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Clinical significance of lncRNA-AWPPH in coronary artery diseases

T.-T. TANG[1], B.-Q. WANG[2]

[1]Department of Laboratory Medicine, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China
[2]Intensive Care Unit, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China

Abstract. – OBJECTIVE: The aim of this study was to determine serum level of long non-coding RNA (lncRNA)-AWPPH in coronary artery disease (CAD) patients and its clinical significance as a serum marker.

PATIENTS AND METHODS: Serum levels of lncRNA-AWPPH in 132 CAD patients and 50 controls were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Based on medical history of statin therapy, differential expressions of lncRNA-AWPPH in CAD patients were examined. Then, the correlation between lncRNA-AWPPH level and clinical data of CAD patients was analyzed. Moreover, risk factors influencing prognosis of CAD were assessed by multivariate logistic regression analysis.

RESULTS: It was found that lncRNA-AWPPH was highly expressed in serum of CAD patients, especially those receiving rosuvastatin therapy. LDL-C, hs-CRP, and serum lncRNA-AWPPH were independent risk factors for CAD, while HDL-C was favorable to CAD.

CONCLUSIONS: LncRNA-AWPPH is highly expressed in serum of CAD patients, which can be reduced by statin therapy, and it may be a potential serum marker for predicting the prognosis of CAD.

Key Words:
Coronary artery disease (CAD), LncRNA-AWPPH, Clinical significance.

Introduction

Coronary artery disease (CAD) is resulted by excessive deposition of lipid in the arterial intima, leading to stenosis and blocking blood flow, which further develops into myocardial ischemia or necrosis[1,2]. For the middle-aged and elderly, the incidence of CAD increases with age because of vascular elasticity decline[3].

Long non-coding RNAs (lncRNAs), a type of non-coding RNAs with over 200 nt long, have been verified to be critical regulators in biological processes[4,5]. They could regulate gene expressions on epigenetics, transcription, and post-transcription. Accumulating evidence[6,7] has shown the extensive involvement of lncRNAs in tumor development. Recently, multiple lncRNAs involved in CAD have been identified[8,9]. It is reported that human chromosome 9p21.3 region is mostly linked to cardiovascular diseases. ANRIL is one of the few transcripts found in chromosome 9p21 that is associated with atherosclerotic vascular disease (ASVD) sensitivity[10]. Yan et al[11] proposed that UCA1 is downregulated in most of acute myocardial infarction (MI) patients and UCA1 level is related to the disease onset.

LncRNA-AWPPH, located on chromosome 2, is the host of MIR4435-2[12], and it exhibits carcinogenic properties in hepatocellular carcinoma[13], bladder cancer[14], and triple-negative breast cancer[15]. By interacting with YBX1, lncRNA-AWPPH promotes progression of hepatocellular carcinoma[16]. In bladder cancer, lncRNA-AWPPH regulates proliferation, autophagy, and metastasis[17]. In this paper, the serum level of lncRNA-AWPPH in CAD patients was detected, and its clinical significance in CAD was analyzed.

Patients and Methods

Clinical Data of Patients

A total of 132 CAD patients and 50 controls in The First Affiliated Hospital of Jinzhou Medical University from May 2016 to 2018 were enrolled. Stenosis of the left main trunk, anterior descending branch, circumflex artery, right coronary artery or its branches for more than 50% revealed on coronary angiography was defined

Corresponding Author: Baoquan Wang, MM; e-mail: tiantiantang@cumt.edu.cn

as CAD. Subjects in control group were excluded for CAD by performing coronary angiography. In CAD group, there were 91 men and 41 women, with an average age of 59 years (35-89 years). All patients received conventional treatment after admission. They were administrated with low-molecular-weight heparin, nitrate drugs, β-blocker drugs, calcium antagonists or aspirin according to the symptoms. In particular, 66 randomly selected CAD patients were additionally administrated orally with Atorvastatin, 10 mg, qd (Jialin Pharmaceutical, Beijing, China; Medicine: H20093819). The other 66 CAD patients were additionally administrated orally with Rosuvastatin, 5 mg, qd (Simcere Dongyuan, Nanjing, China, Medicine: H20113246). One course of medication lasted for 8 consecutive weeks. Therapeutic efficacy was evaluated after one course. This study was approved by the ethics committee of The First Affiliated Hospital of Jinzhou Medical University.

Serum Collection

Elbow vein blood of each subject was collected, placed at 4°C for 30 min, and centrifuged at 3000 r/min for 15 min. The upper layer supernatant was subpacked in non-RNA enzyme cryogenic vials, and stored at -80°C.

RNA Extraction

250 μL of serum was added in 750 μL of TRIzol (Invitrogen, Carlsbad, CA, USA), and the mixture was let stand for 5 min. Subsequently, 200 μL of chloroform was applied, let stand for 10 min, and centrifuged at 12 000 r/min for 10 min. Then, the supernatant was added in the same volume of isopropanol, let stand for 3 min, and centrifuged at 12 000 r/min for 10 min. Next, the precipitant was washed in 1 ml of 75% ethanol and centrifuged at 12 000 r/min for 2 min, and the precipitant was air dried. The extracted RNA was dissolved in 10 μL of diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China). At last, the concentration and purity of RNA were determined using NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Through reverse transcription of RNA, the extracted complementary deoxyribose nucleic acid (cDNA) was used for PCR detection by SYBR Green method (TaKaRa, Otsu, Shiga, Japan) with an ABI7900 Fast Real-Time System (Applied Biosystems, Foster City, CA, USA). With glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference, the relative level of target gene was measured in triplicate and calculated as $RQ=2^{-\Delta\Delta Ct}$. The primer sequences are listed as follows: LncRNA-AWPPH forward: 5′-CTGGATGGTCGCTGCTTTTTA-3′ and reverse: 5′-AGGGGGATGAGTCGTGATTT-3′, GAPDH forward: 5′-GAAGGTGAAGGTCGGAGTC-3′ and reverse: 5′-GAAGATGGTGATGGGATTTC-3′.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean ± SD (standard deviation). The *t*-test was used for analyzing differences between two groups. Multivariate logistic regression analysis was conducted to assess risk factors for CAD. $p<0.05$ indicated the significant difference.

Results

Clinical Data and Laboratory Indicators

By collecting clinical data of 132 CAD patients and 50 controls, no significant differences in sex, age, smoking, and family history of hypertension were found between CAD group and control group. Nevertheless, significant differences in BMI and family history of CAD were pronounced ($p<0.05$, Table I). In addition, higher levels of LDL-C and hs-CRP, and lower level of HDL-C were identified in CAD group than those of control group (Table II). It is suggested that BMI, family history of CAD, and dyslipidemia may be potential risk factors influencing the development of CAD.

LncRNA-AWPPH was Highly Expressed in CAD

Compared with that in controls, serum level of lncRNA-AWPPH was higher in CAD patients, indicating its potential involvement in disease progression ($p<0.05$, Figure 1).

Influences of Different Statins on AWPPH

To explore the influences of different statins on AWPPH, CAD patients were randomly classified into Atorvastatin group (n=66) and Rosuvastatin group (n=66). No significant difference in serum level of AWPPH was observed before medication between the two groups ($p>0.05$). After one course of treatment, serum level of AWPPH was markedly reduced in both groups, and it was more

Table I. Clinical data of enrolled subjects in control group and CAD group.

Variable	n	Control (n=50)	CAD (n=132)	χ^2	*p*
Sex					
male	68	19	49	0.012	1.000
female	114	31	83		
Age					
<60	71	21	50	0.259	0.614
≥60	111	29	82		
BMI/(kg/m²)					
<24	79	35	44	19.847	<0.001
≥24	103	15	88		
Smoking					
No	83	25	58	0.537	0.507
Yes	99	25	74		
Family history of hypertension					
No	77	26	51	1.568	0.244
Yes	105	24	81		
Family history of CAD					
No	81	37	44	28.369	<0.001
Yes	101	13	88		
Dyslipidemia					
No	55	22	23	11.61	0.001
Yes	127	28	99		

BMI: Body Mass Index; *p<0.05.

Table II. Clinical data of enrolled subjects in control group and CAD group.

Variable	Control (n=50)	CAD (n=132)	*p*
TG (mmol/L)	1.65±0.51	1.79±0.69	0.194
TC (mmol/L)	4.48±0.94	4.55±0.89	0.642
LDL-C (mmol/L)	2.19±0.33	2.95±0.59	<0.001
HDL-C (mmol/L)	1.99±0.59	1.58±0.61	<0.001
hs-CRP (mg/L)	3.54±0.61	3.79±0.78	0.043
Lp(a) (mg/L)	239.21±98.25	268.66±102.37	0.082

TG: triglyceride; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; hs-CRP: high-sensitive C- reactive protein; Lp(a): lipoprotein a.

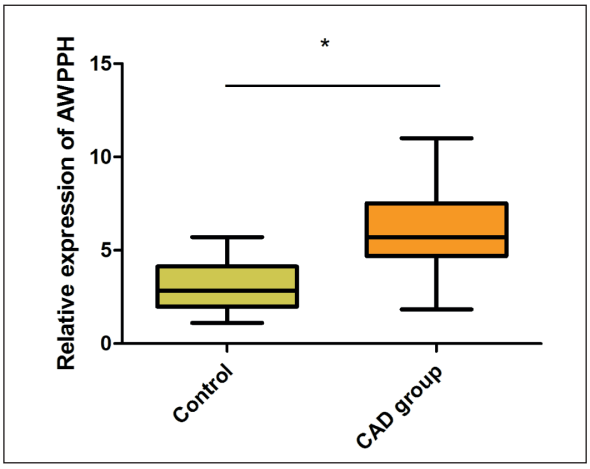

Figure 1. LncRNA-AWPPH is highly expressed in CAD.

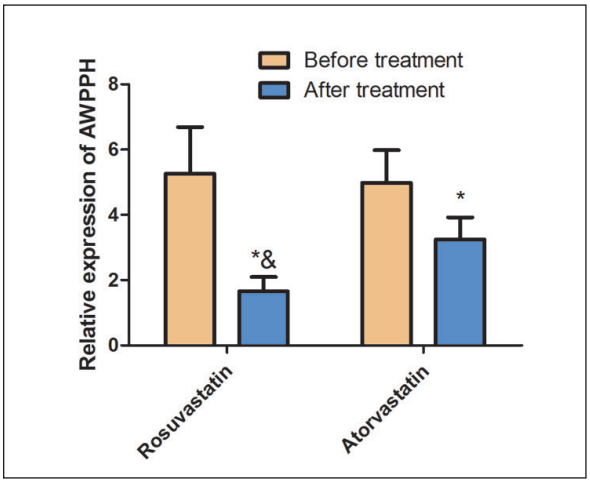

Figure 2. Influences of statins on lncRNA-AWPPH level.

Table III. Multivariate logistic regression analysis on risk factors for coronary artery diseases.

Variable	OR (95% CI)	p	OR (95% CI)*	p*
LDL-C (mmol/L)	2.761 (1.923-5.336)	0.008	2.067 (1.258-3.989)	0.029
HDL-C (mmol/L)	0.589 (0.321-0.962)	0.032	0.682 (0.436-0.839)	0.039
hs-CRP (mg/L)	1.624 (1.227-2.987)	0.038	1.127 (1.026-1.998)	0.042
AWHHP	3.221 (1.597-6.227)	0.015	3.017 (1.467-4.201)	0.022

*Adjustment on BMI, family history of CAD and dyslipidemia. LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; hs-CRP: high-sensitive C-reactive protein.

pronounced in Rosuvastatin group (p>0.05, Figure 2). Collectively, statins could significantly inhibit AWPPH level, and the inhibitory effect of Rosuvastatin was superior than that of Atorvastatin.

Multivariate Logistic Regression Analysis on Risk Factors for CAD

Multivariate logistic regression analysis on risk factors for CAD was conducted after adjusting BMI, family history of CAD, and dyslipidemia. The data showed that LDL-C, hs-CRP, and serum lncRNA-AWPPH were independent risk factors for CAD, while HDL-C was favorable to CAD (Table III).

Discussion

Artery stenosis or occlusion caused by atherosclerosis in coronary vessels leads to CAD. CAD could be classified into angina pectoris, MI, asymptomatic MI (also known as occult CAD), ischemic heart failure (also known as ischemic heart disease), and sudden death[18]. Pathological factors of CAD include changeable risks (i.e., dyslipidemia, hypertension, obesity, diabetes) and unchangeable risks (i.e., family genetic, cytomegalovirus infection, *Helicobacter pylori* infection)[19,20]. At present, the incidence of CAD increases with the aging society[21]. Effective prevention and treatment of CAD are necessarily required.

LncRNAs are mainly distributed in the cytoplasm or nucleus, which are of significance during live activities[22]. LncRNAs directly affect the development of atherosclerosis by changing phenotypes of vascular smooth muscle cells. Meanwhile, they are closely linked to vital risk factors for atherosclerosis, that is, lipogenesis and lipidosis. Sun et al[23] discovered 175 lncRNAs related to lipogenesis, adipocyte differentiation, and phenotype changes by analyzing differentially expressed transcriptomes in mouse immature and mature adipocytes. Furthermore, they identified 26 highly expressed lncRNAs in brown and white adipose tissues. A large-scale case-control study[24] found a gene transcript located on 22q12.1, MIAT, a lncRNA associated with MI.

LncRNA-AWPPH exerts a carcinogenic effect in human cancers[13]. It aggravates ovary cancer by activating the Wnt/β-catenin pathway[25]. In non-small cell lung cancer (NSCLC), lncRNA-AWPPH induces apoptosis and inhibits proliferative ability[26]. In this paper, lncRNA-AWPPH was upregulated in CAD patients, which was markedly reduced following the treatment of either Atorvastatin or Rosuvastatin. Notably, the inhibitory effect of Rosuvastatin on AWPPH level was better than that of Atorvastatin in CAD patients. In addition, LDL-C, hs-CRP, and serum lncRNA-AWPPH were independent risk factors for CAD, while HDL-C was favorable to CAD. It was believed that lncRNA-AWPPH could be a serum marker for diagnosing and monitoring CAD.

Conclusions

This study shows that lncRNA-AWPPH is highly expressed in serum of CAD patients, which can be reduced by statin therapy, especially Rosuvastatin medication, and it may be a potential serum marker for predicting the prognosis of CAD.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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