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COMMENTARY

Deciphering the β-adrenergic response in human embryonic stem cell-derived-cardiac myocytes: closer to clinical use?

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Human embryonic stem cells (hESCs) are a pluripotent cell type considered to have high potential for the treatment of cardiovascular disease by cell replacement therapies. Several groups have shown that hESCs can be differentiated *in vitro* into cardiac myocytes, which may be used to facilitate tissue regeneration by injection directly into damaged myocardium. However, several hurdles still need to be overcome before these cells can be used in clinical trials. In particular, because transplanted hESC-cardiac myocytes should integrate fully within the damaged heart, these cells must be functionally compatible with the host myocardium. To assess this aspect of hESC-cardiac myocytes, Brito-Martins *et al.* (2008) in this issue of the *BJP*, describe the responses of hESC-cardiac myocytes to β-adrenoceptor stimulation, compared to those of myocytes from adult human hearts. Data obtained using specific β-adrenoceptor agonists showed good compatibility of hESC-cardiac myocytes with adult human myocardium in terms of β-adrenoceptor response.
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Abbreviation: hESC, human embryonic stem cell

Although substantial benefits have been achieved with the current pharmacotherapies, cardiovascular disease still remains one of the leading causes of mortality and morbidity worldwide (Mark *et al*., 2007). The development of novel therapeutic approaches for improving cardiac function in the damaged heart is therefore a critical goal.

A major challenge in the treatment of the damaged heart, following myocardial infarction and cardiac ischaemia, is the irreversible loss of adult cardiac myocytes, due to their limited proliferative potential. A promising new strategy to counter this loss is the use of cell-based therapies by engraftment of functional cardiac myocytes and thus to restore full contractility to the damaged tissue (Hassink *et al*., 2004). However, to accomplish this, a major hurdle to overcome is the identification of suitable cell types. A number of cell types such as mesenchymal stem cells, skeletal muscle myoblasts or haematopoietic stem cells have been tested (Lapidos *et al*., 2004; Pittenger and Martin, 2004), but unfortunately their limited potential for differentiation into cardiac myocytes or side effects intrinsic to their biological properties has demonstrated their unsuitability for cardiac regeneration. On the other hand, the use of human embryonic stem cells (hESCs) holds great potential for myocardial replacement therapy. So far, several different hESC cell lines have been derived from the inner cell mass of frozen blastocyst stage embryos that were set aside from *in vitro* fertilization procedures (Thomson *et al*., 1998). They are characterized by a differentiation potential that varies from cell line to cell line and are now available from several institutions throughout the world. hESCs are pluripotent and have self-renewal properties that enable them to differentiate indefinitely into various cellular types (Pera and Trounson, 2004). In fact, hESCs can differentiate into a cardiac-like cell syncytium composed of single spontaneously contracting cells with properties similar to post-natal cardiac myocytes, such as pacemaker activity, propagation of action potential (Kehat *et al*., 2001; Mummery *et al*., 2003; Satin *et al*., 2004) and a quasi mature cell Ca^{2+}-handling apparatus (Dolnikov *et al*., 2006; Liu *et al*., 2007). However, before these cells can be successfully used for tissue engrafting in clinical trials, a more extensive characterization is needed. In particular, the assessment of inotropic and lusitropic properties in response to fundamental external stimuli, such as β-adrenergic and muscarinic agonists, is of utmost importance. In addition, comparison of these features in hESC-cardiac myocytes with those in

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adult ventricular myocytes from both normal and failing hearts is critical for the prediction of their function after implantation. In the current issue of the *British Journal of Pharmacology*, Brito-Martins *et al*. (2008) report their detailed pharmacological analysis of the β-adrenoceptor response in developing hESC-cardiac myocytes. Their main findings can be summarized as follows: (1) stable response of hESCs during cardiac differentiation to the mixed β_1- and β_2-adrenoreceptor agonist, isoprenaline, from day 10 to 79; (2) an isoprenaline concentration for half-maximal response similar to that of adult ventricular cardiac myocytes, even though the type of response was closer to that obtained from failing rather than normal adult human heart; (3) responses of both inhibition of isoprenaline-induced contraction and relaxation and sensitivity to muscarinic inhibition, which were similar to those of adult ventricular myocytes; and (4) contribution of both β_1- and β_2-adrenoreceptors to inotropic responses similar to adult cardiac myocytes but a substantial dependence of β_1-adrenoreceptor for the lusitropic response, suggesting that β_1-adrenoreceptors play a major role compared with β_2-adrenoreceptors in this context.

Overall, the current study (Brito-Martins *et al*., 2008) is an important step forward in the understanding of molecular mechanisms underlying the nature of hESC-cardiac myocytes. The hESC-cardiac myocytes responded to β-adrenoreceptor stimulation similarly to adult ventricular myocytes, thus strengthening the possibility of successful physiological integration of differentiated hESC-cardiac myocytes into the host myocardium by cell grafting. In the context of the rapidly increasing knowledge in the stem cell field, we would consider the use of hESC-cardiac myocytes to be probable candidates for cell-therapy applications and in particular, for the treatment of heart failure.

However, caution must be exercised when considering stem cells for heart failure treatment and many issues still need to be resolved before the clinical use of hESC-cardiac myocytes becomes a reality. An essential prerequisite for the practical application of hESC-cardiac myocytes is a technical one, the scaling up of hESC-cardiac myocyte yield, especially if large areas of the myocardium have to be salvaged. Due to differences between human and mouse ESC-cardiac myocytes, it is a real challenge to achieve the same high yields of hESC-cardiac myocytes, as can be obtained from the more extensively characterized mouse ESC-cardiac myocytes and more work is needed to define the optimal culture conditions for hESC-cardiac myocytes to maximize the yield. However, some encouraging results have recently been described by Caspi *et al*. (2007). In addition, effective application of hESC-cardiac myocytes requires more defined and reproducible strategies aimed at the development of an efficient cardiac myocyte differentiation system, such as a more straightforward differentiation process. A better knowledge of the biochemical processes involved in the differentiation of hESC-cardiac myocytes will certainly improve the technical conditions necessary for achieving this task. Another serious issue that needs to be solved is the immunological compatibility between donor and grafted tissues, lack of which leads to ES cell rejection after transplantation. Recent work has yielded encouraging results on this point, demonstrating that lower levels of the class I major histocompatibility complex on hESC might make the derived cardiac myocytes less immunogenic (Drukker *et al*., 2006). Furthermore, the development of chemically defined culture media supplemented with recombinant cytokines and growth factors is a crucial requirement if these cells are to have clinical applications. A last but no less important question to consider is the limitation that current legal and ethical issues impose on the potential therapeutic use of embryonic cells in many countries. In conclusion, we believe that within the next few years, we will experience an acceleration of our knowledge in this exciting and important field, driven primarily by the prospect of clinical application of hESC-derived cells.

References

Brito-Martins M, Harding SE, Ali NN (2008). β_1- and β_2-adrenoceptor responses in cardiomycytes derived from human embryonic stem cells: comparison with failing and non-failing adult human heart. *Br J Pharmacol* **153**: 752–760 (this issue).

Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A *et al.* (2007). Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol* **50**: 1884–1893.

Dolnikov K, Shilkrut M, Zeevi-Levin N, Gerecht-Nir S, Amit M, Danon A *et al.* (2006). Functional properties of human embryonic stem cell-derived cardiomyocytes: intracellular Ca^{2+} handling and the role of sarcoplasmic reticulum in the contraction. *Stem Cells* **24**: 236–245.

Drukker M, Katchman H, Katz G, Even-Tov Friedman S, Shezen E, Hornstein E *et al.* (2006). Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells* **24**: 221–229.

Hassink RJ, Passier R, Goumans MJ, Mummery CL, Doevendans PA (2004). New and viable cells to replace lost and malfunctioning myocardial tissue. *Minerva Cardioangiol* **52**: 433–445.

Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A *et al.* (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* **108**: 407–414.

Lapidos KA, Chen YE, Earley JU, Heydemann A, Huber JM, Chien M *et al.* (2004). Transplanted hematopoietic stem cells demonstrate impaired sarcoglycan expression after engraftment into cardiac and skeletal muscle. *J Clin Invest* **114**: 1577–1585.

Liu J, Fu JD, Siu CW, Li RA (2007). Functional sarcoplasmic reticulum for calcium-handling of human embryonic stem cell-derived cardiomyocytes: insights for driven maturation. *Stem Cells* [e-pub ahead of print].

Mark DB, Van de Werf FJ, Simes RJ, White HD, Wallentin LC, Califf RM *et al.* (2007). Cardiovascular disease on a global scale: defining the path forward for research and practice. *Eur Heart J* **28**: 2678–2684.

Mummery C, Ward-van Oostwaard D, Doevendans P, Spijker R, van den Brink S, Hassink R *et al.* (2003). Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation* **107**: 2733–2740.

Pera MF, Trounson AO (2004). Human embryonic stem cells: prospects for development. *Development* **131**: 5515–5525.

Pittenger MF, Martin BJ (2004). Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* **95**: 9–20.

Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I *et al.* (2004). Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. *J Physiol* **559**: 479–496.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS *et al.* (1998). Embryonic stem cell lines derived from human blastocysts. *Science* **282**: 1145–1147.