Decreased FENDRR and LincRNA-p21 expression in atherosclerotic plaque

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Abstract

Objective: Cardiovascular diseases are the most important cause of mortality worldwide, particularly atherosclerosis. Recently, lncRNAs affecting atherosclerotic progression have been reported in vascular smooth muscle cells, endothelial cells, and monocytes, suggesting that lncRNAs play an important role in atherosclerosis.

Methods: In recent clinical studies, nowadays, it was determined that internal mammary bypass grafts are closest to ideal grafts in coronary artery bypass surgery. In this study, we used tissue samples taken from atherosclerotic coronary arteries and the internal mammary artery (IMA) during coronary artery bypass surgery. Using RT-PCR, we investigated the role of two lncRNAs, FENDRR and LincRNA-p21, by comparing their expression levels in coronary artery plaques and normal mammary arteries of 20 atherosclerotic patients.

Results: We found that the FENDRR and LincRNA-p21 expressions decreased by approximately 2 and 7 fold in coronary artery plaques, respectively, compared with those in IMA, which is known to have no plaque development.

Conclusion: This study was the first to use mammary artery tissues of the same patients as a control and to study FENDRR expression. Our data may provide helpful insights regarding the association of lncRNAs and atherosclerosis. *(Anatol J Cardiol 2018; 19: 131-6)*

Keywords: atherosclerosis, lncRNA, FENDRR, lincRNA-p21, coronary artery plaque, internal mammary arteries

Introduction

Atherosclerosis, a complex vascular disease, is the most important cause of mortality worldwide. Atherosclerosis can be identified as an inflammatory response to a vascular wall injury (1). This response is characterized by the migration of mononuclear lymphocytes to the vascular wall, proliferation of vascular smooth muscle cells (VSMC), and aggregation of extracellular matrix factors (1, 2). Many enviromental as well as genetic factors contribute to atherosclerotic progression.

lncRNAs is a group of noncoding RNAs that is known to play a role in diverse biological processes, including control of enzyme activity, inhibition of transcription regulators, determination of splicing pattern, and regulation of mRNA transcription (3-5). It has been suggested that lncRNAs regulate cardiac development and play important roles in cardiac insufficiency (6). Epigenetic mechanisms such as DNA methylation as well as noncoding RNA (ncRNA) regulation have been thought to be associated with many diseases including atherosclerosis. Among lncRNAs, FENDRR is found to be associated with heart development (7). It also regulates the chromatin structure and gene activity via binding polycomb repressive complex 2 (PRC2) and trithorax group/mixed lineage leukemia complexes (TrxG/MLL) (7). Although FENDRR is found to be essential for proper heart and body wall development in mice, there is no clear evidence about the role of FENDRR in cardiovascular diseases (CVD) (7). Our study is the first to investigate this relationship. LincRNA-p21 is another example of well-studied lncRNA in atherosclerosis. It was shown that lincRNA-p21 can bind p53 repressor, MDM2, and it regulates cell proliferation and apoptosis in VSMC (8, 9). In this study, we investigated the expression patterns of FENDRR and LincRNA-p21 in atherosclerotic coronary artery tissue and internal mammary artery (IMA) tissue of the same patients to explore whether there is a relationship between plaque development and lncRNA expression.

Methods

Study population

Human tissue samples of atherosclerotic coronary tissues were obtained from 20 patients subjected to coronary endarterectomy and bypass grafting due to occlusive atherosclerosis. Samples were taken from patients who underwent the bypass surgery

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Accepted Date: 20.12.2017 **Available Online Date:** 29.01.2018

DOI:10.14744/AnatolJCardiol.2017.8081

Anatol J Cardiol 2018; 19: 131-6
DOI:10.14744/AnatolJCardiol.2017.8081

Table 1. Study population characteristics

Parameters	Patient (n %)	Parameters	Patient (n %)
Total	20 (100%)	NYHA classification	
Age, median	62 (40-84)	Class 2, n (%)	8 (40%)
Gender, male	17 (85%)	Class 3, n (%)	12 (60%)
Smokers	17 (85%)	Coronary arteries stenosis	
Cholesterol	9 (45%)	Right coronary artery, median (range)	80% (60-100)
Diabetes	8 (40%)	Left anterior descending artery, median (range)	80% (40-90)
Family history	13 (65%)	Circumflex artery, median (range)	65% (0-100)
Hypertension	16 (80%)	Bypass (at least 1), n (%)	20 (100%)
LDL	11 (55%)	Left ventricular ejection fraction, median (range)	46% (25-56)
Previous MI	8 (40%)		
Triglyceride	18 (90%)		

Table 2. FENDRR, LincRNA-p21 and glyceraldehyde-3-phosphate dehydrogenase primer sequences used in RT-PCR

Gene	Forward (5'-3')	Amplicon size (bp)
FENDRR	Forward 5'-TCTTGTCTTTGTAATCAGGCAG-3'	460
	Revers 5'-GGAGGTATTTCAGTTCTGTCGT-3'	
LincRNA-p21	Forward 5'-CAGGGTGCCAGAAGAGTGAG-3'	222
	Revers 5'-AGACTAAAGCTCCTACTTCAGCAG-3'	
GAPDH	Forward 5'-TGCACCACCAACTGCTTAGC- 3'	87
	Revers 5'-GGCATGGACTGTGGTCATGAG-3'	

bp- base pair, GAPDH- glyceraldehyde-3-phosphate dehydrogenase

Table 3. Fold change and expression rate of internal mammary artery tissues and coronary artery tissues are measured with RT2 profiler RT-PCR Array Data Analysis Programme

	Expression rate	Fold change	*P* value (*P*<0.05)
FENDRR	0.4635	-2.16	0.264389
LincRNA-P21	0.1493	-6.7	0.705267
GAPDH	1	1	0

GAPDH- glyceraldehyde-3-phosphate dehydrogenase

take into account clinic and laboratory data and risk stratification. From the same patients, IMA samples were obtained during bypass surgery and were used as controls. Patients were routinely operated cardiopulmonary bypass while stopping their hearts. Coronary endarterectomy practice to vessel which is external diameter is 2.0 mm while coronary vessel is completely blocked and in case of no endarterectomy coronary endarterectomy

All surgical procedures were performed at the Department of Cardiovascular Surgery of Cumhuriyet University. The study has been approved by the Ethics Committee of Cumhuriyet University (file number: 2015e01/18) and is in accordance with the Declaration of Helsinki; all patients signed written informed consent.

A total of 20 patients (3 females and 17 males) with atherosclerosis were enrolled in this study. The mean age of patients was 62.60±7.68 years. Table 1 shows the demographic data of patients.

RNA Isolation and qRT-PCR

First, 40 mg of coronary artery plaques and IMA tissues were obtained from each patient and homogenized at 7000 rpm for 30 s using MagnaLyser. The total RNA was extracted using miRNeasy Mini kit (Qiagen Inc., Valencia, CA, USA) and reverse-transcribed into cDNA using RT2 First Strand Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. The RNA concentration was measured using Flowmeter (Qbit ver 3.0).

Real-time PCR was performed using RT2 SYBR Green Mastermix kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocols. The reaction conditions included denaturation at 95°C for 10 s, 45 cycles at 95°C for 15 s, and 60°C for 60 s. Nonspecific amplification was not determined by the dissociation curve. Primer sequences used in the study are given in Table 2.

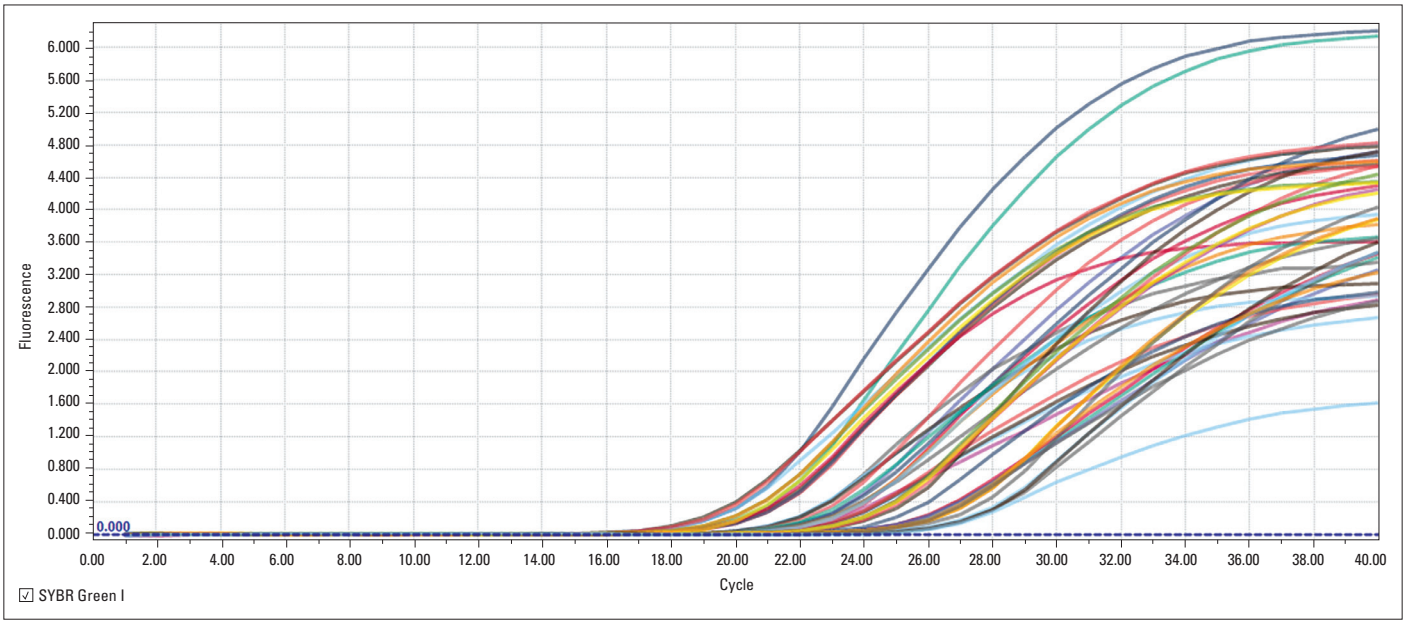

Figure 1. Amplification curve of FENDRR in internal mammary artery tissues. Each sample was studied in triplicate

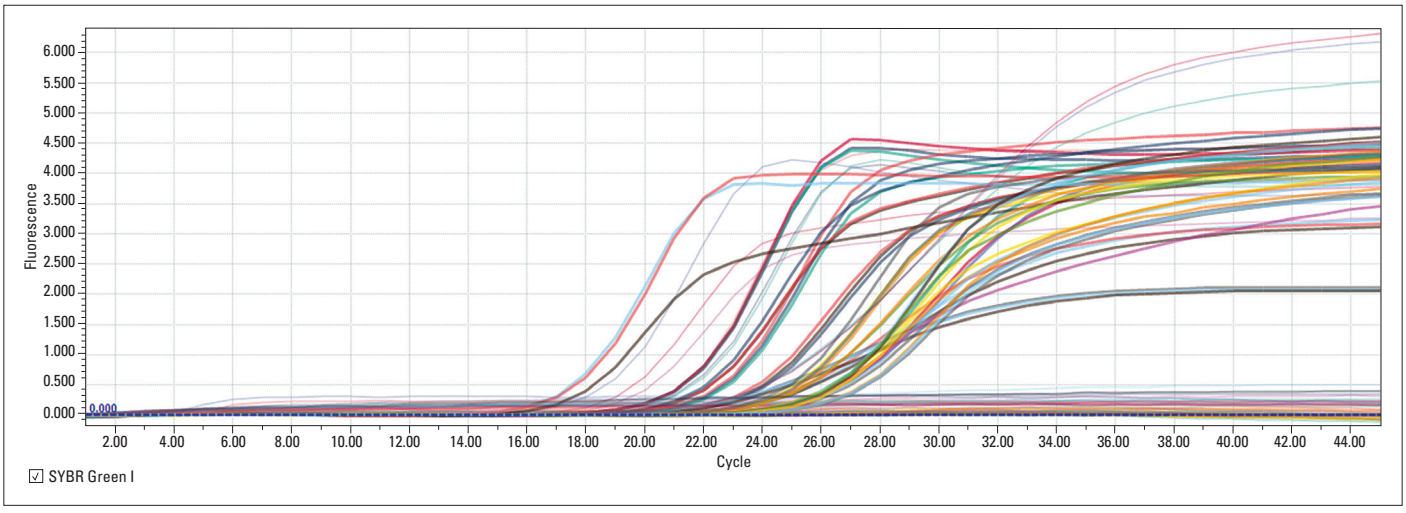

Figure 2. Amplification curve of LincRNA-p21in internal mammary artery tissues. Each sample was studied in triplicate

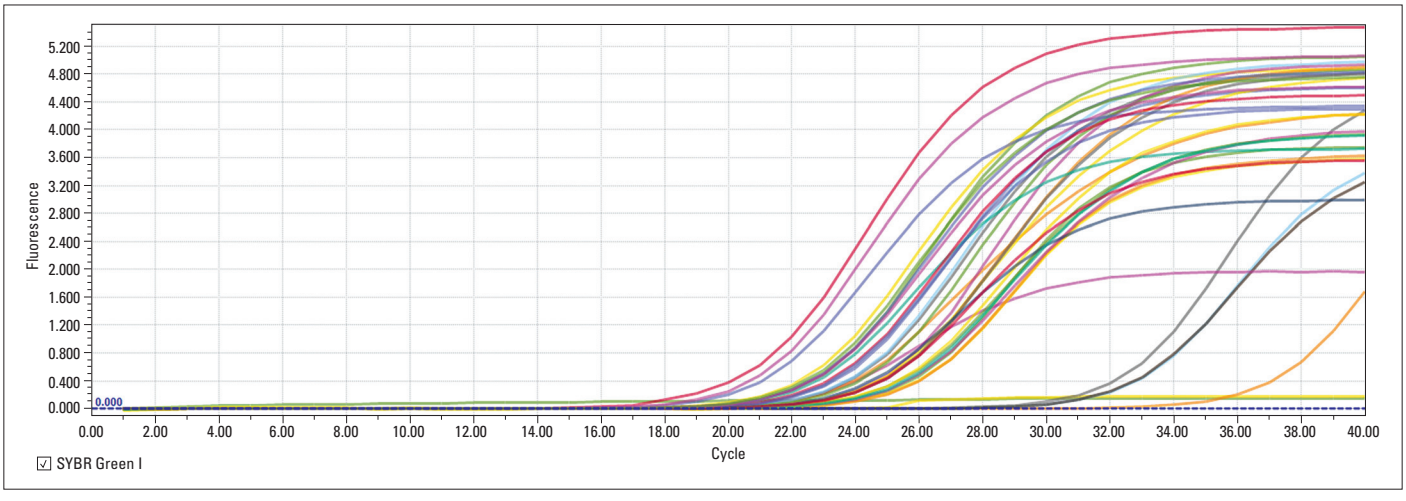

Figure 3. Amplification curve of glyceraldehyde-3-phosphate dehydrogenase in internal mammary artery tissues. Each sample was studied in triplicate

Anatol J Cardiol 2018; 19: 131-6
DOI:10.14744/AnatolJCardiol.2017.8081

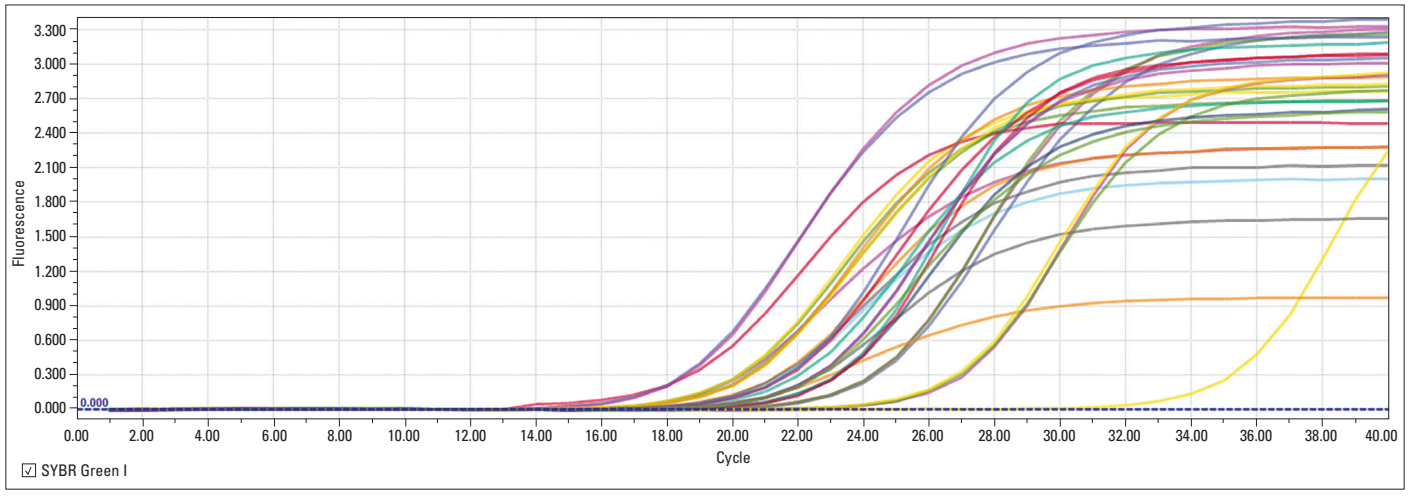

Figure 4. Amplification curve of FENDRR in coronary artery plaque tissues. Each sample was studied in triplicate

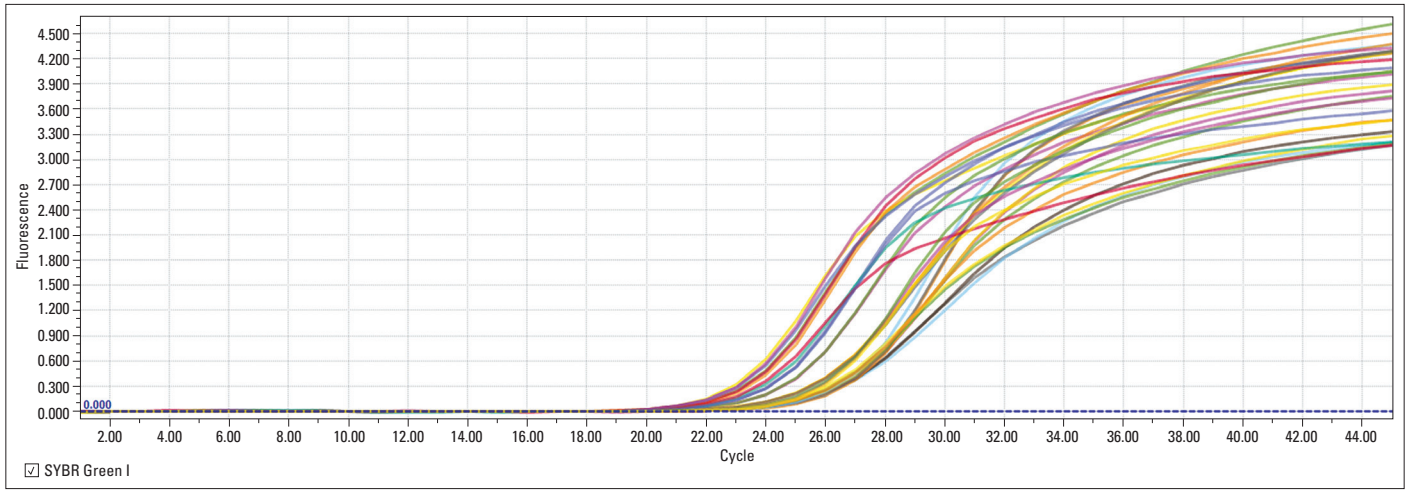

Figure 5. Amplification curve of LincRNA-p21 in coronary artery plaque tissues. Each sample was studied in triplicate

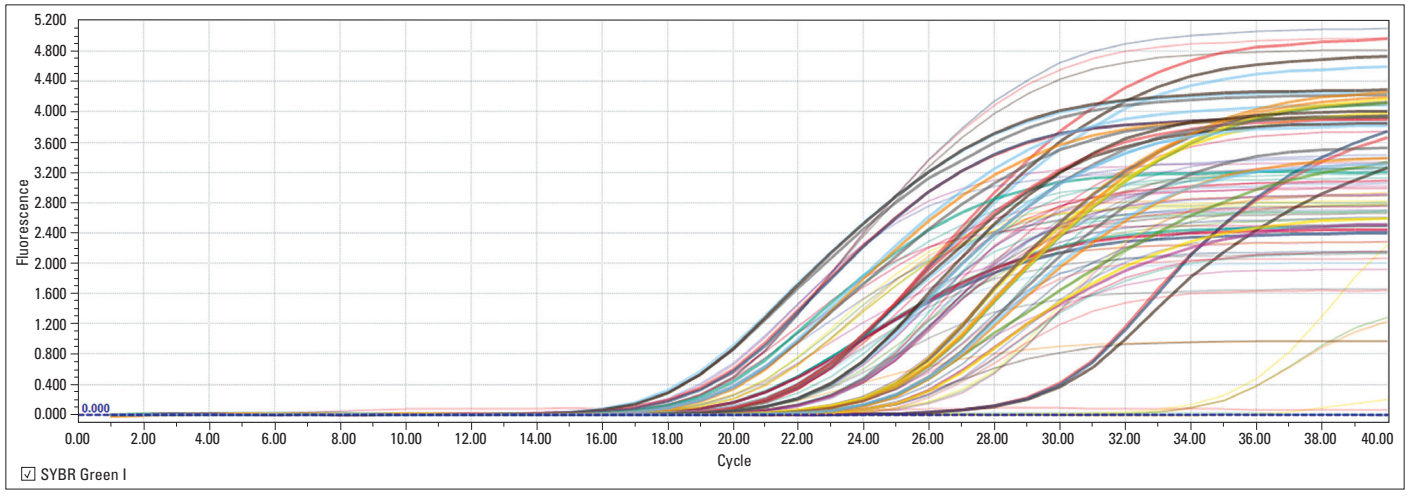

Figure 6. Amplification curve of glyceraldehyde-3-phosphate dehydrogenase in coronary artery plaque tissues. Each sample was studied in triplicate

The quantification of expression levels was performed by the ΔΔCt (threshold cycle) method using RT2 profiler RT-PCR Array Data Analysis Programme (Qiagen Inc., Valencia, CA, USA, version 3.5) (http://pcrdataanalysis.sabiosciences.com/ pcr/array-analysis. php), and gene expression levels were normalized to GAPDH transcript levels using the following expression: (primer efficiency)$^{-\Delta\Delta Ct}$, where ΔΔCt means ΔCt (target gene) –ΔCt (reference gene). P<0.05 was considered as significant.

Results

LincRNA-p21 and FENDRR levels were analyzed by comparing their expression patterns in coronary artery plaques and IMA tissues that were normalized to GAPDH levels. The results of expression levels are given in Table 3. Compared with control IMA tissues, FENDDR and LincRNA-p21 expressions downregulated 2 and 7 fold, respectively. No significant difference regarding the expression of these two lncRNAs was observed between the coronary artery plaques and IMA tissues ($p>0.05$). The amplification curves of FENDRR, LincRNA-p21, and GAPDH in coronary artery plaques and IMA tissues are shown in Figures 1, 2, 3, 4, 5 and 6.

Discussion

Our study was the first to compare coronary artery plaque tissues and internal mammary arteries of the same patients to observe the expressions of FENDRR and LincRNA-p21. Our data showed that the atherosclerotic coronary artery plaques have decreased FENDRR (2.16 fold) and lincRNA-p21 (6.7 fold) expression levels compared with the IMA tissues.

In recent years, regulation mechanism of some lncRNAs have been enlightened, and some of the lncRNA were found to be differentially expressed in human CVD. It has also been reported that these differential expressions could be used as predictive markers of cardiovascular disease (10, 11). Although there are many studies performed using miRNA expression patterns in atherosclerosis, only a few studies can be found in the literature for lncRNA and CVD. FENDRR and LincRNA-p21 are two important lncRNAs that are asscoiated to the development and phsysiology of heart (7, 9). Therefore, their expression levels could be an indicator of CVD pathophysiology. To explore this idea, FENDRR and LincRNA-p21 expression levels were analyzed in 20 atherosclerotic patients in this study.

FENDRR is a regulator of two important histon-modifing complexes, PRC2 and TrxG/MLL (7); by binding to PRC2 complex, FENDRR induces Histone H3 Lysine 27 trimethylation (H3K27Me3) that transcriptionally inhibits many genes (12). Wierda et al. (12) have shown that H3K27Me3 modification levels were decreased in advanced atherosclerotic plaque vessels. However, the loss of H3K27Me3 was found to be associated with cell proliferation and decreased H3K27Me3 expression, which promotes atherosclerotic plaque development (12). In another study that investigated H3K27Me3 modification level in VSMC in dietary hypercholestrolemic ApoE (-/-) mouse cell, the global H3K27Me3 expression level was specifically decreased (13). Our 2.16-fold decreased FENDRR expression data may correlate with that of Alkemade et al. (13), and it may explain the reduced level of H3K27Me3, which may cause cell proliferation in atherosclerosis. During atherosclerotic plaque development, the proliferation of VSMC resembles cancer cell proliferation. Although there are limited reports about FENDRR in the literature, one extensive study done in gastric cancer (14), wherein blocked FENDRR expression caused upregulated fibronectin expression. In turn, this upregulation was implicated in invasion and metastasis. About two-fold downregulated expression of FENDRR in our data was not statistically significant; however, with sufficient number of patients, much more accurate and convincing results can be obtained, especially with the tissues of same individuals. Moreover, it is important to use the same patients' tissue material because of the genetic variations among individuals. Most of the comparison studies have been performed using samples from different individuals. This may cause conflicting results as there are many genetic variations between individuals. Thnerefore, we suggest using samples from same individuals.

In this study, we also showed that lincRNA-p21 expression is reduced about 7-fold in atherosclerotic plaque tissues, but it was not statistically significant. This result is correlated with the results of Wu et al. (9). They showed decreased lincRNA-p21 expression in atherosclerotic plaques of mice and reported it as a key regulator of cell proliferation and apoptosis during atherosclerosis. It was also recently reported that p53 regulates lincRNA-p21 expression. The authors suggested that the downregulation of lncRNA may cause cell proliferation during plaque deveolpment through p53-mediated apoptotic pathway as lincRNA-p21 represses cell proliferation and activates apoptosis. Our results also support this observation in mice.

This study show that decreased FENDRR and LincRNA-p21 expressions are associated with atherosclerosis. Further studies with more patients may provide more statistically significant data.

Study limitations

The number of samples was limited (n=20), given the heterogeneity of atherosclerotic plaques. The major weakness of the study is to be not obtained more patients tissues.

Conclusion

In conclusion, our results with decreased FENDRR and LincRNA-p21 expressions were the first preliminary important findings. More studies with a larger sample size are needed to find out the role of lncRNAs in atherosclerosis.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – A.Ö., S.A.; Design – N.Ç., S.A.; Supervision – S.A., E.P.; Fundings – N.Ç., S.G.; Materials – N.Ç., A.Ö.; Data collection &/or processing – S.G., Ö.B.; Analysis &/or interpretation – S.G., Ö.B.; Literature search – N.Ç., A.Ö.; Writing – N.Ç., E.P.; Critical review – E.P., Ö.B.

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Anatol J Cardiol 2018; 19: 131-6
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