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COMMENTARY

Cell Therapy for Heart Regeneration: Learning from the Past to Build a Brighter Future

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Cell therapy as a means to cure ischemic heart disease and end-stage heart failure has been under investigation for several years. Many clinical studies have been conducted with different cell types: skeletal myoblasts (SM), bone marrow-derived mononuclear cells (BM-MNC), mesenchymal stromal cells (MSC), and resident cardiac stem cells (CSC), to name a few [1, 2]. The efficacy results obtained so far are inconclusive, and cell therapy has never entered into clinical practice. In particular, there are still a few issues hampering an effective translation from the promising preclinical results to the bedside. Among the challenges, the most relevant are the low engraftment rate and the incapacity of the cells to differentiate into fully mature cardiomyocytes, resulting in a lack of heart regeneration which was the original goal that scientists aimed to accomplish when they started testing cell therapy. Despite this apparent unsuccess, much has been learned from the first clinical trials, and these now well-established concepts must drive our choices when designing new cell therapy protocols if we want to eventually succeed in repairing failing hearts.

The most recent lesson comes from an observational study, appearing in this issue of STEM CELLS Translational Medicine, in which the (very) long-term fate of SM transplanted into a human heart is described [3]. I like to think about this work as the natural closure of a circle, as the senior author, Philippe Menasché, was the first to transplant SM in a patient back in 2001 [4]. Thanks also to this new study, we now have enough elements to critically retrace the history of SM use for heart disease and to draw final conclusions. The history started in 1996, when Murry and colleagues demonstrated for the first time that neonatal SM could graft into an injured mouse heart and that the engrafted cells initially proliferate and then begin to form multinucleated myotubes that, with time, can differentiate into mature myofibers [5]. They also showed that it was possible to stimulate the contraction of the newly formed muscle ex vivo. However, in vivo, the myotube grafts were isolated from the remaining myocardium and failed to form electrical connections. Based on this positive preclinical evidence (but in hindsight underestimating the negative), Menasché's group performed the first intramyocardial injection of SM in a human failing heart during an aortic coronary artery bypass graft (CABG) intervention [4]. The reassuring safety data yielded by this pilot study led to the execution of the placebo-controlled, double-blind MAGIC trial [6], in which 97 patients with heart failure were randomly assigned to receive either a low or a high dose of autologous SM or placebo. At 6 months, the high-dose cell group showed a significant decrease in left ventricle volumes compared with the placebo group. However, the study did not achieve its primary endpoint, which was the improvement in left ventricular ejection fraction (LVEF). Moreover, concerns were expressed regarding a potential pro-arrhythmic effect played by SM therapy, likely due to the lack of electrical coupling. Meanwhile, other clinical trials tested the use of SM transplant with results similar to those reported in the MAGIC [7]. For these reasons, the development of therapies based on SM was basically abandoned. Despite this unhappy ending, thanks to the foresight of a few investigators who collected and analyzed the hearts of patients (when they died or received transplant) previously treated with SM, we have learned important concepts from this journey.

In 2003, two independent studies described the fate of SM at an intermediate [8] and at a long-time point [9] after intramyocardial transplantation. The first study was a phase I clinical trial investigating the feasibility and safety of autologous SM transplant in patients affected by ischemic heart disease undergoing left ventricular assist device (LVAD) implantation as a bridge to orthotopic heart transplantation. Four hearts were collected at the time of transplant, performed after an average time of 4 months from LVAD implant. Few areas of engrafted SM were identified in trichrome-stained sections and confirmed by immunohistochemistry for the skeletal muscle-specific myosin heavy chain. Additionally, evidence of SM differentiation with expression of slow-twitch myosin isoform was reported. Even though the cells were located in large scarred areas, the majority demonstrated healthy morphology and was mostly aligned in parallel with

the host myocardial fibers. Unfortunately, the presence of cardiac-specific proteins was not verified, including connexin 43. Of note, in some cases there was a significant increase in the number of blood vessels associated with the graft sites. The second study described the case of a patient enrolled in the MAGIC trial who died 1.5 years after the treatment with SM. The grafted cells showed the morphology of well-developed multinuclear skeletal myotubes with a morphologically developed contractile apparatus. The grafts were identified inside scarred tissue and not surrounded by inflammatory cells. Approximately 35% of myotubes stained positive for the fast-twitch myosin isoform and 32% stained positive for the slow myosin, while 33% coexpressed the slow and fast-twitch isoforms. Importantly, staining for the cardiac-specific antigens pan-cadherin and connexin 43 was negative. The absence of gap junction with recipient cardiomyocytes make unlikely, if not impossible, the hypothesis that the grafted myotubes can contract synchronously with the rest of the heart and actively contribute to cardiac function.

Menasché's group now reports new histological findings from another patient enrolled in the MAGIC trial [3, 6]. This case report was made possible because the patient, 16 years after receiving CABG and SM implantation, underwent heart transplant for end-stage heart failure. Of note, 1 year after the intervention the cardiac performance of the patient was remarkably improved, with the LVEF increasing from 22% to 45%. Most importantly, the patient was asymptomatic and in good cardiovascular compensation for approximately 9 years before his clinical condition and heart performance started to decline. The most relevant finding of this work is that a few engrafted cells were still present after all those years and displayed the typical pattern of myotubes, most of them expressing the fast isoform of myosin heavy chain and only a few expressing the slow isoform. The immunohistochemistry analysis showed that the myotubes were not connected to the neighboring cardiomyocytes and did not express connexin 43, thus confirming the absence of electrical connection with the recipient's myocardium. As the authors correctly stated in their conclusions, these findings support the concept that, if the engrafted SM contributed (in combination with CABG) to the initial improvement of heart function, they did it through paracrine mechanisms. Strong evidence from preclinical studies and secretome profiling of SM is in agreement with this hypothesis [7]. Like in the case of other cell types, cardioprotection, neovascularization, improved cardiac metabolism, prevention of LV negative remodeling, and stimulation of endogenous cardiac regeneration are the main reparative mechanisms activated by progenitor cells' secretome [1, 2, 10, 11].

Overall, the take-home messages of these studies are (a) SM are capable of permanently engrafting in the human heart, even though the percentage of cells surviving is extremely low, (b) SM fail to transdifferentiate into mature cardiomyocytes, (c) SM fail in forming electro-mechanical couplings with native cardiomyocytes and do not integrate with the rest of the contracting cardiac mass, and (d) the positive effects reported by the MAGIC and other clinical trials in terms of ventricular remodeling and revascularization are very likely mediated by paracrine effects.

Keeping in mind these messages will be very important to develop more effective cell therapies. The issue of poor cell engraftment is common to all the other cell types tested in clinical trials: BM-MNC, MSC, and CSC [1]. Several approaches have been proposed to overcome this hurdle, from the overexpression of protective genes to cell preconditioning [2]. More recently, administration of cells together with degradable biomaterials or the use of other tissue engineering techniques has been tested in animal models with promising results [1, 2, 12]. Also, the incapacity to robustly differentiate into cardiomyocytes is a problem shared with BM-MNC, MSC, and CSC [1, 2, 13]. This is obviously the most relevant limitation for heart regeneration. There are a couple of possibilities that scientists are considering in order to overcome this problem: either optimize the use of pluripotent cells, such as embryonic stem cells (ESC) [14] and induced pluripotent stem cells (iPSC) [15], or develop effective strategies to potentiate the limited innate regenerative capacity of the heart [1, 2, 16]. In particular, iPSCs are not burdened by ethical issues and have been shown to differentiate into cardiac-like cells both in vitro and in vivo [15]. The characteristics of these cardiac cells are similar, but not identical, to human cardiomyocytes also in their ion channel apparatus, as demonstrated by disease-modeling studies [17, 18]. Efforts to obtain standardized protocols to produce iPSC lines possibly committed toward cardiac lineage and to induce the maturation of iPSC-derived cardiomyocytes are needed. As shown by several investigators, induction of endogenous CSC proliferation/differentiation and of cardiomyocyte replication can be obtained through administration of progenitor cell's secretome, proteins, or noncoding RNA [11, 16, 19–21]. These strategies also need to be optimized and their efficacy improved. As already shown, the profiling and comparison of cell secretome may certainly be helpful to identify putative molecules with cardioreparative potential [13, 22, 23].

In conclusion, the hope of using SM to cure heart failure has probably vanished, but the possibility of regenerating broken hearts is still the dream of many scientists and clinicians who are continuously learning important lessons from the past to build a brighter future.

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