Role of cardiac myocyte CXCR4 expression in development and left ventricular remodeling after acute myocardial infarction

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Materials and Methods:

Immunostaining: Mixed cellular population (containing cardiac myocytes, endothelial cells and fibroblast cells) of Neonatal heart was isolated from the pups (1-3 days old) of MLC2vCRE+/-CXCR4floxflox parents with Neomytis Kit (Cellutron Life Technology Cat # nc-6031) and plated on the 35mm plate. The cells were cultured for 24 hours and then fixed in 2% paraformaldehyde, blocked with 1% BSA, 1% donkey serum solution and then stained for CXCR4 (primary antibody 1:100 from abcam Catalog#7199 overnight at 4°C, secondary 1:1000- anti rabbit 594 from abcam for 1 hour at room temperature).

Immunostaining in tissue was performed on the hearts harvested 48 hours post MI and 21 days post MI from MCM+/-CXCR4floxflox mice with and without tamoxifen treatment. The hearts were fixed in formalin and embedded in paraffin blocks. 6μ sections were made and stained as described1. Forty eight hours post AMI tissue were stained for CXCR4 (Primary - NB100-74396 (Novus Biologicals), secondary- donkey anti rabbit IgG Alexa Fluor 488 from molecular probes) and 21 days post AMI tissue was stained to calculate vessel density with isolectin (Cat # FL-1201 from Vectorlabs) and wheat germ agglutin to quantify cardiac myocyte density (Cat #RL-1022 from Vectorlabs).

Western Blotting - Western blotting was done on the 48 hours post infarcted tissue homogenate of CXCR4floxflox and MCM+/-CXCR4floxflox mice (with tamoxifen administration) as described1. Briefly, the infarcted tissue was homogenized in PBS with 0.1% Triton X-100 supplemented with PMSF (100 mM), leupeptin (10 μg/ml) and aprotinin (10 μg/ml). Total protein (50 μg) from each sample was prepared in 4x lammli buffer (200 mM Tris HCl (pH 6.8), 8% SDS, 0.1% bromophenol blue, 40% glycerol), subjected to 10% SDS PAGE and transferred on PVDF membrane. The blot was finally probed with primary antibody (1:500 in 5% milk in 1xTBST) against CXCR4 (Abcam, cat# 2074) followed by incubation with peroxidase-conjugated anti-mouse secondary antibody (1:4000 in 5% milk in 1xTBST). Chemiluminescence (Amersham Biosciences) was used to visualize the bands. Microtek scanner was used to scan the blots and and the density of the bands was analysed using the NIH software, Image J.

Cardiac remodeling – Mice were anesthetized and their hearts were perfusion fixed with 10% phosphate-buffered formalin under normal pressure 21 days after LAD ligation. The hearts were embedded in paraffin and 5μm sections were cut from the apex to the level just below ligation and alternating sections were stained with Masson trichrome as described2. 5X images were taken to measure infarct size. Interstitial fibrosis was measured using the viable posterior wall of non infarcted ventricle. Cardiac myocyte diameter was measured along the shortest diameter across the nucleus. 60 such readings were taken. All parameters were measured using Image-Pro Plus (Media Cybernetics). Formula used for calculating infarct size - %infarct size = (epicardial infarct length/ total epicardial circumference) X 100.

Echocardiography: Baseline 2D-echocardiography was performed on MLC2v-Cre+/-CXCR4floxflox, MCM+/-CXCR4floxflox and CXCR4 floxflox mice at 6 weeks of age using 15MHz linear array transducer interfaced with a Sequoia C256 and GE vision 7 as described previously3. Fourteen days post tamoxifen administration echocardiography was performed on MCM+/-CXCR4floxflox mice to determine the baseline function. Doppler and myocardial straining analysis was done on MLC2v-Cre+/-CXCR4floxflox mice at 6 weeks of age. Echocardiography was also performed 3 and 21 days post AMI. EF was calculated as the (LVEDarea-LVESarea)/LVEDarea x 100 where the LVESarea and the LVEDarea are the the end systolic and end diastolic areas of the left ventricle obtained in the parasternal long axis view.40, 41
**Left Anterior Descending (LAD) artery ligation:** LAD ligation was performed on 8-10 weeks old MLC2vCRE<sup>+/−</sup>CXCR4<sup>flox/flox</sup>, MCM<sup>+/−</sup>CXCR4<sup>flox/flox</sup> (post tamoxifen injection) and CXCR4<sup>flox/flox</sup> mice as previously described. Briefly, the animals were anesthetized with Xylazine/ketamine, intubated and ventilated with room air at 105 breaths per minute using a rodent ventilator (Harvard Apparatus). Sternotomy was performed and LAD was identified with the help of surgical microscope (Leica M500). LAD was ligated by using 7-0 prolene. Immediate blanching and anterior wall dysfunction revealed a successful ligation. The chest and skin were closed using 6-0 prolene. The animals were removed from the ventilator and kept under oxygen until they recover from anesthesia. Only the animals which survived first 24 hours of ligation were considered for the study.

**References**