Apelin-13 increases myocardial progenitor cells and improves repair of post-myocardial infarction
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BACKGROUND
1. Apelin is an endogenous ligand for the angiotensin-like 1 receptor (APJ) and has beneficial effects against myocardial ischemia or reperfusion injury.
2. Accumulating evidence indicates that apelin has a critical role in the regulation of bone marrow derived vascular progenitor cells and hematopoietic stem cell regeneration and mobilization.
3. Although the involvement of apelin/APJ in the regulation of angiogenesis and the protection of myocardial ischemia or reperfusion injury have been characterized, the role of apelin/APJ on the homing of vascular progenitor cells (PCs) in ischemic heart failure and post-MI is less clear.

OBJECTIVE
The present study investigates whether apelin-13 affects PCs homing to the injured hearts thereby mediating repair and functional recovery post-MI.

METHODS AND RESULTS
1. Experimental animal model: C57BL/6J mice were anesthetized with ketamine plus xylazine, intubated, and artificially ventilated with room air. An 8-0 nylon suture was placed around the LAD. Myocardial ischemia was achieved by ligation of the LAD. Sham controls underwent surgery without the LAD.
2. Systemic administration of apelin-13 in the experimental mice: The experimental mice received intraperitoneal (i.p.) apelin-13 (1mg/kg/d) daily for 3 days prior to surgery. After surgery, the mice continued to receive i.p.apelin-13 for 14 days prior to sacrifice.
3. SDF-1α, CXCR-4, VEGF, apelin, eNOS, Akt, Jagged1 and Notch3 expression were measured by western blot analysis.
4. Analysis of myocardial capillary and arteriolar densities: Sections were cut and incubated with fluorescein-labeled Griffonia Bandeiraea Simplicifolia Isolectin B4 (IB4) and Cy3-conjugated anti-smooth muscle actin (SMA).
5. Fibrosis: Sections were stained with Masson's trichrome (MT, Sigma).
6. Apelin: Heart tissue sections were stained with transferase deoxyuridine nick end labeling TUNEL (Promega, WI).
7. Cardiac function: A 1.4-Fr pressure–conductance catheter (SPR-839, Millar Instrument, Houston, TX) was inserted into the left ventricle (LV) to record baseline cardiac hemodynamics of the hearts.
8. The method was based on measuring the time-varying electrical conductance signal of two segments of blood in the left ventricle from which total volume is calculated.

Figure 1. Apelin-13 promotes vascular progenitor cell homing into the ischemic hearts.
A. Representative images of co-localization of CD133 with c-kit in mouse infarcted hearts at 14 days of MI. CD133+ (red), c-kit cells (green) and nuclei were stained by DAPI (blue, 40X). CD133+ and c-kit cells were recruited into ischemic area of mouse hearts at day 14 post-MI.
B. Immunofluorescence images showing co-localization of Sca1 and c-kit in the ischemic mouse hearts. Sca1+ (green), c-kit cells (red) and nuclei were stained by DAPI (blue, 40X). Merged images showed that Sca1 co-localized with c-kit (yellow) in the mouse hearts of post-MI and the increase was more dramatic in the post-MI animals receiving apelin-13.

Figure 2. Apelin-13 enhances myocardial neovascularization in ischemic hearts.
A. Treatment of ischemic mice with apelin-13 significantly increased capillary formation (IB4 staining) compared to ischemic control.
B. Myocardial ischemia significantly increased myocardial arteriolar density (SMA staining) in ischemic control compared to sham control mice at 14 days. Treatment of ischemic mice with apelin-13 caused a significant increase in arteriolar formation compared to ischemic control.

CONCLUSION
Apelin-13 increases angiogenesis and improves cardiac repair following MI by a mechanism involving upregulation of SDF-1α/CXCR4 and homing of vascular progenitor cells.

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