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Returns of the Living Dead

Therapeutic Action of Irradiated and Mitotically Inactivated Embryonic Stem Cells

Joseph D. Gold, Joseph C. Wu

From the time of their initial isolation, scientists have been fascinated by one particular characteristic of pluripotent stem cells: their ability to differentiate into specific cell types. Initially, this property was exploited to explore the changes in gene expression that accompany cell fate choices, and a vast amount of knowledge was added to the developmental biology literature, based both on *in vitro* studies and the generation of mice with specific alterations in crucial loci. With the advent of human embryonic stem cells (ESCs), an additional possibility arose: the chance that these cells might be the source of a new type of therapy, one in which absent or diseased cells were replaced by healthy versions derived from human ESCs. Cardiovascular disease presented an appealing target; the shortage of donor hearts, coupled with the tantalizing possibility of generating unlimited supplies of new myocytes, attracted a great deal of attention.

Article, see p 1286

Of course, there were always other possible candidate cells to be used in this type of therapy. Skeletal myoblasts,¹ which are readily obtainable from both allogeneic and autologous sources, as well as cells isolated from the bone marrow,² bone marrow stroma,³ and other tissue sources,⁴ were evaluated as possible therapeutic cells. In many cases, these cells were isolated and injected into various types of preclinical models of heart damage with the hope that something about the damaged heart environment would trigger a fundamental change in the nature of the input cells. In fact, in many cases, the injected cells quickly vanished from the injected animals, likely because of immune rejection or the simple stress of transplantation. The observed impermanence of these transplanted cells created a puzzle: why did so many of these cells actually improve heart function and not merely in preclinical models but in human trials?⁵

At the same time, other investigators focused on the cell types that could easily generate cardiomyocytes *in vitro*, namely ESCs and induced pluripotent stem cells.⁶ Sometimes

these cells were injected in the undifferentiated state, in the hopes that the local environment would steer them into exclusively cardiac cell fates. In other cases, investigators generated cardiomyocytes *in vitro* and injected the differentiated cells. It was soon demonstrated that in the absence of *in vitro* pretreatments, undifferentiated ESCs had the unfortunate tendency to generate teratomas upon injection.⁷⁻⁹ And while injected human ESC-derived cardiomyocytes survived and engrafted to varying degrees in animal models and often improved heart function compared with nontransplanted controls,^{10,11} the reason behind the improvement was far from obvious. The ESC-derived cardiomyocytes were clearly electrically and phenotypically immature.⁶ Even if they were mature, could they beat at the 400 to 650 bpm rate of rat or mouse hearts? In addition, the ventricular wall is a highly organized tissue—could an injection of dissociated cells really engraft and organize in a way that allowed the new tissue to contribute contractile function to the damaged heart?

It is now evident that the most obvious way in which cells could restore function to a damaged heart is not necessarily the correct one or even the most relevant one. Although the direct replacement of dead muscle by the transplanted cells is a possibility, other mechanisms are at least equally plausible (Figure). The other functions cells might provide include structural reinforcement (the repopulation of stiff scar tissue with viable, noncontracting cells); the production of factors that might salvage hibernating myocardium (possibly through induction of new blood vessels); the elaboration of factors that might trigger regeneration of muscle by endogenous cardiac progenitor cells (or recruitment of extracardiac host cells to repair the damage); and, in the case of apoptotic cells, the stimulation of anti-inflammatory cytokines that might limit scar formation (the dying stem cell hypothesis).¹²

In this issue of *Circulation Research*, Burt et al¹³ show us that injected cells need not even be viable to improve cardiac function in preclinical models of acute myocardial infarction. In a clever twist on standard attempts at cell therapy, Burt et al irradiated undifferentiated ESCs (murine and human) and, after demonstrating that the cells are not only mitotically inactivated but are clearly dying *in vitro*, injected the cells into the hearts of immunocompetent mice immediately after 1 hour of ischemia. One month after injection, the animals that received either ESCs or irradiated ESCs showed significant improvements in systolic blood pressure, cardiac contractility, cardiac relaxation, and had much smaller scars. Although a sizeable fraction (30%) of immunocompetent mice that received nonirradiated cells developed teratomas, none was detected in animals that received irradiated cells (this was true in both immunocompetent and immunodeficient mice). Similar results were seen when

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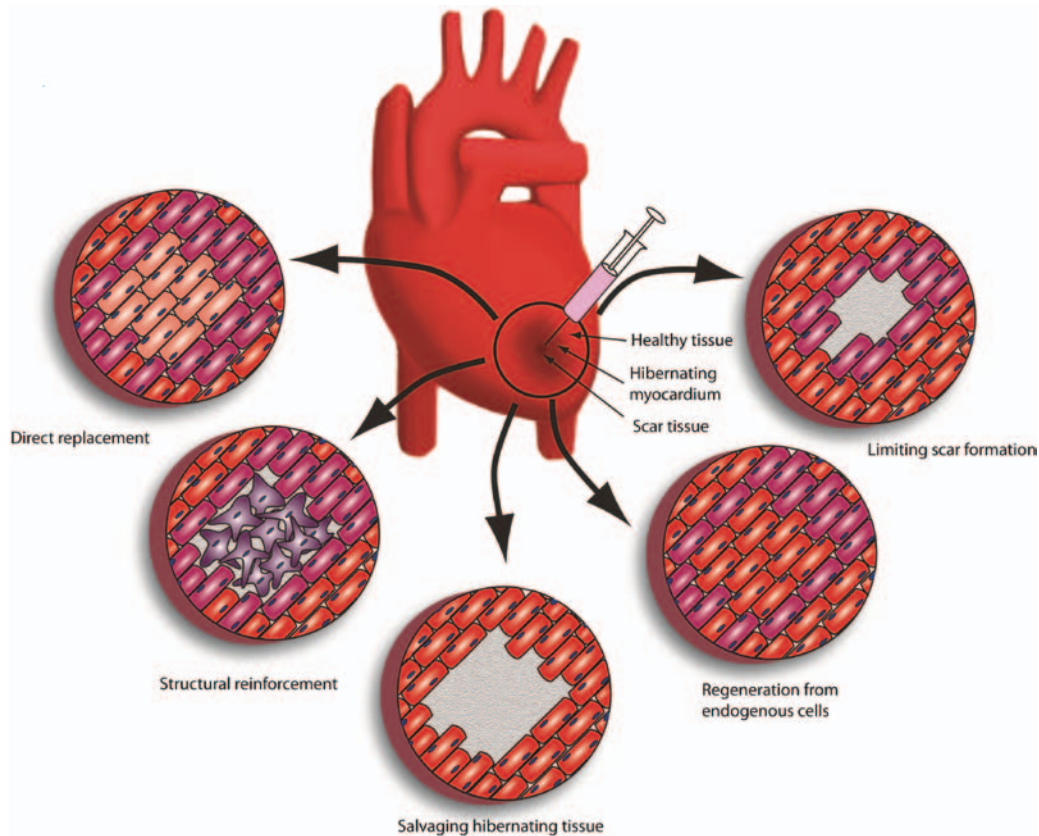


Figure. Potential mechanisms of action of injected cells for treatment of acute myocardial infarction: direct replacement of dead cardiomyocytes; structural reinforcement of the damaged ventricle by noncontracting but healthy cells that preserve normal heart geometry; converting hibernating myocardium back to contractile tissue; stimulation of regeneration from endogenous cells (whether cardiac-resident or extracardiac); limiting scar formation by induction of immunomodulatory cytokines as the host responds to the presence of apoptotic cells.

irradiated human ESCs were injected into rhesus monkeys immediately after 50 minutes of ischemia.

What mechanisms might be involved in the increased cardiac function detected in the animals? Histological examination of mice that received the irradiated cells showed insignificant levels of ESC engraftment, indicating that persistence of the cells was not required. No improvements were seen in animals that were injected with conditioned medium from the ESCs, suggesting a requirement for at least transitory presence of the cells. Microarray analysis showed transient differences in gene expression between hearts receiving conditioned medium and irradiated cells, in keeping with the notion that the cells supplied more than a burst of secreted factors. Bromodeoxyuridine (BrdU) injections revealed a 6-fold higher incorporation of the nucleoside in the myocardium adjacent to the cell injection sites than that seen in infarcted control mice, supporting the notion that a response from the endogenous cardiomyocytes might be responsible for the improvements. Indeed, the irradiated ESCs functioned as a feeder layer for adult mouse cardiomyocytes, resulting in increased surviving primary cells after 48 hours of coculture.

Can we conclude from this that the transplanted cells function by stimulating the generation of new host cardiomyocytes? Not necessarily, because the BrdU results do not distinguish between increased proliferation of host cardiomyocytes and increased DNA repair (or multinucleation). And while the authors attempted to control for the general effects

of transplanting apoptotic cells into the heart (induction of immunosuppressive cytokines that might affect cell survival and fibrosis) by showing that injection of irradiated mouse embryonic fibroblasts had no effects on heart function, irradiated mouse embryonic fibroblasts have been used for decades as feeder layers for pluripotent stem cells at least partially because they are resistant to radiation-induced apoptosis. The observation that nonirradiated ESCs similarly improved heart function does suggest that there may not be a need to induce apoptosis before cell injection; it would be instructive to understand the degree of apoptosis the transplanted nonirradiated cells underwent either as a result of the harvest and injection or exposure to the immediate postischemic environment, because only 3 mice receiving these cells developed intracardiac teratomas. It will also be important to understand whether these cells still improve cardiac function when they are injected at later time points. Although the current model shows beneficial effects when the cells are introduced immediately after the onset of ischemia, it is not clear whether the same mechanisms will be relevant in subacute or chronic settings. Finally, if the real therapeutic requirement is for apoptotic cells, other, less controversial cell sources with even less risk of teratomas or other ectopic structures might be chosen. These questions may serve as fertile grounds for future investigations.

One often-cited benefit of employing human ESCs as a therapeutic cell is that they are immortal and infinitely

expandable, which was typically seen as an advantage when the next step was to use them to generate specific differentiated cell types. In contrast to the challenges involved in generating large batches of homogeneous differentiated cells from the human ESCs, it would be comparatively easy to expand the undifferentiated human ESCs, irradiate them, demonstrate nonproliferation, and cryopreserve them until needed. If confirmed, the returns in cardiac function generated by these lethally irradiated cells represent a highly novel and intriguing new possibility for the treatment of ischemic heart disease.

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Disclosures

None.

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