Induced Pluripotent Stem Cell Transplantation in the Treatment of Porcine Chronic Myocardial Ischemia

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Background. **This study was designed to test the effects of induced pluripotent stem cell (iPSC) in the treatment of chronic myocardial ischemia.**

Methods. **The reprogramming of passage 3 myocardial fibroblasts was performed by using the lentiviral vector containing 4 human factors: OCT4, SOX2, KLF4, and c-MYC. The iPSC colonies at P12-17 were allogeneically transplanted into ischemic myocardium of 10 swine by direct injection. Cohorts of 2 animals were sacrificed at 2, 4, 6, 8, and 12 weeks after injection.**

Results. **No signs of graft versus host disease were evident at any time points. At 2 weeks, clusters of SSEA-4-positive iPSCs were detected in the injected area. At 4 to 8 weeks, these cells started to proliferate into small spheres surrounded by thin capsules. At 12 weeks the cell clusters still existed, but decreased in size and numbers. The cells inside these masses were homogeneous with no sign of differentiation into any specific lineage. Increased smooth muscle actin or vWF positive cells were found inside and around the iPSC clusters, compared with non-injected areas. By real-time polymerase chain reaction, the levels of VEGF, basic FGF, and ANRT expression were significantly higher in the iPSC-treated myocardium compared with untreated areas. These results suggest that iPSCs contributed to angiogenesis.**

Conclusions. **Allogeneically transplanted pig iPSCs proliferated despite an ischemic environment in the first 2 months and survived for at least 3 months in immunocompetent hosts. Transplanted iPSCs were also proangiogenic and thus might have beneficial effects on the ischemic heart diseases.**

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Marrow-derived stem or progenitor cells have been used for patients with ischemic myocardial diseases since 2003 [1–8]. Functional improvement has been reported by most clinical trials; however, no evidence has shown that marrow-derived cells can differentiate into beating myocytes in vitro, even though several studies claimed myocyte-like characteristics or markers after treating these cells with certain chemicals or factors [9–11]. Thus, most investigators believe that the beneficial improvement is mainly due to paracrine effects of implanted cells [12–16]. Induced pluripotent stem cells (iPSCs) have been recently generated in murine, human, and several other species since 2006 [17–19]. These iPSCs have become exciting tools for understanding the mechanisms of diseases and for potentially treating diseases through cell replacement therapy. In order to apply this new technology for future clinical use it needs to be tested in a large animal model. Recently, pig iPSCs have been derived from fetal fibroblasts by ectopic expression of 4 human transcriptional factors, OCT4, SOX2, KLF4, and c-MYC using lentiviral vectors [20]. These pig iPSCs showed pluripotency through in vitro differentiation assays and teratoma formation assays. However, these cells have failed to differentiate into beating myocytes in vitro. Recent studies have suggested that iPSCs seem to retain epigenetic memory because they have a higher tendency to differentiate into the lineages from which they were reprogrammed [21–23]. Therefore, we sought to derive iPSCs from porcine myocardial fibroblasts and hope these iPSCs can facilitate our efforts to generate cardiomyocytes. In this study we performed allogeneic transplantation of the cardiac fibroblast-derived pig iPSCs into chronic ischemic myocardium, and compared their effects with fetal fibroblast-derived iPSCs [20]. The results were compared with those using bone marrow-derived mesenchymal stem cell (MSC) previously reported by our laboratory [24, 25].

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Abbreviations and Acronyms

ANRT = aryl hydrocarbon receptor nuclear translocator
bFGF = basic fibroblast growth factor
DMEM = Dulbecco's Modified Eagle Medium
ESC = embryonic stem cell
FBS = fetal bovine serum
iPSC = induced pluripotent stem cell
MEF = mouse embryonic fibroblasts
MSC = mesenchymal stromal cell
NHLBI = National Heart, Lung, and Blood Institute
RT-PCR = real-time polymerase chain reaction
VGEF = vascular endothelial growth factor

Material and Methods

Pig Myocardium-Derived Fibroblasts Preparation and Culture

A tissue explant method was used for the myocardium-derived fibroblast culture. Briefly, a full length of left ventricular tissue (1 × 1 cm) was obtained from a healthy adult donor (Yorkshire, 3 months old). After washing 3 times in serum-free ×2 penicillin-streptomycin solution containing Dulbecco's Modification of Eagle's Medium (DMEM), the tissue was cut into small pieces (2-mm cube) and then washed twice. The small tissue clumps were transferred into a 10-cm culture dish, and cultured with DMEM supplemented with 10% fetal bovine serum, ×1 penicillin-streptomycin solution. Fibroblasts began to migrate out of the tissue fragments and into the surrounding area after 3 to 7 days in culture. These fibroblasts were harvested by trypsinization and passaged in DMEM with 10% fetal bovine serum (Figs 1A–1D).

Pig iPSC Derivation and Culture

Passage 3 myocardium-derived fibroblasts were seeded in 6-well culture plates at a density of approximately 1×10^5 per well. On the next day they were transduced with StemCCA lentiviral vectors (Millipore, Billerica, MA) containing the 4 human factors, OCT4, SOX2, KLF4, and c-MYC, following the manufacturer provided protocol. After viral transduction, the fibroblasts were cultured in DMEM medium with 10% fetal bovine serum for 5 more days and then trypsinized and plated on 10-cm tissue culture dishes with irradiated mouse embryonic fibroblasts (MEF). The next morning, the culture medium was switched to standard human embryonic stem cell (ESC) medium and changed daily afterward. The iPSC-like colonies began to appear 5 days after switching to ES medium (Figs 1E, 1F). These colonies were manually picked about a week later into 24-well plates and subsequently passed and expanded following standard human ESC/iPSC cultural protocol. Passage 12–17 pig iPSCs were used for the allogeneic transplantation study.

Animals

Yorkshire domestic pigs, initially weighing 15 to 20 kg were used as allogeneic iPSC recipients for this study. The experimental protocol was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, and all procedures conformed to the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996, Washington, DC).

Ameroid Placement and iPSC Transplantation

To create a chronic ischemic model, all animals underwent ameroid constrictor placement around the proximal left circumflex coronary artery [26]. Four weeks later, a second left thoracotomy was performed on each animal;

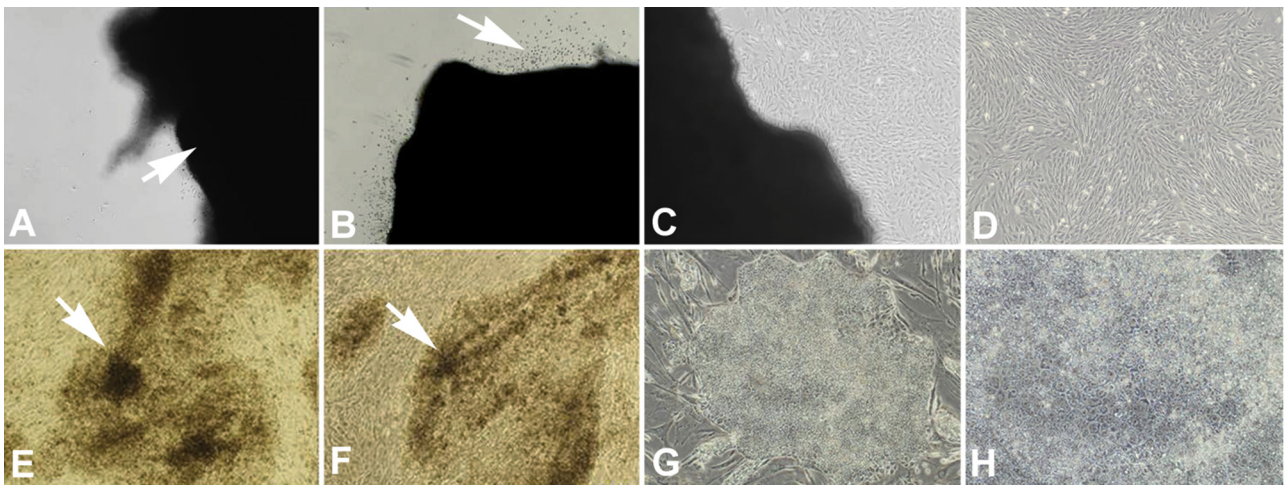

Fig 1. Top panel shows tissue explant method to obtain myocardial fibroblasts: (A) Arrow indicating a tissue clump attached to the culture plate; (B) arrow indicating cells migrating out from tissue clump after 3 days in culture; (C) migrated cells starting to proliferate to localized confluence; (D) cells derived from the myocardial tissue clump showing homogeneous fibroblast morphology after splitting and subculturing. Bottom panel shows reprogramming of myocardial fibroblasts; (E) and (F) arrows indicating preliminary colonies 5 days after transfection; (G) a mature induced pluripotent stem cell colony at passage 5 at day 44; (H) higher magnification of (G). Magnifications: (A)–(G) = ×100; H = ×200.