Hypoxic Preconditioning Enhances the Benefit of Cardiac Progenitor-Cell Therapy for Treatment of Myocardial Infarction by Inducing CXCR4 Expression

Yao Liang Tang, MD, PhD[*], Wuqiang Zhu, MD, PhD[†], Min Cheng, MD[†], Lijuan Chen, MD[*], John Zhang, MS[*], Tao Sun, MD[*], Raj Kishore, PhD[†], M. Ian Phillips, PhD Ds.c[*], Douglas W. Losordo, MD[†], Gangjian Qin, MD[†‡]

[*]Stem Cell Biology, Keck Graduate Institute, Claremont, CA, 91711, USA
[†]Feinberg Cardiovascular Research Institute, Northwestern University, Chicago, IL, 60611, USA

Supplemental Material

Supplemental Figures

Normoxia	Hypoxia
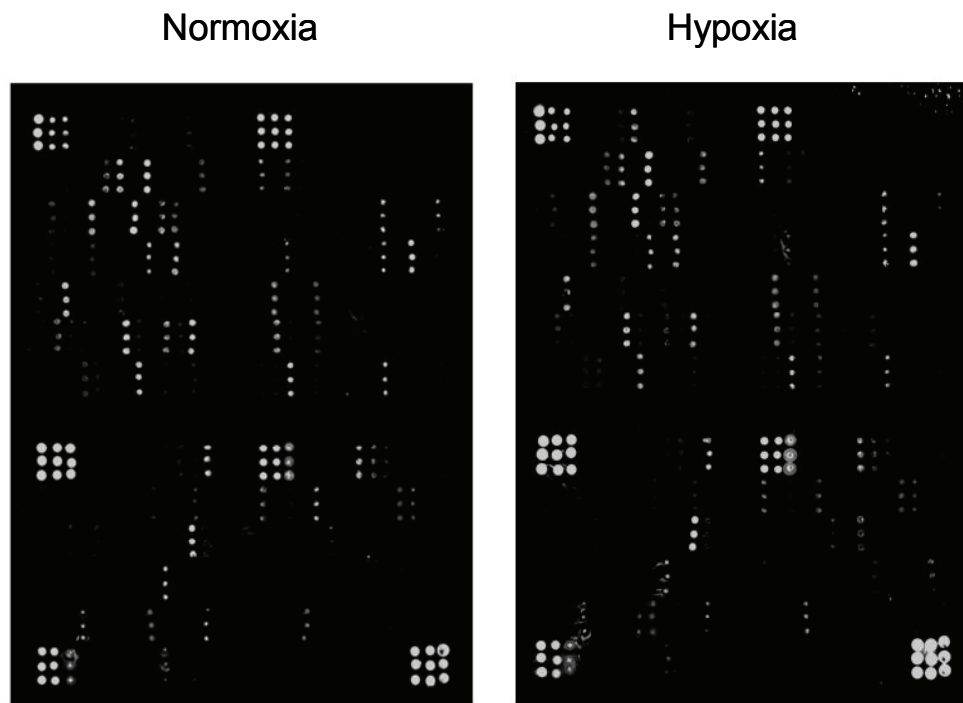
Online Figure I: Protein-array assessments of paracrine-factor secretion. CLK cells (passages 7-8) were cultured to 90% confluency in standard media, then transferred to a standard incubator or hypoxia chamber (0.1% O_2) and cultured in DMEM containing 0.2% FBS. Twelve hours later, the media were collected, concentrated by using the Millipore

Amicon Centricon YM-3 system (Bedford, MA), and protein array assays (L Series 308, Ray Biotech, Inc) were performed as directed by manufacturer's instructions. After developing, the membranes were scanned, and the images were processed with the analysis-tool software (Ray Biotech, Inc). The mean signal intensity for each cytokine was obtained from triplicate measurements in each chip and normalized to the internal controls, then the ratio of cytokine levels in hypoxia-preconditioned and normoxia-cultured cells was calculated. Representative images of arrays performed with the media from normoxia-cultured (left) and hypoxia-preconditioned (right) cells are displayed here, and the paracrine factors analyzed are provided in Online Table I.
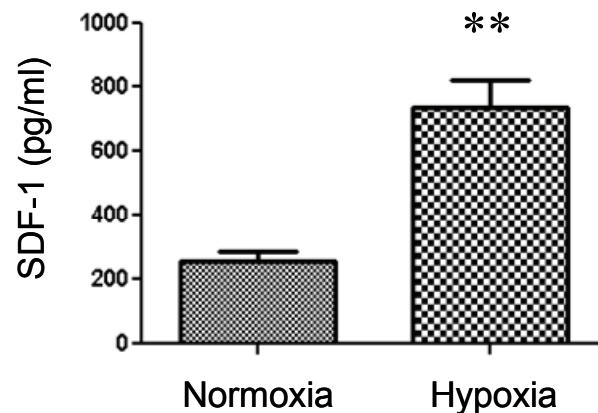
Online Figure II: Hypoxic preconditioning increases SDF-1 secretion from CLK cells. CLK cells (passages 7-8) were cultured to 90% confluency in standard media, then transferred to a standard incubator or hypoxia chamber (0.1% O_2) and cultured in DMEM containing 0.2% FBS. Six hours later, the media were collected, and SDF-1 concentrations were measured with high-sensitivity mouse CXCL12/SDF-1 ELISA kits (Quantikine, R&D Systems, Minneapolis, MN) as directed by the manufacturer's instructions. Values are presented as mean±SEM of 4 experiments (**p=0.0015, Student's t-test).

Supplemental Table

Online Table I: Paracrine-factor secretion from hypoxia-preconditioned and normoxia-cultured CLK cells.

	Hypoxia-CLK	Normoxia-CLK	Ratio
Activin A	3,990.00	1,000.68	**3.99**
TIMP-1	1,535.33	396.96	**3.87**
TNF RI / TNFRSF1A	1,126.33	370.22	**3.04**
Progranulin	1,944.33	671.41	**2.90**
TGF-beta RII	1,006.00	366.16	**2.75**
VEGF	687.00	299.15	**2.30**
PF-4	1,010.67	509.31	**1.98**
TCA-3	575.67	318.78	**1.81**
TMEFF1 / Tomoregulin-1	649.33	376.99	**1.72**
6Ckine	1,146.67	672.76	**1.70**
TGF-beta RI / ALK-5	542.33	324.54	**1.67**
Lefty-1	614.33	379.02	**1.62**
PDGF R beta	519.33	322.84	**1.61**
VCAM-1	516.00	327.58	**1.58**
Epigen	2,200.00	1,425.38	**1.54**
Osteopontin	17,689.00	11,466.36	**1.54**
WISP-1 / CCN4	539.67	351.61	**1.53**
IL-3 R beta	1,093.00	716.08	**1.53**
RAGE	468.67	308.63	**1.52**
Frizzled-7	506.67	335.03	**1.51**
AgRP	1,001.00	662.61	**1.51**
OX40 Ligand / TNFSF4	530.33	352.62	**1.50**
TGF-beta 2	639.67	428.43	**1.49**
P-Selectin	499.33	336.04	**1.49**
Resistin	491.67	332.66	**1.48**
PDGF R alpha	504.67	342.81	**1.47**
FGF-21	505.00	345.86	**1.46**
TECK	539.33	369.54	**1.46**
PIGF-2	564.00	387.48	**1.46**
CXCR4	589.00	405.42	**1.45**
DAN	510.67	352.29	**1.45**
RANTES	507.00	349.92	**1.45**
TNF-alpha	563.67	390.19	**1.44**
TFPI	514.67	357.02	**1.44**
RELM beta	458.67	319.12	**1.44**
TRAIL R2 / TNFRSF10B	477.67	334.35	**1.43**
Pentraxin3 / TSG-14	518.67	363.45	**1.43**
b FGF	459.00	321.83	**1.43**
IL-17BR	995.67	698.14	**1.43**
Granzyme G	489.67	346.87	**1.41**
Chordin-Like 2	483.33	343.15	**1.41**
TNF-beta / TNFSF1B	547.33	390.53	**1.40**

HGF R	634.67	453.13	**1.40**
CXCR6	540.33	386.13	**1.40**
TRANCE / TNFSF11	475.33	340.10	**1.40**
EDAR	476.00	341.12	**1.40**
WIF-1	510.00	365.82	**1.39**
TWEAK / TNFSF12	467.00	335.03	**1.39**
VE-Cadherin	438.67	315.74	**1.39**
IL-3	690.33	497.12	**1.39**
IL-2 R beta	662.33	477.50	**1.39**
VEGF R1	554.00	401.02	**1.38**
TL1A / TNFSF15	523.00	378.68	**1.38**
VEGF R2	536.00	388.16	**1.38**
Csk	497.33	362.44	**1.37**
Soggy-1	533.67	389.85	**1.37**
AR (Amphiregulin)	503.67	370.56	**1.36**
TLR1	497.67	366.16	**1.36**
Kremen-1	483.33	356.01	**1.36**
Leptin R	546.67	402.71	**1.36**
IL-24	520.33	383.42	**1.36**
Dkk-3	502.67	371.57	**1.35**
TARC	463.67	343.15	**1.35**
MFG-E8	694.33	515.40	**1.35**
TWEAK R / TNFRSF12	430.67	319.80	**1.35**
Cerberus 1	484.33	359.73	**1.35**
Dkk-4	597.67	444.67	**1.34**
PDGF C	461.00	343.49	**1.34**
DPPIV / CD26	485.00	361.42	**1.34**
IL-1 R9	494.00	368.53	**1.34**
Frizzled-1	480.33	358.38	**1.34**
IL-1 RII	1,105.33	826.06	**1.34**
Tie-2	478.33	357.70	**1.34**
Activin RIB / ALK-4	692.33	518.11	**1.34**
DKK-1	572.67	429.10	**1.33**
HVEM / TNFRSF14	600.00	449.75	**1.33**
TSLP R	469.67	352.96	**1.33**
Prolactin	524.33	394.25	**1.33**
EGF R	518.67	390.19	**1.33**
Fractalkine	568.67	428.43	**1.33**
TCCR / WSX-1	509.00	384.77	**1.32**
Angiopoietin-like 3	581.00	441.29	**1.32**
FGF R3	467.33	355.33	**1.32**
MAdCAM-1	506.33	385.11	**1.31**
IL-10 R alpha	447.67	340.78	**1.31**
MMP-3	497.00	378.68	**1.31**
TIMP-4	506.00	386.46	**1.31**
IL-3 R alpha	496.33	379.36	**1.31**
CD40 Ligand / TNFSF5	511.00	390.86	**1.31**
BAFF R / TNFRSF13C	530.67	406.09	**1.31**
uPAR	521.33	399.32	**1.31**
Kremen-2	532.33	408.12	**1.30**
TIMP-2	505.67	387.82	**1.30**
Coagulation Factor III / Tissue Factor	616.67	474.45	**1.30**

S100A10	438.00	337.06	**1.30**
IL-6 R	494.67	381.05	**1.30**
Integrin beta 2 / CD18	438.67	338.41	**1.30**
Erythropoietin (EPO)	523.00	404.06	**1.29**
IFN-alpha / beta R2	480.33	371.91	**1.29**
Growth Hormone R	498.67	386.13	**1.29**
IL-20 R alpha	527.33	408.46	**1.29**
TACI / TNFRSF13B	477.67	370.22	**1.29**
Urokinase	454.33	352.29	**1.29**
IL-18 R alpha/IL-1 R5	452.33	351.27	**1.29**
IL-13 R alpha 2	546.67	425.04	**1.29**
IL-4	460.33	358.04	**1.29**
IL-12 R beta 1	508.00	395.60	**1.28**
Artemin	562.67	438.24	**1.28**
Lymphotactin	526.33	410.15	**1.28**
CD40	578.67	451.10	**1.28**
MIP-3 alpha	579.00	451.44	**1.28**
CCR7	448.33	350.59	**1.28**
IL-31 RA	519.00	406.43	**1.28**
TGF-beta 3	427.00	335.03	**1.27**
CD14	494.33	388.16	**1.27**
FLRG (Follistatin)	468.00	367.51	**1.27**
IL-17E	637.00	500.51	**1.27**
CD27 Ligand / TNFSF7	512.67	403.72	**1.27**
B7-1/CD80	467.33	368.19	**1.27**
SIGIRR	470.00	370.56	**1.27**
G-CSF	542.33	428.43	**1.27**
MMP-2	509.00	402.71	**1.26**
CCL7 / MCP-3 / MARC	21,480.00	17,009.86	**1.26**
Axl	605.00	479.19	**1.26**
IL-21 R	471.33	373.94	**1.26**
TSLP	522.00	414.55	**1.26**
Fit-3 Ligand	666.67	529.61	**1.26**
Glut2	928.33	737.73	**1.26**
GM-CSF	626.67	499.49	**1.25**
IFN-beta	462.00	369.54	**1.25**
SCF	529.67	423.69	**1.25**
IL-7 R alpha	468.00	374.62	**1.25**
TGF-beta 1	617.00	494.08	**1.25**
IL-22	808.67	647.72	**1.25**
IL-15	768.67	615.91	**1.25**
Osteoactivin / GPNMB	589.67	472.76	**1.25**
ALCAM	502.67	403.05	**1.25**
TREM-1	454.00	364.13	**1.25**
TLR4	553.67	444.67	**1.25**
FGF R4	965.67	776.65	**1.24**
GDF-5	542.67	437.23	**1.24**
Neuregulin-3 / NRG3	460.67	371.24	**1.24**
MCP-5	549.00	442.64	**1.24**
IGFBP-6	562.67	454.15	**1.24**
IL-17C	492.67	397.97	**1.24**
DR3 / TNFRSF25	756.00	612.18	**1.23**

ICAM-2 / CD102	718.67	582.40	**1.23**
IL-20	773.00	627.41	**1.23**
ICAM-5	459.67	373.27	**1.23**
CCR10	582.67	475.80	**1.22**
MMP-24 / MT5-MMP	424.67	346.87	**1.22**
CXCR2 / IL-8 RB	584.00	477.50	**1.22**
EG-VEGF / PK1	456.00	372.93	**1.22**
ICAM-1	557.33	455.84	**1.22**
Decorin	516.67	423.01	**1.22**
Eotaxin-2	552.00	454.15	**1.22**
SCF R / c-kit	461.67	380.71	**1.21**
LIF	577.33	476.14	**1.21**
IL-17RD	556.33	458.88	**1.21**
KC	592.33	489.34	**1.21**
MMP-12	452.33	374.28	**1.21**
IGFBP-5	579.00	479.19	**1.21**
VEGFC	430.00	356.01	**1.21**
BTC (Betacellulin)	444.67	369.21	**1.20**
CD30 L	619.67	515.40	**1.20**
IL-21	518.00	431.47	**1.20**
Fas Ligand	626.00	521.49	**1.20**
Adiponectin / Acrp30	623.00	519.80	**1.20**
CTLA-4 / CD152	804.67	675.81	**1.19**
FAM3B	599.67	503.89	**1.19**
Insulin	618.33	520.48	**1.19**
Spinesin Ectodomain	454.67	383.42	**1.19**
CCL8 / MCP-2	555.33	468.36	**1.19**
GITR Ligand / TNFSF18	597.33	503.89	**1.19**
TLR3	484.67	409.14	**1.18**
IGF-II	542.67	458.55	**1.18**
Activin C	616.67	521.15	**1.18**
Cripto	595.33	503.22	**1.18**
IL-2	523.33	442.98	**1.18**
ICK	488.00	414.21	**1.18**
IL-9 R	1,235.33	1,048.73	**1.18**
IL-12 p40/p70	580.33	492.73	**1.18**
Thrombospondin	531.67	451.44	**1.18**
Ubiqultin	636.33	540.44	**1.18**
GFR alpha-3 / GDNF R alpha-3	577.67	491.03	**1.18**
Endoglin / CD105	579.00	492.73	**1.18**
Frizzled-6	2,089.00	1,778.35	**1.17**
CD30	525.33	447.72	**1.17**
Epiregulin	590.67	503.55	**1.17**
Cardiotrophin-1	563.00	480.20	**1.17**
MDC	558.67	478.17	**1.17**
Neurturin	482.33	413.20	**1.17**
MIG	479.00	410.83	**1.17**
VEGF R3	496.67	426.40	**1.16**
TPO	565.00	485.28	**1.16**
CD27 / TNFRSF7	500.33	430.12	**1.16**
LEPTIN(OB)	521.33	449.07	**1.16**
LIGHT / TNFSF14	481.00	415.23	**1.16**

IL-17D	525.67	454.49	**1.16**
NOV / CCN3	483.33	417.94	**1.16**
CXCR3	665.67	576.65	**1.15**
IL-15 R alpha	746.00	650.42	**1.15**
SDF-1	461.00	403.05	**1.14**
IFN-gamma	514.67	450.09	**1.14**
GFR alpha-2 / GDNF R alpha-2	573.00	503.22	**1.14**
Cryptic	547.33	481.90	**1.14**
NGF R / TNFRSF16	547.33	481.90	**1.14**
IL-10	660.33	585.79	**1.13**
I-TAC	654.67	581.73	**1.13**
IL-13	915.67	817.26	**1.12**
Shh-N	539.67	483.93	**1.12**
L-Selectin	645.00	580.37	**1.11**
IL-17F	455.00	412.18	**1.10**
IL-17RC	587.67	533.67	**1.10**
Thymus Chemokine-1	734.00	669.71	**1.10**
beta-Catenin	532.33	485.96	**1.10**
Galectin-3	8,177.67	7,519.14	**1.09**
MMP-9	606.00	560.41	**1.08**
MIP-1 alpha	591.67	548.90	**1.08**
Follistatin-like 1	557.67	517.77	**1.08**
FCrRIIB / CD32b	752.00	700.17	**1.07**
CRP	581.00	542.81	**1.07**
E-Selectin	770.00	719.46	**1.07**
Angiopoietin-like 2	568.67	531.98	**1.07**
Serum Amyloid A1	503.00	470.73	**1.07**
MFRP	528.33	494.76	**1.07**
IL-1 R6 / IL-1 R rp2	778.00	736.38	**1.06**
IL-23	795.33	754.32	**1.05**
GITR	513.67	487.65	**1.05**
Dtk	2,533.00	2,413.54	**1.05**
IL-31	910.67	868.36	**1.05**
IL-17	557.00	532.66	**1.05**
IL-5	578.33	553.30	**1.05**
BCMA / TNFRSF17	694.33	664.98	**1.04**
Osteoporotegerin	994.00	959.39	**1.04**
LRP-6	534.00	516.75	**1.03**
MIP-3 beta	563.67	546.87	**1.03**
Gremlin	3,213.00	3,170.91	**1.01**
GDF-1	628.00	619.97	**1.01**
VEGF-D	474.33	468.36	**1.01**
CXCL16	2,215.33	2,188.16	**1.01**
CCR6	1,940.00	1,922.51	**1.01**
IGFBP-2	821.00	814.55	**1.01**
TROY	2,072.00	2,058.89	**1.01**
IL-22BP	1,177.00	1,170.22	**1.01**
MCP-1	837.67	839.93	**1.00**
IFN-alpha / beta R1	622.33	624.03	**1.00**
Internal control	48,289.33	48,825.85	**0.99**
CTACK	15,464.00	15,800.38	**0.98**
IL-5 R alpha	553.33	567.18	**0.98**

FADD	2,786.67	2,870.74	**0.97**
IFN-gamma R1	573.67	593.23	**0.97**
CCL4 / MIP-1 beta	551.67	571.58	**0.97**
IL-7	575.33	596.62	**0.96**
GFR alpha-4 / GDNF R alpha-4	1,157.33	1,200.68	**0.96**
Granzyme D	618.67	648.73	**0.95**
GDF-9	595.00	624.03	**0.95**
MIP-1 gamma	1,904.00	2,031.48	**0.94**
Lungkine	950.33	1,017.94	**0.93**
MIP-2	1,080.33	1,170.90	**0.92**
CD11b	717.33	779.02	**0.92**
Eotaxin	644.67	701.52	**0.92**
IL-6	577.67	642.98	**0.90**
CCL1 / I-309 / TCA-3	2,201.33	2,450.43	**0.90**
CCR3	2,201.33	2,450.43	**0.90**
IL-2 R alpha	1,332.67	1,485.62	**0.90**
TRAIL / TNFSF10	1,028.33	1,147.89	**0.90**
LIX	847.00	948.56	**0.89**
Lymphotoxin beta R / TNFRSF3	1,811.33	2,034.18	**0.89**
MMP-14 / LEM-2	762.00	856.18	**0.89**
IL-1 RI	3,182.33	3,602.72	**0.88**
IGF-I	955.33	1,085.62	**0.88**
SLPI	558.33	639.26	**0.87**
HGF	2,070.33	2,379.02	**0.87**
Fas / TNFRSF6	3,466.00	4,004.75	**0.87**
IL-4 R	618.00	719.12	**0.86**
IGFBP-3	634.00	738.07	**0.86**
IL-28 / IFN-lambda	1,494.33	1,803.39	**0.83**
TLR2	1,181.33	1,427.75	**0.83**
BLC	641.67	784.44	**0.82**
IL-1 beta	848.67	1,047.72	**0.81**
TNF RII	1,809.33	2,247.38	**0.81**
IL-27	3,409.00	4,244.34	**0.80**
Granzyme B	746.67	930.97	**0.80**
Crossveinless-2	3,666.67	4,649.76	**0.79**
Common gamma Chain / IL-2 R gamma	1,253.67	1,591.88	**0.79**
CRG-2	634.33	822.34	**0.77**
Endocan	790.67	1,030.46	**0.77**
FGF R5 beta	978.67	1,299.83	**0.75**
GDF-3	712.33	952.29	**0.75**
SPARC	1,836.00	2,471.75	**0.74**
IL-23 R	3,980.33	5,377.68	**0.74**
IL-12 p70	629.67	853.81	**0.74**
M-CSF	1,779.33	2,439.60	**0.73**
IL-1 R4 / ST2	3,874.67	5,383.43	**0.72**
Endostatin	543.33	795.60	**0.68**
IGFBP-rp1 / IGFBP-7	5,945.33	8,725.57	**0.68**
VEGF-B	948.67	1,401.70	**0.68**
IL-17R	1,771.67	2,625.73	**0.67**
IL-16	3,540.00	5,278.19	**0.67**
IL-9	1,148.33	1,738.08	**0.66**
CXCL14 / BRAK	1,897.33	2,889.69	**0.66**

CCL28	3,373.33	5,188.51	**0.65**
CCR4	3,373.33	5,188.51	**0.65**
IL-11	3,446.67	5,352.98	**0.64**
CCR9	1,232.33	2,064.30	**0.60**
IGFBP-1	1,364.33	2,436.89	**0.56**
GDF-8	3,463.67	6,307.97	**0.55**
IL-1 alpha	1,467.33	3,314.05	**0.44**

Supplemental methods

CLK-cell gene expression

Flow cytometry

Flow cytometry analyses of cultured CLK cells were performed with a BD LSRII flow cytometer and BD FACSDivaTM software as previously described.[1] Briefly, CLK cells were blocked with 5% rat serum and stained with a panel of conjugated antibodies, including anti-c-kit-APC, anti-Sca-1-PE, anti-CD34-PE, anti-CD31-biotin, anti-CD45-Per-CP, anti-CXCR4-PE (BD Biosciences, San Jose, CA), and anti-Flk-1-biotin (Novus Biologicals, Inc., Littleton, CO), or isotype-matched control antibodies. Anti-CD31-biotin and anti-Flk-1-biotin antibodies were resolved via secondary staining with streptavidin-APC-Cy7 and streptavidin-FITC, respectively.

Immunocytochemistry

Immunofluorescent analyses on cultured slides and tissue sections were performed as previously described.[1] CLK cells were cultured on slides and fixed with cold 4% paraformaldehyde, then stained with primary antibodies specific for GATA4, Nkx2.5, HIF-1α (Santa Cruz Biotechnology, Inc.), mouse CD184 (CXCR4) (BD Biosciences), or with isotype-matched IgG control antibodies. Pertinent secondary antibodies were conjugated with Alexa Fluor 488 or Alexa Fluor 555. Nuclei were counterstained with DAPI (Vector Laboratories, Burlingame, CA). Sections were examined under a Leica TCS confocal microscope (Leica Microsystems Inc., Bannockburn, IL).

Quantitative real-time RT-PCR

Real time RT-PCR were performed by following standard techniques.[2] Briefly, total RNA was extracted using the RNA Stat-60 reagent (Tel-Test, Friendswood, TX). RNA was reverse transcribed with the Taqman Multiscribe RT kit (Applied Biosystems). Real-time PCR was performed in duplicate with cDNA from 10 ng of RNA using the Lightcycler hybridization Probes Master Mix (Roche). For each probe set, a negative control without a template was included. Relative gene expression was calculated using the Ct method with normalization to

GAPDH. The primer sequences for mouse CXCR4 were 5'-CCTCGCCTTCTTCCACTGTT-3' (forward) and 5'-CTGGGCAGAGCTTTTGAACTTG-3' (reverse).

Western blotting

Western blotting was performed with 30 μg of whole-cell lysate by following standard techniques.[2] HIF-1α and CXCR4 proteins were probed with rabbit polyclonal anti-HIF-1α (Santa Cruz Biotechnology, Inc.) and biotinylated anti-mouse CD184 (CXCR4) (BD Biosciences) antibodies. GAPDH protein was probed with an HRP-conjugated antibody (Abcam Inc., Cambridge, MA) as an internal control. The antigen-antibody complexes were visualized with enhanced chemiluminescence (Amersham plc, Buckinghamshire, UK). Band intensities were determined densitometrically with Image J software, and the relative levels of HIF-1α and CXCR4 were normalized to GAPDH levels.

CLK-cell migration

CLK-cell migration was measured with QCM™ Chemotaxis 96-Well Cell Migration Assay (Millipore, Billerica, MA), consisting of two chambers separated by a ChemoTx filter (8-μm pore size). CLK cells were gently detached from the culture dish with harvesting buffer and adjusted to a concentration of 4×10^5 cells/mL in DMEM medium. CLK cells were then seeded into the upper chamber (4×10^4 cells/well) and the lower chamber was filled with recombinant human SDF-1 (125 ng/mL) (R&D Systems Inc., Minneapolis, MN). The chambers were incubated at 37°C for 4 hours under hypoxic or normoxic conditions, then the CLK cells that had migrated to the lower surface of the filter were detached with cell detachment buffer, lysed, and stained with CyQuant GR dye (Chemicon International, Inc., Temecula, CA), which fluoresces strongly when bound to cellular nucleic acids. Fluorescence was measured with SpectraMax Gemini EM Dual-Scanning Microplate Spectrofluorometer with a 480/520-nm filter set (Molecular Devices, Sunnyvale, CA).

Echocardiography

Echocardiographic measurements were performed 1, 2, and 4 weeks after MI with a Vevo 770TM high-resolution ultrasound biomicroscope (VisualSonics Inc., Toronto, Ontario, Canada) and Vevo analysis software (Vevo 2.2.3, [VisualSonics Inc.]) as described

previously.[3] Mice were lightly anesthetized with inhaled 2% Isofluorane, and heart rates were maintained at 400 to 500 beats per minute. M-Mode short axis images were recorded at the level of the mid-papillary muscle, and B-Mode long axis images were recorded in a plane containing the aortic and mitral valves. Left-ventricular internal diameter during systole (LVIDS) and diastole (LVIDD), and left-ventricular end systolic volume (LVESV) and end diastolic volume (LVEDV) were measured and used to calculate fractional shortening (FS = [LVIDD-LVISD] / LVIDD] and ejection fraction (EF = [EDV-ESV] / EDV).

Histological assessments

Twenty-eight days after surgically induced MI and intravenous injection of CLK cells, the vasculature was labeled by injecting 50 µL Lectin I (Vector laboratories) into the tail vein, and mice were euthanized 10 min later. The hearts were harvested, and body weights and heart weights were measured, then cardiac tissues were fixed in 4% paraformaldehyde for 4 hours, incubated overnight in 30% sucrose, embedded in OCT compound (Sakura Finetek U.S.A., Inc., Torrance, CA), snap-frozen in liquid nitrogen, and cut into 5-µm sections. Serial cryosections were cut beginning 1 mm below the suture used to ligate the LAD and proceeding to the apex, with three consecutive sections cut per mm to allow for quantitative pathohistological analysis at each level. To evaluate infarct area, Masson Trichrome elastic tissue staining was performed as described previously.[4] The infarct wall thickness and infarct size was analyzed using a computerized digital image analysis system (Image Pro, version 4.5; [MediaCybernetics, Inc., Bethesda, MD]). Infarct wall thickness was measured at three points (the two ends and the middle of the infarct area) and reported as the mean value. Infarct size was reported as the ratio of the length of fibrotic area to the length of the left-ventricular inner circumference.[4] Vascular density was evaluated via immunofluorescent staining with an anti-Lectin I antibody (Vector Laboratories)[4]; 3 sections per ischemic heart and 6 fields per section were examined, and capillaries were recognized as tubular structures positive for Lectin I. All surgical procedures and pathohistological analyses were performed by investigators blinded to treatment assignment.

Online Table II. Oligonucleotides and Primers Used to Create Backbone Plasmids and siRNA

Mouse CXCR4 shRNA	sense: 5'–CCGGCCAAAGCCTGAAATGGTGGCTCGAGCCACCATTTCAGGCTTTGGTTTTTG–3' antisense: 5'–AATTCAAAAACCAAAGCCTGAAATGGTGGCTCGAGCCACCATTTCAGGCTTTGG–3'
human Nkx2.5 genomic DNA	Forward primer: 5'-CTCGAGAAGCTTGATACTCCCGGATGTAGCCTCGATGT-3' Reverse primer: 5'-CGACGTGCGCTCCGGACAGAGGAGGGGGACTCAGCCT-3'
mouse HIF-1α siRNA	sense: 5'–GGAUACAAGCUGCCUUUUUtt–3' antisense: 5'–AAAAAGGCAGCUUGUAUCCtc–3'

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