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PRE-CLINICAL RESEARCH

Micro-RNA-34a Contributes to the Impaired Function of Bone Marrow-Derived Mononuclear Cells From Patients With Cardiovascular Disease

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Objectives This study evaluated the regulation and function of micro-RNAs (miRs) in bone marrow–mononuclear cells (BMCs).

Background Although cell therapy with BMCs may represent a therapeutic option to treat patients with heart disease, the impaired functionality of patient-derived cells remains a major challenge. Small noncoding miRs post-transcriptionally control gene expression patterns and play crucial roles in modulating cell survival and function.

Methods Micro-RNAs were detected by miR profiling in BMCs isolated from healthy volunteers (n = 6) or from patients with myocardial infarction (n = 6), and the results were confirmed by polymerase chain reaction (PCR) in a larger cohort (n = 37). The function of selected miRs was determined by gain-of-function studies in vitro and by locked nuclear acid (LNA) modified inhibitors in vitro and in vivo.

Results We identified several miRs that are up-regulated in BMCs from patients with myocardial infarction compared with BMCs from healthy controls, including the pro-apoptotic and antiproliferative miR-34a and the hypoxia-controlled miR-210. Inhibition of miR-34 by LNA-34a significantly reduced miR-34a expression and blocked hydrogen peroxide–induced cell death of BMC in vitro, whereas overexpression of miR-34a reduced the survival of BMCs in vitro. Pre-treatment of BMCs with LNA-34a ex vivo significantly increased the therapeutic benefit of transplanted BMCs in mice after acute myocardial infarction (AMI).

Conclusions These results demonstrate that cardiovascular disease modulates the miR expression of BMCs in humans. Reducing the expression of the pro-apoptotic miR-34a improves the survival of BMCs in vitro and enhances the therapeutic benefit of cell therapy in mice after AMI. (BMC Registry, NCT00962364; Progenitor Cell Therapy in Dilative Cardiomyopathy, NCT00284713) (J Am Coll Cardiol 2012;59:2107–17) © 2012 by the American College of Cardiology Foundation

Cell therapy is a promising option to improve neovascularization and repair after ischemia. Transplantation of various adult and embryonic stem cells was shown to augment contractile function after acute myocardial infarction (AMI). Particularly, several subsets of bone marrow–derived cells (BMCs), including hematopoietic progenitor cells, endothelial progenitor cells, and mesenchymal stem cells, improved recovery after ischemia (1–4) most likely due to an effect on vascularization of the ischemic tissue (5). Based on these studies, clinical trials tested the effects of BMCs as well as selected subpopulations to treat patients with ischemic disease. Intracoronary infusion of BMCs in patients with AMI improved ejection fraction and prevented left ventricular remodeling in most, but not all, studies (6). However, the extent of improvement was variable between trials, and the benefit of cell therapy with BMCs particularly in chronically ill patients was modest (7). One reason underlying the modest improvement of cardiac function by cell therapy may relate to the use of the patients' own cells, which are partially compromised in function by the exposure to risk factors. Age, concomitant disease

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Abbreviations and Acronyms

AMI = acute myocardial infarction
ANOVA = analysis of variance
BMC = bone marrow–derived mononuclear cell
CDK = cyclin-dependent kinases
DCM = dilated cardiomyopathy
ICM = ischemic cardiomyopathy
LNA = locked nuclear acid
miR = micro-RNA
MSP = methylation-specific PCR
PCR = polymerase chain reaction
SDF = stromal cell–derived factor
WMSI = wall motion score index

(e.g., diabetes), and heart failure were shown to impair the functionality of the patients' own cells (8–10). The mechanisms underlying the dysfunction are multiple and, for example, include the inhibition of endothelial nitric oxide synthase expression and dysregulation of the balance between nitric oxide and reactive oxygen species (11,12) and the activation of protein kinases (such as p38 mitogen-activated protein kinase) in diabetic patient-derived cells (13).

Micro-RNAs (miRs) are small noncoding RNAs that recently emerged as key epigenetic regulators that control self-renewal and differentiation of stem cells (14,15) and modulate the function of pro-angiogenic BMCs (16). Moreover, they play a crucial role particularly during pathophysiological stress conditions in the heart as well as in the vasculature (for review, see [17]). Micro-RNAs bind to up to hundreds of target genes and induce mRNA degradation or block translation of the targeted mRNA, thereby modulating the gene expression patterns.

We determined the regulation of the miR expression profile in BMCs isolated from patients with heart disease compared with healthy volunteers and elucidated the function of the dysregulated miRs. We demonstrated that several pro-apoptotic and senescence associated miRs, such as miR-34a (18,19) and let-7 family members (20), as well as the hypoxia-controlled miR-210 (21), are increased in BMCs isolated from patients with acute myocardial infarction or chronic heart failure. Inhibition of 1 of the dysregulated miRs, namely miR-34a, reduced cell death of BMCs in vitro and improved the functional capacity of BMCs to restore cardiac function in a mouse model of AMI in vivo.

Methods

Patient characteristics. Bone marrow aspirates were obtained from healthy volunteers without any evidence of coronary artery disease by history and physical examination, or from patients undergoing intracoronary infusion of BMCs for the treatment of AMI or ischemic cardiomyopathy (ICM) (time from last myocardial infarction >3 months), or heart failure without coronary artery disease (dilated cardiomyopathy [DCM]) within our clinical trials or an ongoing registry. The ethics review board of Goethe University (Frankfurt, Germany), approved the protocols, and the studies are registered with www.clinicaltrials.gov (NCT00962364 and NCT00284713). Written informed consent was obtained from each donor and patient.

Cell isolation. BMCs were isolated by Ficoll density gradient centrifugation as previously described (7,22).

Micro-RNA array. Total RNA was isolated with miRNeasy kits from Qiagen (Hilden, Germany). The expression of mature human miRs was determined by human miR microarrays (DNAvision, Charleroi, Belgium). Murine miRs isolated from BMCs of old (18 month) or young (10 weeks) mice were detected by the Agilent microarray (DNAvision). For confirmation of the results, real-time polymerase chain reaction (PCR) was performed using commercially available Taq man PCR primers on an Applied Biosystems StepOnePlus (Carlsbad, California). Expression was normalized to small nucleolar RNA U48. The relative expression is calculated using the formula $2^{-\Delta Ct}$.

Micro-RNA inhibition and overexpression. Micro-RNAs were inhibited by locked nucleic acid (LNA) modified antisense miRs ("in vivo LNA microRNA Inhibitors"; Exiqon, Vedbæk, Denmark) with the following sequences: LNA-Co: ACGTCTATACGCCCA; and LNA-34a: AGCTAAGACACTGCC. LNA inhibitors were incubated with BMCs without any transfection reagent. For overexpression, BMCs were transfected with pre-miR-34a (5 nM) or pre-miR-negative control (5 nM) (Ambion, Carlsbad, California) using the Nucleofector Device (Amaxa, Gaithersburg, Maryland).

Invasion. After incubation of BMCs with LNA, a total of 1×10^6 BMCs were resuspended in 250 μl medium and placed in the upper chamber of a modified Boyden chamber filled with Matrigel (BioCoat invasion assay, 8-μm pore size; Becton Dickinson, Franklin Lakes, New Jersey). Then, the chamber was placed in a 24-well culture dish containing 500 μl x-vivo 10 medium. For some experiments, 100 ng/ml stromal cell–derived factor (SDF)-1 was added to the lower chamber. After 24 h of incubation at 37°C, transmigrated cells were counted. Invasion assays were run in duplicates.

Viabililty. BMCs (2×10^6) were incubated with the miR inhibitors or phosphate-buffered saline, and cell death was induced with 200 μM hydrogen peroxide for 12 hours. In the starvation-induced cell death study, 2×10^6 BMCs were incubated with the miR inhibitors or phosphate-buffered saline with or without 2% fetal calf serum (FCS) for 48 h. Dead cells were counted after incubation with Trypan blue. The percentage of dead cells was calculated by dividing the number of Trypan blue-positive cells by the number of total cells. Alternatively, pre-miR-34a was overexpressed, and cell death was quantified using the same method. Representative pictures of Trypan blue-positive cells were taken with a light microscope at 20× magnification.

DNA methylation. Micro-RNA-34a promoter DNA methylation was determined using methylation specific primers after bisulfite conversion of DNA (23). In brief, bisulfite treatment of 500 ng gDNA was performed using