Human cardiac regeneration

New cells in old hearts revisited

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Trials are underway to explore the use of cardiac stem cells as a source of cardiomyocytes, but a simpler approach may be to induce the heart's intrinsic capacity to regenerate as a means to reverse heart failure in patients.

A 2013 article in the *New England Journal of Medicine* discussed how a mouse heart could regenerate;[1] the 'Holy Grail' of genetics is to replicate this in humans. We are not there yet, but we are at a stage where studies using human cardiac tissue have firmly challenged the dogma that the human heart is a terminally differentiated organ.

The adult human heart contains about three billion cardiomyocytes and when a patient experiences a large myocardial infarction up to 25% of these cells are eliminated. Survival has improved in patients with coronary artery disease but, despite our best medical therapy, many patients still develop heart failure.

Although cardiomyocytes readily proliferate during fetal development, soon after birth they are believed to exit the cell cycle leaving little opportunity for replication and regeneration. Following the discovery that bone marrow-derived stem cells (BMCs) possess cardiomyogenic potential, cardiac regeneration via stem cell therapy has garnered much interest.[2] This article outlines the state of stem cell therapy for cardiac regeneration in 2017.

Tissue and embryonic stem cells

Over the past decade, multiple human studies have been performed with the aim of generating functionally integrated cardiomyocytes through the infusion of stem cells derived from peripheral blood, bone marrow and skeletal myoblasts. Of these, BMCs have been of most interest because of the relative ease of collection; more than 3000 patients worldwide have undergone treatment with BMCs.[3] However, the outcomes of these trials have been disappointing, with only modest and inconsistent benefits, which reflect the lack of standardised delivery protocols and the heterogeneous populations of BMCs.[4]

Key points

- **The human heart is no longer regarded as an aplastic organ; there are low levels of cardiomyocyte turnover throughout life.**
- **Human trials with cardiac stem cells have largely been disappointing and are yet to show engraftment.**
- **Some of the perceived improvements in cardiac function with stem cell infusions may be due to stimulation of existing cardiomyocytes to re-enter the cell cycle and divide.**
- **The key to one day reversing heart failure in patients will rely on the translation of animal models to human cardiac regeneration.**

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Embryonic stem cells and induced pluripotent stem cells are emerging sources of cardiomyocytes that have demonstrated engraftment, electrical coupling and enhancement of contractile function in simian models of myocardial infarction.[5,6] However, several hurdles remain to the use of these pluripotent cells in humans, including the risk of teratoma formation and their immunogenicity. With current differentiation protocols, these 'generated' cardiomyocytes are also phenotypically immature and arrhythmogenic because of their incomplete coupling to the host myocardium.

Cardiac stem cells

A more attractive approach is to stimulate intrinsic mechanisms of regeneration within the human heart. Recent studies on cardiac growth and proliferation using human tissue have revealed astonishing results. Human hearts do possess proliferating cardiomyocytes. Carbon-14 dating of healthy adult hearts has demonstrated that about 1% of cardiomyocytes are renewed each year in young people (20 years), declining to about 0.5% per year by age 75 years.[7] This implies that about 50% of cardiomyocytes are replaced over a normal human lifespan. Therefore, human cardiomyocyte renewal must be a relatively slow process, because it cannot overcome the myocyte loss seen in patients with heart failure. Regeneration, therefore, requires the awakening of dormant, resident cardiac stem cells (CSCs) or the induction of terminally differentiated cardiomyocytes to re-enter the cell cycle.

CSCs are stem cells localised to within a cardiac niche. They are capable of differentiating into one of the three major cell types of the myocardium:

- cardiomyocytes
- endothelial cells
- vascular smooth muscle cells.

There are encouraging results from trials involving the transplantation of human CSCs, such as c-Kit-positive CSCs, cardiosphere-derived stem cells, stem cell antigen-1-positive stem cells and epicardium-derived cells. Preclinical studies that infused CSCs into an infarcted heart show relative benefit for smaller animals; however, the salubrious effects seem to also extend to humans.[8] Early reports suggested significant improvements in tangible endpoints such as left ventricular ejection fraction and a reduction in scar tissue as demonstrated by cardiac MRI.[9,10]

Despite initial excitement about CSCs as an endogenous source of cardiomyocyte renewal, contrary evidence led to the current consensus that both externally derived-CSCs and resident CSCs, although they bear cardiomyogenic potential, do not meaningfully contribute to cardiomyocyte renewal under basal conditions.[11] Direct intracoronary or intramyocardial delivery of stem cells of all types have been relatively ineffective in regenerating myocardial mass, with most delivered cells perishing within a week of delivery.[12,13] Any potential benefit in the clinical realm (as alluded to above) likely can be attributed to paracrine effects rather than actual electromechanical coupling of the infused cells (i.e. engraftment).[14] Despite more than 15 years of intense focus on cardiac regeneration, a cellular candidate with efficient and effective cardiomyogenic potential and a low side effect profile has yet to emerge.

Second-generation stem cell treatment

'Second-generation' stem cell treatment seeks to circumvent the limitations of CSCs through approaches to enhance stem cell engraftment and survival, including stress preconditioning, combinatory stem cells, pretreatment with growth and transcription factors, and gene editing.[15-17] Engineered scaffolds and patches can provide mechanical and trophic support for CSCs and have been utilised in the Autologous Human Cardiac-derived Stem Cell to Treat Ischemic Cardiomyopathy (ALCADIA) trial for cardiosphere-derived cells (www.clinicaltrials.gov/ct2/show/NCT00981006). The Combination of Mesenchymal and C-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure (CONCERT-HF) trial of combined c-Kit-positive CSCs and bone marrow mesenchymal stem cells for ischaemic cardiomyopathy is currently underway (https://clinicaltrials.gov/ct2/show/NCT02501811).

Using the robust cardiac repair process of lower organisms such as zebrafish and mice, lineage-tracing experiments have shown that progenitor cells are rarely involved in cardiomyogenesis.[1,19] Mature cardiomyocytes undergo a process of de-differentiation and hyperplasia to reconstitute injured myocardium. There is emerging recognition that non-human mammalian hearts have excellent regenerative capacity during development. This decreases after birth, but it may be reactivated in postnatal life, particularly given the stimulus of myocardial injury.[18,19] Induction of this intrinsic regenerative capacity has been achieved in small animals through overexpression and knock-down of cell cycle regulators and replication of developmental signalling.[20]

Translation to human hearts

Unfortunately, data from the humble mouse model of heart disease does not always translate to humans.[21] The differences in genetic and phenotypic make-up mean that elucidating pathways such as those of inflammation or immune response is fraught with overinterpretation with respect to human applicability. The molecular mechanisms and conditions underlying both terminal differentiation and de-differentiation of human cardiomyocytes remain unknown, and the cardiomyocyte population responsible for proliferation is uncharacterised. By elucidating human-specific pathways that direct myocyte proliferation, intrinsic cardiac regeneration can be enhanced. However, other approaches such as fibroblast reprogramming and exosome-based cardioprotection can also be improved.[22,23] Both these approaches are cell-free therapies that rely on transmission of molecular signals to induce regeneration. Clearly, more translational research on human cardiac tissue is needed before clinical trials can be conducted, and this requirement also extends to stem cell-based therapies.[24]

Available evidence now firmly challenges the tenet that the human heart is aplastic and that its growth is driven only by hypertrophy rather than proliferation. It is increasingly likely that proliferation

contributes strongly to developmental heart growth. This raises the possibility that the loss of myocytes in a failing heart may be countered by stimulating the regeneration of new, functional myocardium from remaining (relatively healthy) cardiac cells. The advantage here is that the progeny will inherit the correct electromechanical connections, meaning that the new cells are less prone to induce fatal arrhythmias. In addition, it is likely that the extent and duration of cell proliferation can be better controlled. Given that the parent cardiomyocytes are terminally differentiated cells, this means that downstream malignant transformation is less likely.

Conclusion

Taken together, the above observations point to stimulation of cardiomyocyte proliferation as a promising therapeutic approach for treating heart failure. Determining the exact means by which this can be achieved will undoubtedly require further translational studies using human cardiac models. **CT**

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