Editorial

Enlightened Stem Cells in the Heart
More Efficient and Safer Reporter Gene Imaging

Gabriel Courties, Matthias Nahrendorf

Heart failure remains a major cause of death worldwide, with 6 million patients in the United States alone. Short of transplanting a new organ, there are currently limited options to overcome the poor prognosis of end-stage heart failure. This urgent clinical need drives the exploration of cardiac repair with stem cells. Although the ultimate goal of replacing dead myocytes in patients’ hearts is yet to be accomplished, many efforts aim to use the regenerative properties of stem cells for strategies to repair injured myocardium. The therapeutic potential of bone marrow–derived stem cells was evaluated first. Both cytokine-induced mobilization of bone marrow progenitor cells and their direct injection into the myocardium improved cardiac function, potentially by giving rise to new cardiomyocytes and vascular structures in preclinical experimentation. It was also discussed whether the effects were paracrine in nature. However, some clinical trials using bone marrow cells failed to achieve long-lasting beneficial effects. Currently, the field focuses on expanding cardiac progenitor cells, embryonic stem cells, and the use of induced pluripotent stem cells derived from differentiated cells, such as fibroblasts. No matter which cells are transplanted, key questions to be resolved include the mode of administration, sufficient cell homing, efficient engraftment, long-term survival, differentiation into cardiac cells, their functional integration, and last but not least safety issues as transplanted embryonic stem cells may form teratomas.

The reporter gene addition was successfully achieved in iPSCs to safely and effectively engineer stem cells before transplantation into the mouse heart. In summary, the authors provide convincing proof of concept for applying a promising strategy in hESCs and iPSCs to safely and effectively engineer stem cells before gene imaging. Here, stem cells are transfected with a specific reporter gene before cell injection into the heart. Only if the cell is alive and expressing the reporter gene, injected imaging agents will be activated (ie, luciferin will be activated if luciferase is expressed by a transfected stem cell) or retained. Previously, the reporter genes were randomly integrated into the stem cells’ genome. The article by Wang et al in this issue of Circulation Research takes stem cell reporter gene imaging to the next level by simultaneously making it more efficient and safer.

Wang et al report the specific targeted addition of a reporter gene cassette in the genome of human pluripotent stem cells as a tool to track their fate after in vivo administration. To explore the fate of transplanted stem cells, they designed a reporter construct (an ubiquitin promoter–driven expression cassette) that contains 3 different reporter systems enabling subsequent in vivo cell fate tracking using different modalities, including fluorescence (mRFP), bioluminescence (Fluc), and positron emission tomography (HSVtk) imaging.

Genome editing using zinc finger nucleases (ZFNs) was used to drive the targeted integration of the donor construct into human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). ZFNs are synthetic enzymes that can be engineered by fusing DNA-binding proteins (ie, zinc finger proteins) to the effector domain of the endonuclease FokI acting like molecular scissors. ZFNs represent a powerful method enabling efficient and precise genetic integration by generating double-strand breaks putatively at any desired location within the genome. Here, the AAVS1 locus, which is known to be risk-free, was chosen as the site for targeted integration of the transgene cassette. To avoid potential inselctional mutagenesis occurring in retroviral- and lentiviral-derived iPSCs, the authors used a minicircle DNA vector for the derivation of transgene-free iPSCs from adult adipose stromal cells.

The reporter gene addition was successfully achieved in both hESCs and iPSCs without altering the cells’ differentiation potential. A robust and constant reporter gene activity was measured in ZFN-edited cells over 2 months in vitro. After subcutaneous cell injection in mice, the firefly luciferase reporter activity could be quantified by bioluminescence imaging from as little as 10 000 injected cells. Furthermore, neither ZFN-mediated integration nor subsequent expression of reporter genes altered the differentiation potential of hESCs into both cardiomyocytes and endothelial cells compared with unmodified differentiated cells. Finally, both hESC-derived cell types could be imaged for up to 1 month after cell transplantation into the mouse heart.

In summary, the authors provide convincing proof of concept for applying a promising strategy in hESCs and iPSCs to safely and effectively engineer stem cells before...
transplantation. Techniques as the one developed here will allow for more sensitive in vivo imaging of stem cell fate in mice, and hopefully one day in human patients, as the inserted gene contained a positron emission tomography reporter. High sensitivity and quantitative cardiac positron emission tomography/computerized tomography or positron emission tomography/magnetic resonance imaging10 could thus follow the location and number of transplanted cells in clinical trials. Careful analysis of stem cell fate after transplantation, using tools such as the one described here by Wu et al,6 may shed light on how to harness the regenerative power of stem cells for heart failure therapy.

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**References**


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