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Endothelial-specific Fibroblast Growth Factor Receptor 1 and 2 Deletion Impairs Vascular Remodeling and Recovery in an *In Vivo*, Closed-Chest Model of Cardiac Ischemia-Reperfusion Injury

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BACKGROUND AND OBJECTIVES

Fibroblast growth factor (FGF) signaling is cardioprotective in various models of myocardial infarction. FGF receptors (FGFRs) are expressed in multiple cell types in the adult heart, but the cell type-specific FGFR signaling which mediates different cardioprotective endpoints is currently unknown.

METHODS

Mice: To create a conditional ablation of FGFR1 and FGFR2 in endothelial cells, mice transgenic for Cre recombinase driven by the Tie2Cre promoter (Tie2Cre) were crossed with mice with both *Fgfr1* and *Fgfr2* genes flanked by *loxP* sites (*Fgfr1$^{f/f}$, Fgfr2$^{f/f}$*). This resulted in endothelial-specific ablation of FGFR1 and FGFR2 (Tie2Cre FGFR1/2 DCKO). Wildtype controls for these experiments are double flox –flox or Tie2Cre controls with wildtype FGFRs.

Mouse Model of Closed-chest Cardiac Ischemia-Reperfusion Injury: The mouse model of closed-chest cardiac ischemia-reperfusion injury was performed in the Mouse Cardiovascular Phenotyping Core at Washington University in St. Louis School of Medicine. Mice were anesthetized with ketamine/xylazine (100mg/kg and 10 mg/kg, i.p.), prepped and ventilated through a tracheostomy. Mice were then subjected to a left mini-thoracotomy and the pericardium was dissected. An 8-0 polypropolene suture was passed under the proximal LAD artery, and the two ends of the suture were threaded through a 0.5mm piece of PE-10 tubing forming a loose snare around the LAD (Figure 4B). The suture was then exteriorized through each side of the chest wall and the chest wall was closed (Figure 4B). The mouse was removed from the respirator and allowed to recover for seven days. After this recovery time, mice were re-anesthetized but not ventilated, and only the skin above the chest wall was reopened. Mice were taped to an EKG board to observe ST segment changes during ischemia and reperfusion. The suture ends were pulled apart gently until ST segment elevation appeared on the EKG showing LAD occlusion and was continued for 90 minutes of ischemia time (Figure 4A). To induce reperfusion, the sutures were cut close to the chest wall releasing the tension, and reperfusion was confirmed with resolution of ST segment elevation. The surgeon was blinded to mouse genotype for all interventions.

Echocardiography: Mouse echocardiography was performed using a Visual Sonics Vevo2100 High-Resolution *In vivo* Imaging System. To obtain images, mice were anesthetized with Avertin (2,2,2-tribromoethanol, 250mg/kg, i.p.) which was chosen due to its lack of cardiovascular effects. Ejection fraction was calculated by measuring the end systolic and diastolic dimensions from long-axis images using the following formula: 100 * ((LV end diastolic volume – LV end systolic volume / LV end diastolic volume). Hypokinetic area was calculated by examining serial short-axis images (1 mm apart) spanning from the base of the left ventricle (level of aortic valve) to the apex. The percent area of the ventricle that was hypokinetic was calculated by determining the percent of the ventricle with wall motion abnormalities.

Histology: Hearts were halted in diastole in potassium chloride saturated phosphate buffered saline, fixed in 10% formalin, and transferred to 70% ethanol the next day. Hearts were paraffin-embedded and then sectioned transversely (5–10 µm). To visualize cardiomyocyte size, heart setctions were stained with wheat germ agglutinin (1:4 dilution, Sigma). To evaluate arteriole and venule remodeling, sections were labeled with Cy3-conjugated anti-smooth muscle acting antibody (1:500 dilution, Sigma) and counterstained with DAPI (Vector Laboratories). To visualize capillary remodeling, sections were labeled with anti-CD31 antibody (1:20 dilution, Dianova) and Cy3 anti-rat IgG (1:5000 dilution, Sigma) and counterstained with DAPI. Fluorescence was visualized using a Zeiss ApoTome microscope, and digital images were captured and processed with AxioVision Release 4.8 software. Images were analyzed with NIH Image J software.

Statistical Analysis: All values are expressed as mean ± standard error of the mean. Echocardiography data for LV wall motion abnormalities and ejection fraction were compared using analysis of variance with a post-hoc Student's t-test. The remaining data were compared using a Student's t-test. Data with a p<0.05 were considered statistically significant.

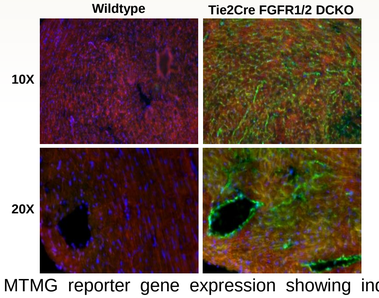

Figure 1: MTMG reporter gene expression showing induction of GFP expression in endothelium in Tie2Cre FGFR1/2 DCKO hearts but not wildtype control hearts.

RESULTS

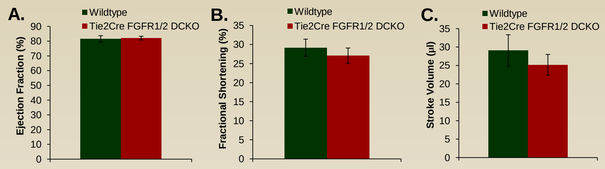

Figure 2: Echocardiographic determination of baseline functional characteristics of Tie2Cre FGFR1/2 DCKO mice. There are no alterations in ejection fraction **(A)**, fractional shortening **(B)**, or stroke volume **(C)** in Tie2Cre FGFR1/2 DCKO mice in the abcense of injury. n=5-6.

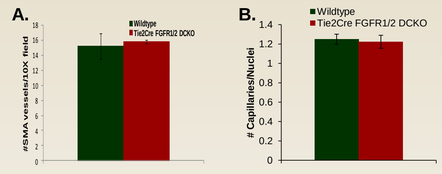

Figure 3: Baseline quantitation of SMA positive vessel density **(A)** and capillary density **(B)** in non-ischemic hearts shows no difference between Tie2Cre FGFR1/2 DCKO and wildtype control hearts. n=4.

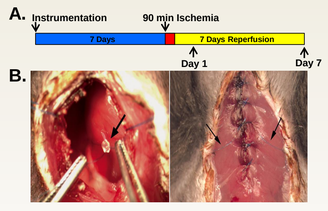

Figure 4: Schematic **(A)** and images **(B)** of *in vivo*, closed-chest, regional cardiac ischemia-reperfusion model.

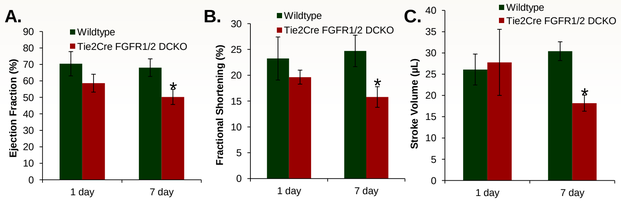

Figure 5: Echocardiographic determination of cardiac function at 1 day and 7 days after *in vivo* IR injury. Tie2Cre FGFR1/2 DCKO hearts show reduced cardiac function including ejection fraction **(A)**, fractional shortening **(B)** and stroke volume **(C)** compared to wildtype control hearts at 7 days but not 1 day after IR injury. n=5-6, *p<0.05 vs. wildtype.

RESULTS

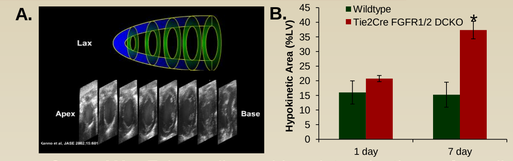

Figure 6: **(A)** Echocardiographic determination of wall motion abnormalities at 1 day and 7 days after *in vivo* IR injury. **(B)** Tie2Cre FGFR1/2 DCKO hearts show increased hypokinetic area compared to wildtype control hearts at 7 days but not 1 day after IR injury. n=5-6, *p<0.05 vs. wildtype.

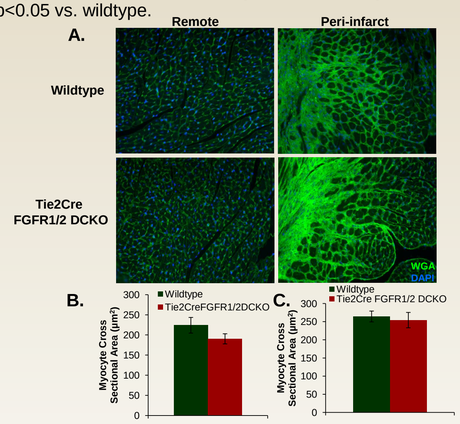

Figure 7: A) Representative images of wheat germ agglutinin staining of the peri-infarct and remote myocardium of wildtype and Tie2Cre FGFR1/2 DCKO hearts after 7 days of reperfusion. There is no significant difference in the cardiac hypertrophic response after IR injury in the remote **(B)** or peri-infarct area **(C)**. n=5-6.

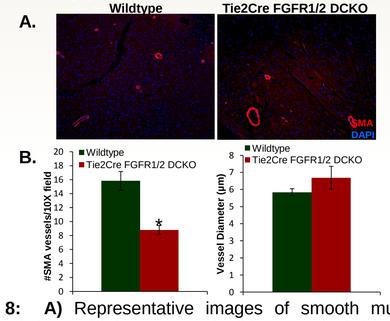

Figure 8: **A)** Representative images of smooth muscle actin (SMA) staining of the peri-infarct area of wildtype and Tie2Cre FGFR1/2 DCKO hearts after 7 days of reperfusion. **B)** Ablation of FGFR1 and FGFR2 in endothelium results in decreased vessel density but no change in vessel size after IR injury. n=5-6. *p<0.05 vs. wildtype.

RESULTS

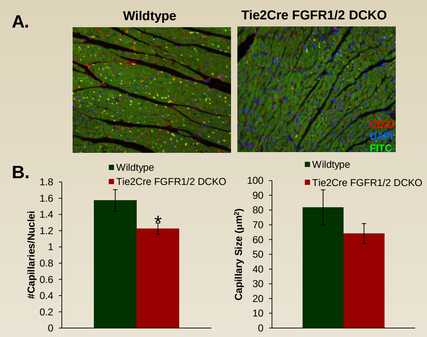

Figure 9: A) Representative images of CD31 staining capillaries in the peri-infarct area of wildtype and Tie2Cre FGFR1/2 DCKO hearts after 7 days of reperfusion. **B)** Ablation of FGFR1 and FGFR2 in endothelium results in decreased capillary density but no change in capillary size after IR injury. n=5-6. *p<0.05 vs. wildtype.

SUMMARY

- Ablation of FGFR1 and FGFR2 in endothelial cells has no baseline effect on cardiac function or vessel density (both arteriole and capillary).
- After *in vivo*, closed-chest cardiac IR injury, FGFR1 and FGFR2 ablation in endothelial cells resulted in reduced cardiac function and increased wall motion abnormalities at 7 days but not 1 day of reperfusion.
- Ablation of FGFR1 and FGFR2 in endothelial cells does not effect the cardiac hypertrophic response to IR injury.
- Vascular remodeling after IR injury is impaired in mice with endothelial-specific ablation of FGFR1 and FGFR2.

CONCLUSION

Ablation of FGFR1 and FGFR2 in endothelial cells results in impaired vascular remodeling, worsened cardiac functional recovery, and increased infarct size without affecting the cardiac hypertrophic response in an *in vivo*, closed-chest model of cardiac ischemia-reperfusion injury.

FUNDING

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