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Expression of lncRNA-ANRIL in patients with coronary heart disease before and after treatment and its short-term prognosis predictive value

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Abstract. – OBJECTIVE: This study was designed to explore the expression of LncRNA-ANRIL in patients with coronary heart disease before and after treatment and its short-term survival prediction value.

PATIENTS AND METHODS: Eighty-three patients with coronary heart disease who came to our hospital undergoing interventional therapy were selected as a research group, 81 healthy volunteers who came to our hospital for normal physical examination during the same period were selected as a control group, and LncRNA-ANRIL of subjects in the two groups before and after treatment were detected by RT-PCR. Levels of Gensini score, lactate dehydrogenase (LDH), creatine kinase isoenzyme (CK-MB), creatine kinase (CK), and BNP of patients in research group before treatment were evaluated and detected, and the correlation between those and LncRNA-ANRIL was analyzed. Then, the effective treatment of LncRNA-ANRIL for patients with coronary heart disease and the predictive value of poor prognosis were analyzed.

RESULTS: The expression of LncRNA-ANRIL in patients with coronary heart disease was lower than that of normal subjects ($p<0.05$), and the expression levels of Gensini score, LDH, CK-MB, CK, and BNP gradually increased with the increased number of their diseased vessels ($p<0.05$). The expression of LncRNA-ANRIL was negatively correlated with expressions of Gensini score, LDH, CK-MB, CK, and BNP ($p<0.05$); ROC of LncRNA-ANRIL in predicting effective treatment, and poor prognosis of patients with coronary heart disease was over 0.9, as well as smoking; LncRNA-ANRIL, Gensini score, LDH, CK-MB, CK, and BNP were independent risk factors for the occurrence of MACE.

CONCLUSIONS: LncRNA-ANRIL expresses low in the serum of patients with coronary heart disease, and it has high predictive value both for effective treatment and poor prognosis of them. Also, lncRNA-ANRIL is also an independent risk factor for their poor prognosis.

Key Words:
LncRNA-ANRIL, Coronary heart disease, Treatment, Prognosis, Survival, Prediction.

Introduction

Coronary heart disease, as one of the most common cardiovascular diseases, is also one of the main causes of death in cardiovascular diseases at present[1]. In recent years, with the improvement of living standards, the morbidity and mortality of coronary heart disease are also increasing, posing a great threat to human life and health[2].

Coronary atherosclerosis, as the pathological basis of coronary heart disease, has a relatively complicated mechanism of occurrence, among which neogenesis of endangium and proliferation of vascular smooth muscle are the pathological mechanisms leading to coronary atherosclerosis[3]. However, with the development of molecular biology in recent years, it has also been found that abnormal expression of certain genes in myocardial cells is also one of the causes of myocardial injury in coronary heart disease[4]. LncRNA is a long-chain non-coding RNA, which can affect the biological function of cells by regulating gene expression and chromatin structure[5]. At present, studies on LncRNA mostly involve in tumor, but there are relatively few reports on its role in cardiovascular diseases[6]. LncRNA-ANRIL is a LncRNA located in chromosome 9q21 region, which expresses in various tissues of human body[7]. At first, LncRNA-ANRIL was found to regulate the proliferation, apoptosis, and other biological functions of tumor cells[8], but in recent years, some investigations have discovered that LncRNA seems to play an important role in cardiovascular diseases, for example, Shu et al[9] have observed that LncRNA-ANRIL can protect hypoxia-induced myocardial cell H9c2 by targeting miR-7-5p/SIRT1 axis, and Tan et al[10] have verified that LncRNA-ANRIL can inhibit cell senescence of vascular smooth muscle by regulating miR-181a/SIRT1 axis. These studies make us begin to think about the clinical significance of LncRNA-ANRIL in cardiovascular diseases.

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Therefore, we analyzed the expression level and clinical significance of LncRNA-ANRIL before and after treatment for patients with coronary heart disease and the relationship between LncRNA-ANRIL and the short-term prognosis of patients to provide new molecular targets for predicting their efficacy and prognosis.

Patients and Methods

General Data

Eighty-three patients with coronary heart disease who visited our hospital from July 2016 to October 2018 and underwent interventional therapy were selected as research group, including 47 male patients and 36 female patients. The average age of all patients was (64.62±8.97) years. Eighty-one healthy volunteers with normal physical examination in our hospital during the same period were selected as control group, including 46 male volunteers, 35 female volunteers, and the average age of all volunteers was (64.88±8.82) years. Inclusion criteria were as follows: patients diagnosed as coronary heart disease by coronary angiography. Exclusion criteria were as follows: patients with malignant tumors, severe liver, and kidney dysfunction, other infectious or immune diseases, and communication and cognitive dysfunction. All patients and their families agreed to participate in the experiment and sign an informed consent. This research was approved by the Hospital Ethics Committee.

Detection of Indicators

ORT-PCR Detection of LncRNA-ANRIL Expression

Altogether 5 ml venous blood was respectively drawn from all subjects on an empty stomach, centrifuged at 3000 r/min for 5 min after drawing, and the supernatant was taken for detection after centrifugation. TRIzol was added into the serum to extract total RNA, and the purity, concentration, and integrity of total RNA were detected by ultraviolet spectrophotometer and agarose gel electrophoresis. According to the kit instructions (TransGen Biotech, Beijing, China), cDNA reverse transcription, and ANRIL detection were performed. ANRIL amplification system was as follows: cDNA 1 μL, upstream and downstream primers 0.4 μL each, 2×SYBR Green mixture 10 μL, Passive Reference Dye (50X) 0.4 μL, and ddH_2O supplemented to 20 μL. Amplification conditions: PCR reaction conditions were as follows: 95° pre-denaturation for 60 s, 95° denaturation for 5 s, 60° annealing extension for 15 s, a total of 40 cycles, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as internal reference. The primer sequence was shown in Table I, and the experiment was repeated 3 times.

Detection of Other Relevant Indicators

Other biochemical indicators related to coronary heart disease of patients in research group were detected. First, 3 ml venous blood was drawn on an empty stomach, sodium citrate was added to the test tube for anticoagulation, and all biochemical indicators were detected by Hitachi 7600 automatic biochemical analyzer. The biochemical indicators included lactate dehydrogenase (LDH), creatine kinase isoenzymes (CK-MB), creatine kinase (CK), etc. The level of BNP (Shanghai Yaji Biotechnology Co., Ltd., CL06818) was detected by full-automatic immunofluorescence chemiluminescence analyzer (IMMULITE system) and original kit.

Observation Indicators

(1) Expression of serum LncRNA-ANRIL of subjects in the two groups before and after treatment was detected and compared. (2) Patients in research group were divided into single-vessel disease group, double-vessel disease group, and multi-vessel disease group according to the conditions of coronary artery disease, and the serum LncRNA-ANRIL, other indicators related to coronary heart disease and Gensini score[11] before and after treatment of patients in the three groups were compared. (3) The correlation between LncRNA-ANRIL, degree of coronary lesions, and other indicators of coronary heart disease was analyzed. (4) The predictive value of LncRNA-AN-

Table I. Primer sequence table.

Factor	Upstream primer	Downstream primer
ANRIL	5-TGCTCTATCCGCCAA TCAGG-3’	5’-GGGCCTCAGTGGCACA TACC-3’
GAPDH	5'-TGTGGGCATCAATGGATT TGG-3	5'-ACACCATGTATTCCGGGTCA AT-3'

RIL in the efficacy of patients was analyzed. (5) Major adverse cardiovascular events (MACE) within 6 months after treatment in research group were recorded. (6) The independent risk factors of MACE occurred in patients were analyzed.

Statistical Analysis

The collected data were statistically analyzed by SPSS 19.0 (IBM, Armonk, NY, USA) software package, the required pictures were drawn by GraphPad 6 software (San Diego, CA, USA) package, the comparison between the two groups was conducted by independent-samples *t*-test, the comparison among multiple groups was performed by one-way analysis of variance (ANOVA) test, back testing was performed by LSD/*t*-test, Pearson was used for correlation analysis, Logistic regression model was used to analyze the risk factors of the occurrence of MACE, and a *p*-value lower than 0.05 was considered significant.

Results

General Data

There was no significant difference in gender, age, and BMI of subjects between the two groups ($p>0.05$), but there were differences in related indicators of diabetes mellitus, hypertension, and blood fat ($p<0.05$), as shown in Table II.

Expression of LncRNA-ANRIL Before and After Treatment

Before treatment, the LncRNA-ANRIL expression in research group was significantly lower than that in control group ($p<0.05$). After treatment, the LncRNA-ANRIL expression in research group was significantly higher than that before treatment, but still lower than that in normal people ($p<0.05$), as shown in Figure 1.

Comparison of Other Relevant Indicators of Patients in the Research Group

Patients were divided into single-vessel disease group (30 cases), double-vessel disease group (31 cases), and multi-vessel disease group (22 cases) according to the conditions of coronary artery disease. After comparing the serum LncRNA-ANRIL, other related indicators of coronary heart disease and Gensini score of patients in the three groups before treatment, it was found that the LncRNA-ANRIL level of patients in single-vessel disease group was significantly higher than that in double-vessel disease group and multi-vessel disease group, and the levels of Gensini score, LDH, CK-MB, CK and BNP of patients in single-vessel disease group were significantly lower than those in double-vessel disease group and multi-vessel disease group ($p<0.05$). The LncRNA-ANRIL level of patients in double-vessel disease group was significantly higher than that in multi-vessel

Table II. Comparison of general data.

Factor	Research group (n=83)	Control group (n=81)	X^2	*p*
Gender			0.000	0.9483
Male	47 (56.63)	46 (51.90)		
Female	36 (43.37)	35 (43.21)		
Age (years)			0.014	0.906
≥64	53 (63.86)	51 (62.92)		
<64	30 (36.14)	30 (37.08)		
BMI			0.000	0.980
≥22	48 (58.83)	47 (58.02)		
<22	35 (42.17)	34 (41.98)		
Family history			0.007	0.933
Yes	22 (26.51)	21 (25.93)		
No	61 (73.49)	60 (74.07)		
Diabetes			16.10	<0.001
Yes	46 (55.42)	20 (24.69)		
No	37 (44.58)	61 (75.31)		
Hypertension			10.17	0.001
Yes	45 (54.22)	24 (29.63)		
No	38 (45.78)	57 (70.37)		
TG (mmol/L)	1.85±0.21	1.62±0.22	6.849	<0.001
TC (mmol/L)	4.11±0.23	4.29±0.25	4.800	<0.001
HDL (mmol/L)	1.47±0.33	1.12±0.24	7.752	<0.001

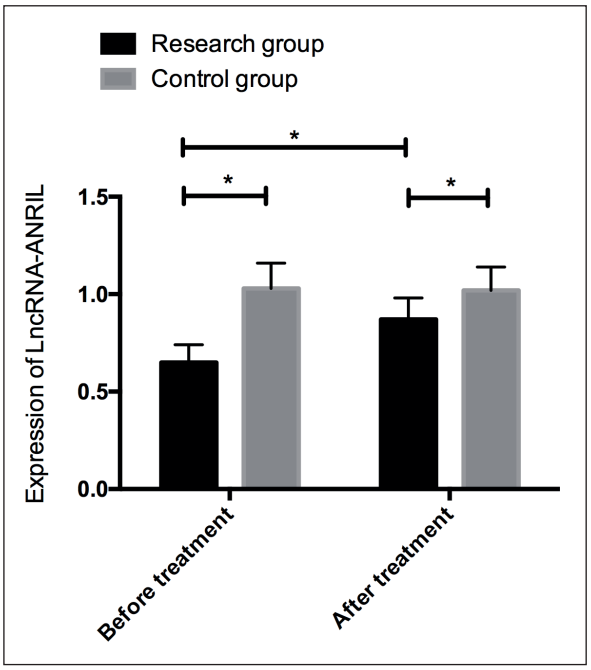

Figure 1. Expression of LncRNA-ANRIL before and after treatment * signified $p<0.05$.

disease group. The levels of Gensini score, LDH, CK-MB, CK, and BNP of patients in single-vessel disease group were significantly lower than those in multi-vessel disease group ($p<0.05$). More details were shown in Table III.

Correlation Analysis of Serum LncRNA-ANRIL and Other Indicators of Coronary Heart Disease in Patients With Coronary Heart Disease

There was a negative correlation between the expression of LncRNA-ANRIL in serum of patients with coronary heart disease and the number of diseased vessels (r=-0.820, $p<0.05$). There was a negative correlation between serum LncRNA-ANRIL and the levels of Gensini score, LDH, CK-MB, CK and BNP in coronary heart disease (r=-0.875, $p<0.05$; r=-0.886, $p<0.05$; r=-0.829, $p<0.05$; r=-0.872, $p<0.05$; r=-0.818, $p<0.05$). More details were shown in Figure 2.

Predicative Value of LncRNA-ANRIL in the Efficacy of Patients

Patients were divided into effective group (71 people) and ineffective group (12 people) according to the efficacy of patients. The expression of LncRNA-ANRIL of patients in the two groups was compared. After ROC analysis of the efficacy of patients, it was found that the expression of LncRNA-ANRIL of patients in effective group was significantly higher than that in ineffective group ($p<0.05$). The sensitivity, specificity, and AUC of LncRNA-ANRIL in patients with coronary heart disease were 91.67%, 74.65%, and 0.905 respectively after drawing ROC curve, which had higher predictive value. More details were shown in Figure 3.

Comparison of LncRNA-ANRIL in Patients With Different Prognosis

We divided patients into MACE group (32 cases) and non-MACE group (51 cases) according to the occurrence of MACE within 6 months after treatment, and compared the serum LncRNA-ANRIL of patients in the two groups before treatment. The results showed that the serum LncRNA-ANRIL of patients in MACE group was significantly lower than that in non-MACE group ($p<0.05$). The sensitivity, specificity, and AUC of LncRNA-ANRIL for patients with coronary heart disease were 94.12%, 71.88%, and 0.962 respectively after drawing ROC curve, which showed high predictive value. More details were shown in Figure 4.

Table III. Comparison of other related indicators of patients in the research group.

Indicators	Single-vessel disease group (n=30)	Double-vessel disease group (n=31)	Multi-vessel disease group (n=22)	F	p
LncRNA-ANRIL	0.73±0.04	0.64±0.04	0.55±0.06	97.64	<0.001
Gensini score	17.25±2.37	26.15±5.91	35.42±7.52	70.37	<0.001
LDH (IU/L)	153.23±25.61	261.95±28.45	336.16±33.67	403.70	<0.001
CK-MB (IU/L)	36.06±4.97	69.33±20.79	169.46±45.26	166.70	<0.001
CK (IU/L)	186.13±16.52	233.16±39.77	336.73±50.62	107.50	<0.001
BNP (pg/ml)	75.34±5.15	132.62±19.47	254.82±42.71	329.50	<0.001

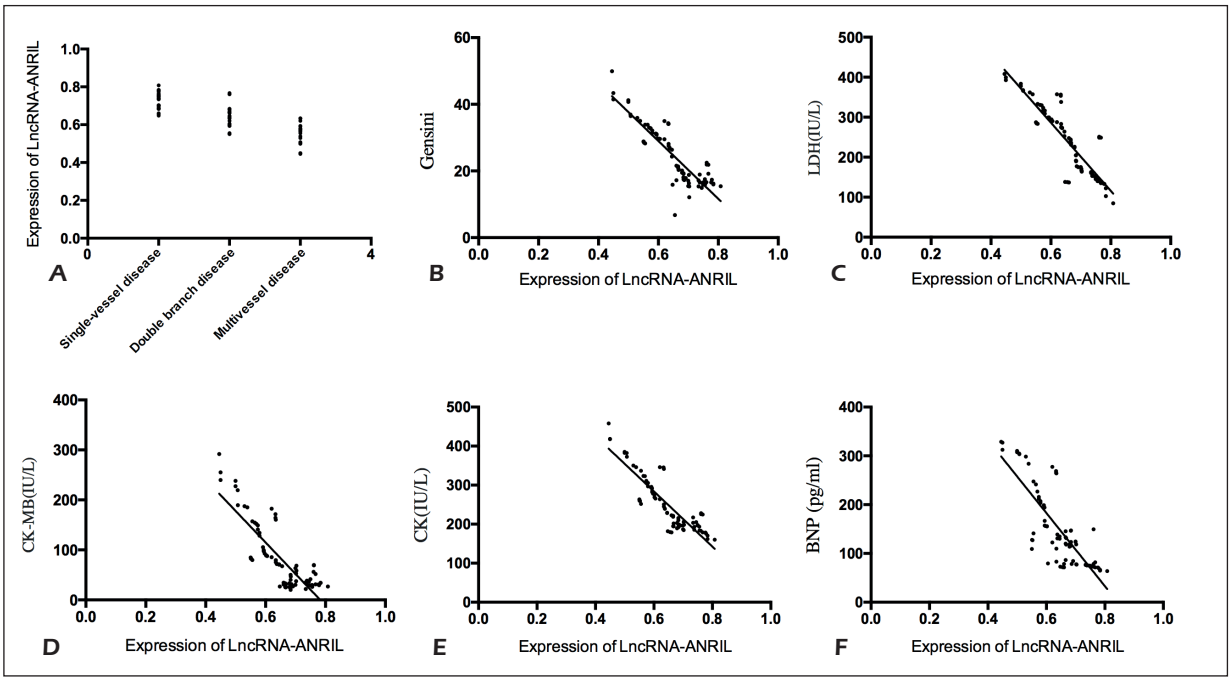

Figure 2. Correlation analysis of serum LncRNA-ANRIL, and other indicators of coronary heart disease in patients with coronary heart disease. **A**, There was a negative correlation between the expression of serum LncRNA-ANRIL in patients with coronary heart disease and the number of diseased vessels (r=-0.820, p<0.05). **B**, There was a negative correlation between serum LncRNA-ANRIL and Gensini score of patients with coronary heart disease (r=-0.875, p<0.05). **C**, There was a negative correlation between serum LncRNA-ANRIL and LDH levels in patients with coronary heart disease (r=-0.886, p<0.05). **D**, There was a negative correlation between serum LncRNA-ANRIL and CK-MB levels of patients with coronary heart disease (r=-0.829, p<0.05). **E**, Serum LncRNA-ANRIL and CK levels of patients with coronary heart disease were negatively correlated (r=-0.872, p<0.05). **F**, Serum LncRNA-ANRIL and BNP levels of patients with coronary heart disease were negatively correlated (r=-0.818, p<0.05).

Single Factor Analysis of MACE in Patients

After single factor analysis of patients in MACE group and non-MACE group, it was found that there was no significant difference in gender, age, hypertension, diabetes and other aspects of patients between the two groups (p>0.05). There was significant difference in the levels of history of smoking, LncRNA-ANRIL, Gensini score, LDH, CK-MB, CK, and BNP (p<0.05). More details were shown in Table IV.

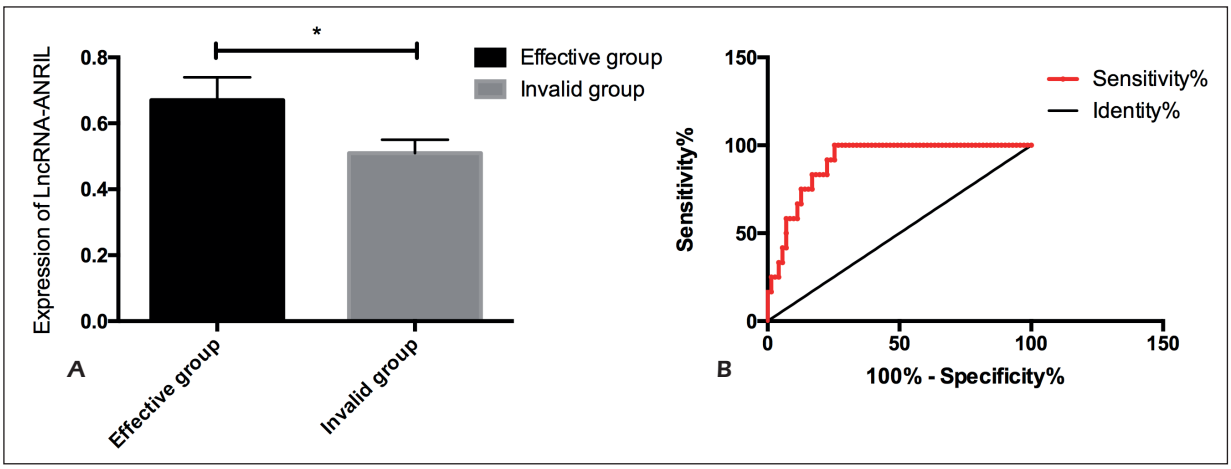

Figure 3. Predictive value of LncRNA-ANRIL in the efficacy of patients. **A**, Expression of LncRNA-ANRIL of patients in the effective group was higher than that in the ineffective group. **B**, ROC curve of LncRNA-ANRIL for treatment of patients with coronary heart disease * signified p<0.05.

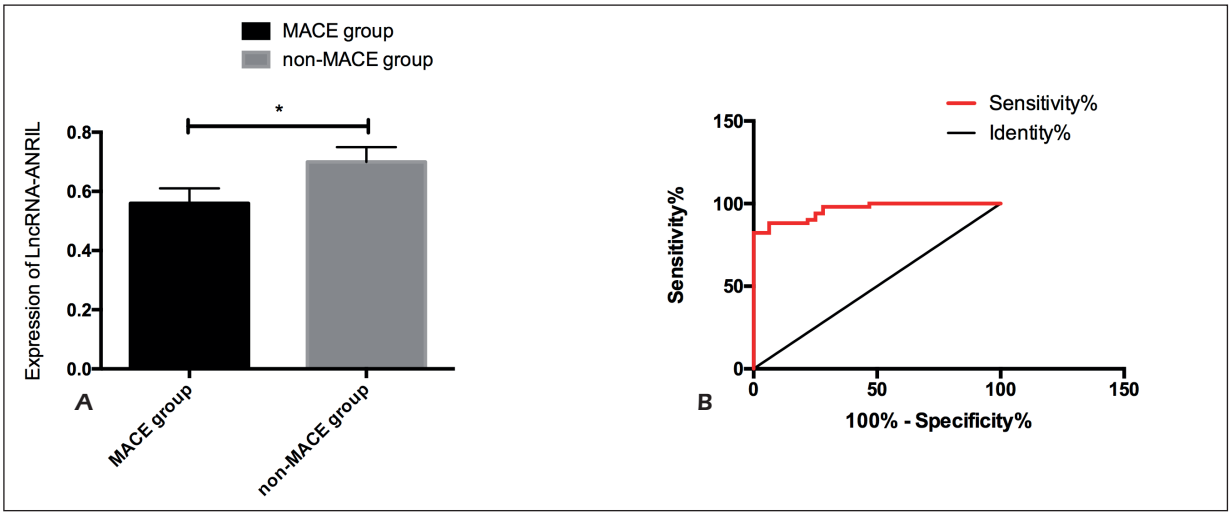

Figure 4. Predicative value of LncRNA-ANRIL in poor prognosis of patients. **A**, Expression of LncRNA-ANRIL in non-MACE patients was higher than that in MACE patients. **B**, ROC curve of LncRNA-ANRIL for predicting poor prognosis of patients with coronary heart disease * signified $p<0.05$.

Multiple Factor Analysis of MACE in Patients

Smoking, LncRNA-ANRIL, Gensini score, LDH, CK-MB, CK, and BNP were involved in the analysis of results, and they were listed as dependent variables for assignment (Table V). Having MACE or not was taken as the dependent variable, Logistic regression model was used for multiple factor analysis, and the results showed that smoking, LncRNA-ANRIL, Gensini score, LDH, CK-MB, CK, and BNP were independent risk factors for patients to develop MACE (Table VI).

Discussion

Coronary heart disease is a cardiovascular disease with complicated pathological mechanism caused by many complicated factors[12]. During the occurrence and development of coronary heart disease, chronic inflammation and immune disorder are the pathological basis of its genetics, which is also one of the main causes of vascular endothelial damage in coronary heart disease[13]. With the development of molecular biology, LncRNA, as a long-chain non-coding RNA that can regulate gene expression, is revealed to exert in diseases, mostly

Table IV. Single factor analysis of MACE in patients.

Factor	MACE group n=32	Non-MACE group n=51	t/χ^2	*p*
Gender (n(%))				
Male	18 (56.25)	29 (56.85)	46 (55.42)	
Female	14 (43.75)	22 (43.14)		
Age (years)			0.041	0.839
≥64	20 (62.50)	33 (64.71)		
<64	12 (37.50)	18 (43.14)		
Hypertension	17 (53.13)	28 (54.90)	0.025	0.874
Diabetes mellitus	14 (43.75)	23 (45.10)	0.040	0.842
Smoking history	22 (68.75)	21 (41.18)	5.988	<0.05
LncRNA-ANRIL	0.57±0.05	0.70±0.05	11.563	<0.001
Gensini score	24.65±2.85	30.26±6.12	4.857	<0.001
LDH (IU/L)	210.34±31.81	259.33±51.28	4.845	<0.001
CK-MB (IU/L)	76.59±13.54	134.94±49.16	6.547	0.506
CK (IU/L)	221.06±34.78	296.54±61.27	6.348	<0.001

Note: Smoking history is defined as at least one cigarette per day for one year straight.

Table V. Assignment table.

Factor	Assignment
Smoking history	Yes =1; No =2
LncRNA-ANRIL	The data belong to continuous variables and are analyzed with original data
Gensini score	The data belong to continuous variables and are analyzed with original data
LDH	The data belong to continuous variables and are analyzed with original data
CK-MB	The data belong to continuous variables and are analyzed with original data
CK	The data belong to continuous variables and are analyzed with original data
BNP	The data belong to continuous variables and are analyzed with original data

Table VI. Multiple factor analysis of poor prognosis in patients with heart failure.

Factor	β	S.E	Wald	OR	95%CI	p
Smoking history	0.069	0.011	3.169	1.225	1.079-1.411	<0.05
LncRNA-ANRIL	0.031	0.109	0.055	1.423	1.113-2.425	<0.05
Gensini score	1.009	0.335	9.412	2.744	1.443-5.221	<0.05
LDH	0.210	0.421	7.179	1.224	1.174-4.421	<0.05
CK-MB	1.167	0.531	4.795	3.213	1.132-9.164	<0.05
CK	0.013	0.061	3.695	1.421	1.051-1.721	<0.05
BNP	0.221	0.513	7.348	1.310	1.211-4.762	<0.05

in tumor[14,15]. In recent years, the role of LncRNA in angiocarpy is gradually uncovered. For example, studies[16] have found that LncRNA-ANRIL can regulate the proliferation and migration of vascular endothelial cells. However, the clinical significance of LncRNA-ANRIL in patients with coronary heart disease has not been studied and discussed.

LncRNA-ANRIL is a kind of LncRNA existing in human chromosome 9q21 region. We detected that the expression of LncRNA-ANRIL in serum of patients with coronary heart disease is lower than that of normal subjects, and found that the expression will gradually increase after treatment, which suggests that LncRNA-ANRIL may be used as a factor for predicting the efficacy of them. For this reason, we further divided patients into effective group and ineffective group according to their efficacy, and analyzed the predicative value of LncRNA-ANRIL in efficacy. The results showed that the expression of LncRNA-ANRIL in patients with effective treatment was higher than that in patients with ineffective treatment, and its predicted AUC for patients with coronary heart disease was more than 0.9, which suggested that we might adjust the treatment plan of patients by detecting LncRNA-ANRIL, so that they could get more appropriate treatment plans. Previous studies did not analyze the predictive value of LncRNA-ANRIL in the treatment of coronary heart disease, so further follow-up investigations are needed to prove it. To further analyze the clinical significance of LncRNA-ANRIL in coronary heart disease, we tested other factors related to coronary heart disease. BNP, as one of the products of embryonic genes, can effectively reflect the myocardial function of patients[17], while LDH, CK-MB, and CK are classical myocardial injury indicators, which can effectively reflect the myocardial injury of patients[18]. However, myocardial injury also plays a key role in the pathological process of coronary heart disease, which makes us pay attention to the relationship between LncRNA-ANRIL and myocardial injury in patients with coronary heart disease. Our results showed that the expression of LncRNA-ANRIL gradually decreased with the increased number of diseased vessels, while the levels of Gensini score, LDH, CK-MB, CK, and BNP gradually increased. After correlation analysis, it was found that LncRNA-ANRIL was negatively correlated with the number of diseased vessels, the levels of Gensini score, LDH, CK-MB, CK, and BNP of patients, which suggested that LncRNA-ANRIL might be one of the molecular indicators for evaluating the condition of patients with coronary heart disease, and might be involved in the process of myocardial injury of patients. He et al[19] proved that LncRNA-ANRIL was highly correlated with the risk of patients with coronary heart disease, but the specific molecular mechanism was still unclear.

Subsequently, to further analyze the clinical value of LncRNA-ANRIL in coronary heart disease, we made statistics on patients with MACE within 6 months after treatment, and analyzed the predictive value of LncRNA-ANRIL in short-term prognosis of patients with coronary heart disease. It was found that the expression of LncRNA-ANRIL in MACE event group was lower than that in non-MACE event group. After ROC analysis, it was found that AUC in patients with coronary heart disease treated by LncRNA-ANRIL with poor prognosis after treatment was over 0.9, which had higher predictive value. This suggested that we might predict the prognosis of patients by detecting LncRNA-ANRIL, thus preventing it in advance and reducing the occurrence of MACE in patients. Then, we further carried out single factor and multiple factor analysis on the factors causing MACE. The results showed that smoking, LncRNA-ANRIL, Gensini score, LDH, CK-MB, CK, and BNP were independent risk factors for patients with MACE. The above results indicated that the decrease of LncRNA-ANRIL might be one of the markers for predicting the occurrence and prognosis of MACE in patients with coronary heart disease. Duran et al[20] observed that smoking was an independent risk factor for poor prognosis of patients with coronary heart disease. Gensini score is a score that reflects the coronary artery disease of patients. LDH, CK-MB, CK, and BNP can all reflect the degree of myocardial injury. Scholars[21,22] also indicated that they were independent risk factors for poor prognosis of patients with coronary heart disease, which was consistent with our conclusion. Zhuang et al[23] also found that p15INK4b methylation was associated with coronary artery disease and the expression of ANRIL, and both were affected by Shr9p21 gene polymorphism. Yari et al[24] discovered that the expression variation of long non-coding RNA ANRIL was closely related to the susceptibility to coronary heart disease. All these indicated that the expression of LncRNA ANRIL was related to coronary artery. However, the research on LncRNA in coronary heart disease is still at an initial stage, and several experiments and data are still needed to support the research results. Therefore, there are still some shortcomings in this investigation. For example, since this study is still an exploratory experiment, the sample size included is relatively small. Secondly, we have not conducted *in vitro* cell researchs. The molecular mechanism of LncRNA-ANRIL in coronary heart disease is still unclear, which requires us to explore further.

Conclusions

LncRNA-ANRIL expresses low in the serum of patients with coronary heart disease, and it has high predictive value for both effective treatment and poor prognosis of them. Also, it is an independent risk factor for their poor prognosis.

Acknowledgments

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Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Faridi KF, Rymer JA, Rao SV, Dai D, Wojdyla D, Yeh RW, Wang TY. Ad hoc percutaneous coronary intervention in patients with stable coronary artery disease: a report from the National Cardiovascular Data Registry CathPCI Registry. Am Heart J. 2019 Jul 13; 216: 53-61. doi: 10.1016/j.ahj.2019.07.004. [Epub ahead of print].
2) Karaman S, Coskun A. Do MCHC, MPV, and procalcitonin levels determine prognosis in acute coronary syndrome? Emerg Med Int 2019; 2019: 6721279.
3) AlBadri A, Eshtehardi P, Hung OY, Bouchi Y, Khawaja S, Mercado K, Corban MT, Mehta PK, Shaw LJ, Samady H. Coronary microvascular dysfunction is associated with significant plaque burden and diffuse epicardial atherosclerotic disease. JACC Cardiovasc Interv 2019; 12: 1519-1520.
4) Toraih EA, El-Wazir A, Alghamdi SA, Alhazmi AS, El-Wazir M, Abdel-Daim MM, Fawzy MS. [PROVISIONAL] Association of long non-coding RNA MIAT and MALAT1 expression profiles in peripheral blood of coronary artery disease patients with previous cardiac events. Genet Mol Biol. 2019 Jun 10. pii: S1415-47572019005020101. doi: 10.1590/1678-4685-GMB-2018-0185. [Epub ahead of print].
5) Liu L, Wang HJ, Meng T, Lei C, Yang XH, Wang QS, Jin B, Zhu JF. LncRNA GAS5 inhibits cell migration and invasion and promotes autophagy by targeting miR-222-3p via the GAS5/PTEN-signaling pathway in CRC. Mol Ther Nucleic Acids 2019; 17: 644-656.
6) Zhang M, Wang X, Yao J, Qiu Z. Long non-coding RNA NEAT1 inhibits oxidative stress-induced vascular endothelial cell injury by activating the

miR-181d-5p/CDKN3 axis. Artif Cells Nanomed Biotechnol 2019; 47: 3129-3137.

7) Yap KL, Li S, Muñoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou MM. Molecular interplay of the noncodingRNA ANRIL and methylated histone H3 lysine 27 by Polycomb CBX7 intranscriptional silencing of INK4a. Mol Cell 2010; 38: 662-674.

8) Wang Jun-Bin, Jin Yan, Wu Peng et al. Tumor suppressor PLZF regulated by lncRNA ANRIL suppresses proliferation and epithelial mesenchymal transformation of gastric cancer cells.[J] .Oncol. Rep., 2019, 41: 1007-1018.

9) Shu L, Zhang W, Huang C, Huang G, Su G, Xu J. LncRNA ANRIL protects H9c2 cells against hypoxia-induced injury through targeting the miR-7-5p/SIRT1 axis. J Cell Physiol. 2019 Jul 1. doi: 10.1002/jcp.29031. [Epub ahead of print].

10) Tan P, Guo YH, Zhan JK, Long LM, Xu ML, Ye L, Ma XY, Cui XJ, Wang HQ. LncRNA-ANRIL inhibits cell senescence of vascular smooth muscle cells by regulating miR-181a/Sirt1. Biochem Cell Biol 2019; Biochem Cell Biol 2019; 97: 571-580.

11) Mantovani A, Bonapace S, Lunardi G, Canali G, Dugo C, Vinco G, Calabria S, Barbieri E, Laaksonen R, Bonnet F, Byrne CD, Targher G. Associations between specific plasma ceramides and severity of coronary-artery stenosis assessed by coronary angiography. Diabetes Metab. 2019 Aug 3. pii: S1262-3636(19)30118-1. doi: 10.1016/j.diabet.2019.07.006. [Epub ahead of print].

12) Wang LJ, Mohebali J, Goodney PP, Patel VI, Conrad MF, Eagleton MJ, Clouse WD. The effect of clinical coronary disease severity on outcomes of carotid endarterectomy with and without combined coronary bypass. J Vasc Surg. 2019 Aug 7. pii: S0741-5214(19)31157-7. doi: 10.1016/j.jvs.2019.03.074. [Epub ahead of print].

13) Caidahl K, Hartford M, Ravn-Fischer A, Lorentzen E, Yndestad A, Karlsson T, Aukrust P, Ueland T. Homeostatic chemokines and prognosis in patients with acute coronary syndromes. J Am Coll Cardiol 2019; 74: 774-782.

14) Mou E, Wang H. LncRNA LUCAT1 facilitates tumorigenesis and metastasis of triple-negative breast cancer through modulating miR-5702. Biosci Rep 2019; 39. pii: BSR20190489.

15) Yang Y, Fei M, Zhou X, Li Y, Jin D. The potential value of lncRNA-BC050642 in osteosarcoma origination and outcomes. Artif Cells Nanomed Biotechnol 2019; 47: 1859-1866.

16) Congrains A, Kamide K, Katsuya T, Yasuda O, Oguro R, Yamamoto K, Ohishi M, Rakugi H. CVD-associated non-coding RNA, ANRIL, modulates expression of atherogenic pathways in VSMC. Biochem Biophys Res Commun 2012; 419: 612-616.

17) Sergeeva IA, Hooijkaas IB, Van Der Made I, Jong WM, Creemers EE, Christoffels VM. A transgenic mouse model for the simultaneous monitoring of ANF and BNP gene activity during heart development and disease. Cardiovasc Res 2014; 101: 78-86.

18) Amini Negin, Sarkaki Alireza, Dianat Mahin et al. Protective effects of naringin and trimetazidine on remote effect of acute renal injury on oxidative stress and myocardial injury through Nrf-2 regulation. [J] .Pharmacol Rep, 2019, 71: 1059-1066.

19) He S, Gu W, Li Y, Zhu H. ANRIL/CDKN2B-AS shows two- stage clade-specific evolution and becomes conserved after transposon insertions in simians. BMC Evol Biol 2013; 13: 247.

20) Duran M, Elçik D, Murat S, Öksüz F, Çelik ÐE. Risk factors for coronary artery disease in young patients with stable angina pectoris. Turk J Med Sci 2019; 49: 993-998.

21) Feistritzer HJ, Reinstadler SJ, Klug G, Reindl M, Wöhrer S, Brenner C, Mayr A, Mair J, Metzler B. Multimarker approach for the prediction of microvascular obstruction after acute ST-segment elevation myocardial infarction: a prospective, observational study. BMC Cardiovasc Disord 2016; 16: 239.

22) Missov E, Boularan A M, Bonifacj C Descomp B, Chaptal PA, Albat B. Prognostic value of myocardial lactate dehydrogenase subunit ratio in heart transplant recipients. J Heart Lung Transplant 1998; 17: 959-968.

23) Zhuang J, Peng W, Li H, Wang W, Wei Y, Li W, Xu Y. Methylation of p15INK4b and expression of ANRIL on chromosome 9p21 are associated with coronary artery disease. PLoS One, 2012; 7: e47193.

24) Yari M, Bitarafan S, Broumand MA, Fazeli Z, Rahimi M, Ghaderian SMH, Mirfakhraie R, Omrani MD. The relationship between long non-coding RNA ANRIL expression variation and susceptibility to coronary heart disease. INT J Mol Cell Med 2018; 7: 1-7.