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MicroRNA 199a Is Downregulated in Patients After Coronary Artery Bypass Graft Surgery and Is Associated with Increased Levels of Sirtuin 1 (SIRT 1) Protein and Major Adverse Cardiovascular Events at 3-Year Follow-Up

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Background: The cardioprotective protein SIRT1 is elevated in patients with coronary artery disease (CAD) to compensate for the disease-related adverse effects, but less is known about the prognostic role of SIRT 1 regulating microRNAs in patients after coronary artery bypass graft (CABG) surgery.

Material/Methods: The expression of the SIRT 1-specific microRNAs miR-199a and miR-195 was analyzed using real-time PCR in 68 patients referred for CABG surgery and 34 control patients undergoing heart valve surgery. In CABG patients, major adverse cardiac and cerebrovascular events (MACCEs), including all-cause death, myocardial infarction (MI), re-vascularization, heart failure symptoms ≥NYHA II, re-hospitalization for any cardiovascular reason, and stroke, were analyzed at a median follow-up (FU) of 3.2 years (range: 3.0–3.6).

Results: The level of miR-199a in patients with CAD was significantly reduced compared to the control group (relative expression: 0.89±0.49 *vs.* 1.90±0.90, p=0.001), while SIRT 1 protein was markedly enhanced (p<0.001). In patients undergoing CABG who had MACCEs, miR-199a was significantly lower compared to patients with an uneventful FU (0.71±0.25 *vs.* 0.98±0.53, p=0.007). Heart failure status, death, and total MACCEs rate were inversely correlated with the amount of miR-199a (p=0.039) at 3-year FU.

Conclusions: Altered expression of miR-199a in myocardial tissue was found to be associated with SIRT 1 upregulation in patients with CAD undergoing CABG and was associated with an increased MACCEs rate at mid-term follow-up.

MeSH Keywords: Coronary Artery Bypass • MicroRNAs • Sirtuin 1 • Ventricular Function, Left

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Background

Sirtuin 1 (SIRT 1) is a cardioprotective protein involved in the regulation of angiogenesis, prevention of endothelial dysfunction, and counteraction of deleterious effects of ischemia reperfusion injury [1–4]. At the molecular level, it promotes DNA stability by binding and deacetylating several substrates; it upregulates endothelial nitric oxide synthase (eNOS) and manganese-dependent superoxide dismutase (MnSOD) expression and activates FoxO1-dependent pathways [1–4].

Thus, it is not surprising that SIRT1 protein levels are markedly increased in patients with a history of myocardial infarction (MI) or coronary artery disease (CAD), which might be the result of a compensatory mechanism to counteract the adverse effects of oxidative stress/hypoxia in patients with cardiovascular disease (CVD) [5,6].

The 2 cardiomyocyte-specific microRNAs, miR-195 and miR-199a, are primary involved in the regulation of SIRT1 expression in cardiac tissue [7]. MicroRNAs (miRs) are small ribonucleic acids (RNAs) that negatively regulate gene expression on the post-transcriptional level by inhibiting mRNA translation or promoting mRNA degradation [8]. As SIRT 1 has a key role in cardiac pathophysiology, it would be of great value to know if its regulating microRNAs predict adverse events in patients with cardiovascular disease at an early stage.

So far, a few studies have demonstrated that some miRs are involved as key regulators in several pathological processes linked to cardiac diseases [8–11]. Further, changes in the concentration of miRs were related to disease outcome and improved risk prediction for cardiac events beyond traditional risk factors [12,13]. Lower levels of miR-199a were associated with an increased risk of a cardiovascular-related re-hospitalization in patients with chronic heart failure (CHF) during an 18-month follow-up (FU) period [12]. These results were supported by the finding that lower concentrations of miR-199a were associated with high levels of several biochemical markers related to inflammation, angiogenesis, and endothelial dysfunction, which are all involved in the development and progression of atherosclerosis [12]. A study by Jansen et al. has shown that decreased miR-199a expression in circulating microvesicles predicted cardiovascular events in patients with CAD at long-term FU [14]. In addition, miR-199a-3p was the best predictor of deterioration of renal function in patients who were hospitalized due to acute heart failure [15].

What might be the primary mechanism, which is responsible for the activation of the miR 199a- SIRT1 cascade, yielding to increased SIRT 1 levels in several cardiac diseases. In contrast to miR-195, hypoxia suppresses the expression of miR-199a with subsequent induction of SIRT1, which in turn downregulates prolyl hydroxylase 2 (PHD2), which stabilizes HIF-1α, the key transcription factor of hypoxia signaling [16]. As tissue hypoxia in patients with CVD is common, it was postulated that miR-199a is the denominator of hypoxia-triggered pathways and is mainly responsible for the prevention of deleterious effects of hypoxia in patients with CAD or heart failure [16].

Despite research about the prognostic value of miR-199a in patients with CAD and/or CHF [14–16], little is known about its association with adverse cardiac events after coronary artery bypass graft (CABG) surgery. One study has demonstrated that circulating miR-199a might be used as a novel biomarker for identifying perioperative myocardial infarction in cardiac surgery [17], while other studies have shown a potential predictive role of miR-199a in the development of post-CABG atrial fibrillation (AF) [18,19]. As known from previous studies, several predictors for re-hospitalization and adverse outcome after CABG include length of stay in the intensive care unit, severe non-cardiac complications, duration and severity of preoperative cardiac symptoms, intra-aortic balloon insertion, preoperative resting angina, female sex, advanced age, diabetes, and surgical procedure [20,21].

According to recent evidence, multiple variables such as age, extent of vessel disease (VD), left main disease, impaired left ventricular ejection fraction, and the presence of peripheral artery disease (PAD) were independent predictors of post-CABG mortality [22].

Thus, the goal of this study was to find novel, common risk markers and pathways for outcome prediction in patients after CABG, who always represent a heterogeneous collective characterized by diverse comorbidities and heart failure symptoms. Here, we investigated the role of miR-199a in prediction of adverse events at mid-term FU in patients undergoing CABG surgery.

Material and Methods

Sixty-eight patients undergoing isolated CABG surgery and 34 patients undergoing valve surgery were recruited. The operations were performed at the Bezmialem Vakif University Hospital and at the Mehmet Akif Ersoy Heart Hospital, Istanbul. Severe hepatic, renal, or pulmonary disease were determined as exclusion criteria.

The ethics criteria of the Declaration of Helsinki were followed during the study. The study was approved by the Committee for Medical Research Ethics of the Bezmialem Vakif University and all patients signed an informed consent before inclusion.

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After cardiopulmonary bypass (CPB) at mild hypothermia (34°C), cross-clamping (ACC) was performed and coronary anastomoses were attached.

The control group consisted of patients who underwent isolated valve repair or replacement without coronary artery disease. Thirteen concomitant mitral and tricuspid valve repairs, 9 concomitant aortic and mitral valve replacements, 9 isolated aortic valve replacements, and 3 isolated mitral valve replacements were performed.

Patients were routinely observed at the Intensive Care Unit and furthermore monitored at the ward until discharge. Thereafter, outpatient visits were conducted every year, including assessment of the functional capacity via NYHA classification, physical examination, and echocardiography at 1 and 3 years. The electrocardiogram-guided echocardiographic examination, using a transthoracic approach, was performed by an experienced sonographer using Philips Envisor C HD (Philips Medical Systems, Andover, MA, USA) echocardiography devices and 2- to 4-MHz phase transducers. All measurements were obtained as per the criteria recommended by the current guidelines [23]. Left ventricular ejection fraction (LVEF) from apical 4- and 2-chamber views were measured sing the modified Simpson method [23].

Atrial biopsies

Myocardial tissue samples were obtained during right atrial cannulation. After sufficient heparinization, a purse string was placed around the right atrial appendage and was then tightened. Samples were excised from the right atrial appendage during the insertion of the cannulas but before starting the cardiopulmonary bypass circulation. Then, samples were immediately frozen in liquid nitrogen and kept at –80°C.

miRNA expression

Fifty mg tissue per sample was homogenized and RNA was isolated using the mirVana miRNA Isolation Kit (Life Technologies, AM1560). RNA quality was controlled by the Implen NanoPhotometer (Implen). After equalization of RNA samples to a concentration of 2 ng/µl, the miRNA reverse transcription polymerase chain reaction was performed with the TaqMan MicroRNA Reverse Transcription Kit (Life Technologies, 4366596). RNA quantification by real-time PCR was performed with a system from Applied Biosystems (Applied Biosystems, 7500). Subtracting the crossing point of the investigated miRNA from the average crossing point of normalization miRNAs revealed the level of miRNA expression, presented as the normalized crossing point. After normalization of the miRNA to the endogenous control RNU43, relative expression levels were calculated using the CT (cycle number) method.

Western blot analysis of SIRT1 protein expression

Myocardial tissue samples belonging to the same group (first group: CABG n=6 *vs.* control n=6; second group MACCE (–) CABG n=6 *vs.* MACCE (+) CABG n=6) were used for Western blot analysis. The CABG patients in the first group were selected from patients without any MACCEs. The control group included patients with all types of valve disease.

Each sample was homogenized separately. After treatment with lysis buffer (RIPA Lysis Buffer, Thermo Fisher Scientific, Cat. No. 89900) and protease inhibitor and phosphatase inhibitor cocktail, the protein concentration was measured with the Quibit® Fluorimeter (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA). Forty µg protein was size-fractionated by 4–12% NuPAGE electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes using the iBlot® Dry Blotting System (Invitrogen, Life Technologies Corporation). After blocking the membrane in 5% nonfat milk in 50 mm Tris-buffered saline for 1 h at room temperature, it was incubated overnight with rabbit polyclonal anti-sirt1 (rabbit polyclonal, 07-131, Millipore). Thereafter a peroxidase-conjugated goat anti-rabbit (Amersham, GE Health Care UK Limited, Buckinghamshire, England) antibody was used as the second antibody. To test the reproducibility, Western blot analysis was performed for each sample 3 times.

Protein loading was controlled using the rabbit monoclonal GAPDH antibody (rabbit monoclonal antibody, 14C10, Cell Signaling). The ECL-Advanced Western Blotting Detection kit (Amersham, GE Health Care UK, Limited) was used for blot development and the Bio-Rad ChemiDoc XRS (Bio-Rad Laboratories, Inc) was used for visualization. Protein quantification was conducted densitometrically with the ImageJ program and corrected with values determined on GAPDH blots. The expression was given as relative values compared with the control or MACCE negative- CABG groups. The *t* test was used for statistical analysis. The results are shown as mean ± standard deviation (SD). * $p<0.05$. ** $p<0.001$.

Major adverse cardiovascular and cerebral events (MACCEs)

Clinical endpoints analyzed included the composite of all-cause death, myocardial infarction (MI), re-vascularization, heart failure symptoms ≥NYHA II, re-hospitalization for any cardiovascular reason, heart failure, and stroke at a median follow-up (FU) period of 3.2 years (range 3.0–3.6).

Statistical analysis

All continuous variables were tested for normality using the Kolmogorov-Smirnov test. Data are presented as percentages, mean ± standard deviation (SD), or median (interquartile

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Table 1. General characteristics of the first study groups.

	CABG (N=68)	Control (N=34)	p
Age (y)	59.7±9.0	55.7±12.5	0.072
Male gender (n)	54 (79.4%)	25 (73.5%)	0.065
Current smoker at operation (n)	27 (39.7%)	7 (20.6%)	<0.001**
Positive family history for CAD (n)	57 (83.8%)	17 (50%)	<0.001**
Arterial hypertension (n)	32 (47.1%)	3 (8.8%)	<0.001**
Diabetes mellitus (n)	38 (55.9%)	4 (11.8%)	<0.001**
Dyslipidemia (n)	26 (38.2%)	7 (20.6%)	<0.001**
BMI (kg/m^2)	28.9±4.9	27.4±3.7	0.148
History of MI (n)	25 (36.8%)	1 (2.9%)	<0.001**
History of stroke (n)	4 (5.9%)	0	<0.001**
Carotid artery disease (n)	3 (4.4%)	0	<0.001**
Chronic heart failure NYHA Functional Class NYHA ≥II (n)	11 (11.8%)	6 (17.6%)	0.504
Chronic kidney disease (n)	6 (8.8%)	0	<0.001**
Preop LA volume (ml)	55.9±23.3	56.6±11.5	0.908
Preop LA volume index (ml/m^2)	29.7±12.7	37.5±16.6	0.010*
Preop. LVEF (%)	53.7±11.8	56.5±11.5	0.776
Preop LVEF <40%	12 (17.6%)	6 (17.6%)	1.0
Glucose (mg/dl)	161.5±82.2	105.15±26.4	0.001*
Hematocrit (%)	41.3±5.9	40.6±5.4	0.533
WBC (x10^3/ml)	9.2±2.6	7.8±2.6	0.012*
CRP (mg/dl)	7.3±4.6	2.0±2.3	0.002*
Total Cholesterol	163.9±96.2	104.9±115.6	0.034*
Low density lipoprotein (mg/dl)	121.5±68.1	83.6±71.9	0.027*
High density lipoprotein (mg/dl)	33.6±17.4	24.9±29.6	0.183
Triglycerides (md/dl)	178±149.5	83.5±93.6	0.001*
Creatinine (mg/dl)	1.1±1.2	0.8±0.3	0.083

n – number of individuals. Differences in continuous variables were tested using Student's t test. Categorical variables were compared by Chi-square test. The results are shown as mean ± Standard Deviation (SD). * p<0.05; ** p<0.001.

range). Chi-square analysis was used for comparing categorical variables between groups. Differences in continuous variables were tested with the *t* test or Mann-Whitney U test for parametric and non-metric variables, respectively. Pearson's correlation test was also used to determine the relation between parameters of interest. All tests were 2-sided and P<0.05 was regarded as significant. Statistical analysis was performed using SPSS version 2.0 (SPPS Inc., Chicago, IL, USA).

Results

Demographic and clinical analysis

General characteristics of the first study groups (CABG *vs.* Control) are summarized in Table 1. The average age in both groups was comparable (CABG 59.7±9.0 *vs.* Control 55.7±12.5, p=0.072) with a male predominance (CABG 79.4% *vs.* Control 73.5%).

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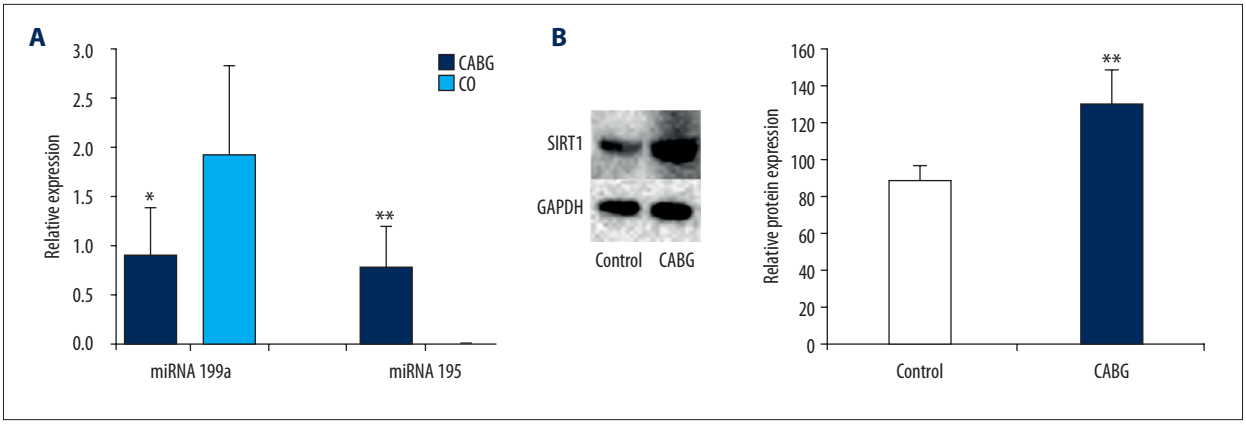

Figure 1. (**A**) Relative expression of microRNAs 199a and 195 in cardiac tissue probes of the study groups. Statistical evaluation by *t* test. The results are shown as mean ± standard deviation (SD). * p<0.05, ** p<0.001. (**B**) Representative Western blot analysis of SIRT1 protein (CABG n=6; CO n=6). The relative SIRT1 expression was normalized against GAPDH. Statistical evaluation by *t* test. The results are shown as mean ± standard deviation (SD). ** p<0.001.

The frequency of cardiovascular risk factors such as smoking, positive family history for CAD, arterial hypertension, diabetes mellitus, and dyslipidemia was significantly higher in the CABG group (p<0.001 for each risk factor). More than 1/3 of the patients in the CABG group had a history of myocardial infarction (MI) (CABG 36.8% *vs.* Control 0%, p<0.001), 5.9% had stroke (p<0.001), 4.4% had carotid artery disease (p<0.001), and 8.8% had chronic kidney disease (p<0.001). There was no difference in the distribution of chronic heart failure (NYHA ≥II) between groups (CABG 11.8% *vs.* Control 17.6%, p=0.504).

The preoperatively determined left atrial (LA) volume index was higher in the Control than in the CABG group (CABG 29.7±12.7 ml/m^2 *vs.* Control 37.5±16.6 ml/m^2, p=0.01). The left ventricular ejection fraction (LVEF) was comparable in both groups (CABG 53.7±11.8 *vs.* Control 56.5±11.5, p=0.776).

Fasting blood glucose, total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG) concentrations and CRP values were significantly higher in the CABG group (for each parameter p<0.05), while the hematocrit and creatinine values were similar in both groups.

MicroRNA expression in CABG *vs.* control patients

MicroRNA 199a levels were significantly decreased in patients with CABG compared to the Control group (CABG 0.89±0.49 *vs.* Control 1.90±0.90, p=0.001) In contrast, miR-195 was less common in the Control group compared to the significantly higher levels in the CABG group (CABG 0.78±0.41 *vs.* Control 0.006±0.003, p<0.001) (Figure 1A).

Table 2. Frequency of major adverse cardiac and cerebrovascular events (MACCEs) in the CABG group.

Total MACCEs (patient based) (n)	20 (29.4%)
Myocardial infarction (n)	7 (10.3%)
Re-vascularization (n)	3 (4.4%)
Chronic heart failure NYHA Functional Class ≥II (n)	18 (26.5%)
Stroke (n)	2 (2.9%)
Re-hospitalization (n)	18 (26.5%)
Death, all cause (n)	6 (8.8%)

SIRT 1 protein expression in CABG *vs.* control patients

SIRT1 protein was significantly induced in tissue probes of the CABG group compared to the Control group (relative protein expression Control 89.61%±7.74 *vs.* CABG 131.06%±18.16, p<0.001) (Figure 1B).

Major adverse cardiac and cerebrovascular events (MACCEs) in the CABG group

Seven patients had myocardial infarction during a follow-up period of 3.2 years and 3 of them were revascularized with percutaneous coronary intervention. Eighteen (26.5%) patients had a functional status NYHA ≥II. Stroke occurred in 2 patients and 18 patients were re-hospitalized for any cardiac-related cause. Six patients died: 1 died due to a myocardial infarction, 1 died due to major stroke with occlusion of the right middle cerebral artery, and 4 died due to advanced heart failure with reduced LVEF <35% and a NYHA functional status III-IV (of these latter 4 patients, 3 had been re-hospitalized previously) (Table 2).

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Clinical characteristics of CABG patients with and without MACCEs

Patients with MACCEs were older, with a mean age of 62.8±8.2 years compared to MACCEs-negative patients with a mean age of 57.9±8.8 (p=0.039). The cardiovascular risk profile and comorbidity status was similar in both groups, without significant differences. The preoperatively determined LVEF was comparable in both groups (MACCEs-negative 53.5±11.6 *vs.* MACCEs-positive 54.1±12.5, p=0.849). Nine patients (18.8%) in the MACCEs-negative and 3 patients (17.6%) in the MACCEs-positive group had LVEF <40% (p=0.958). The functional class was comparable in both groups, with 1.17±0.37 in the MACCEs-negative and 1.10±0.30 in the MACCEs-positive group (p=0.451). At 3-year FU, the LVEF tended to decrease in the MACCEs-positive group (49.3±13.8), while the LVEF in the MACCEs-negative group remained high (55.4±11.6) (p=0.105). Four patients (23.5%) in the MACCEs-positive group and 6 patients (14%) in the MACCEs-negative group had LVEF <40% (p=0.374). The NYHA functional class significantly deteriorated in the MACCEs-positive group (MACCEs-negative 1.30±0.48 *vs.* MACCEs-positive 2.5±1.0, p<0.001). Forty-three patients (89.7%) in the MACCEs-negative group and 15 patients (75%) in the MACCEs-positive group displayed a stable or increasing LVEF. The percentage of patients with reduced LVEF in FU was higher in the MACCEs-positive group (25%) compared to the MACCEs-negative group (10.4%), without significant differences between groups (p=0.254). Total graft number, LIMA-use, RCA-graft use, and pre-and postoperative medication were comparable in both groups, without marked differences (Table 3).

Expression of microRNA 199a and SIRT 1 protein in CABG patients with and without MACCEs

The expression of miR-199a was significantly decreased in tissue samples of patients with MACCEs (MACCEs-negative 0.98±0.53 *vs.* MACCEs-positive 0.71±025, p=0.007) (Figure 2A). The concentration of SIRT 1 protein was significantly higher in patients with MACCEs compared to those without adverse events (p=0.013) (Figure 2B).

Correlation analysis of miR-199a expression with MACCEs and LVEF

We detected a positive correlation between miR-199a expression and preoperative LVEF (r=0.276; p=0.026). This correlation maintained after 3-year FU, with increasing significance (r=0.322; p=0.011). There was no correlation between miR-199a expression and preoperative functional status, while miR-199a was significantly correlated with the postoperative NYHA status (r=–0.362; p=0.003). There was also a negative correlation between miR-199a and death (r=–0.244; p=0.045) and between miR-199a and total MACCEs (r=–0.252; p=0.039) (Table 4).

Discussion

Several studies have shown that SIRT 1-regulating miR-199a predicted adverse events in patients with cardiovascular disease [12,14,15], but less is known about the prognostic role of miR-199a in patients with CAD undergoing CABG surgery. Patients selected for CABG surgery generally have various comorbidities, multi-vessel disease, and reduced LVEF due to previous MI or ischemia-induced myocardial stunning. Thus, postoperative clinical status and cardiac function are dependent on many variables and are often unpredictable [20–22]; while some patients benefit from CABG surgery in terms of improved functional status and cardiac function, others do not [20–22].

In light of these considerations, we investigated the prognostic role of the cardiomyocyte-specific microRNA 199a in patients undergoing CABG surgery. We demonstrated that miR-199a was markedly downregulated in samples of patients with CAD compared to patients with valve disease, which was paralleled by the induction of its target protein SIRT 1 (Figure 1A, 1B). The cardioprotective protein SIRT 1 exerts an important role in the regulation of vascular homeostasis, angiogenesis, endothelial senescence, and endothelial function through the modulation of eNOS activity and other pathways involved in oxidative stress [1–4]. Further, it participates in biological processes related to the development of heart failure (HF), including the regulation of energy production, oxidative stress, autophagy, and cell death/survival, mainly by playing a protective role in failing hearts [24]. The insulin-like growth factor-1 (IGF-1) propeptide (mIGF-1) helps the heart to recover from infarction through the induction of SIRT1 expression in cardiomyocytes, exerting protection from hypertrophy and oxidative stress [25]. Oxidative stress and tissue hypoxia are among the most important trigger factors for the development or aggravation of cardiovascular diseases. It is reported that SIRT 1 is induced in several cardiac diseases to overcome the disease-related adverse events, which are mainly driven by hypoxia and oxidative stress [6,26].

As miR-199a is known as the master regulator of hypoxia-induced pathways, and it can be assumed that there is cross-talk between disease-induced oxidative stress, miR-199a, and SIRT 1.

Another SIRT 1-specific miR-195 seems to play a less important role in the development of cardiac disease, as its expression profile markedly diverges from that of miR-199a (Figure 1). MicroRNA 195 induces apoptosis by increasing ROS production [27] but it is not involved in hypoxia or ischemia-triggered pathways [16].

Compared to MACCEs-negative patients, miR-199a expression was significantly lower in patients with MACCEs; accordingly,

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Table 3. Clinical characteristics of patients undergoing coronary artery bypass surgery (CABG) with and without MACCEs at 3 years follow up. Differences in continuous variables were tested using Student's t test. Categorical variables were compared by Chi-square test.

	MACCEs (–) (N=48)	MACCEs (+) (N=20)	p
Age (y)	57.9±8.8	62.8±8.2	0.039*
Male gender (n)	39 (81.2%)	15 (75.0%)	0.564
Current smoker at operation (n)	21 (43.8%)	6 (30.0%)	0.640
Positive Family history for CAD (n)	39 (81.2%)	18 (90.0%)	0.375
Arterial Hypertension (n)	23 (47.9%)	9 (45.0%)	0.827
Diabetes mellitus (n)	26 (54.2%)	12 (60.0%)	0.661
Dyslipidemia (n)	19 (39.6%)	7 (35.0%)	0.725
BMI (kg/m^2)	29.3±4.7	28.9±5.6	0.867
History of MI (n)	18 (37.5%)	7 (35.0%)	0.847
History of Stroke (n)	4 (8.3%)	0	0.187
Carotid artery disease (n)	2 (4.2%)	1 (5.0%)	0.880
Chronic kidney disease (n)	2 (4.2%)	4 (20%)	0.768
Preop. LVEF (%)	53.5±11.6	54.1±12.5	0.849
Preop. LVEF <40%	9 (18.8%)	3 (17.6%)	0.958
Preop. NYHA Functional Class	1.17±0.37	1.10±0.30	0.451
LVEF at 3 y FU (%)	55.4±11.6	49.3±13.8	0.105
LVEF <40%	6 (14.0%)	4 (23.5%)	0.374
NYHA Functional Class at 3 y FU	1.3±0.48	2.5±1.0	<0.001**
LVEF development at 3 y FU			0.254
Stable LVEF	35 (72.9%)	13 (65%)	
Increasing LVEF	8 (16.7%)	2 (10%)	
Reduced LVEF	5 (10.4%)	5 (25%)	
Total graft number, n	3.4±1.0	3.1±0.6	0.076
LIMA use, n	48 (100%)	19 (95%)	0.121
RCA graft use, n	31 (64.6%)	13 (65%)	0.974
Preop. Beta blockers	23 (47.9%)	11 (55%)	0.597
Preop. ACE-inhibitors/angiotensin receptor blockers	20 (41.7%)	8 (40%)	0.671
Preop. statins	11 (22.9%)	4 (20%)	0.793
Postop. Beta blockers	21 (43.8%)	14 (70%)	0.720
Postop. ACE-inhibitors/angiotensin receptor blockers	23 (47.9%)	15 (53.6%)	0.680
Postop. statins	27 (56.3%)	10 (50%)	0.790

The results are shown as mean ± Standard Deviation (SD). * p<0.05; ** p<0.001. LVEF – left ventricular ejection fraction; FU – follow up; LIMA – left internal mammary artery; RCA – right coronary artery.

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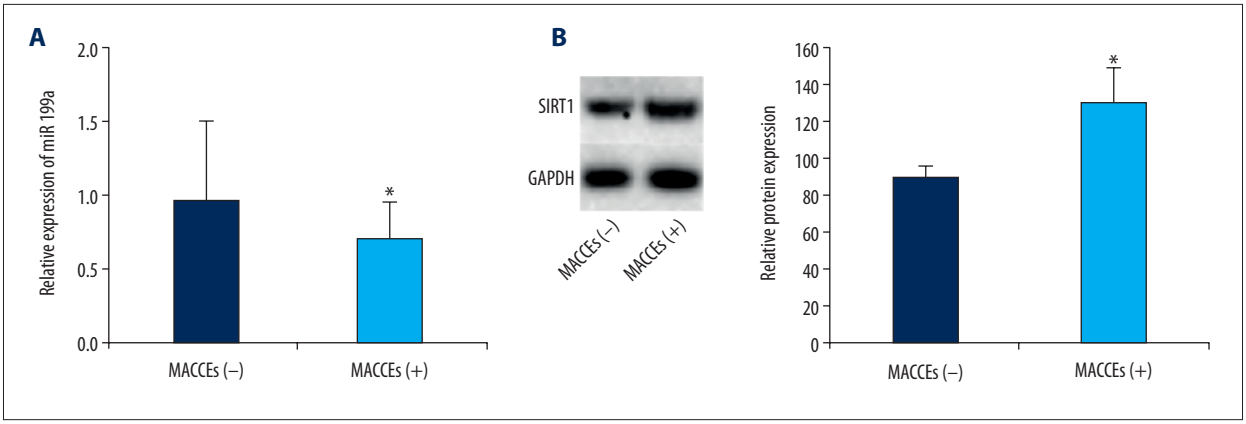

Figure 2. (**A**) Relative expression of microRNAs 199a in CABG patients with and without MACCEs. Statistical evaluation by *t* test. The results are shown as mean ± standard deviation (SD). * p<0.05 (p= 0.007). (**B**) Representative Western blot analysis of SIRT1 protein (MACCEs-negative n=6; MACCEs-positive n=6). The relative SIRT1 expression was normalized against GAPDH. Statistical evaluation by *t* test. The results are shown as mean ± standard deviation (SD). * p<0.05.

SIRT 1 was elevated (Figure 2A, 2B) but this was not the case for miR-195. It is reasonable to assume that oxidative stress, either induced by coronary ischemia or heart failure, is increased in patients suffering from MACCEs.

Almost 30% of the patients developed MACCEs at 3-year FU (Table 2), whereas 26.5% were re-hospitalized. In an earlier study, 23% of CABG patients were re-hospitalized in the first 6 months following surgery [28]. More recently, 33% of CABG patients were re-hospitalized within the first 2 years after surgery, with the most common reasons for re-admission being acute myocardial infarction, arrhythmia, angina, and heart failure [29].

In the present study, 18 patients (26.5%) from among 20 MACCEs-positive patients had a functional status ≥NYHA II. While the functional class remained stable in MACCEs-negative patients (NYHA=1.3±0.48) during the 3-year FU, the functional capacity significantly deteriorated in the MACCEs-positive group (NYHA=2.5±1.0) (Table 3). Six patients died, 4 of them due to advanced heart failure with LVEF less than 35% and a functional status NYHA III–IV.

These results are in line with the development of LVEF, as the percentage of patients with deteriorating LVEF in FU was higher in the MACCEs-positive group (25%) compared to the MACCEs-negative group (10.4%). More than 23% of the patients displayed a LVEF <40% in the MACCEs-positive group, while LVEF was <40% in only 14% in the MACCEs-negative group (Table 3). Preoperative low LVEF is one of the clearest predictors of post-CABG morbidity and mortality [20–22]. According to these observations, there is a positive correlation between miR-199a and preoperative LVEF, which was maintained at 3-year FU (Table 4).

Table 4. Pearson's correlation analysis of miR-199a expression with Major Adverse Cardiac and Cerebrovascular events (MACCEs) and left ventricular ejection fraction (LVEF).

	miR-199a	
	r	p
Preop. LVEF	0.276	0.026*
Preop. NYHA	–0.113	0.357
Postop. LVEF	0.322	0.011*
Postop. NYHA	–0.362	0.003*
MACCE	–0.252	0.039*
Myocardial infarction	0.005	0.969
Revascularization	0.027	0.828
Stroke	–0.085	0.489
Rehospitalization	–0.181	0.139
Death	–0.244	0.045*

* p<0.05.

Intriguingly, the decrease in functional status in the MACCEs-positive group was more evident than the decrease in LVEF (Table 3), as was the correlation between miR-199a and NYHA status. This suggests that in addition to left ventricular dysfunction (LVD), functional capacity is related to the altered expression of miR-199a after surgery. Functional capacity is dependent on many factors: age, psychosocial factors, comorbidities, and genetic factors affect the ability to participate in regular physical activity and affect ability to respond with an increase in physical fitness if needed [20]. Thus, functional capacity does not always reflect cardiac function *per se* and might be affected by the interaction of various variables. According to a study in which 60% of patients experienced dyspnea before CABG surgery, 54% of these were completely relieved of dyspnea,

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22% reported some improvement, and 18% had no improvement at 6 months after surgery; 9% of the patients reported more dyspnea following surgery, with more than 50% of these reports being from patients without dyspnea pre-surgery [28].

To summarize, there is considerable heterogeneity in prognosis among patients with CAD with and without HF amenable to CABG, and the benefit from surgical re-vascularization is not uniform. Thus, reduced levels of miR-199a are associated with worse outcome in patients with deteriorating or even stable reduced LVEF after surgery (Table 4). Irrespective of the LVEF, the functional capacity of these patients was NYHA 1 or 2 before surgery, but they displayed a worse functional status or even died during FU. Thus, in patients with low LVEF, a preoperatively measured miR-199a might help to stratify individual patient risk for adverse events, including death. However, it should be kept in mind that the present patient groups were not specifically selected and consisted of all-comers with and without heart failure.

Although the study population included patients with a wide range of LVEF values from normal to reduced and different LVEF development after surgery (constant, increasing, and decreasing), the correlation between miR-199a and LVEF was consistent during the 3-year FU. The interaction of the SIRT 1 system and LVEF was also shown in another study, where after myocardial infarction with successful percutaneous coronary intervention (PCI), LVEF was negatively correlated with SIRT 1 at 1-year FU [6].

The potential crosstalk between oxidative stress, miR-199a, and SIRT 1 might be causally linked to the progression of ischemic heart failure after CABG surgery. This hypothesis is supported by the lacking correlation between miR-199a and NYHA status in patients undergoing heart valve surgery, although the percentage of heart failure patients was comparable in both study groups.

Thus, the oxidative stress – miR-199a- SIRT 1 axis might be a specific pathway for ischemia-driven heart failure and left ventricular dysfunction. Rane et al. demonstrated that knockdown of miR-199a during hypoxia-induced apoptosis, whereas knockdown of miR-199a before hypoxia surprisingly imitated pre-conditioning and protected cardiomyocytes against hypoxic damage via SIRT1 induction [16]. Further, atorvastatin provided cardioprotective effects against ischemia reperfusion injury via increasing GSK-3β through inhibition of miR-199a-5p [30]. The induction of the miR-199a target protein SIRT 1 under statin treatment was also observed [6].

In all these studies, miR-199a downregulation under disease conditions, specifically ischemia, was linked to cardio-protection, probably to compensate for the deleterious effects of ischemic injury.

Conclusions

Altered expression of miR-199a in human myocardium was found to be mainly responsible for SIRT 1 upregulation in patients with CAD undergoing CABG and was associated with an increased MACCEs rate at mid-term follow-up. miR-199a should be determined in patients referred to CABG surgery as a predictor of future adverse events and to optimize patient care and improve patient outcomes. Clinicians need to have improved ability to determine which patients are likely to achieve a morbidity and mortality benefit from CABG.

Study limitations

The size of our study groups was small, especially for evaluating the association of miR-199a with ischemic events after surgery. An objective test, like the 6-minute walk test, and brain natriuretic peptide measurements might be conducted to objectively assess functional status.

Conflict of interest.

None.

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