



Analysis of Coronary Artery Disease Using Serum/Plasma Data in a Quantum Microrna Language with Artificial Intelligence (MIRAI)

Yoichi Robertus Fujii*

Kawada-Cho, 106-6, Atsuta-Ku, Nagoya, 456-0065, Japan

*Corresponding author: Yoichi Robertus Fujii, Kawada-Cho, 106-6, Atsuta-Ku, Nagoya, 456-0065, Japan; Tel: +81-52-682-7003; E-mail: fatfujii@hotmail.co.jp

Abstract

Background: Noninvasive microRNA (miRNA) biomarker panels have been investigated in plenty of human diseases. In addition, using data from the miRNA panel, many therapeutic target genes in coronary artery disease (CAD) have been shown in silico. To further elucidate the integrated molecular mechanism of CAD, which is also a metabolic disease, the quantum miRNA language with artificial intelligence (MIRAI) was used and the etiology was precisely simulated and statistically validated.

Methods: Data of miRNA panels in serum or plasma of patients with CAD was extracted from the database. A miRNA entangling target sorter (METS) with MIRAI, a bioinformatic algorithm based on quantum theory, was used to analyze etiology in CAD as described previously.

Result: Two biological processes involved in CAD therapeutic targets: 1) cardiac pacemakers and 2) inflammation of the intima in the arteries. Hyperpolarization activated cyclic nucleotide gated potassium channel 4 (HCN4) and potassium voltage-gated channel subfamily H member 2 (KCNH2) were increased by down regulation of the miR-133b hub. Cell cycle related proteins, cyclin D1 (CCND1), cyclin D3 (CCND3), cyclin E1 (CCNE1) and cyclin-dependent kinase 6 (CDK6) were enhanced by down regulation of the miR-424-5p hub. Further, hypoxia inducible factor 1 subunit alpha (HIF1A) was also increased by down regulation of the miR-424-5p hub. An area under the curve (AUC) of these CAD therapeutic targets was 97.19% (accuracy: 94.4, precision: 99.04).

Conclusion: For the first time, we found a CAD therapeutic target for a cardiac pacemaker. High levels of HCN4 expression increase funny currents (I_f) and rapid spontaneous pulsations that can cause arrhythmia. Increased KCNH2 is related with ventricular fibrillation through increased I_{Kr} current, a major cause of sudden cardiac death. The other was the target of atherosclerosis. Activation of cell cycle-related proteins in G1/S phase was implicated in the proliferation of endothelial and smooth muscle cells. Further, HIF1A augmentation is involved in macrophage proliferation, including incorporation of oxidized low-density lipoprotein (oxLDL) into atherogenic hypoxic lesions. Two different etiologies occurred simultaneously in different lesions. Therefore, miR-133b and miR-424-5p mimics may be potential CAD prophylaxis candidates as state-of-the-art treatments. MIRAI is a useful tool for decoding layered-miRNA codes.

Keywords: microRNA; Quantum miRNA language; Artificial intelligence; CAD; CVD; Atherosclerosis; pacemaker

Introduction

Coronary artery disease (CAD) mechanically involves the narrowing of coronary arteries and the restricting of blood flow to the heart, and results in cardiovascular disease (CVD), such as angina pectoris, myocardial infarction (MI) or sudden death. The prevalence of CVD in the United State of America (USA) was seriously an estimated 126.9 million adults over 20 years old

(49.2% overall population) in 2018 data [1]. CAD includes acute coronary syndrome (ACS) subtypes as unstable angina (UA), acute myocardial infarction (MI) with ST-segment elevation (STEMI), and MI without ST elevation (NSTEMI) and stable CAD. The risk factors of CAD are smoking, low physical activity, high body mass index (BMI), high calorie diet, high blood pressure, high blood lipids, glycemia, genetic factors and

Received date: 28 November 2021; Accepted date: 02 December 2021; Published date: 07 December 2021

Citation: Robertus Fujii Y (2021). Analysis of Coronary Artery Disease Using Serum/Plasma Data in a Quantum Microrna Language with Artificial Intelligence (MIRAI). SunText Rev Med Clin Res 2(4): 143.

DOI: <https://doi.org/10.51737/2766-4813.2021.043>

Copyright: © 2021 Robertus Fujii Y. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

infections, etc. Protein biomarkers, such as natriuretic peptides, soluble suppression of tumorigenicity 2 (sST2), growth differentiation factor-15 (GDF-15), high-sensitivity cardiac troponins (hs-cTn), C-reactive protein (CRP), interleukin 6 (IL-6), and myeloperoxidase, etc., have been used for patients of stable CAD [2]. In the case of ACS, cardiac troponin T (CTnT), cardiac troponin I (CTnI), creatine kinase muscle/brain subtype (CK-MB) has been used [3]. These biomarkers are well known to have some limitations, such as slow first appearance and increasing of chronic kidney disease (CKD). Therefore, sudden death by CAD is not easy for early accurate diagnosis with current histopathologic technology. It is well known that CAD is caused by atherosclerosis and atherosclerosis is driven by a chronic inflammatory milieu that induces plaque development with accumulation of oxidized low-density lipoprotein (oxLDL) into macrophages (oxLDL-macrophages) and endothelial cells [4]. Despite these data about the mechanisms of CAD, the impact of atherosclerosis has not yet cleared by the histochemical and immunological pathophysiology because atherosclerosis is a complex multifactorial disease. The patients with increases in the base-line levels of CRP as the inflammation index have significantly been correlated with acute CAD, and anti-cholesterol agent, statin has decreased CVD risk but not changed in the LDL profile of patients [5]. In addition, by double-blind trial of canakinumab, anti-interleukin 1 β (IL-1 β), recurrent risk of CVD has been observed to reduce in patients with previous MI and high CRP level of >2 mg/L [6]. Therefore, residual heightened inflammation might be associated with risk of CDA but oxLDL reduction has not curtailed to contribute for CAD risk [7]. As an evidence, the foam cells in the arterial intima absorb LDL; however, the molecular mechanisms controlling atherogenesis are still not fully understood [8]. Because increasing rates of LDL transcytosis across endothelial cells remain unclear during hypercholesterolaemia [9]. Processes of modified LDL particles are multiple, such as proteolysis, phospholipolysis, oxidation, hydrolysis and proteoglycan interactions. Although the modified LDL particles can trigger proinflammatory reactions, it is uncertain whether activation of arterial intimal cells or inflammation via the intima injury is the first event. A VLDL-ligand ApoE-knockout (KO) mice with high-fat diet is commonly used to examine atherosclerotic events; however, suppression of polyunsaturated fatty acids (PUFAs) with high fat diet has not provoked ApoE-deficiency mediated atherosclerosis plaque formation in the double KO mouse (fads2 $^{-/-}$ x apoe $^{-/-}$) model experiment [10-12]. As the similar results were obtained in LDL receptor deficient double KO mice, $\Delta 6$ -fatty acid desaturase (fads2) related $\omega 6/\omega 3$ -polyunsaturated fatty acids would be more closely implicated in the plaque formation than LDL. PUFA is a source of precursors for immunomodulators, such as prostaglandin and leukotriene [13]. Even murine model

experiments, the data suggests that inflammation would be the first cause of atherosclerosis. The comprehensive results in the dietary studies of atherosclerosis suggest that LDL particles in histopathological data of atherosclerosis might have not been corresponding to the cause of disease as the amyloid plaque hypothesis in Alzheimer's disease. MiRNA has an important role for controlling of the pathogenesis in CAD [14]. Cholesterol homeostasis, reverse cholesterol transport, plaque initiation and progression, plaque rupture and plaque neovascularisation related miRNAs in atherosclerosis have been precisely documented in several reviews [14-20]. Circulating miRNAs in the plasma or serum have been available for non-invasive and early diagnosis to prevent sudden death by CAD [18]. But the key of the CAD aetiology related with miRNAs remains unclear. A bioinformatic technique, miRNA entangling target sorting (METS) analysis with quantum miRNA language plus artificial intelligence (MIRAI) has revealed the cancer aetiology and therapeutic targets of breast, lung, colorectal, pancreatic, esophageal, gastric and liver cancers [21-24]. Etiologies of infectious diseases, such as hepatitis B and C viruses (HBV and HCV), human immunodeficiency virus (HIV-1) and severe acute respiratory syndrome human coronavirus 2 (SARS-CoV-2), have also been computed by METS with MIRAI [24-28]. These data suggested that miRNA biomarker panels in diagnosis is useful for pathophysiologic search to investigate therapeutic targets. Plenty of successful METS analysis data showed that miRNA gene code of human could be decoded by an algorithm of quantum miRNA language, and it is very distinct from protein gene code because miRNA gene code follows the basis of quantum mechanics [29]. Since the RNA wave 2000 model has been enough validated by previous lots of reports; 1) the miRNA gene is a mobile genetic element that induces transcriptional and posttranslational silencing through network processes, 2) the RNA information supplied by miRNA genes expands to intracellular, intercellular, intraorgan, interorgan, intraspecies, and interspecies under the life cycles in the global environment, 3) mobile miRNAs self-proliferate and 4) cell contain resident and genomic miRNAs, most of all biological processes including atherosclerosis are controlled by miRNAs [29]. In this paper, the etiology of coronary artery disease (CAD) from circulating profiles in plasma/serum was investigated by using METS/MIRAI. The factor of cardiac pacemaker in CAD was firstly found as a cause of CAD and the controversial data of atherosclerosis in CVD was discussed.

Materials and Methods

Databases

Google Scholar (<https://scholar.google.co.jp>) were firstly used for data extraction from miRNA panels and miRNA profiles in the

plasma or serum. Total information contents were 181,427 in CAD, 157,794 in atherosclerosis and 2,716,846 in cardiovascular disease. The gene function of protein was searched by GeneCards. Protein ontology was investigated by GO enrichment analysis in Geneontology. Data of multi-targets to a miRNA and multi-miRNAs to a target were obtained from TargetScan Human 7.2, DIANA-TarBase and miRTarBase Ver. 8.0 or mirtarbase data for miRTarBase release 6.1, which was re-built up by Excel file of the package in the GitHub. Protein/protein interaction search and cluster analysis were performed by using STRING Ver. 11.0. The RNA secondary structures of the artificial stem loop were computed by RNA Fold.

METS network analysis

The METS network analysis was performed with MIRAI from the miRNA memory package (MMP), of which data is statistically extracted from clinical miRNA biomarkers and miRNA profiles as previously described [24-26, 30-32]. Data

mining about miRNA panels was performed by 1) data from serum or the plasma, 2) cleared in expression levels of up- and down-regulation, 3) data was statistically analysed by receiver operating characteristic (ROC) and the cut off value of an area under the curve (AUC) about biomarker profiling is 0.7 (Table 1). As the quantum miRNA language, single nexus score (SNS) and double nexus score (DNS) in the matrix algorithm were computed as previously described [29]. The values of electric field tangent score (EFT) were computed in microRNA memory package (MMP) from miRNA biomarker panels of CAD to use weighting of the DNS value as described previously [33].

MIRAI

AI was used with the quantum miRNA language for METS analysis as previously described. The area under the curve (AUC) in receiver operating characteristic (ROC), accuracy, and precision were calculated as the percentage by using previous integrated data pool [24].

Table 1: Table 1: MMP from plasma/serum data in CAD.

miRNA	Level	Source	SNS	AUC data		Reference no.
				Stable CAD	Acute CAD	
miR-133b	down	Plasma	3	0.800		34
miR-499a-5p	up	Plasma	5	0.713		35
miR-765	up	Plasma	11	0.959	0.972	36
miR-149-5p	down	Plasma	4	0.938	0.977	
miR-29b-3p	down	Serum	4	0.930		37
miR-208a-3p	up	Serum	5	0.847		
miR-215-5p	up	Serum	4	0.913		
miR-424-5p	down	Plasma	4	0.919	0.960	38, 39
miR-502-5p	up	Serum	5	0.867		37

Bold: hub miRNAs

Table 2: Validation of CAD etiology with MIRAI.

	CAD
AUC	97.19
Accuracy	94.40
Precision	99.04
Recall	94.50
F value	96.71

Quantum energy levels of CAD

Energy levels of DNS and EFT were computed and depicted by using MMPs of CAD. Unique radar chart was observed according to weighting of EFT values. Levels of DNS and EFT in the hub miRNAs were 12 and 439875.5 in AD. The hub miRNAs experienced the high value of electric field intensity; therefore, CAD-related hub miRNA forced high electric intensity (EFT value). On the other hand, DNS of the hub miRNA in CAD was quite low because the quantum energy layers of DNS for the

matrix of MMP in CAD was shown as the quantum core region (QCR) of 10-20. But the frequency of DNS was high in the QCR of 0-20. It suggests that CAD-related miRNAs are stable in quantum state but electrically active (Figure 1).

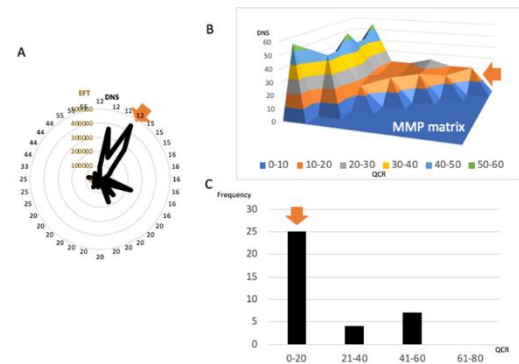


Figure 1: Quantum energy levels of miRNAs in CAD. The DNS and EFT values of MMP for CAD were depicted in the radar chart (A). The matrix

SUNTEXT REVIEWS

of MMP was shown as the DNS values and the quantum core region (QCR) layers were determined (B). Frequencies of the DNS value in CAD in layers of QCR, 0-20, 21-40 and 41-60 were represented (C). QCR containing a DNS of two hub miRNAs were shown as arrows (amber).

MMPs of CAD

Nine miRNAs were extracted as an MMP of coronary artery disease (CAD) from circulating profiles in plasma/serum upon a meta-analysis [34-39]. Four miRNAs, miR-133b, miR-149-5p, miR-29b-3p and miR-424-5p have been down regulated, and five miRNAs, miR-449a-5p, miR-765, miR-208a-3p, miR-215-5p and miR-502-5p were up regulated. AUC on CAD diagnosis of 8 miRNAs was >0.8 in stable CAD vs. non-coronary artery (NCA) people except for miR-449a-5p (AUC: 0.713). Three miRNAs, miR-765, miR-149-5p and miR-424-5p were common ones of stable and unstable CAD with the AUC of >0.96. While the seed of miR-133a-3p has the complete same nucleic acid sequences in miR-133b except for the 5' end (g/a), miR-133a-3p and miR-449a-5p, which were unregulated in the tissues, have shown as the biomarker of sudden cardiac death by using with samples of autopsy cardiomyocyte tissues [40]. Further, miR-133b (down regulation) has been as circulating biomarker in early prediction of CAD [34]. When METS computing of CAD biomarker was performed, the GO analysis of miRNA targets showed two possible biological processes: 1) SA node cell to atrial cardiac muscle cell signaling (GO: 0086018), 2) epithelial cell maturation (GO: 0002070). The data indicated two separate biological pathways. The former would be implicated in heart pacemaker and the latter would be associated to inflammation followed by atherosclerosis. Therefore, the etiological investigation of CAD by METS was involved into two parts of the organ, the heart and the coronary arteries. It is suggested that two different etiologies are simultaneously occurring in two different lesions (Figure 2).

The cardiac pacemaker in stable CAD

MiR-133b has been statistically used as a biomarker of stable CAD. Heart rate is initiated by spontaneous depolarization of the sinoatrial node as pacemaker cells [41]. An increased heart rate has been associated with coronary atherosclerosis in animal models and patients [42]. Although it has been thought that an elevated heart rate would be due to several stresses, such as increasing plaque formation in the arterial endothelial walls, oxidative stress and inflammation of the coronary artery, our METS analysis showed that hyperpolarization activated cyclic nucleotide gated potassium channel 4 (HCN4) and potassium voltage-gated channel subfamily H member 2 (KCNH2) were increased by down regulation of miR-133b hub along with miR-6511b-5p, miR-4748 plus miR-557, and with miR-7-5p. Calcium voltage-gated channel auxiliary subunit gamma 7 (CACNG7) was

suppressed by up regulation of miR-765 in combination with miR-7-5p.

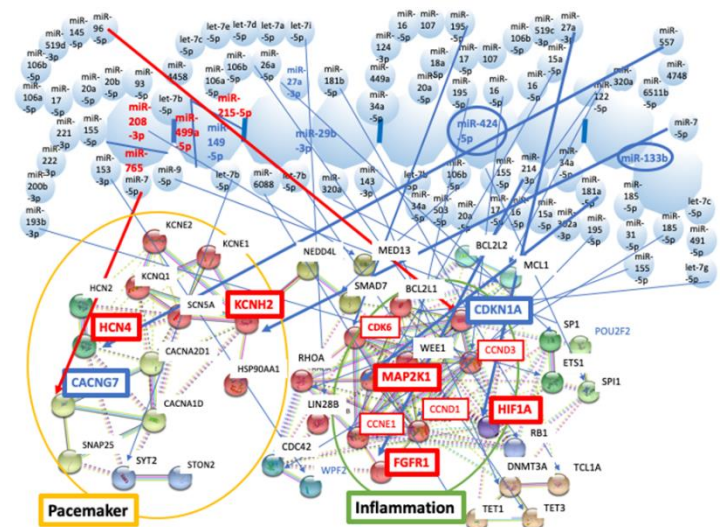


Figure 2: Network diagram of METS computer simulation in MMP of CAD. The linkage among protein clusters and miRNA/miRNA as presented by METS. miRNAs and proteins in red: up regulation, in blue: down regulation. Small circles in blue are the hub miRNAs. Large circles in amber (pacemaker) and in green (inflammation) are presented as GO protein cluster functions.

In mouse embryonic stem cell-derived cardiomyocytes, HCN4-overexpression have increased funny currents (If) and rapid spontaneous beating [43]. Since elevating of If have augmented pacemaker activity of the sinoatrial node and heart rate in mice, HCN4 increasing by miR-133b hub down regulation would be implicated in heart rate elevating in CAD. On the contrary, KCNH2 (the human ether-a-go-go-related channel, hERG) overexpression has increased IKr currents and accelerated re-entry frequency or fibrillation in neonatal rat ventricular myocyte monolayers but been undetectable pacing frequencies [44,45]. It is suggested that KCNH2 elevating by miR-133b hub down regulation would be related to ventricular fibrillation, which causes a major cause of sudden cardiac death. As arterial and ventricular myocytes in ischemic cardiomyopathy have reduced expression of CACNG7, down regulation of CACNG7 by up regulation of miR-765 may be a cause of CAD have showed that heart failure-associated miRNAs target to the sinoatrial node (SAN) automaticity-associated proteins, HCN1, HCN4 and solute carrier family 8 member A1 (SLC8A1) from comprehensive transcriptomic analysis of pure human SAN pacemaker tissue. This report from the human tissue with heart failure strongly supported our data in the etiologic analysis with METS/MIRAI from circulating miRNA panel [46,47].

Inflammation in the arterial intima

A biomarker of miR-424-5p was used as both acute and stable CAD states. Cell cycle related proteins, cyclin D1 (CCND1), cyclin D3 (CCND3), cyclin E1 (CCNE1) and cyclin-dependent kinase 6 (CDK6) were enhanced. For details, CDK6 was up regulated by miRNA hub miR-29b-3p or miR-424-5p down regulation long with miR-34a-5p, miR-449a, miR-124-3p, miR-16-5p, miR-107 and miR-195-5p. CCNE1 was increased by down regulation of miR-424-5p with miR-16-5p and miR-15a-5p. CCND3 and CCND1 were enhanced by miR-424-5p with miR-16-5p, and with let-7b-5p, miR-34a-5p, miR-503-5p, miR-20a-5p, miR-17-5p, miR-16-5p, miR-15a-5p, miR-302a-5p and miR-195-5p, respectively. Cell migration of atherosclerosis occurs at G1/S phase of the cell cycle, and CCND1 plus CCND3 controlled by CDK6 and CCNE1 are implicated in G1/S transition has showed in their bioinformatics analysis that CDK6 is a key regulator of atherosclerosis. Since human endothelial cells and vascular smooth muscle cells produce proinflammatory mediators in atherosclerosis, augmentation of cell cycle related proteins would progress angiogenic inflammation with proinflammatory cytokines. Fibroblast growth factor receptor 1 (FGFR1) was increased by miR-424-5p down regulation with miR-214-3p. FGFR1 expression of endothelial cells in patients with atherosclerosis has been decreased [48-50]. In the mouse apoE-/- model, atherosclerotic lesions have expressed FGFR1 and activated FGF/FGFR1 signalling pathways that promotes atherosclerosis development [51]. When human carotid atherosclerotic plaques were analysed, expression of basic fibroblast growth factor (bFGF) and FGFR1 has been increased in vascular smooth muscle cells (VSMCs) [52]. Both bFGF and FGF-2 has been detected in human atherosclerotic plaques and been synthesized by endothelial cells, vascular smooth muscle cells and macrophages, and FGFR1 been implicated in cell growth of endothelial and vascular smooth muscle cells [53,54]. These data strongly supported our computer simulation data from miRNA biomarker panels that FGFR1 elevation by miR-424 hub down regulation would induce CAD through atherosclerosis. Foam cell formation is initiated by accumulation of oxidized low-density lipoprotein (oxLDL) into macrophages, and its dysfunction with endothelial cells at lesion-prone sites in the walls of arteries causes inflammation of the arterial intima [9]. The thickness increasing of the arterial walls by inflammation induces hypoxic conditions in the atherosclerotic lesion; therefore, hypoxia-inducible factor 1 alpha (HIF1A) is related with atherosclerosis. HIF1A expression has been a major factor of angiogenesis in above cellular response in the hypoxic legions [55]. HIF1A was augmented by down regulation of miR-424-5p with miR-20a-5p, miR-18a-5p, miR-17-5p, miR-107, miR-106b-5p, miR-519c-3p and miR-27a-3p. Since hypoxia has enhanced lipid uptake into macrophages, up regulation of HIF1A would be deeply implicated in CAD progression [56]. Furthermore, HIF1A

molecule is modulated by phosphorylation with extracellular signal-regulated kinase 1/2 (ERK1/2), mitogen-activated protein kinase (MAPK) and protein kinase B (PKT) [57]. However, in our analysis, MAP2K1 as the ERK activated kinase was increased by suppression of miR-424-5p with miR-34a-5p and miR-181a-5p. Therefore, up regulation of MAP2K1 would be related with CAD progression via ERK1/2 activation. In the case of a mouse model, ERK1/2, MAPK and AKT has proliferated oxLDL incorporated-macrophages (oxLDL-macrophages) and CDKN1A (p21cip) inhibition by short interfering RNA (siRNA) has suppressed proliferation of oxLDL-macrophages via granulocyte/macrophage colony-stimulating factor (GM-CSF) suppression [58]. Virtually, CDKN1A was suppressed by up regulation of miR-208-3p long with miR-93-5p, miR-20b-5p, miR-20a-5p, miR-17-5p, miR-106a-5p, miR-106b-5p, miR-519d-5p, miR-145-5p and miR-96-5p; therefore, suppression of CDKN1A would be cooperated with increasing of oxLDL-macrophages in the atherosclerotic lesion. However, the oxLDL particles can trigger proinflammatory reactions and canakinumab, anti-interleukin 1 β (IL-1 β) reduced recurrent risk of patients in CVD [6]. In addition, as obesity and overweight induces a serious inflammatory condition that contributes to atherosclerosis through increasing of chemoattractant macrophages, oxidative stress and abnormal lipid metabolism, hypertension and sympathetic nerve activation, anti-inflammatory adipokines and weight management decrease atherosclerotic risk [59,60]. Therefore, HIF1A increasing expression may be related to proliferation of inflammatory macrophages in the hypoxic regions of the arterial intima at first, then accumulation of oxLDL.

First-line treatment of CAD

Medications for the treatment of stable CAD are statin, beta blockers and aspirin in the evidence rating of A (consistent, good-quality patient-oriented evidence) [61]. Although statin treatment is a therapy of lipid-lowering to decrease LDL, inflammation might be associated with risk of CDA but oxLDL reduction has not curtailed to contribute for CAD risk [7]. By using statin, many patients of hypercholesterolemia can reduce LDL levels; however, statins have pleiotropic effects, such as suppression of inflammation, inhibition of oxidative stress, regulating angiogenesis and improvement of endothelial function [62,63]. Therefore, it is unknown whether decreasing risk of CAD is due to effect of lowering LDL by stain or not, or combined effects. It has been shown from meta-analysis that in the first two years after MI, beta blockers can double the reduction in cardiovascular events compared with other antihypertensive agents [64]. However, it has recently been reported that beta-blockers have little or no effect on the short-term risk of a reinfarction and mortality [65]. In further recent meta-analysis, beta blockers have not showed the benefit for patients with stable CAD without prior

MI or left ventricular dysfunction to prevent cardiovascular disease [66]. The use of aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) is widely recommended for the secondary prevention of atherosclerosis in all patients. Aspirin inhibits cyclooxygenase 1 and 2, reducing prostaglandin and thromboxane-A production and preventing platelet aggregation while aspirin also have pleiotropic effects [67]. Further, aspirin is connected to increasing internal bleeding as the harmful side effect that would outweigh its cardio protective properties in some patients [68]. Therefore, recent randomized controlled trials have challenged the primary prevention of atherosclerotic CVD and using meta-analysis, in patients with percutaneous coronary intervention and stenting, assigned to a strategy of early aspirin discontinuation vs. dual antiplatelet therapy, the risk of death and ischemic events has not been significantly different [69,70]. These data statistically indicate that the therapeutic targeting of CAD would still be not enough to make strategy of precious medicine treatment according to its pathophysiologic mechanisms. Thus, the etiologic computer simulation of CAD from circulating profiles in plasma/serum was useful for precious medicine to find new therapeutic targets.

Data validation and network analysis

In our METS simulation with AI, miR-133b down regulation was involved into arrhythmia and fibrillation and miR-424-5p suppression was implicated in atherosclerosis. Data of CAD showed 97.19% of AUC, 94.4% of accuracy, 99.04% of precision, 94.5% of recall and 96.71% of F value (Table 2).

MiR-133b reduction has been observed in human infarcted tissues, which contributed to arrhythmogenesis [71]. MiR-424-5p has been found as a circulating biomarker of future acute MI prediction; however, miR-133b and miR-424-5p pathophysiologic protein targets for CAD have not yet been shown. Plenty of CAD therapeutic target genes have been outcome in silico by the integrated network analysis, such as homeobox A5 (HOXA5), HOXB5, HOXC6, HOXC8, HOXB7, collagen type I alpha 1 chain (COL1A1), CCND1, c-c motif chemokine ligand 2 (CCl2), haptoglobin (HP), twist family BHLH transcription factor 1 (TWIST1), smad family member 4 (SMAD4), toll like receptor 4 (TLR4), sp1 transcription factor (SP1), estrogen receptor 1 (ESR1), interferon regulatory factor 2 (IRF2), cell death inducing DFFA like effector B (CIDEB), proopiomelanocortin (POMC), and calreticulin (CALR) genes; however, with the software, for example, Gene Set Enrichment Analysis (GSEA) (<http://software.broadinstitute.org/gsea/index.jsp>), the relation among miRNAs is not computed at all and the statistical validation of the results has not been done. Additionally, by meta-analysis in 2021, caveolin 1 (CAV1) and heat shock transcription factor 2 (HSF2) genes have been extracted as the CAD therapeutic target; therefore, the results of more CAD therapeutic

targets were complicated and the pinpointed therapeutic target for CAD has still been uncertain. It is the reason that a miRNA can direct to multi-protein mRNA 3'UTR, and a 3'UTR of mRNA is targeted to multi-miRNAs [72-78]. To solve this mathematical problem, algorithm between miRNA and miRNA interaction is needed with AI. On the other hand, as the matrix algorithm between multi-miRNAs based on the quantum miRNA language was used in our METS analysis with AI, above problem is cleared. Therefore, this is the first report statistically validated that HCN4 plus KCNH2, and cell cycle-related proteins plus HIF1A plus FGFR1 are the certain therapeutic target of heart pacemaker, and atherosclerosis for patients with CAD, respectively (Figure 3).

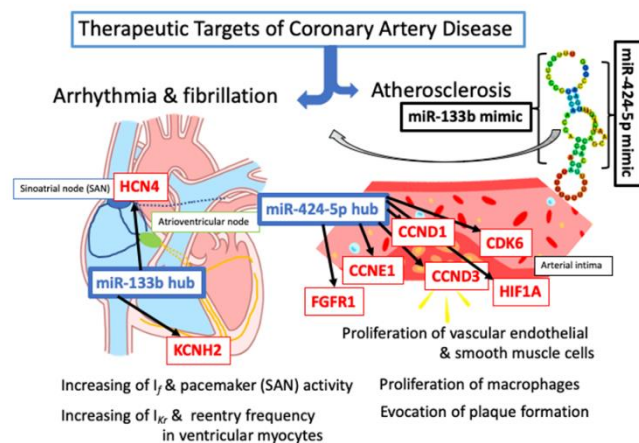


Figure 3: Therapeutic target miRNAs and proteins in CAD. Molecular mechanisms in CAD were depicted in right and left panels. Up regulated proteins and pathways were in red, down regulated miRNAs were in blue. An artificial stem loop is containing miR-133b and miR-424-5p mimics and both miRNA hub mimics may use for treatment of CAD.

Thus, an artificial stem loop agent including miR-133b and miR-424-5p hub mimics may provide the benefit for individuals with stable CAD. However, CAD pathogenic experiments have been prepared from mouse or rat model except for human clinical miRNA data. To remove rodent bias, statistically significant sample size in human was still limited for more precious diagnosis and prediction of CAD. Further clinical data with circulating miRNAs would be needed.

Conclusion

The current systematic review and meta-analysis showed to challenge the treatment of CAD with the first- and second-line drugs and it can reduce the risk of CAD. There was no clear evidence of an association between CAD therapeutic agents use and adverse cardiovascular outcomes. To further understand the CAD therapeutic target, we used circulating miRNA biomarker panels. A therapeutic target of CAD about cardiac pacemaker was

firstly found in silico. HCN4, a potassium channel in SAN, was up regulated by down regulation of miR-133b hub with miR-6511b-5p, miR-4748 plus miR-557. Since HCN4 increasing induces funny currents (If) and rapid spontaneous beating, augmentation of HCN4 levels may evoke arrhythmia. Further, KCNH2, a potassium voltage-gated channel in ventricular myocytes, was also increased by down regulation of miR-133b hub with miR-7-5p. While KCNH2 up regulation increases IKr currents and then re-entry frequency, KCNH2 enhancing may be implicated in ventricular fibrillation that induces sudden cardiac death. Another therapeutic target of CAD with atherosclerosis was secondarily found by METS analysis. Endothelial and smooth muscle cells would be proliferated in the arterial intima by acceleration cell cycle. G1/S phase-related proteins, CCND1, CCND3, CCNE1 and CDK6 were up regulated by down regulation of miR-424-5p hub with miR-15 family. In addition, HIF1A was augmented by down regulation of miR-424-5p hub. Therefore, miR-133b and miR-424-5p mimics would be the possible CAD preventing agent candidate as a state-of-the-art treatment. MIRAI is useful for decoding layered-miRNA codes and finding therapeutic targets for human diseases.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

- Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart disease and stroke statistics-2021 update. *Circulation*. 2021; 143: 254-743.
- McCarthy CP, McEvoy JW, Januzzi JL. Biomarkers in stable coronary artery disease. *Am Heart J*. 2018; 196: 82-96.
- Mushtaque RS, Hameed S, Mushtaque R, Idrees M, Siraj F. Role of cardio-specific micro-ribonucleic acids and correlation with cardiac biomarkers in acute coronary syndrome: a comprehensive systematic review. *Cureus*. 2019; 11: 5878.
- Rodriguez AD, Palacios GB, Gonzales PA. Increased heart rate and atherosclerosis: potential implications of ivabradine therapy. *World J Cardiol*. 2011; 3: 101-104.
- Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med*. 2001; 344: 1959-1965.
- Ridker PM, Everett BM, Thuren ET, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017; 377: 1119-1131.
- Alfaddagh A, Martin SS, Leucker TM, Michos ED, Blaha MJ, Lowenstein CJ, et al. Inflammation and cardiovascular disease: from mechanisms to therapeutics. *Am J Prev Cardiol*. 2020; 4: 100130.
- Boren J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2020; 41: 2313-1330.
- Pentikainen MO, Oorni K, Korpela MA, Kovanen PT. Modified LDL-trigger of atherosclerosis and inflammation in the arterial intima. *J Int Med*. 2000; 247: 359-370.
- Poznyak AV, Bezsonov EE, Popkova T, Starodubova AV, Orekhov AN. Immunity in atherosclerosis: Focusing on T and B cells. *Int J Mol Sci*. 2021; 22: 8379.
- Tang M, Hong L, Li H, Chen W, Tai L, Minshall R, et al. Stiffness of aortic and carotid arteries increases in ApoE-knockout mice with high-fat diet: evidence from echocardiography. *Am J Transl Res*. 2021; 13: 1352-1364.
- Stoffel W, Binczek E, Soltau IS, Brodessaer S, Wegner I. High fat/high cholesterol diet does not provoke atherosclerosis in the ω 3- and ω 6-polyunsaturated fatty acid synthesis-inactivated Δ 6-fatty acid desaturase-deficient mouse. *Mol Met*. 2021; 54: 101335.
- Du C, Fujii YR, Ito M, Harada M, Morikawa E. Dietary polyunsaturated fatty acids suppress acute hepatitis, alter gene expression and prolong survival of long-Evans Cinamon rats, a model of Wilson disease. *J Nutr Biochem*. 2004; 15: 273-280.
- Fard SG, Gholipour M, Taheri M. Role of microRNAs in the pathogenesis of coronary artery disease. *Front Cardiovasc Med*. 2021; 8: 632392.
- Matute JM, Rotllan N, Aranda JF, Hernando CF. MicroRNAs and atherosclerosis. *Curr Atheroscler Rep*. 2013; 15: 322.
- Solly EL, Dimasi CG, Bursill CA, Psaltis PJ, Tan JTM. MicroRNAs as therapeutic targets and clinical biomarkers in atherosclerosis. *J Clin Med*. 2019; 8: 2199.
- Churov A, Summerhill V, Grechko A, Orekhova V, Orekhov A. MicroRNAs as potential biomarkers in atherosclerosis. *Int J Mol Sci*. 2019; 20: 5547.
- Fazmin IT, Achercouk Z, Edling CE, Said A, Jeevaratnam K. Circulating microRNA as a biomarker for coronary artery disease. *Biomol*. 2020; 10: 1354.
- Sheikh MS, Alduraywish A, Almaeen A, Alruwali M, Alruwaili R, Alomair BM, et al. Therapeutic value of miRNAs in coronary artery disease. *Oxid Med Cell Longev*. 2021; 2021: 8853748.
- Mir R, Elfaki I, Khullar N, Waza AA, Jha C, Mir MM, et al. Role of selected miRNAs as diagnostic and prognostic biomarkers in cardiovascular diseases, including coronary

- artery disease, myocardial infraction and atherosclerosis. *J Cardiovasc Dev Dis.* 2021; 8: 22.
21. Fujii YR. The quantum language of the microRNA gene and anti-cancer: with a dynamic computer simulation of human breast cancer drug resistance. *Integr Mol Med.* 2018; 5: 1-13.
 22. Fujii YR. Cancer simulation from stage minus one by quantum microRNA language: lung, colorectal and pancreatic cancers. *Med One.* 2019; 4: 190023.
 23. Fujii YR. Quantum microRNA network analysis in gastric and esophageal cancers: xenotropic plant microRNAs cure from cancerous paradox via *Helicobacter pylori* infection. *Gastroenterol Hepatol Endosc.* 2019; 4: 1-18.
 24. Fujii YR. The quantum microRNA immunity in human virus-associated diseases: virtual reality of HBV, HCV and HIV-1 infection, and hepatocellular carcinogenesis with AI machine learning. *Arch Clin Biomed Res.* 2020; 4: 89-129.
 25. Fujii YR. The etiology of COVID-19 in silico by SARS-Cov-2 infection with the quantum microRNA language-AI. *Viro Immunol J.* 2020; 4: 000243.
 26. Fujii YR. The COVID-19 deadly risk assessment upon the updated etiologic computer simulation by quantum microRNA language in SARS-CoV-2 infection in eo. *Int J Clin Case Stud Rep.* 2020; 3: 142-154.
 27. Fujii YR. In silico study by quantum microRNA language for the development of anti-COVID-19 agents: COVID-19 is prevented by rice MIR2097-5p through suppression of SARS-Cov-2 viral microRNAs plus HIPK2 target proteins. *Viro Immunol J.* 2020; 4: 256.
 28. Fujii YR. Quantum microRNA assessment of COVID-19 RNA vaccine: hidden potency of BNT162b2 SARS-CoV-2 spike RNA as microRNA vaccine. *Adv Case Stud.* 2021; 3: 552.
 29. Fujii YR. The microRNA 2000: from HIV-1 to healthcare. Scientific Research Publishing. 2017.
 30. Fujii YR. The RNA gene information: retroelement-microRNA entangling as the RNA quantum code. *Methods Mol Biol.* 2013; 936: 47-67.
 31. Yoshikawa M, Fujii YR. Human ribosomal RNA-derived resident microRNAs as the transmitter of information upon the cytoplasmic cancer stress. *Biomed Res Int.* 2016; 2016: 7562085.
 32. Fujii YR. Quantum language of microRNA: application for new cancer therapeutic targets. *Methods Mol Biol.* 2018; 1733: 145-157.
 33. Ozone T, Yoshikawa M, Fujii YR. MicroRNA memory II: A novel scoring integration model for prediction of human disease by microRNA/microRNA quantum multi-interaction. *J Adv Med Phar Sci.* 2016; 5: 1-18.
 34. Kumar D, Narang R, Sreenivas V, Rastogi V, Bhatia J, Saluja D, et al. Circulatory miR-133b and miR-21 as novel biomarkers in early prediction and diagnosis of coronary artery disease. *Genes.* 2020; 11: 164.
 35. Wang W, Li T, Gao L, Li Y, Sun Y, Yao HC. Plasma miR-208b and miR-499: potential biomarkers for severity of coronary artery disease. *Dis Markers.* 2019; 2019: 9842427.
 36. Sheikh MS, Xia K, Li F, Deng X, Salma U, Deng H, et al. Circulating miR-765 and miR-149: potential noninvasive diagnostic biomarkers for geriatric coronary artery disease patients. *BioMed Res Inter.* 2015; 2015: 740301.
 37. Wang J, Pei Y, Zhong Y, Jiang S, Shao J, Gong J. Altered serum microRNAs as novel diagnostic biomarkers for atypical coronary artery disease. *PLoS One.* 2014; 9: 107012.
 38. Sayed AS, Xia K, Li F, Deng X, Salma U, Li T, et al. The diagnostic value of circulating microRNAs for middle-aged (40-60-year-old) coronary artery disease patients. *Clinics.* 2015; 70: 257-263.
 39. Fang Q, Liao Y, Xu Z, Li J, Zhang X, Wang Y. The diagnostic value of circulating microRNAs as biomarkers for coronary artery disease: a meta-analysis. *Anatol J Cardiol.* 2020; 24: 290-299.
 40. Yan F, Chen Y, Ye X, Zhang F, Wang S, Zhang L, et al. miR-3113-5p, miR-223-3p, miR-133a-3p, and miR-499a-5p are sensitive biomarkers to diagnose sudden cardiac death. *Diag Pathol.* 2021; 16: 67.
 41. Kennedy A, Finlay DD, Guldenring D, Bond R, Moran K, McLaughlin J. The cardiac conduction system: generation and conduction of the cardiac impulse. *Crit Care Nurs Clin North Am.* 2016; 28: 269-279.
 42. Custodis F, Schirmer SH, Baumhake M, Heusch G, Bohm M. Vascular pathophysiology in response to increased heart rate. *J Am Coll Cardiol.* 2010; 56: 1973-1983.
 43. Saito Y, Nakamura K, Yoshida M, Sugiyama H, Ohe T, Kurokawa J, et al. Enhancement of spontaneous activity by HCN4 overexpression in mouse embryonic stem cell-derived cardiomyocytes—a possible biological pacemaker. *PLoS One.* 2015; 10: 0138193.
 44. El Houry N, Mathieu S, Marger L, Ross J, El Gebeily G, Ethier N, et al. Upregulation of the hyperpolarization-activated current increases pacemaker activity of the sinoatrial node and heart rate during pregnancy in mice. *Circulation.* 2013; 127: 2009-2020.
 45. Hou L, Deo M, Furspan P, Pandit SV, Mironov S, Auerbach DS, et al. A major role for hERG in determining frequency of reentry in neonatal rat ventricular myocyte monolayer. *Circ Res.* 2010; 107: 1503-1511.
 46. Gronich N, Kumar A, Zhang Y, Efimov IR, Soldatov NM. Molecular remodeling of ion channels, exchangers and pumps in atrial and ventricular myocytes in ischemic cardiomyopathy. *Channels.* 2010; 4: 101-107.

SUNTEXT REVIEWS

47. Li N, Artiga E, Kalyanasundaram A, Hansen BJ, Webb A, Pietrzak M, et al. Altered microRNA and mRNA profiles during heart failure in the human sinoatrial node. *Sci Rep*. 2021; 11: 19328.
48. Andres V. Control of vascular cell proliferation and migration by cyclin-dependent kinase signaling: new perspectives and therapeutic potential. *Cardio Res*. 2004; 63: 11-21.
49. Kang L, Jia H, Huang B, Lu S, Chen Z, Shen J, et al. Identification of differently expressed mRNAs in Atherosclerosis reveals CDK6 is regulated by circHIPK3/miR-637 axis and promotes cell growth in human vascular smooth muscle cells. *Front Genet*. 2021; 12: 596169.
50. Chen PY, Qin L, Baeyens N, Li C, Afolabi T, Budatha M, et al. Endothelial-to-mesenchymal transition drives atherosclerosis progression. *J Clin Invest*. 2015; 125: 4514-4528.
51. Raj T, Kanellakis P, Pomilio G. Inhibition of fibroblast growth factor receptor signaling attenuates atherosclerosis in apolipoprotein E-Deficient mice. *Arterioscler Thromb Vasc Biol*. 2006; 26: 1845-1851.
52. Sigala F, Savvari P, Lontos M, Sigalas P, Pateras IS, Papalampros A, et al. Increased expression of bFGF is associated with carotid atherosclerosis plaques instability engaging the NF- κ B pathway. *J Cell Mol Med*. 2010; 14: 2273-2280.
53. Lindner V, Lappl DA, Majack RA, Reldy MA. Role of basic fibroblast growth factor in vascular lesion formation. *Circ Res*. 1991; 68: 106-113.
54. Yang X, Liaw L, Prudovsky I, Brooks PC, Vary C, Oxburgh L, et al. Fibroblast growth factor signaling in the vasculature. *Curr Atheroscler Rep*. 2015; 17: 509.
55. Aarup A, Pedersen TX, Junker N, Christoffersen C, Bartels ED, Madsen M, et al. Hypoxia-inducible factor-1 α expression in macrophages promotes development of atherosclerosis. *Atheroscler Thromb Vasc Biol*. 2016; 36: 1782-1790.
56. Crucet M, Wust SJA, Spielmann P, Luscher TF, Wenger RH, Matter CM, et al. Hypoxia enhances lipid uptake in macrophages: role of the scavenger receptors Lox1, SRA, and CD36. *Atherosclerosis*. 2013; 229: 110-117.
57. Gessi S, Fogli E, Sacchetto V, Merighi S, Varani K, Preti D, et al. Adenosine modulates HIF-1 α , VEGF, IL-8, and Foam cell formation in a human model of hypoxic foam cells. *Arterioscler Thromb Vasc Biol*. 2010; 30: 90-97.
58. Ishii N, Matsumura T, Kinoshita H, Motoshima H, Kojima K, Tsutsumi A, et al. Activation of AMP-activated protein kinase suppresses oxidized low-density lipoprotein-induced macrophage proliferation. *J Biol Chem*. 2009; 284: 34561-34569.
59. Henning RJ. Obesity and obesity-induced inflammatory disease contribute to atherosclerosis: a review of the pathophysiology and treatment of obesity. *Am J Cardiovasc Dis*. 2021; 11: 504-529.
60. Cassar A, Holmes Jr DR, Rihal CS, Gersh BJ. Chronic coronary artery disease: diagnosis and management. *Mayo Clin Proc*. 2009; 84: 1130-1146.
61. Do MP, Winslow BT, PharmD KM, Dauber IM. Medical treatment of stable coronary artery disease. *Am Fam Physician*. 2011; 83: 819-826.
62. Davignon J. Pleiotropic effects of pitavastatin. *Br J Clin Pharmacol*. 2012; 73: 518-535.
63. Sahebkar A, Kiaie N, Gorabi AM, Mannarino MR, Bainaconi V, Jamialahmadi T, et al. A comprehensive review on the lipid and pleiotropic effects of pitavastatin. *Prog Lipid Res*. 2021; 84: 101127.
64. Law MR, Morris JK, Wald NJ. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: met-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ*. 2009; 338: 1665.
65. Safi S, Sethi NJ, Nielsen EE, Feinberg J, Jakobsen JC, Gluud C. Beta-blockers for suspected or diagnosed acute myocardial infarction. *Cochrane Database Syst Rev*. 2019; 12: 12484.
66. Arero AG, Farahani AV, Soltani D. Meta-analysis of the usefulness of beta-blockers to reduce the risk of major adverse cardiovascular events in patients with stable coronary artery disease without prior myocardial infarction or left ventricular dysfunction. *Am J Cardiol*. 2021; 158: 23-29.
67. Hybiak J, Broniarek I, Kiryczynski G, Los LD, Rosik J, Machaj F, et al. Aspirin and its pleiotropic application. *Eur J Pharmacol*. 2020; 866: 172762.
68. McEvoy L, Carr DF, Pirmohamed M. Pharmacogenomics of NSAID-induced upper gastrointestinal toxicity. *Front Pharmacol*. 2021; 12: 684162.
69. Nudy M, Cooper J, Ghahramani M, Ruzieh M, Mandrola J, Foy AJ, et al. Aspirin for primary atherosclerotic cardiovascular disease prevention as baseline risk increases: a meta-regression analysis. *Am J Med*. 2020; 133: 1056-1064.
70. Wiebe J, Ndrepepa G, Kufner S, Lahmann AL, Xhepa E, Kuna C, et al. Early aspirin discontinuation after coronary stenting: a systematic review and meta-analysis. *J Am Heart Assoc*. 2021; 10: 018304.
71. Bostjancic E, Zidar N, Stajer D, Glavac D. MicroRNA miR-1 is up-regulated in remote myocardium in patients with myocardial infarction. *Folia Biol*. 2010; 56: 27-31.
72. Bye A, Rosjo H, Nauman J, Silva GJ, Follestad T, Omland T, et al. Circulating microRNAs predict future fatal myocardial infarction in healthy individuals-the HUNT study. *J Mol Cell Cardiol*. 2016; 97: 162-168.
73. Tan L, Xu Q, Wang Q, Shi R, Zhang G. Identification of key genes and pathways affected in epicardial adipose tissue from



SUNTEXT REVIEWS

- patients with coronary artery disease by integrated bioinformatics analysis. *Peer J.* 2020; 8: 8763.
74. Xiao SJ, Zhou YF, Wu Q, Ma WR, Chen ML. Uncovering the differentially expressed genes and pathways involved in the progression of stable coronary artery disease to acute myocardial infarction using bioinformatics analysis. *Eur Rev Med Pharm Sci.* 2021; 25: 301-312.
 75. Zhang G, Shi H, Wang L, Zhou M, Wang Z, Liu X, et al. MicroRNA and transcription factor mediated regulatory network analysis reveals critical regulators and regulatory modules in myocardial infarction. *PLoS One.* 2015; 10: 0135339.
 76. Xu X, Li H. Integrated microRNA-gene analysis of coronary artery disease based on miRNA and gene expression profiles. *Mol Med Rep.* 2016; 13: 3063-3073.
 77. Wang W, Xu Z, Zhu X, Chang X. Mining the potential therapeutic targets for coronary artery disease by bioinformatics analysis. *Mol Med Rep.* 2018; 18: 5069-5075.
 78. Liu L, Zhang J, Wu M, Xu H. Identification of key miRNAs and mRNAs related to coronary artery disease by meta-analysis. *BMC Cardiovasc Disord.* 2021; 21: 443.