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Case Report

# The Role of miR-320 Family in Atherosclerosis

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## ABSTRACT

Coronary Artery Disease (CAD), characterized by the development of atherosclerosis and the rupture of plaques, is the leading cause of morbidity and mortality around the global. miR-320a/b/c/d/e belongs to miR-320 family, and accumulating evidence suggested the important roles of miR-320 family in the regulation of Cardio Vascular Diseases (CVD), such as atherosclerosis. Here, we reviewed the functional significance of miR-320 members in atherosclerosis and aimed to provide new insights into the prevention, treatment and the screening of therapeutic targets for CAD.

**Keywords:** Coronary artery disease; Endothelial cells/macrophage; Atherosclerosis; Functional significance; Therapeutic targets

## INTRODUCTION

Coronary Artery Disease (CAD), characterized by the initiation and development of atherosclerotic plaques, is a kind of chronic and complex disease and become one of major life-threatening public health issues.

MicroRNAs (miRNAs) are endogenous, small and non-coding RNAs, which play pivotal roles in a variety of cellular processes by the degradation of their mRNA targets and/or the inhibition of mRNA translation. miR-320 family consists of 5 members including miR-320a, miR-320b, miR-320c, miR-320d, and miR-320e. The consensus sequence for mature miR-320 family members was shown in Figure 1. Recently, we have found that hsa-miR-320b was significantly up-regulated in CAD patients compared with health controls. Meanwhile, we demonstrated that miR-320b inhibits macrophage cholesterol efflux and promotes atherosclerosis by targeting ABCG1 and EEPD1. In this mini-review, we summarized the current knowledge on the role of miR-320 family in atherosclerosis.

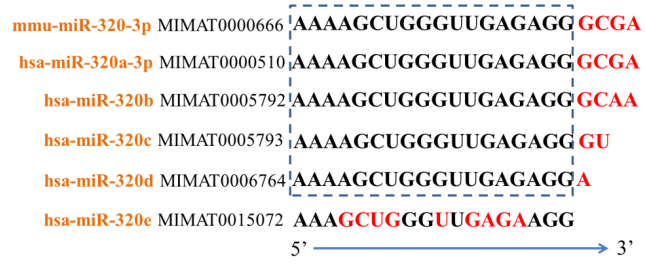


**Figure 1:** The mature sequence of 5 members of miR-320 family.

## CASE PRESENTATION

### Differentially expressed profiles of miR-320 family in atherosclerosis

At present, circulating miRNAs are emerged as new, non-invasive biomarkers in CAD [1] as they remain stable in peripheral blood under normal physiological condition. A subset of dysregulated miRNAs was screened in CAD during past decades, including miR-320 family members.

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Previously, Chen, et al. have identified that hsa-miR-320a was highly expressed in the peripheral blood of patients with CAD (n=10) compared with that in CAD high-risk individuals (n=10) or healthy controls (n=10), indicating that miR-320a is a potential biomarker for atherosclerosis and CAD [2]. In our study, we conducted miRNA microarray analysis in the Peripheral Blood Mononuclear Cells (PBMCs) of 24 patients with CAD (8 samples with acute myocardial infarction, 8 samples with unstable angina and 8 samples with stable angina) and 7 healthy controls and found that miR-320b was increased in CAD patients [3]. In the validation stage, the results from a total of 123 patients and 104 healthy controls confirmed that miR-320b was up-regulated in PBMCs of CAD patients [3]. A recent report has showed that miR-320e was also remarkably elevated in the plasma from 203 patients with CAD in comparison to 144 age-matched controls (126 high-risk controls and 18 healthy volunteers) [4]. Moreover, Area under Curve (AUC) for miR-320e was calculated and the value was 0.767, suggesting it might be a convincing biomarker for CAD diagnosis [4]. Xu et al. performed a miRNA microarray analysis in 5 patients with hyperlipidemia and 5 control subjects, and discovered that miR-320b and miR-320e were significantly up-regulated by 14.7 and 9.6 Fold Change (FC), respectively [5]. However, two studies have showed that the plasma miR-320b was down-regulated in patients with acute myocardial infarction [6] and ischemic cerebrovascular diseases [7]. The reasons for these conflict results of miR-320b [3,6,7] might be the following: i) the experimental approach and ii) the various cohorts recruited in different studies. Further work was required to verify the expression of miR-320 family in a large-scale population or by using meta-analysis.

### The effects of miR-320 family on atherosclerosis

The process of atherosclerosis, characterized by inflammation and lipid deposition in the arteries, involves complicated cell-cell cross-talk containing endothelial injury and activation, monocyte adhesion and trans-endothelial migration, Vascular Smooth Muscle Cells (VSMCs) phenotype switching, the transformation of monocyte-derived macrophages into foam cells, etc.

In cultured endothelial cells, the over-expression of miR-320a could attenuate cell proliferation and induced cell apoptosis by targeting Serum Response Factor (SRF) [2]. Under the hyperlipidemic state, oxidative low density lipoprotein (ox-LDL) would ultimately cause atherosclerosis by inducing endothelial dysfunction. Xu et al. determined miR-320 levels in Human Umbilical Vein Endothelial Cells (HUVECs) stimulated by ox-LDL, and observed that miR-320 was decreased after treatment with ox-LDL for 24 h and 48 h [8]. Another work demonstrated that miR-320a was significantly enhanced in HUVEC treated with Oxidized Palmitoyl-Arachidonoyl-PhosphatidylCholine (OxPAPC), a critical determinant in atherosclerosis [9]. Previously, Gidlof et al. showed that platelet miR-320b could be taken up by endothelial cells and decrease the expression of intercellular adhesion molecular 1 (ICAM1) [10]. Given the fact that endothelial dysfunction is recognized as an early marker in the pathology of atherosclerosis, miR-320a/b has the potential to regulate inflammatory responses in CAD.

Macrophage lipid homeostasis is a key factor to atherosclerosis, and cholesterol efflux from macrophage is regarded as an athero-protective step. In our study [3], human monocyte derived macrophages and mouse RAW264.7 cell lines were transfected with miR-320b mimics or inhibitor and cellular cholesterol uptake and efflux assay were performed. The results showed that miR-320b suppressed macrophage cholesterol eflux to High Density Lipoprotein (HDL) and apolipoprotein A1 (apoA1) partly *via* ATP Binding Cassette Subfamily G member 1 (ABCG1) and Endonuclease/Exonuclease/Phosphatase Family Domain containing 1 (EEPD1), while it has no effect on cholesterol uptake. It is noteworthy that miR-320b inhibits endothelial activation and cholesterol efflux from macrophage simultaneously, indicating that miR-320b has double-edged role during the early stage of atherosclerosis, and the *in vivo* study is warranted.

Abnormal blood lipid metabolism is an independent risk factor of atherosclerosis, and high-fat diet fed Apoe-/- mice were commonly used for establishing atherosclerotic models. As reported, the replenish of miR-320a resulted in an increase in plasma Total Cholesterol (TC), Triglyceride (TG) and Low Density Lipoprotein Cholesterol (LDL-C) and a reduction in HDL-C levels [2]. Apoe-/- mice with miR-320a over-expression developed markedly lager atherosclerotic lesions when compared to control mice [2]. Conversely, mice with miR-320a knock-down displayed opposite phenotypes. Although miR-320b is expressed in human, not in rodents, the binding sites of miR-320b in its direct targets ABCG1 and EEPD1 3'-UTR are consensus in human and mouse. And Adeno-Associated Virus (AAV) mediated miR-320b over-expression attenuated cholesterol efflux from peritoneal macrophages and increased atherosclerotic plaque size and macrophage content in lesion [3]. Meanwhile, plasma LDL-C levels were significantly up-regulated when HDL-C levels were reduced. In conclusion, miR-320a/b could promote the development of atherosclerosis *in vivo* and provide a potential therapeutic target for CAD.

### Potential signaling pathway in CVD that miR-320 participate in

Numerous studies indicated that miR-320 family plays essential roles in CVD. Chen et al. found that miR-320a induced pro-inflammatory associated pathway in atherosclerosis [2]. They also proved that inhibition of miR-320a ameliorate doxorubicin induced endothelial cells impairment through Vascular Endothelial Growth Factor (VEGF) signaling pathway [11]. Later, the same group showed that nuclear miR-320a-argonaute 2 (Ago2)/cluster of differentiation 36 (CD36) pathway induced lipotoxicity by promoting lipid uptake without affecting fatty acid oxidation in diabetic cardiomyopathy [12,13]. In our previous study [3], miR-320b controls cholesterol efflux from macrophage and the progression of atherosclerosis at least two pathways: i) liver X receptors (LXRα)-ABCA1/ABCG1 pathway; ii) nuclear factor-κB (NF-κB) related pathway. However, the underlying mechanisms by which miR-320a/b modulates atherosclerosis are still not fully understood and require further work.

## DISCUSSION

Nowadays, advances in technologies have made a group of miRNAs sound candidates as therapeutics (in the form of miRNA mimics or inhibitors) [14]. miR-320 family members, previously described in the broader context of cancer, have hardly been elucidated within atherosclerosis related disease. In this mini-review, we summarized the diagnostic and therapeutic potential for atherosclerosis and CAD by targeting miR-320.

Several studies have identified miR-320a, miR-320b and miR-320e as differentially expressed miRs by using microarray or qPCR in various CAD cohorts. Here, a summary table was provided to better illustrate miR-320 family mentioned in the text (Table 1). Huang et al. found that miR-320b was significantly decreased in CAD patients from Chinese population, implying that miR-320b could serve as a protective indicator in atherosclerosis [6]. However, recent studies have observed that miR-320b was abnormally over-expressed in patients with hyperlipidemia or CAD [3,5]. In addition, miR-320b was reported to be increased in diabetic hearts relative to controls [15]. All of these findings suggested that miR-320a/b/e was highly expressed in atherosclerosis related individuals and may contribute to athero-genesis. Consistent with the hypothesis, *in vitro* and *in vivo* experiments confirmed the critical promoting effect for miR-320a/b in atheroma formation. Some limitations are also existed, for instance, the widely used Apoe-/- mice have impaired HDL metabolism. In that case, Ldlr-/- mice are idealized atherosclerotic models for further investigation. Moreover, the effect of miR-320a/b on atheroma formation is limited on certain tissue and cell types, not fully investigated by the network regulation of multiple organs. In the future, the extensive mechanistic insights of miR-320a/b in atherosclerosis should be provided with additional experiments.

**Table 1:** The expression profiles of miR-320 family in atherosclerosis related diseases

| miRNA | Objectives | Change | Cell models (functional validation) | Animal models (functional validation) | Reference |
|---|---|---|---|---|---|
| miR-320a | Patients with CAD | Up | Human and mouse cultured endothelial cells | High fat diet fed Apoe$^{-/-}$ mice | 2 |
| miR-320a | HUVECs treated with ox-LDL | Down | HUVECs | N/A | 8 |
| miR-320a | HUVECs treated with oxPAPC | Up | HUVECs | N/A | 9 |
| miR-320b | Patients with CAD | Up | Human and mouse macrophages | High fat diet fed Apoe$^{-/-}$ mice | 3 |
| miR-320b | Patients with AMI | Down | N/A | N/A | 6 |
| miR-320b | The platelets from patients with STEMI | Down | Endothelial cells | N/A | 10 |
| miR-320b | Patients with carotid atherosclerosis | Down | N/A | N/A | 7 |
| miR-320b<br>miR-320e | Patients with hyperlipidemia | Up<br>Up | N/A | N/A | 5 |
| miR-320e | Patients with CAD | Up | N/A | N/A | 4 |

**Abbreviations:** CAD: Coronary Artery Disease; HUVECs: Human Umbilical Vein Endothelial Cells; ox-LDL: Oxidative Low Density Lipoprotein; AMI: Acute Myocardial Infarction; STEMI: ST-Elevation Myocardial Infarction

## CONCLUSION

Up to now, a number of studies confirmed that miR-320a/b/e was significantly elevated in CAD patients, and biological functional studies revealed that re-express miR-320a/b could accelerate atherosclerotic lesion size. Overall, miR-320a and miR-320b are key modulators contributing to various aspects of atherosclerosis and might be promising therapeutic targets for CAD.

## REFERENCES

1. Kaur A, Mackin ST, Schlosser K, Wong FL, Elharram M, Delles C, et al. Systematic review of microRNA biomarkers in acute coronary syndrome and stable coronary artery disease. Cardiovasc Res. 2020; 116: 1113-1124.

2. Chen C, Wang Y, Yang S, Li H, Zhao G, Wang F, et al. MiR-320a contributes to atherogenesis by augmenting multiple risk factors and down-regulating SRF. J Cell Mol Med. 2015; 19: 970-985.

3. Lu X, Yang B, Yang H, Wang L, Li H, Chen S, et al. MicroRNA-320b modulates cholesterol efflux and atherosclerosis. J Atheroscler Thromb. 2021; 28: 1-21.

4. Su M, Niu Y, Dang Q, Qu J, Zhu D, Tang Z, et al. Circulating microRNA profiles based on direct S-Poly(T)Plus assay for detection of coronary heart disease. J Cell Mol Med. 2020; 24: 5984-5997.

5. Xu J, Chen Z, Wang Y, Wang X, Chen L, Yuan T, et al. Several circulating miRNAs related to hyperlipidemia and atherosclerotic cardiovascular diseases. Lipids Health Dis. 2019; 18: 104.

6. Huang S, Chen M, Li L, He M, Hu D, Zhang X, et al. Circulating MicroRNAs and the occurrence of acute myocardial infarction in Chinese populations. Circ Cardiovasc Genet. 2014; 7: 189-198.

7. Zhang R, Qin Y, Zhu G, Li Y, Xue J. Low serum miR-320b expression as a novel indicator of carotid atherosclerosis. J Clin Neurosci. 2016; 33: 252-258.

8. Xu X, Ma C, Liu C, Duan Z, Zhang L. Knockdown of long noncoding RNA XIST alleviates oxidative low-density lipoprotein-mediated endothelial cells injury through modulation of miR-320/NOD2 axis. Biochem Biophys Res Commun. 2018; 503: 586-592.

9. Schrottmaier WC, Oskolkova OV, Schabbauer G, Afonyushkin T. MicroRNA miR-320a modulates induction of HO-1, GCLM and OKL38 by oxidized phospholipids in endothelial cells. Atherosclerosis. 2014; 235: 1-8.

10. Gidlöf O, van der Brug M, Ohman J, Gilje P, Olde B, Wahlestedt C, et al. Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. Blood. 2013; 121: 3908-3917; S1-26.

11. Yin Z, Zhao Y, Li H, Yan M, Zhou L, Chen C, et al. miR-320a mediates doxorubicin-induced cardiotoxicity by targeting VEGF signal pathway. Aging (Albany NY). 2016; 8: 192-207.

12. Li H, Fan J, Zhao Y, Zhang X, Dai B, Zhan J, et al. Nuclear miR-320 mediates diabetes-induced cardiac dysfunction by activating transcription of fatty acid metabolic genes to cause lipotoxicity in the heart. Circ Res. 2019; 125: 1106-1120.

13. Tong M, Sadoshima J. Nuclear miR-320 controls lipotoxicity. Circ Res. 2019; 125: 1121-1123.

14. Rupaimoole R, Slack FJ. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017; 16: 203-222.

15. Costantino S, Paneni F, Lüscher TF, Cosentino F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. Eur Heart J. 2016; 37: 572-576.