LncRNA *TARID* Induces Cell Proliferation Through Cell Cycle Pathway Associated With Coronary Artery Disease

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Abstract

Background/Aim:

Long noncoding RNA *TARID* (lncRNA *TARID*) can activate the tumor suppressor *TCF21* in tumorigenesis by inducing promoter demethylation. However, the impact on lncRNA *TARID* and its variants of coronary artery disease (CAD) are poorly understood.

Methods

We performed a case-control study enrolling 949 case patients and 892 controls to assess genotype. Five variants were genotyped by TaqMan assay. 20 case patients and 20 controls were used to evaluate the expression of lncRNA *TARID*. The qRT-PCR and cell cycle analysis were applied to examined cell proliferation and cell distribution.

Results

This study indicated that rs2327433 GG genotype was associated with CAD risk adjusting for traditional risk factors (OR=2.74, 95%CI: 1.10-6.83, $P=0.03$). Our results analyses revealed that the genotype of rs2327433 was related to the proportion of CAD patients with left anterior descending artery disease and left circumflex artery disease ($P=0.025$ and $P=0.025$, respectively). The results showed that the minor allele frequency of rs2327433 was significantly correlated with the severity of the disease ($P=0.029$). The eQTL analysis showed that rs2327433 may affect the transcription factors *TCF21* regulated by lncRNA *TARID*. We found that *TARID* silencing regulated the cell proliferation and altered cell cycle progression by induced upregulation of CDK1 and PCNA.

Conclusions

SNP rs2327433 in lncRNA *TARID* was associated with CAD risk and the severity of CAD in Chinese Han population. Furthermore, SNP rs2327433 may affect the expression of atherosclerosis-related transcription factor *TCF21* regulated by lncRNA *TARID*. Finally, our study provided a new lncRNA-dictated regulatory mechanism participating in cell proliferation.

1. Introduction

Coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide[1]. This disease is caused by the interaction of genetic and environmental factors[2]. Its etiology and pathogenesis have not yet been fully elucidated. Genetic factors play a decisive role in the pathogenesis of CAD.

In recent years, CAD related single nucleotide polymorphism (SNP) sites confirmed by GWAS are located in non-protein coding genes[3] [4] [5]. Long non-coding RNA (lncRNA), a type of transcripts comprising >200 nucleotides, were involved in the etiology of diverse human disorders through epigenetic, transcriptional and post-transcriptional regulations[6]. Interestingly, it was appeared that lncRNAs also participated in the development of cardiovascular diseases, including heart failure, cardiac hypertrophy, cardiac metabolic diseases and myocardial infarction[7]. The well-known 9p21 chromosome region has been reported[8]. The SNP in the lncRNA (Long non-coding RNA) *ANRIL* of this region is significantly related to CAD risk[9]. In our previous study, we also found that the SNP on lncRNA *ANRIL* is not only significantly related to the susceptibility of CAD[10], but also significantly related to the severity of CAD[11]. A large number of lncRNAs have been found to be abnormally expressed in CAD[12] or myocardial infarction[13], which indicates that this non-coding RNA molecule may play a very important role in the occurrence of CAD. Therefore, the impact of lncRNA and its genetic variation on the sensitivity and severity of CAD needs further study.

Mechanistically, long non-coding RNA (lncRNA) are large intergenic noncoding RNAs lacking of protein-coding function which are involved in diverse cell functions, such as proliferation, apoptosis and migration[14]. lncRNA *TARID* is located in the 6q23.2 region of the chromosome. The GWAS study found that the chromosome region 6q23.2 is significant related to CAD susceptibility[15], which indicates that *TARID* may be related to the occurrence and development of coronary heart disease[16]. Studies have found that this lncRNA is involved in the demethylation of *TCF21* promoter to promote its expression[17]. In the latest research, the protein-coding gene *TCF21* was discovered to regulate smooth muscle cell phenotypic transformation and inhibit atherosclerosis[18]. Accordingly, the function of lncRNA *TARID*, and the relationship between the *TARID* variants and the CAD susceptibility in Chinese Han population deserves further exploration.

2. Methods

2.1 Study population

The study population was composed of 949 case patients and 892 age- and sex-frequency-matched controls. The another population was made up of 20 patients and 20 controls. All the enrolled subjects were from Chongqing City. Approval was obtained from the ethics committee of Chongqing Medical University in Chongqing, China (No.2021043). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. All subjects signed written informed consent.

Inclusion criteria for CAD cases has been described elsewhere[10]. The control group chooses people who have undergone health examinations during the same period. Structured questionnaires were accomplished by trained interviewers. The questionnaire collected information about demographic variables, medical history, drug use and lifestyle factors.

2.2 SNP selection

According to the tag SNP information in Hapmap and 1000 genome databases, the previous CAD-related research of the Chongqing population and the results of GWAS research reported at home and abroad, this study selected five non-coding gene *TARID* SNPs. Five SNPs were selected on the *TARID* gene located on human chromosome 6, namely rs1966248, rs2327429, rs2327433, rs12190287 and rs6569912.

The selected SNPs met the following inclusion criteria: 1) minor allele frequency (MAF) ≥ 0.1 in Chinese Han population of the HapMap project (the Phase II database); 2) Hardy Weinberg equilibrium (HWE) test P value ≥ 0.05 .

2.3 Genotyping

Fasting venous blood was collected from each participant in 5-ml EDTA tubes, and the genomic DNA was isolated with a Tiangen DP319-02 kit (Tiangen Company, Beijing, China). Genotyping was performed with the TaqMan Genotyping Assay Systems and ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA), in 384-well format. Genotypes were automatically detected by analysis software version 2.2.1 (SDS 2.2.1).

2.4 Cell culture and RNA interference

The human umbilical vein endothelial cell was cultured in DMEM with 10% FBS and 1% penicillin/streptomycin. The human myeloid leukemia mononuclear cell was cultured in RPMI 1640. Cells passaged less than 7 times were used for all experiments.

Small-interfering RNA duplexes (siRNA) against *TARID* (si *TARID*-1) and siRNA control (negative control, NC) were designed by the website(<http://biodev.extra.cea.fr/DSIR/DSIR.html>) and synthesized from Shanghai Sangon Biotech Co., Ltd (China). The sequence of si *TARID*-1 and NC were shown in **supplementary 1**.

Transient transfection into THP-1 or HUVEC were performed using Lipofectamine®2000 siRNA Transfection Reagent (Invitrogen Inc.). According to the manufacturer's instructions, cells were harvested at 48h post-transfection.

2.5 Cell cycle analysis

DNA analysis by flow cytometry was employed to measure cell distribution[19]. The cells were seeded in 6-well plates at a density of 70,000 cells/well and cultured at 37°C overnight. At 48h after transfection, viable cells were gathered and fixed at 4°C overnight. The fixed cells were then stained with PI at 37°C for 30 min in the dark. The cell cycle analysis of different groups was evaluated using a flow cytometer (CytoFLEX, American).

2.6 RT-qPCR

The total RNA was extracted from cells using RNAi plus Reagent (TaKaRa, Japan) and the cDNA was reverse transcribed by using 2×Fast PCR Master Mix(MedChemExpress Inc.) or PrimeScript™ RT Master Mix (TaKaRa, Japan). QPCR was performed using random primers and SYBR Green qPCR Master Mix Kit

(MedChemExpress Inc.) on the BioRad ® 96 instrument Real-Time PCR System. The PCR reaction condition were as follows: denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 5s, annealing at 60°C for 10s, and a final extension at 72°C for 30s. The sequences of primers were all listed in supplementary 1. GAPDH was used as a reference internal control. The fold change of gene expression was calculated as the $2^{-\Delta\Delta C_T}$ method.

2.7 Statistical analysis

The measurement data is expressed as the mean $\pm$ standard deviation (mean $\pm$ SD), and the count data is expressed as the percentage of the number of cases. The 1-Sample K-S test was used to test the normality of continuous variables. The data that obeyed the normal distribution was analyzed by the F test. The data that did not meet the normal distribution was analyzed by the Kruskal-Wallis rank sum test. The Chi-square test was used to the Hardy-Weinberg equilibrium test of each SNPs and the count data.

Binary unconditional logistic regression was used to analyze the relationship between the genotype of each SNP and the risk of CAD. All statistical analysis tests are two-sided tests. $P < 0.05$ is considered statistically significant. Haploview 4.2 software was used to map the linkage relationship between each SNP. SHEsis (<http://analysis.bio-x.cn/myAnalysis.php>) online tool was used to perform haplotype frequency estimation of SNP.

3. Results

3.1 Characteristics of the subjects and variants

This study adopted the method of case-control study. This study included 949 CAD cases with a mean age of 66.2 ± 10.7 years and 892 controls with a mean age of 65.6 ± 12.7 years. Cases and controls were frequency matched according to age and gender. The basic clinical data of the study subjects were shown in **Table 1**. The pairwise LD of the five SNPs was calculated for the 949 case patients and 892 age- and sex-frequency-matched controls. The haplotype block was generated from the Haploview software (**supplementary 2**).

Table 1. General characteristics of CAD cases and controls

Variants	Case(N=949)	Control(N=892)	p-value
Male/female, %	49.3/50.7	49.8/50.1	0.82
Age(years)	66.2±10.7	65.6±12.7	0.30
BMI (kg/m ²)	23.4±3.0	23.6±3.2	0.50
Smoking, (yes/no)	25.2/74.8	16.0/84.0	<0.01
Drinking, (yes/no)	11.1/88.9	17.9/82.1	<0.01
FPG (mmol/L)	6.7±2.8	6.0±2.5	<0.01
SBP	136.0±19.4	131.3±18.4	<0.01
DBP	76.6±12.4	80.0±10.8	<0.01
TC (mmol/L)	4.3±1.2	4.8±1.1	<0.01
TG (mmol/L)	1.6±1.2	1.5±1.1	0.10
HDL (mmol/L)	1.2±0.4	1.3±0.5	<0.01
LDL (mmol/L)	2.5±1.0	2.9±1.0	<0.01
Hypertension, (yes/no)	31.9/68.1	56.1/43.9	<0.01
Diabetes, (yes/no)	70.0/30.0	86.8/13.2	<0.01
Coronary angiography	378	4	...
0-vessel disease, %	143	4	...
1-vessel disease, %	115	...	...
2-vessel disease, %	64	...	...
3-vessel disease, %	56	...	...
LM, %	19	...	...
LAD, %	217	...	...
RAD, %	112	...	...
LCX, %	109	...	...

Abbreviations: FPG, fasting plasma glucose; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TC, Total cholesterol; TG, triglycerides; HDL, High density lipoprotein; LDL, Low-density lipoprotein; LM, left main coronary artery disease; LAD, left anterior descending artery disease; LCX, left circumflex artery disease; RAD, right coronary artery disease.

3.2 Association between SNPs and CAD risk

The distribution of genotype and allele frequency in the case group and the control group were shown in Table 2. The results of univariate analysis showed that there was no statistical difference in the frequency distribution of the five SNPs between the case-control groups. Considering that confounding factors may bias the results of the study, we further adopted a binary unconditional logistic regression model. As represented in the table, SNP rs2327433 in *TARID* was associated with CAD after adjusting for sex, age, smoking, drinking, diabetes and five SNPs. The rs2327433 GG genotype was found as a risk factor for CAD in adults (OR=2.74, 95%CI: 1.10-6.83, $P=0.03$). As shown in **supplementary 3**, stratified analysis indicated a significant association between SNPs (rs2327433 and rs1966248) and risk of CAD. Our results showed that genotype GG of the SNP rs2327433 was associated with risk of CAD in the male's group and the drinking group (OR =3.37, 95%CI: 1.22-9.32, $P=0.02$ and OR=9.72, 95%CI: 1.14-82.83, $P=0.04$, respectively). The results indicated that genotypes AA carriers of rs1966248 had significant association with CAD risk in non-diabetes subjects (OR=1.45, 95%CI: 1.04-2.03, $P=0.03$).

Table 2

Association of 5 SNP in TARID gene with CAD Cases and Controls in the Chinese Han population

SNPs	Genotype	Case (n, %)	Control (n, %)	Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value*
rs2327429	TT	235(25.4)	206(23.9)	1.00			
	TC	466(50.4)	457(53.0)	0.89(0.71-1.12)	0.33	0.63(0.33-1.19)	0.15
	CC	223(24.1)	199(23.1)	0.98(0.75-1.28)	0.90	0.44(0.16-1.17)	0.10
	Allele						
	T	912(49.4)	855(49.6)	1.00			
	C	936(50.6)	869(50.4)	0.99(0.87-1.13)	0.88		
rs2327433	AA	594(66.3)	579(69.5)	1.00			
	AG	271(30.2)	236(28.3)	1.12(0.91-1.38)	0.29	1.19(0.85-1.67)	0.31
	GG	31(3.5)	18(2.2)	1.68(0.93-3.03)	0.09	2.74(1.10-6.83)	0.03
	Allele						
	A	1459(81.4)	1394(83.7)	1.00			
	G	333(18.4)	272(16.3)	1.17(0.98-1.40)	0.08		
rs12190287	CC	315(36.5)	285(34.7)	1.00			
	CG	411(47.6)	419(51.0)	0.89(0.72-1.10)	0.27	1.21(0.75-1.97)	0.44
	GG	137(15.9)	118(14.4)	1.05(0.78-1.41)	0.74	1.48(0.65-3.41)	0.35
	Allele						
	C	1041(60.3)	989(60.2)	1.00			
	G	685(39.7)	655(39.8)	0.99(0.87-1.14)	0.93		
	AA	170(18.8)	139(16.6)	1.00			
	AT	424(46.9)	391(46.8)	1.07(0.87-1.32)	0.44	1.17(0.83-1.65)	0.38

* The P values and OR (95% CI) were calculated from logistic regression analyses adjusting for sex, age, smoking, drinking, diabetes and five SNPs.

SNPs	Genotype	Case (n, %)	Control (n, %)	Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value*
rs1966248	TT	310(34.3)	306(16.6)	1.21(0.92-1.59)	0.49	1.29(0.74-2.24)	0.37
	Allele						
	T	1044(57.7)	1003(60.0)	1.00			
rs6569912	A	764(42.3)	669(40.0)	1.10(0.96-1.26)	0.18		
	CC	273(30.5)	255(30.6)	1.00			
	CT	434(48.5)	416(49.9)	0.97(0.78-1.21)	0.82	1.20(0.72-1.90)	0.48
	TT	187(20.9)	163(19.5)	1.07(0.82-1.41)	0.62	1.37(0.61-3.08)	0.45
	Allele						
	C	980(54.8)	926(55.5)	1.00			
	T	808(45.2)	742(44.5)	1.03(0.9-1.18)	0.68		
* The P values and OR (95% CI) were calculated from logistic regression analyses adjusting for sex, age, smoking, drinking, diabetes and five SNPs.							

The results of GMDR model analysis for the entire case-control data were shown in Table 3, which showed that the best model was the four-factor model. This model was adjusted for smoke. There were significant interactions with polymorphisms in the four genes in *TARID*.

Table 3
The models to predict risk of CAD by GMDR.

Factor	Training Bal. Acc	Testing Bal. Acc	Sign Test(P)*	CV consistency
[rs1966248]	0.5199	0.4875	3(0.9453)	6/10
[rs1966248 rs6569912]	0.5329	0.4662	0(1.0000)	5/10
[rs1966248rs12190287 rs6569912]	0.5559	0.4922	4(0.8281)	6/10
[rs1966248 rs2327433 rs12190287 rs6569912]	0.5876	0.5189	9(0.0107)	10/10
[rs1966248 rs2327429 rs2327433 rs12190287 rs6569912]	0.6018	0.5036	7(0.1719)	10/10
*Adjusted for smoking. GMDR: Versatile software for detecting gene-gene and gene-environment interactions underlying complex traits.				

We used the eQTL research platform provided by the GTEx (version 8) database to analyze the relationship between SNP rs2327433 and the expression levels of host genes and adjacent CAD-related genes. The eQTL analysis can confirm SNP-related gene expression differences[20]. We have listed the existing organizational data in **supplementary 4**. The results showed that the different genotypes of rs2327433 was significant correlated with the expression level of *TCF21* gene. The results reveal that in the esophageal mucosa (enriched endothelial cells) the expression of *TCF21* is lower in the GG genotype of SNP rs2327433. *TCF21* is a protein-coding gene adjacent to the downstream of *TARID*. By performing eQTL analysis in aortic tissue from the STARNET database²⁵ on this larger set of SNPs, studies found that the CAD risk imparted by rs2327433 was also correlated with lower *TCF21* expression from that allele[21]. Reduced *TCF21* expression is associated with increased coronary disease risk[21].

3.3 Association between CAD-related variant rs2327433 and the severity of CAD

The binary logistic regression analyses revealed that the genotype of rs2327433 was related to the proportion of CAD patients with left anterior descending artery disease (LAD) (Figure 1a) and left circumflex artery disease (LCX) (Figure 1b) after adjusting for traditional risk factors such as age, smoking, gender, drinking ($P=0.025$ and $P=0.025$, respectively). We further assessed the effect of SNPs on the development of CAD. The results showed that the minor allele frequency of rs2327433 was significantly correlated with the severity of the disease ($P=0.029$) (Figure 1c).

3.4 TARID silencing affected cell cycle progression and the expression of cell cycle-related genes

LncRNA *TARID* activates the expression of *TCF21* by inducing promoter methylation[17]. The SNP site rs2327433 of lncRNA *TARID* is related to the expression of *TCF21*. On the esophageal mucosa, the expression of *TCF21* is lower in the GG genotype of SNP rs2327433. We speculate that the GG genotype of SNP rs2327433 affects the expression of lncRNA *TARID*, preventing it from activating *TCF21*. Then the expression of lncRNA *TARID* in patients with coronary heart disease is not clear, we use human peripheral blood mononuclear cells to detect the expression level of lncRNA *TARID*. The results showed that after normalization with housekeeping gene (18s), compared with healthy subjects, the expression level of *TARID* in peripheral blood mononuclear cells of CAD cases was significantly reduced ($P < 0.01$) (Figure 2a).

Studies have shown that *TCF21* affects cell cycle progression of cells[22]. However, it is not clear whether the silencing of lncRNA *TARID* can affect the cell cycle through the *TCF21* pathway. As displayed in Figure 2b, the expression of *TARID* RNA in THP-1 or HUVEC cells transfected with si *TARID*-1 were significantly lower than in NC group, respectively. To assess how *TARID* silencing affects cell cycle progression, we conducted flow cytometry to analyze the distribution of cells across the major phases of the cell cycle in THP-1 cells transfected with *TARID* siRNA. The results revealed that *TARID* silencing resulted in an increase of THP-1 cells in the S-phase from 36.88% to 42.97 %, and decreased accumulation in the G2 phase from 4.85–2.93%, relative to the control group (Figure 2c).

To further study the effect of *TARID* in cell cycle regulation, qRT-PCR was performed to determine cell cycle-related genes. As shown in Figure 2d, *TARID* silencing up-regulated CDK1 and PCNA in THP-1 cells or HUVECs.

4. Discussion

In this study, we selected five SNPs (rs1966248, rs2327429, rs2327433, rs12190287 and rs6569912) in the lncRNA *TARID* gene for the association study with CAD. We used a case-control study method to reveal the relationship between five SNPs and CAD risk in the Chinese Han population. We found that SNP rs2327433 in *TARID* was associated with CAD risk in Chinese population. This SNP was also related with the severity of coronary heart disease.

SNP rs2327433 is located in the promoter region of lncRNA *TARID*. Our results suggested that the genotype of rs2327433 was related to the proportion of CAD patients with LAD and LCX disease. We further assessed the effect of SNPs on the development of CAD. The results showed that the minor allele frequency of rs2327433 was significantly associated with the severity of the disease. In the stratified analysis, we found that the G allele of rs2327433 has an increased risk of CAD in male subgroups and drinking conditions.

To investigate the relationship between SNP genotype and gene expression, we conducted the eQTL analysis. The eQTL analysis results showed that the SNP in the *TARID* gene region was related to the expression levels of *TARID* and *TCF21*. SNP rs2327433 is located in the promoter region of lncRNA *TARID*. *TARID* is a non-protein coding gene located in the 6q23.2 region of chromosome[23]. Studies have found that *TARID* can activate *TCF21* expression by inducing promoter demethylation[17]. It also further clarified the specific mechanism of *TARID* regulating *TCF21*[24]. *TCF21* can regulate the phenotypic transition of vascular smooth muscle to play an anti-atherosclerotic effect[18]. The results showed that the different genotypes of rs2327433 were significantly correlated with the expression level of *TCF21* gene in skeletal muscle or esophageal mucosa. The results showed that the expression of *TCF21* is lower in the GG genotype of SNP rs2327433 in the esophageal mucosa (enriched endothelial cells). Therefore, we speculate that this SNP rs2327433 may play an important role in coronary heart disease by affecting the pathway that *TARID* regulates *TCF21* function.

The SNP rs12190287 is significant related to myocardial infarction in Chinese population[25]. It has been reported that rs12190287 is significantly related to the expression level of the adjacent gene *TCF21*[15]. Studies have further verified the association between rs12190287 and the risk of CAD[26]. These studies indicated that rs12190287 is involved in the miRNA-dependent regulation of *TCF21*[16]. But rs12190287 is not statistically significant in Japanese patients with coronary heart disease[27]. This is a SNP reported in other populations, which is inconsistent with our study. Our study did not find that this SNP rs12190287 is directly related to the risk of CAD in Chinese Han population. This may be attributed to the synergy of multiple parts of coronary heart disease (rs1966248 rs2327433 rs12190287 rs6569912) or the ethnic differences.

We further investigated the expression level of lncRNA *TARID* in the case-control group. The data of expression level in peripheral blood mononuclear cells indicated that knockdown of *TARID* is associated with coronary heart disease. lncRNA is related to many cell functions, such as cell proliferation. Our data suggested that *TARID* silencing could increase the cell proliferation ability. Cell proliferation depends on cell cycle, involving multiple checkpoints[28]. In our study, the increase in S phase and decrease in G2 phase suggested that cell cycle was interrupted in the condition of *TARID*-silencing. In order to identify *TARID* could affect cell proliferation by regulating cell proliferation-related genes. Hence, we checked a series of cell cycle related genes and found that PCNA and CDK1 was up-regulated. Several studies suggested that up-regulated PCNA is related to promoting cell proliferation[29]. PCNA is highly expressed in the S phase of the cell cycle and is an important node for cell inspection[30], [31]. One of the genes essential for cell proliferation and cell cycle regulation is CDK1[32].

There are several limitations of this study that must be admitted. First, the sample size of CAD patients with angiographic evidence is relatively small, thus they may not be representative of the general population. Second, a prospective cohort study with a larger sample size is required to confirm our results.

5. Conclusion

In conclusion, we found that lncRNA *TARID* rs2327433 was significantly related to the severity of coronary artery disease. This finding implies that the rs2327433 may strongly influence the development of CAD. Our experimental results indicated that *TARID* knockdown in the human myeloid leukemia mononuclear cell THP-1 regulated proliferation and altered cell cycling by regulating the PCNA/CDK1 pathways.

Declarations

Authors' contributions

Zheng Cheng contributed to conceptualization, methodology, and writing original article; Yonghong Zhang, Yang Zhuo, Jie Fan, Ying Xu, Mengmeng Li, Hao Chen, contributed to methodology; Li Zhou contributed to review writing and editing, supervision, and funding acquisition.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figures

Figure 1

Association between CAD-related variant rs2327433 and the severity of CAD. a) Proportion of CAD patients with left anterior descending branch disease as a function of genotype. Adjusted for age, smoking, gender, drinking. b) Proportion of CAD patients with left coronary artery circumflex disease as a function of genotype. Adjusted for age, smoking, gender, drinking. c) Association between the frequencies of the rs2327433 minor allele and the progress of CAD. SAP indicates stable angina pectoris; UAP, unstable angina pectoris; MI, myocardial infarction.

Figure 2

IncRNA TARID-mediated cell cycle pathway associated with CAD a) The expression of IncRNA TARID between patients and controls. peripheral blood mononuclear cell was used to measure the expression level of IncRNA TARID. b) Detection of TARID silencing efficiency in THP-1(left) or HUVEC (right). c) TARID silencing affected cell cycle progression of THP-1. Cell cycle distribution of THP-1 cells after THP-1 silencing was analyzed and quantified by ModFit (LT 4.1). d) TARID regulated the expression of cell cycle-related genes THP-1(up) or HUVEC (down). THP-1 or HUVEC were transfected with NC, siTARID-1, siTARID-2 for 48h. The RNA level was evaluated by qRT-QPCR. ** $P < 0.01$, ** $P < 0.01$, * $P < 0.05$, compared with the NC group. The fold change of gene expression was calculated as the $2^{-\Delta\Delta CT}$ method.

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