Enhancing cardiac stem cell differentiation into cardiomyocytes

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This editorial refers to ‘Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields’ by R. Gaetani et al.,3 pp. 411–420, this issue.

Myocardial infarction results in the loss of cardiomyocytes, scar formation, ventricular remodelling, and eventually heart failure. In recent years, cell therapy has emerged as a potential new strategy for patients with ischaemic heart disease. Cell therapy potentially includes embryonic and bone marrow-derived stem cells.1 Recently, the existence of cardiac stem cells that reside in the heart itself was demonstrated. The discovery of cardiac stem cells has sparked intense hope for myocardial regeneration with cells that are obtained from the heart itself and are thereby inherently programmed to reconstitute cardiac tissue. These cardiac stem cells can be detected by several surface markers (e.g. c-kit, Sca-1, MDR1, and Isl-1).2 Both in vitro and in vivo differentiation into cardiomyocytes, endothelial and vascular smooth muscle cells has been demonstrated. Early animal studies showed promising results on improvement or preservation of left ventricular function after cell injections. Gaetani et al.3 present their interesting results in this issue of Cardiovascular Research, introducing a new strategy to enhance cardiac differentiation of stem cells without any pharmacological or genetic manipulation. Studies on stem cell transplantation therapy are mainly focused on the use of undifferentiated stem cells. However, it has been postulated that some degree of cardiomyogenic differentiation of stem cells in vitro, prior to transplantation, would yield better results.4 This approach is associated with an improved safety profile, since it may also alleviate the possibility of spontaneous differentiation of stem cells in vivo into undesired lineages. In the meantime, it was shown that bone morphogenetic proteins are involved in the regulation of processes underlying cardiovascular development, in particular cardiac differentiation.5 This observation may guide alternative approaches for cell therapy such as in vivo reprogramming of cardiac fibroblasts into cardiomyocytes. Ultimately, this approach is attractive, as cardiac fibroblasts already populate the infarcted area of the heart (thus, no cell delivery and rejection issues), and complicated in vitro differentiation steps are superfluous.

It is difficult to obtain terminally differentiated cells in vitro, or even more difficult after transplantation into the heart. Several approaches have been applied, including the use of a special culture milieu, co-culturing with neonatal cardiomyocytes, electrical stimulation, mechanical stimulation, reactive oxygen species application, a heat-shock approach, pharmacological approaches using oxytocin or 5-azacytidin, FGF-2 or TGF-β-stimulation, preconditioning, or combinations thereof.4,6,7 Various groups have isolated and expanded undifferentiated cells from adult heart tissue, based on different stem cell and progenitor antigens and other characteristics. Their most important properties and differentiation protocols are summarized in Table 1.

C-kit+ cardiac stem cells expressed signs of biochemical differentiation into either myocytes (with sarcomere striation and contractile activity), smooth muscle cells, or endothelial cells in specialized differentiation medium (Table 1).8 Also human c-kit+ cardiac stem cells form multicellular clones and differentiate into contracting myocytes.9 Cardiomyocytes can also be isolated by using magnetic anti-biotin microbeads recognizing Sca1+ biotin labelled cells.8 Initially, no contractile proteins were expressed, but some cardiac transcription factors could be detected.10 Upon chemical stimulation cardiac genes and structural proteins were expressed, and differentiation into spontaneously beating cardiomyocytes was shown.12 Recently, our group was able to differentiate Sca1+ cardiomyocyte progenitor cells into mature cardiomyocytes that beat spontaneously upon 5-aza stimulation, also expressing connexin-40 and -43 which is essential for sycinetium formation. This effect was strongly enhanced with the addition of TGF-β.13 Alternatively, cardiac side population cells express α-actin indicating cardiomyocyte differentiation after co-culturing with adult rat cardiomyocytes in specific medium. They also express several transcriptional regulators involved in cell cycling, suggesting a self-renewal capacity as seen in embryonic stem cells. It has been suggested that these cells function as a progenitor cell
population for the development, maintenance, and repair of the heart.14

In the hearts of newborn rodents and humans, cardiac stem cells are found that express the transcription factor Islet-1.15 They also express factors that are known to be involved in the early stage of cardiogenesis (e.g. Nkx2.5 and GATA4). When co-cultured with neonatal myocytes, these cells were able to differentiate and adopt the cardiomyocyte phenotype, including electrical and contractile properties.

Cardiospheres are self-adherent clusters of undifferentiated cells that grow from subcultures of postnatal atrial or ventricular heart tissue. For cardiospheres, both in vitro and in vivo differentiation into the major specialized cell types of the heart has been demonstrated: myocytes (i.e. cells demonstrating contractile activity and/or showing cardiac myocyte markers) and vascular cells (i.e. cells with endothelial or smooth muscle markers).16 In their elegant study presented in this issue, Gaetani et al.3 investigated the hypothesis that the chronic exposure of cardiospheres and cardiosphere-derived cells to extremely low-frequency electromagnetic fields (ELF-EMFs) tuned at 7 MHz, a frequency close to Ca2+ ion cyclotron energy resonance, drives differentiation towards a cardiac-specific phenotype. They reported a significant increase in the expression of cardiac markers after 5 days of exposure to ELF-EMFs, as evidenced at transcriptional, translational, and phenotypical changes. They also reported Ca2+ mobilization among intracellular storages as confirmed by compartmentalized analysis of Ca2+ fluorescent probes after a combination of chronic and acute exposure to 7 MHz ELF-EMFs. Although this study opens new ways to achieving cardiac differentiation, it does not provide answers to some important questions: how can we explain that this technology affects both proliferation and differentiation, which are considered mutually exclusive paths? How does exposure to ELF-EMF induce Ca2+ mobilization, and how do Ca2+ dynamics translate into cardiac stem cell differentiation? These issues will need to be addressed to achieve cardiac cell differentiation in a predictable manner, prior to clinical application.

In summary, multiple studies have demonstrated alternative approaches to enhance cardiac stem cell differentiation into cardiomyocytes. Using ELF-EMFs, Gaetani et al.3 nicely showed that in vitro differentiation of cardiac stem cells is possible in an effective and biotechnically safe manner. The key question is if we really need to differentiate cardiac stem cells prior to transplantation. The alternative would be to enhance in vivo differentiation of transplanted, or even better, resident (stem) cells that are already on target. And even more challenging: do we need cells, or do we only need their specific cytokine/growth factors that are responsible for the so-called paracrine effects? In view of disappointing results of clinical stem cell studies thus far, the optimal regenerative strategy still needs to be determined.

References

### Table 1 Properties and differentiation protocols of various isolated cardiac stem cells

<table>
<thead>
<tr>
<th>Cardiac stem cell</th>
<th>Stem cell markers</th>
<th>Cardiac-specific transcription factor</th>
<th>Differentiation protocol</th>
<th>Cardiac myocyte gene after differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-kit</td>
<td>c-kit</td>
<td>CD34; CD45; lineage</td>
<td>Dulbecco’s MEM with 10% FCS and 10−8 M dexamethasone;5 co-culturing with neonatal rat myocytes9</td>
<td>α-sarcemeric actin, cardiac myosin, desmin, connexin 43</td>
</tr>
<tr>
<td>Sca-1</td>
<td>Sca-1; CD31</td>
<td>c-kit (+); CD34; CD45; lineage</td>
<td>5-azacytidine;10 Oxytocin;12 5-aza + TGF-β13,17</td>
<td>Troponin I, α-sarcemeric actin, cardiac myosin, MHC (α and β), desmin, connexin 43</td>
</tr>
<tr>
<td>Side population</td>
<td>Abcg2; Sca-1</td>
<td>CD31; c-kit (low); CD34; low; CD45 (low)</td>
<td>Co-culturing with cardiac cells in cardiomyocyte specific medium14</td>
<td>Troponin, α-cardiac actinin</td>
</tr>
<tr>
<td>Islet-1</td>
<td>Islet-1</td>
<td>Sca-1; c-kit; CD31</td>
<td>Co-culturing with neonatal myocytes15</td>
<td>Troponin T, α-cardiac actinin</td>
</tr>
<tr>
<td>Cardiospheres</td>
<td>c-kit; Sca-1; MDR</td>
<td>CD34; lineage</td>
<td>Co-culturing with neonatal rat cardiomyocytes;16 extremely low-frequency electromagnetic fields3</td>
<td>Troponin, α – MHC</td>
</tr>
</tbody>
</table>

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