A unique network involving CXCR4 and CXCR7 coordinates cardiac lineage specification and mobilization of induced pluripotent stem cells

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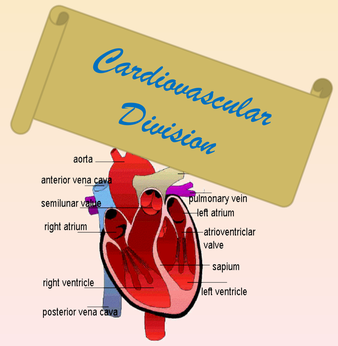

Abstract

Introduction: An adult heart has an intrinsically limited capability to regenerate damaged myocardium. Human embryonic and induced pluripotent stem cell (hESC/hiPSC)-based therapies offer a unique strategy for developing cell replacement treatments for numerous disorders including cardiac diseases.

Hypothesis: The present study identifies a unique signaling network, SDF-1/CXCR4/CXCR7, that regulates cardiac lineage differentiation and migration in human induced pluripotent stem cells (hiPSCs). The fact that SDF-1 binds to CXCR4 and CXCR7 raises a concern how to distinguish the potential contribution of the SDF-1/CXCR7 pathway from SDF-1/CXCR4 pathway in all the processes that were previously attributed to SDF-1/CXCR4. Therefore, we set these studies to disseminate the role of the SDF-1/CXCR4/CXCR7 network in cardiogenic lineage differentiation and migration of hiPSCs with the premise that their improved recruitment could translate into therapeutic benefits.

Methods: Using lentiviral vectors to ablate CXCR4 and/or CXCR7 expression, hiPSC-derived cardiomyocytes (hiPSC-CMs) were tested for phenotypic and functional properties due to gene knockdown.

Results: Gene expression confirmed cardiomyocyte phenotype of differentiated hiPSCs, although reduction of CXCR4 and CXCR7 expression resulted in delayed cardiac phenotype. Only knockdown of CXCR4 reduced the spontaneous beating of hiPSC-CMs. Knockdown of CXCR4 and CXCR7 differentially altered calcium transients and β-adrenergic response in hiPSC-CMs. In engineered cardiac tissues, depletion of CXCR4 or CXCR7 had opposing effects on developed force. The transendothelial migration response to SDF-1 was suppressed by knockdown of either CXCR4 or CXCR7. In contrast, in a trans-well chemotaxis assay, only depletion of CXCR4 reduced hiPSC migration in response to SDF-1.

Conclusions: Both CXCR4 and CXCR7 have distinct roles in the SDF-1/CXCR4/CXCR7 axis as network coordinators of cardiogenic induction and mobilization of hiPSCs. We contend that gaining further insight into the molecular nuances of this phenomenon will provide new insights for optimization of the cardiac repair potential of cell-based therapies.

Hypothesis

We set these studies to disseminate the role of the SDF-1/CXCR4/CXCR7 network in cardiogenic lineage differentiation and migration of hiPSCs with the premise that their improved recruitment could translate into therapeutic benefits. Moreover, it will provide new insights into ventricular cardiomyocyte specification, maturation, and function.

Results

Figure 1A-B. Verification of lentivirus shRNAs against CXCR4 (shCXCR4) (A) and CXCR7 (shCXCR7) (B) in in hiPSC-CMs. Gene expression in hiPSC-CMs is shown at 0, 1, 3, 4, 5, 10, 15 and 20 days after the start of differentiation. A shScramble lentivirus was used as a control. Gene expression normalized to β2-microglobulin (β2M). Experiments were performed in triplicate and statistical analysis done by one-way ANOVA followed by the Bonferroni test *P < 0.05, **P < 0.01, #P < 0.001).

Figure 1C-D. Gene expression was determined by qPCR using primers specific for *OCT4* ©, and *TNNT2* (D). OCT4 is a marker of pluripotency, and TNNT2 is a late cardiac marker; all values were normalized to β2-microglobulin (β2M). Experiments were performed in triplicate and statistical analysis was done by one-way ANOVA followed by the Bonferroni test (*P < 0.05, **P < 0.01, #P < 0.001).

Figure 1E-H. Calcium transients of hiPSC-CMs were measured using the calcium-sensitive fluorophore Fura-2 at 20 days after differentiation (40V, paced at 0.5Hz). Transients were measured either at basal levels or in the presence of 500 nM isoproterenol, a β-agonist (E). Values for baseline (F), amplitude (G), and tau (H) were obtained. Experiments were performed at least in triplicate and statistical analysis was done by two-way ANOVA followed by the Bonferroni post-test (*P < 0.05, **P < 0.01, #P < 0.001, and ns is not significant).

Figure 1I-K. Developed force (mN) was measured in ECTs and normalized to control values (I). Relative beating rate (bpm) was measured following addition of 500 μM isoproterenol and normalized to shScramble values (J). Protein levels of SERCA2a were similar in all conditions in hiPSC-CMs following normalization to GAPDH (K). Experiments were performed in triplicate and statistical analysis done by one-way ANOVA followed by the Bonferroni test *P < 0.05, **P < 0.01, #P < 0.001).

Figure 1L. Transwell migration and Boyden chamber assay of hiPSCs. 8 μm pore size inserts were coated with human umbilical vessel endothelial cells (HUVECs) and upon 100% confluence, 3 x 10^5 infected and/or uninflected hiPSCs (with or without RGDS, integrin receptor inhibitor at 0.2 mM) were added to the inserts and trans-endothelial migration was assessed over 12 h in response to SDF-1 (10 ng/ml). Transmigration was performed similarly in the absence of HUVECs. Each transmigration condition was performed in triplicate. ShScramble-infected hiPSCs was used as a control. Baseline transmigration (no SDF-1) was similar for all groups. Experiments were performed at least in triplicate and statistical analysis was done by two-way ANOVA followed by the Bonferroni post-test (*P < 0.05, **P < 0.01, #P < 0.001).

Conclusion

Collectively, these results indicate that both CXCR4 and CXCR7 are necessary for the transendothelial migration of hiPSCs, but they might act *by* separate mechanisms.

Results