Human embryonic stem cells for cardiac repair: the focus is on refined selection and cardiopoietic programming

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See article on page 1278

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Embryonic stem (ES) cells have taken centre stage in the scientific debate on potentially useful progenitor cell populations for post-infarct cardiac repair. Whereas effective cardiac specification of adult bone marrow-derived progenitor cells remains a matter of intense debate, much of the appeal of pluripotent ES cells stems from their intrinsic capacity to transdifferentiate into endoderm-derived cardiomyocytes^{1–3} and the characterisation of well-defined protocols for in vitro expansion (scalable for potential human application).⁴ The successful generation of expandable, fully differentiated force-generating cardiomyocytes, capable of excitation–contraction coupling,^{3 5–7} renders them a particularly attractive cell type for post-infarction repair. However, irrespective of ethical considerations, major technical hurdles continue to concern researchers and doctors alike, including the risk of uncontrolled neoplastic transformation, potential development of teratomas, immune responses and disruptive growth within the host tissue primed with paracrine signals from ES cell-derived cytokines.⁸

In this issue of *Heart*, Leor and coworkers⁹ report an elaborate number of xenotransplantation experiments, in which they investigate the fate of transplanted human ES cells in normal and infarcted myocardium of immuno-compromised nude rats (see article on page 1278). The authors use two different NIH-approved human ES cell lines to investigate in a first set of experiments the integration, survival and eventual transdifferentiation of these cells in the heart. Of interest, the authors not only examined undifferentiated human ES cells, but also ES cell-derived embryoid bodies (clusters of identifiable beating cells) as well as 10- to 20-day-old beating myocardial tissue specimens. Careful histological evaluation using X and Y chromosome labelling (a tracer for the human origin of the cells) indicated that undifferentiated ES cells and embryoid bodies survive better in a normal myocardial microenvironment than in the hostile milieu of an infarcted myocardial territory. None of the undifferentiated human ES cells injected into the myocardial wall transdifferentiated into a cardiac or endothelial lineage, confirming earlier observations in murine ES cells that the capacity of the foster milieu to guide stem cell specification is limited and that transplantation outside the embryonic environment disrupts their normal differentiation programme.^{10 11}

In contrast, when human ES (hES) cell-derived 10- to 20-day-old cardiomyocytes were implanted in normal cardiac muscle, distinct morphological features of initial cardiac specification were seen, but again these cells failed to survive in infarcted areas. These observations confirm the need for proper guidance cues originating from normal host myocardial cells that can secure and sustain further specification of 10–20-day-old beating myocardial tissue. But even when transplanted into normal myocardial territories, the latter hES cell-derived cardiomyocytes did not acquire a normal growth pattern and were isolated from the host heart by fibrous scar tissue, precluding the formation of a new functional myocardial syncytium. Moreover, in one case the authors observed clear evidence of neoplastic transformation, probably attributable to residual undifferentiated ES cells in the injectate and highlighting the persistent non-cardiogenic potential of ES cells in this setting. Collectively, these sobering data indicate that while hES cells can at best survive in normal myocardium they are unlikely to do so in an ischaemic or acellular environment, in greatest need for cellular cardioplasty.

The major importance of the findings by Leor *et al*⁹ is that they strongly underline the need for proper cardiac specification before transplanting hES cells or ES cell progeny into a hostile milieu. The stringent requirements for hES cell specification before cell transfer contrast with earlier reports using murine ES cells, which showed a stable long-term functional benefit after transplantation of murine embryoid bodies in the infarcted rat heart.¹² Clearly, murine and hES cells differ significantly in their cardiomyogenic differentiation capacity, and unravelling these intricacies is a prerequisite for future regenerative treatments. Fortunately, we are witnessing remarkable scientific progress in our understanding of the molecular cross-talk between ES cells and their host environment. Recently, Behfar and colleagues identified a key role for tumour necrosis factor α in the release of cardio-inductive signals from the intact murine endoderm,¹³ resulting in the generation of murine cardiopoietic cells and yielding truly functional ES cell-derived cardiac progeny. The scientific challenge in the years to come will be to investigate whether comparably effective and safe cardiopoietic programming of human ES cells is feasible for cardiac repair.

In a second approach, Leor *et al*⁹ reported on the first attempt to answer this very question of cardiopoietic programming in a xenotransplantation model using infarcted nude rats. However,

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when prespecified beating hES-derived cardiomyocytes were injected, the authors were unable to detect the cells 3 weeks later, although left ventricular (LV) remodelling was found to be improved with better preserved fractional shortening and a tendency for attenuated scar thinning. The latter data were only obtained in a small number of rats without measurements of diastolic function ($n = 4$) and, hence, need to be confirmed in a larger number of animals, with extended functional evaluation, before we can appreciate their true significance. Although one might envision some alteration of myocardial passive mechanical properties after cell transfer, the failure to document effective cardiogenesis in the infarct zone should invigorate preclinical research, with a specific focus on strategies to enhance cardiopoietic programming and cell survival. Alternate strategies such as heat-shock pretreatment of human ES cells have been shown to reduce significantly early ES cell death after implantation in the infarcted host heart and to increase graft size over time without teratoma formation.¹⁴ In addition, stable grafts of hES cell-derived cardiomyocytes were reported to integrate electromechanically with the surrounding myocardium of immunosuppressed pigs.¹⁵

Many questions obviously remain, most notably about the evidence or not for immune tolerance of ES cells or their progeny in the heart⁸ and about the long-term follow-up after human ES cell transfer. Most preclinical studies only examined a limited time window of events in the foster milieu and hence may fail to detect insidious, potentially prohibitive side effects. Moreover, results were mostly obtained in a xenotransplantation setting involving immuno-compromised hosts, and hence are not directly transposable to allogeneic transplantation protocols. Careful monitoring of transdifferentiation and fusion events as well as disruptive or neoplastic growth patterns will be critically important before ES cell-derived regenerative treatments can be realistically considered. Our molecular understanding of cross-signalling between progenitor cells and the local microenvironment is, however, rapidly expanding and infarcted hearts serve as instructive bioreactors that will provide answers to many of these questions. Sustained research

efforts such as those reported by Leor *et al* will guide further careful development of effective ES cell-based cardiogenesis.

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REFERENCES

- 1 Klug MG, Soonpaa MH, Koh GY, *et al*. Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest* 1996;**98**:216–24.
- 2 Mummery C, Ward-van Oostwaard D, Doevendans P, *et al*. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation* 2003;**107**:2733–40.
- 3 Kehat I, Kenyagin-Karsenti D, Snir M, *et al*. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 2001;**108**:407–14.
- 4 Zandstra PW, Bauwens C, Yin T, *et al*. Scalable production of embryonic stem cell-derived cardiomyocytes. *Tissue Eng* 2003;**9**:767–78.
- 5 He JQ, Ma Y, Lee Y, *et al*. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res* 2003;**93**:32–9.
- 6 Xu C, Police S, Rao N, *et al*. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. *Circ Res* 2002;**91**:501–8.
- 7 Xue T, Cho HC, Akar FG, *et al*. Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: insights into the development of cell-based pacemakers. *Circulation* 2005;**111**:11–20.
- 8 Nussbaum J, Minami E, Laflamme MA, *et al*. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J* 2007;**21**:1345–57.
- 9 Leor J, Gerecht S, Cohen S, *et al*. Human embryonic stem cell transplantation to repair the infarcted myocardium. *Heart* 2007;**93**:1278–84.
- 10 Rohwedel J, Sehlmeier U, Shan J, *et al*. Primordial germ cell-derived mouse embryonic germ (EG) cells in vitro resemble undifferentiated stem cells with respect to differentiation capacity and cell cycle distribution. *Cell Biol Int* 1996;**20**:579–87.
- 11 Foley A, Mercola M. Heart induction: embryology to cardiomyocyte regeneration. *Trends Cardiovasc Med* 2004;**14**:121–5.
- 12 Hodgson DM, Behfar A, Zingman LV, *et al*. Stable benefit of embryonic stem cell therapy in myocardial infarction. *Am J Physiol Heart Circ Physiol* 2004;**287**:H471–9.
- 13 Behfar A, Perez-Terzic C, Faustino RS, *et al*. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *J Exp Med* 2007;**204**:405–20.
- 14 Laflamme MA, Gold J, Xu C, *et al*. Formation of human myocardium in the rat heart from human embryonic stem cells. *Am J Pathol* 2005;**167**:663–71.
- 15 Kehat I, Khimovich L, Caspi O, *et al*. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol* 2004;**22**:1282–9.