 Inc-NEAT1 or miR-125a in the management of cardiac and cerebrovascular diseases in clinical setting.9,32,33 For example, Inc-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 Inc-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 Inc-NEAT1/miR-125a axis and Inc-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 Inc-NEAT1/miR-125a axis presented numerically better value in predicting MACCE risk compared with the single index of Inc-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 Inc-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 Inc-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 | Forward stepwise multivariate Cox’s regression model |
|------------------------------|----------------------------------|-----------------------------------------------|
|                              | p value  | HR   | 95%CI    | P value  | HR   | 95%CI    |
| 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/m²)            | .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| 0.359    | 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| 1.059    | 4.228    |       |        |
| Distal bifurcation involvement| .647    | 1.178| 0.584    | 2.375    |       |        |
| Right CAD involvement        | .474     | 1.349| 0.594    | 3.064    |       |        |

| Items                        | P value  | HR   | 95%CI    |
|------------------------------|----------|------|---------|
| Age (>60 years)              | .003     | 3.521| 1.532   | 8.091   |
| BMI (>25.0 kg/m²)            | <.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 Inc-NEAT1 and miR-125a could lead to the aggravated intima hyperplasia as well as vessels stenosis compared with single effect of Inc-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 Inc-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 Inc-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 Inc-NEAT1 and miR-125a was measured as axis.

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

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