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Research Article

Hypertrophic and antihypertrophic microRNA levels in peripheral blood mononuclear cells and their relationship to left ventricular hypertrophy in patients with essential hypertension

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Abstract

MicroRNAs regulate several aspects of physiological and pathologic cardiac hypertrophy, and they represent promising therapeutic targets in cardiovascular disease. We assessed the expression levels of the microRNAs miR-1, miR-133a, miR-26b, miR-208b, miR-499, and miR-21, in 102 patients with essential hypertension and 30 healthy individuals. All patients underwent two-dimensional echocardiography. MicroRNA expression levels in peripheral blood mononuclear cells were quantified by real-time reverse transcription polymerase chain reaction. Hypertensive patients showed significantly lower miR-133a (5.06 ± 0.50 vs. 13.20 ± 2.15, $P < .001$) and miR-26b (6.76 ± 0.53 vs. 9.36 ± 1.40, $P = .037$) and higher miR-1 (25.99 ± 3.07 vs. 12.28 ± 2.06, $P = .019$), miR-208b (22.29 ± 2.96 vs. 8.73 ± 1.59, $P = .016$), miR-499 (10.06 ± 1.05 vs. 5.70 ± 0.91, $P = .033$), and miR-21 (2.75 ± 0.15 vs. 1.82 ± 0.20, $P = .002$) expression levels compared with healthy controls. In hypertensive patients, we observed significant negative correlations of miR-1 ($r = -0.374$, $P < .001$) and miR-133a ($r = -0.431$, $P < .001$) and significant positive correlations of miR-26b ($r = 0.302$, $P = .002$), miR-208b ($r = 0.426$, $P < .001$), miR-499 ($r = 0.433$, $P < .001$) and miR-21 ($r = 0.498$, $P < .001$) expression levels with left ventricular mass index. Our data reveal that miR-1, miR-133a, miR-26b, miR-208b, miR-499, and miR-21 show distinct expression profiles in hypertensive patients relative to healthy individuals and they are associated with clinical indices of left ventricular hypertrophy in hypertensive patients. Thus, they may be related to heart hypertrophy in hypertensive patients and are possibly candidate therapeutic targets in hypertensive heart disease. J Am Soc Hypertens 2015;9(10):802–810.

Keywords: Left ventricular mass index; miR-1; miR-133a; miR-26b; miR-208b; miR-499; miR-21.

Introduction

Left ventricular (LV) hypertrophy is an initial compensatory mechanism in response to cardiac stress that can degenerate into heart failure and sudden cardiac death, and it is considered as an independent cardiovascular risk factor.[1] Essential hypertension is one of the most important causes of premature death and cardiovascular disease, with considerable morbidity and mortality worldwide,[2] as it results in several cardiac complications, such as heart failure, cardiac dysrhythmias, and sudden death.[3] Chronic exposure to increased blood pressure (BP) induces several structural

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and functional changes in several tissues and organ systems, known as ''target organ damage,'' that are responsible for hypertension-related morbidity and mortality.[4,5] Sustained high BP induces pathologic cardiac hypertrophy initially as a compensatory mechanism to reduce LV wall stress. LV hypertrophy (LVH) is one of the most recognized features of hypertensive heart disease, a marker of subclinical organ damage related to hypertension, and an established risk factor and strong predictor for adverse cardiovascular outcomes in hypertension.[6,7]

MicroRNAs are small noncoding RNAs that are key posttranscriptional regulators of gene expression in all eukaryotic cells. Therefore, their dysregulation often results in impaired cellular function and disease development.[8,9] In the cardiovascular field, microRNAs are emerging as essential modulators of cardiovascular development, physiology, and disease, providing new perspectives on disease mechanisms.[9,10] They control basic functions in virtually all cell types relevant to the cardiovascular system (such as endothelial cells, cardiac muscle, smooth muscle, inflammatory cells, and fibroblasts), and thus, they are directly involved in the pathophysiology of many cardiovascular diseases. MicroRNAs control fundamentally all critical aspects of cardiovascular biology, such as angiogenesis, metabolism, aging, and different components of myocardial remodeling.[10] Thus, they seem to be potential targets for cardiovascular disease prevention, diagnosis, prognostication, and therapy.[10–12]

The implication of microRNAs in heart hypertrophy has been extensively studied in animal models. Some microRNAs, such as miR-1, miR-133, and miR-26, have been shown to have antihypertrophic function, whereas others, such as miR-208, miR-499, and miR-21, have been shown to be agonists of the hypertrophic response.[13] Thus, these microRNAs might play a role in pathologic LVH that develops in hypertensive heart disease. However, there are no data from hypertensive patients to confirm their involvement in the pathophysiology of hypertensive LVH, and therefore, their clinical significance in hypertensive heart disease has not been evaluated.

In the present study, we sought to determine whether cardiac hypertrophy-related microRNAs were differentially expressed in peripheral blood mononuclear cells (PBMCs) of hypertensive patients in relation to LVH. Based on the existing knowledge from experimental animal studies, we selected miR-1, miR-133a, and miR-26b shown to have antihypertrophic function, as well as miR-208b, miR-499, and miR-21 shown to be agonists of the hypertrophic response, as we considered that they could play a role in human hypertensive heart disease, and we studied them in the PBMCs in patients with essential hypertension in relation to LVH. We compared their expression levels in hypertensive patients and healthy individuals, and we further examined if in hypertensive patients they were related to echocardiographic LV mass index, which is a clinical diagnostic marker of the LVH.

Methods

Study Population

One hundred two patients (50 men, mean age 62.51 ± 9.7 years) were recruited from the outpatients' hypertension clinic of our department. Thirty healthy volunteers (14 men, mean age 58.8 ± 8.3 years) without symptoms or signs of cardiovascular disease and with no major cardiovascular risk factors were also enrolled in the study. This group consisted of patients who came to the emergency department with atypical chest pain, but whose clinical and laboratory examinations were normal.

The patient population consisted of individuals who had untreated grade 1 or grade 2 essential hypertension, with no indications of other organic heart disease. Diagnosis of hypertension was based on three outpatient measurements of BP greater than 140/90 mm Hg at intervals no longer than 2 weeks, according to the recommendations of the European Society of Hypertension and European Society of Cardiology.[14] All patients underwent 24-hour ambulatory BP monitoring using an automatic portable device (Spacelabs 90,207, Redmond, USA). Measurements were made every 15 minutes throughout the 24-hour period. The monitoring was performed on a working day, and the subjects were instructed to behave and work as usual, while avoiding any excess physical activity. In addition, patients were asked to describe the quality of their sleep and only those with adequate recordings who reported normal sleep were included. The daytime period was defined according to the patient's sleeping habits. Included participants had at least three readings per hour. The final diagnosis of hypertension and the subjects' enrollment was based on a mean ambulatory BP >135/80 mm Hg.

The following were the criteria for exclusion from the study: heavy smokers; pregnant or lactating women; previous history or medication for hypertension; patients with grade 3 hypertension or secondary hypertension; tachyarrhythmias or bradyarrhythmias; coronary artery disease; ejection fraction <55% cerebrovascular, liver or renal disease, history of drug or alcohol abuse; thyroid gland disease; any chronic inflammatory or other infectious disease during the last 6 months. Vascular, metabolic, or neoplastic conditions were ruled out by careful examination of the history and routine laboratory tests. Subjects were allowed a further 15-minunte supine rest before baseline measurements.

The study was approved by Heraklion University Hospital Ethics Committee, and all participants gave written informed consent to their participation in the study. Baseline data from our patients and controls are presented in Table 1.

Echocardiography

Standard M-mode, two-dimensional echocardiography was performed to all patients using a Vivid 7 (General