News Release

Title

Direct reprogramming of adult adipose-derived regenerative cells toward cardiomyocytes using six transcriptional factors

Key Points

• Adipose-derived regenerative cells (ADRCs) are stem cells in subcutaneous adipose tissue with characteristics similar to those of bone marrow mesenchymal stem cells and will be a promising source of stem and regenerative cells in the treatment of patients with myocardial perfusion defects.

• Nagoya University researchers and colleagues previously reported the clinical outcome, on which autologous ADRC implantation was safe and effective in patients with critical limb ischemia and could repair damaged tissue via its ability to promote angiogenesis and suppress tissue inflammation (Ref. 1).

• In the present study, the research team focused on the direct reprogramming of ADRC to differentiate into cardiomyocyte and examined the cardiac regenerative effect of induced ADRC on the ischemic heart.

• The combination of six unique factors (*Baf60c, Gata4, Gata6, Klf15, Mef2a, and Myocd*) could efficiently differentiate ADRC into cardiomyocytes (6F-ADRC), which expressed multiple cardiac genes, as confirmed by RNA sequencing (RNA-seq) and single-cell RNA sequencing (scRNA-seq).

• *In vivo*, injection of 6F-ADRC into mouse acute myocardial infarcted tissues resulted in the improvement of survival rate, fractional shortening, and reduction of infarction scar area.

• Among stem cell sources, ADRCs are relatively easy to harvest, have low tumorigenicity, and are considered to have few safety and ethical issues (Ref. 2). They continue to investigate direct reprogramming of ADRCs as a new option for cardiac regenerative therapy.

Summary

It is widely accepted that adipose-derived regenerative cells (ADRCs) can differentiate into mesodermal lineage cells. However, reprogramming adult ADRCs into mature cardiomyocytes is challenging. The research team investigated the induction of myocardial differentiation in ADRCs via direct reprogramming using lentiviral gene transfer. First, they identified candidate transcriptional factors by performing RNA sequencing, and ultimately confirmed that the combination of six unique factors (*Baf60c, Gata4, Gata6, Klf15, Mef2a, and Myocd*) could efficiently express enhanced green fluorescent protein (GFP) in ADRCs isolated from adult alpha-myosin heavy chain promoter-driven GFP (αMHC-GFP) transgenic mice. The

GFP-positive ADRCs induced by six factors (6F-ADRCs) expressed multiple cardiac genes and revealed cardiac differentiation in bioinformatic analysis. Moreover, injection of 6F-ADRCs into acute myocardial infarcted tissues in vivo resulted in the improvement of survival rate, fractional shortening, and reduction of infarction scar area. This study provides an alternative method for direct reprogramming of adult ADRCs into cardiomyocytes.

Research Background

World Health Organization (WHO) said that the world's biggest killer is ischaemic heart disease, since 2000, the largest increase in deaths has been for this disease, rising by more than 2 million to 8.9 million deaths in 2019. In this assignment, the research team have been studying regenerative therapies for cardiovascular diseases using stem and progenitor cells including ADRCs. The research team previously reported that stromal cell-derived factor 1 (SDF-1), a chemokine that directs the migration of different cell types, is secreted by implanted ADRCs, promoting the recovery of hindlimb ischemia by directing the migration of recipient cells (Ref. 3). In the mouse myocardial infarction (MI) model, it has been reported that the paracrine effect of angiogenesis-promoting factors vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) from the transplanted ADRCs promoted angiogenesis, reduced the myocardial infarction size, suppressed fibrosis, and improved the survival rate and cardiac function (Ref. 4). They also reported the clinical outcome, on which autologous ADRC implantation was safe and effective in patients with critical limb ischemia and could repair damaged tissue via its ability to promote angiogenesis and suppress tissue inflammation (Ref. 1). Based on the background, the research team conducted a study in which they used a new method of direct programming of ADRC.

Research Results

The research team performed RNA sequencing of adult mouse ADRCs and e11.5 embryonic mouse hearts to find unique combinations of transcriptional factors for transdifferentiating ADRCs into cardiomyocytes. Among the significantly and abundantly expressed genes in the e11.5 mouse heart, they identified 15 myocardium-specific transcription factors by annotation analysis. Among these 15 factors, researchers found that the combination of the six unique factors (*Baf60c, Gata4, Gata6, Klf15, Mef2a, and Myocd*) could efficiently differentiate ADRC into cardiomyocyte like cells (6F-ADRCs). Immunocytochemical staining showed that the induced 6F-ADRCs expressing αMHC-GFP protein were positive for sarcomeric α-actin. They performed RNA sequencing on GFP positive 6F-ADRCs collected three weeks after transduction. This analysis showed that a variety of cardiac genes, including *Myh6, Actc1,* and *Tnnt2,* were highly expressed in GFP^{+}6F-ADRCs, similar to expressions of 12.9 weeks C57BL/6J adult ventricular cardiomyocytes. They also performed Single-cell RNA sequencing and showed that the presence of an GFP expression cluster had the same distribution as the

Myh6, Actc1, and *Tnnt2* clusters. Taken together, GFP$^+$6F-ADRCs have similar characteristics to cardiomyocytes in the pattern of gene expression.

Next, the research team investigated whether 6F-ADRCs transplantation might contribute to cardiac functional recovery by its effect on the acute and chronic phase of MI. They harvested 6F-ADRCs one week after viral induction and injected them into acute MI mouse hearts, whose left anterior descending artery (LAD) was ligated before cell transplantation. The survival rate was improved in the 6F-ADRCs implanted group compared to that in the uninduced ADRCs (Control ADRCs) group. Echocardiography performed at 7, 14, 21, and 28 days after cell transplantation showed that the difference in left ventricular fractional shortening (LVFS) between 6F-ADRCs treated and Control ADRCs treated groups gradually became clearer over time and was statistically sustained in the 6F-ADRC treated group at Days 21 and 28. Cross-sectional tissue examination showed that the scar area and total left ventricle (LV) area were significantly reduced in the 6F-ADRCs group than the Control ADRCs group.

Research Summary and Future Perspective

The researcher team reported the new method that six transcription factors can transdifferentiate adult ADRCs into cardiac lineage cells. They also represented that if implanted in MI, 6F-ADRCs engrafted around the MI border area could stay there for a longer period of time while maintaining the characteristics of cardiomyocytes, which may prevent LV remodeling and impairment of cardiac function by angiogenetic effect. ADRCs are relatively easy to harvest, have low tumorigenicity, and are considered to have few safety and ethical issues. It is hoped that the results of this research will lead to the establishment of new cardiac regeneration therapies via direct reprogramming of ADRC.

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