# 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 infarcted 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-O 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, APJ, eNOS, Akt, Jagged1 and Notch3 expression were measured by western blot analysis.
**4.** Analysis of myocardial capillary and arteriole densities: Sections were cut and incubated with fluorescerin-labeled Griffonia Bandeiraea Simplicifolia Isolectin B4 (IB4) and Cy3-conjugated anti-α smooth muscle actin (SMA).
**6.** Fibrosis: Sections were stained with Masson's trichrome (MT, Sigma).
**7.** Apoptosis: Heart tissue sections were stained with transferase deoxyuridine nick end labeling TUNEL (Promega, WI).
**8.** 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. 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.

Fig 1A

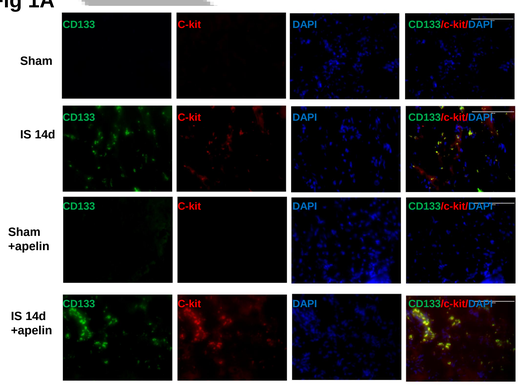


Fig 1B

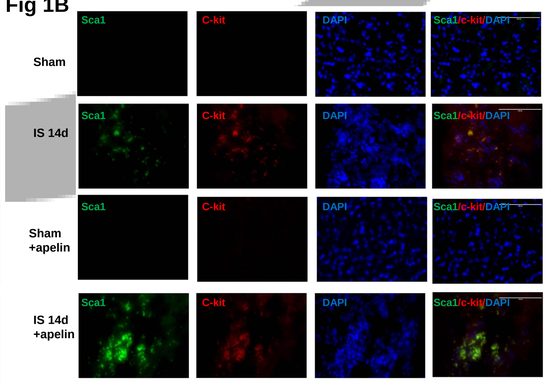


**Figure 1. Apelin-13 promotes vascular progenitor cell home into the ischemic hearts.**
A. Representative images of co-localization of CD133 with c-kit in mouse infarcted hearts at 14 days of MI. CD133$^+$ cells (green); c-kit$^+$ cells (red) and nuclei were stained by DAPI (blue, 40X). CD133$^+$ and 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$^+$ cells (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.

Fig 2A

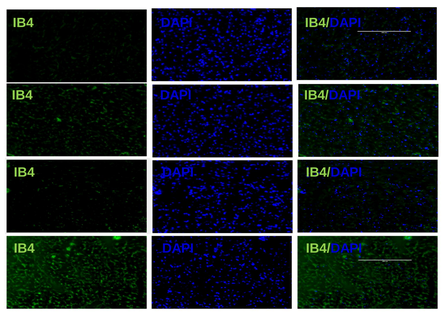


Fig 2B

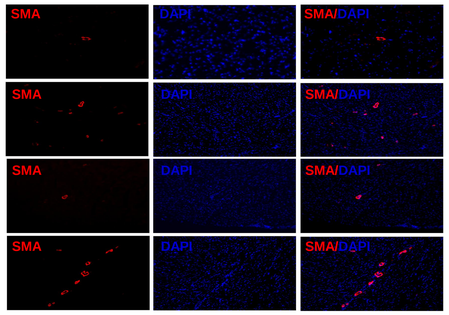


**Figure 2. Apelin-13 enhances myocardial neovascularization in ischemic hearts.**
A. Treatment of ischemic mice with apelin-13 significantly increased capillary formation(IB4 staining ) compared to ischemic control.
B. myocardial ischemia significantly increased myocardial arteriole density (SMA staining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

Fig 3

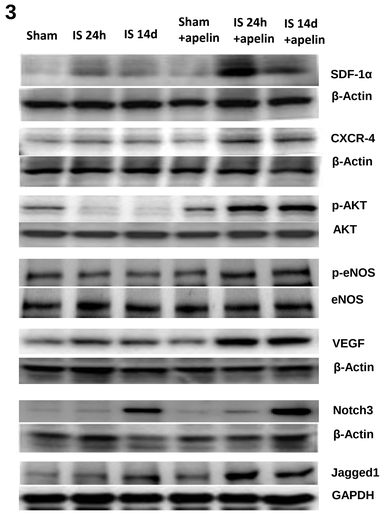


**Figure 3. Apelin-13 increases expression of SDF-1α/CXCR-4, pro-survival and angiogenic growth factors in ischemic hearts.** Apelin-13 significantly upregulated SDF-1α, CXCR-4, VEGF, Jagged1, Notch3 expression and increased Akt and eNOS phosphorylation in the ischemic hearts.

Fig 4

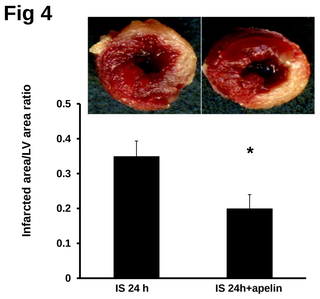


**Figure 4. Apelin-13 reduces myocardial infarct size at 24 hours of ischemia.** The non-infarcted area appears red and the infarct area appears white following TTC staining at 24 hours of ischemia. Myocardial infarct area was significantly reduced in apelin-13-treated mice compared with control MI mice. (n=3, *p<0.05)

Fig 5

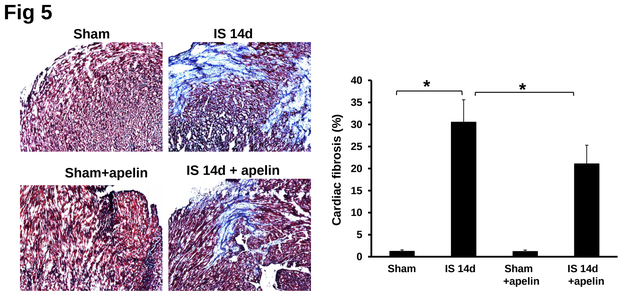


**Figure 5. Apelin-13 reduces cardiac fibrosis.** A significant increase in cardiac fibrosis was observed in mouse hearts after 14 days post-MI. Apelin-13 treatment significantly suppressed cardiac fibrosis in post-MI mice compared to those treated with saline. (n =5 ,*p<0.05)

Fig 6

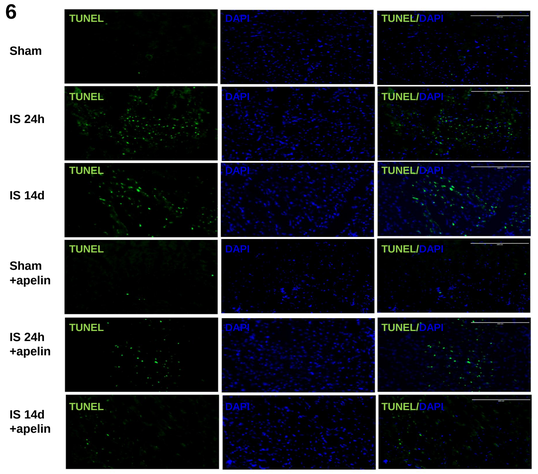


**Figure 6. Apelin-13 attenuates myocardial apoptosis**. TUNEL staining showed increased number of TUNEL$^+$ cells in post MI at 24 hours and 14 days, but in the apelin-13 treated mice fewer TUNEL$^+$ cells were found at each time point.

Fig 7

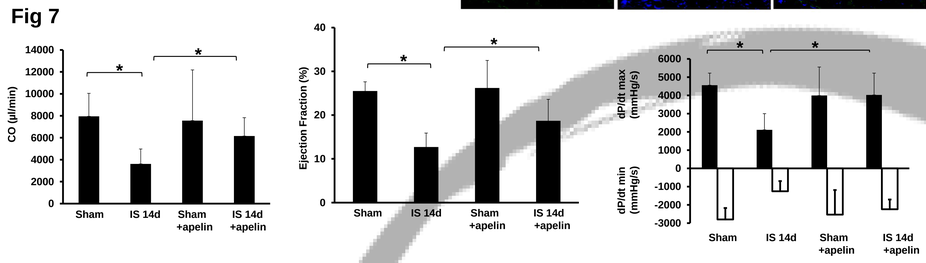


**Figure 7. Apelin-13 improves cardiac functional recovery in post-MI mice.** Cardiac output (CO) and ejection fraction (EF%) were significantly reduced 14 days post MI compared to sham controls . Apelin-13 treatment significantly increases CO and EF% compared to ischemic controls. Left ventricular function was significantly improved in the ischemic mice treated with apelin-13 compare to the saline-treated ischemic mice as showing a higher maximum +dP/dt pressure and a lower minimum -dP/dt pressure at 14 days of ischemia. (n=5-8, *p<0.05)

## 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.

## ACKNOWLEDGEMENTS

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