ANGPTL8 reverses established adriamycin cardiomyopathy by stimulating adult cardiac progenitor cells

Supplementary Material



Supplemental Figure 1. ANGPTL8 protein intramuscular injection reversed established ADM cardiomyopathy. (A) a graphic of fasting plasma human *ANGPTL8* levels. In normal and ADM + saline inject, human *ANGPTL8* was not detectable. Values are presented as mean \pm SEM. *n* =3 per group; **P<0.001 *vs* control groups. ANGPTL8 protein injection at doses of 5µg-20µg-40µg/kg/day for 14 days; (B) a graphic of fractional shortening, (C) a graphic of LV mass, Values are presented as mean \pm SEM. *n* = 3 per group; **P<0.001 and *<0.05 *vs* after ADM.



Supplemental Figure 2. ANGPTL8 protein therapy inducing the activation of ISL-1 (an early cardiac muscle differentiation marker) in epicardium and sub-epicardial layer. (A) normal rat heart, (B) ADM + Saline injection, (C) ADM plus ANGTPL8 protein 5 μ g/kg/day for 14 days, (D) ADM plus ANGTPL8 protein 20 μ g/kg/day for 14 days, (E) ADM plus ANGTPL8 protein 40 μ g/kg/day for 14 days. Scale bar is 25 μ m.



Supplemental Figure 3. Phospho-histone H3 (PHH3) staining shown some epicardial cells and sub-epicardial cardiac muscle cells are in proliferation after ANGPTL8 protein therapy. (A) normal rat heart, (B) ADM + Saline injection, (C) ADM plus ANGTPL8 protein 5 µg/kg/day for 14 days, (D) ADM plus ANGTPL8 protein 20 µg/kg/day for 14 days, (E) ADM plus ANGTPL8 protein 40 µg/kg/day for 14 days. Scale bar is 25 µm.



Supplemental Figure 4. PirB distributed in membrane of ISL-1 positive cardiac progenitor cells. Scale bar is 25 μ m.

Mechanism of Action of ANGPTL8 on Myocardial Regeneration

ANGPTL8 peptide excreted into circulation as a circulatory hormone from liver after ANGPTL8 gene delivery to liver with UTMD.	
ANGPTL8 binding with PirB on membrane of epicardial layer cells that were stimulated into cardiac progenitor cells.	ANGPTL8 binding with PirB on membrane of myocardial cells that up- regulated or recovered PirB expression
Progenitor cells starts to proliferate and differentiate into ISL-1-positive early cardiac progenitor cells and form niches of cardiac progenitor cells in epicardial layer.	and PirB further activates Notch1/FoxO1 pathway to reverse ADM cardiotoxicity
These ISL-1-positive early cardiac progenitor cells migrates from epicardial layer into sub-epicardium.	
The Newly formed cardiac muscle cells to reverse established ADM cardiomyopathy.	

Supplemental Figure 5. Schematic indicating mechanism of action of ANGPTL8 on myocardial regeneration.