SUPPLEMENTARY MATERIAL

Paracrine regulation of cardiac miRNAs by bone marrow mononuclear cell therapy in myocardial infarction

Kazuma Iekushi, MD, PhD; Florian Seeger, MD; Birgit Assums, MD; Andreas M.Zeiher, MD; Stefanie Dimmeler, PhD

From the Institute for Cardiovascular Regeneration, Centre of Molecular Medicine, (K.I., F.S., S.D.), and Dept of Medicine III, Frankfurt University, Frankfurt, (F.S., B.A., A.M.Z.), Frankfurt University, Frankfurt,
**Isolation of Bone Marrow Mononuclear Cells**

Bone marrow aspirates were obtained from healthy volunteers. The Ethics Review Board of the Hospital of the Johann Wolfgang Goethe University of Frankfurt, Germany approved the protocol, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each volunteer. BMCs were isolated as described previously. Briefly, bone marrow aspirates were diluted with 0.9% NaCl (1:5), were filtrated (100 um) and mononuclear cells were isolated by density gradient centrifugation using Ficoll (Cambrex; 800g, 20 minutes, without brake). Mononuclear cells were washed 3 times with 50 mL PBS (800g), were counted, and used for the experiments.

**In situ hybridization**

To detect miR-34a expression, tissue was processed and stained according to Obernosterer et al. 2 10 μm sections were cut and thawed for 30 min at room temperature. After fixation with paraformaldehyde (4%) for 10 min, sections were washed 3 times for 5 min and incubated with protein kinase K (Sigma) for 5 min. After washing 3 times with PBS, sections were incubated with hybridization buffer for 4 h at room temperature. Meanwhile, probes (0.5 μl 3′-DIG labeled LNA probes, Exiqon) were mixed with 150 μl denaturation buffer, heated to 80 °C for 5 min, chilled on ice and added to the sections followed by incubation over night at 56 °C. After incubation for 1 h in 50 % formamide / 1x SSC at 56 °C, 1h in 0.2 SSC at 56 °C and 10 min in solution B1, sections were blocked for 1h at room temperature in blocking reagent. Then, anti-DIG AP (Roche) was added at a dilution of 1:500 for 1h at 37 °C. After washing with solution B1, sections were equilibrated in 1 M Tris, pH=8.3 for 10 min at room temperature and subsequently incubated for 15 min with Fast Red substrate (Dako) containing 1 drop levamisol. After washing in PBS/0.5% Tween-20, sections were mounted in DAPI mounting medium (Vector).


Fig. S1. Real time PCR analysis of cardiac miRs.
(A, B) Real time PCR analysis confirms the regulation of miR-92a (A), miR-29b (B) in response to treatment with BMC compared to treatment with PBS, (S, sham; BZ, border zone of infarcted area).
Fig. S2. Real time PCR analysis of cardiac miRs in the remote area. (A, B, C) MicroRNA expression was measured in the remote area by PCR analysis. miR-21 (A), miR-20a (B), miR-34a (C) in response to treatment with BMC compared to treatment with PBS or sham.
Figure S3. Localization of miR-34a expression in MI.
Representative images showing miR-34a expression in ifarcted mice heart. Sections were counterstained with miR-34a (red), perfused vessels (lectin, green), and nucleus (blue). Scale bar: 20μm.
Fig.S4. survival rate with JB-1 or PBS infusion in treatment with BMC or PBS after MI mice.
Kaplan-Meier analysis with JB-1 or PBS in treatment with BMC or PBS after MI mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
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<tbody>
<tr>
<td>PBS+PBS</td>
<td>56.2%</td>
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<tr>
<td>PBS+JB-1</td>
<td>38.4%</td>
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<tr>
<td>BMC+PBS</td>
<td>75.0%</td>
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<tr>
<td>BMC+JB-1</td>
<td>70.5%</td>
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**Fig. S5. Mir-34a promoter assay.**
The luciferase assay of miR-34a promoter. The arrow above construct P1 indicates the position of the transcription start site. Filled circles show the position of the p53 binding site. Data represent mean ± SEM. Constructed plasmids were provided by Prof. Joshua T. Mendell. (Molecular Cell 2007).