Original Article

Circulating MicroRNAs as Novel Diagnostic Biomarkers for Very Early-onset (≤40 years) Coronary Artery Disease*

DU Ying‡, YANG Sheng Hua‡, LI Sha, CUI Chuan Jue, ZHANG Yan, ZHU Cheng Gang, GUO Yuan Lin, WU Na Qiong, GAO Ying, SUN Jing, DONG Qian, LIU Geng, and LI Jian Jun#

Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Disease, CAMS and PUMC, Beijing 100037, China

Abstract

Objective Very early-onset coronary artery disease (CAD) is a great challenge in cardiovascular medicine throughout the world, especially regarding its early diagnosis. This study explored whether circulating microRNAs (miRNAs) could be used as potential biomarkers for patients with very early-onset CAD.

Methods We performed an initial screening of miRNA expression using RNA isolated from 20 patients with angiographically documented very early-onset CAD and 20 age- and sex-matched normal controls. For further confirmation, we prospectively examined the miRNAs selected from 40 patients with very early-onset CAD and 40 angiography-normal controls.

Results A total of 22 overexpressed miRNAs and 22 underexpressed miRNAs were detected in the initial screening. RT-qPCR analysis of the miRNAs obtained from the initial screening revealed that four miRNAs including miR-196-5p, miR-3163-3p, miR-145-3p, and miR-190a-5p exhibited significantly decreased expression in patients compared with that in controls (P<0.05). The areas under the receiver operating characteristic curve for these miRNAs were 0.824 (95% CI, 0.731-0.917; P<0.001), 0.758 (95% CI, 0.651-0.864; P<0.001), 0.753 (95% CI, 0.643-0.863; P<0.001), and 0.782 (95% CI, 0.680-0.884; P<0.001), respectively, in the validation set.

Conclusion To our knowledge, this is an advanced study to report about four serum miRNAs (miR-196-5p, miR-3163-3p, miR-145-3p, and miR-190a-5p) that could be used as novel biomarkers for the diagnosis of very early-onset CAD.

Key words: MicroRNA; Biomarker; Early-onset coronary artery disease

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INTRODUCTION

Coronary artery disease (CAD) is a major worldwide public health problem and the single largest cause of mortality. CAD, especially early-onset CAD, is also a huge burden for both developing and developed countries. By definition, onset of CAD at a young age (at or before 55 years in men or 65 years in women) is diagnosed as early-onset CAD. Early-onset CAD is different from

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‡The first two authors contributed equally to this study.

#Correspondence should be addressed to LI Jian Jun, MD, PhD, Tel: 86-10-88396077; Fax: 86-10-68331730; E-mail: lijianjun938@126.com

Biographical notes of the first authors: DU Ying, female, born in 1985, PhD in basic research of cardiovascular disease; YANG Sheng Hua, male, born in 1985, MD, specializing in disorders of lipid metabolism and cardiovascular disease.

CAD in certain aspects, such as a higher incidence of smoking, dyslipidemia, and family history in younger patients than in older patients[1-3]. Early-onset CAD also significantly affects the quality of life of patients and leads to morbidity and premature death. It has also been reported that early-onset CAD may result in worse outcomes for the patients and society than those by CAD[3]. Nevertheless, information regarding the effective predictors of this complication is still scarce.

Over the past two decades, remarkable changes in the diagnosis, treatment, and prognosis of cardiovascular disease have led to significant improvement in the diagnosis, treatment, and prognosis of early-onset CAD[4]. However, early-onset CAD is a great challenge in cardiovascular medicine throughout the world, especially regarding its early diagnosis. Therefore, development of innovative biomarkers may be of great clinical interest.

MicroRNAs (miRNAs) are a class of endogenous, noncoding, single-stranded short RNAs of approximately 22 nucleotides in length, which negatively regulate target gene expression at the post-transcriptional level by binding to the 3' untranslated regions of mRNAs[5-6]. They have been identified as key regulators of several physiological and pathophysiological processes of mammalian cardiovascular development and diseases[5,7]. For instance, miR-26 participates in the regulation of cardiovascular repair[8-9] and miR-181 family plays an important role in vascular inflammation[10].

Besides their intracellular functions, recent studies demonstrate that miRNAs can be increasingly found in the systemic circulation of both animals and humans[11]. They can be released by cells and circulate in the blood[12]. Unlike miRNAs in tissues, circulating miRNAs are extremely stable and exist in an RNase-resistant form[4,13]. Multiple freeze-thaw cycles or prolonged room temperature incubation will not affect isolation of circulating miRNAs[4]. Since the levels of circulating miRNAs may significantly vary in different pathological conditions, such as cancer, liver injury, and hepatitis, they have been suggested as great potential biomarkers for diagnosis of these diseases[14-15]. Furthermore, previous studies have shown that miR-423-5p may be used as a clinical diagnostic biomarker for heart failure[16], and miR-29a has been identified as a potential biomarker for myocardial remodeling assessment in hypertrophic cardiomyopathy[17]. More importantly, much evidence has demonstrated that circulating miRNAs can be useful diagnostic and prognostic markers in CAD[18-22], especially in acute coronary syndrome (ACS)[19,23-28]. Nonetheless, whether circulating miRNAs can be useful as diagnostic and prognostic markers in early-onset CAD, especially in very early-onset CAD, remains unknown.

Therefore, the purpose of this study is to search for distinctive miRNA profiles in the serum of patients with angiographically documented very early-onset CAD in comparison with angiography-normal controls. The potential diagnostic significance of circulating miRNAs has been challenged by the ROC curve with the aim of identifying putative specific very early-onset CAD-associated miRNA signatures.

MATERIALS AND METHODS

Study Population

Our study complied with the Declaration of Helsinki and was approved by the hospital ethics review board (Fuwai Hospital & National Center for Cardiovascular Diseases, Beijing, China). Informed written consents were obtained from all patients enrolled in this analysis. A total of 60 patients aged below 40 years with angiographically documented stable CAD and 60 age- and sex-matched controls were enrolled. Based on our previous studies, we diagnosed CAD by the criterion of the presence of coronary lesion ≥50% in at least one major epicardial artery segment assessed by coronary angiography[29-30]. On the basis of general exclusion criteria, patients with the following conditions were excluded from the study: ACS, heart failure, significant hematologic disorders, thyroid disorders, infectious or systematic inflammatory diseases, severe liver and/or renal insufficiency, and malignant disease. In parallel, all the controls were examined by coronary angiography to rule out CAD.

Study Flow

The study flow was shown in Figure 1. A two-phase case-control study was designed to identify serum miRNAs as potential biomarkers for very early-onset CAD. An miScript miRNA PCR Array analysis was performed to screen and select miRNAs that showed significant changes in pooled serum samples between patients with very early-onset CAD (n=20) and controls (n=20). Differentially expressed miRNAs were examined in the same cohort that was used for screening (training set). We then detected