LIGHTS AND SHADOWS IN CARDIAC REGENERATION

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ABSTRACT

Given that the adult human heart has an extremely limited regenerative capacity, diseases characterized by contractile cell loss, as myocardial infarction and cardiomyopathies, lead to ventricular remodeling and heart failure. Hence, diverse strategies to promote myocardial regeneration have been proposed and assessed in animals and humans with ischemic heart disease. Of these, gene transfer and especially stem cell therapy have been used. So far, the overall main outcome is a gross disparity between the promising results obtained in mammalian models and the poor, if any, benefit observed in randomized, controlled clinical trials. Many reasons may account for this disappointing scenario. Some, including flawed trial design and methodology, differences in cell type and dosing as well as in route of administration, erroneous end points selection and heterogeneous patient populations have been extensively discussed in comprehensive reviews. Others, more recently addressed, signal the use of inadequate or non-precise laboratory techniques in cell identification and fate, this leading to precarious or misleading conclusions. We hereby summarize part of the work done and quote some new approaches, like the use of induced pluripotent stem cells and the promotion of self-regeneration by targeting the adult cardiomyocyte cell cycle, that may cast some light in the otherwise shadowy field of cardiac regeneration.

RESUMEN

Dada la limitadísima capacidad regenerativa del corazón humano adulto, las enfermedades caracterizadas por pérdida de tejido contráctil, como el infarto de miocardio y las miocardiopatías, conducen al remodelamiento ventricular y la insuficiencia cardíaca. Por ello, diversas estrategias cardiorregenerativas han sido propuestas y evaluadas en modelos animales y pacientes con cardiopatía isquémica. De ellas, las más usadas han sido la transferencia génica y, especialmente, la terapia con células madre. Hasta aquí, el resultado global es una gran disparidad entre los prometedores resultados obtenidos en modelos animales y los pobres o nulos beneficios observados en los ensayos clínicos. Muchas razones explican este decepcionante escenario. Algunas, tales como imperfecciones de diseño y metodología, diferencias en el tipo y dosis de células así como en la vía de administración, puntos finales erróneamente elegidos, y heterogeneidad en las poblaciones de pacientes, han sido ampliamente discutidos en muy completas revisiones. Otros, más recientemente abordados, señalan el uso de inadecuadas o imprecisas técnicas de laboratorio para identificar el tipo de célula y su destino, conducentes a conclusiones precarias o engañosas. En este artículo resumimos parte del trabajo realizado y citamos algunos nuevos abordajes, tales como el uso de células pluripotentes inducidas y la auto-regeneración por manipulación del ciclo celular del miocardiocito adulto, que podrían arrojar algo de luz al sombrío campo de la regeneración cardíaca.

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Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, accounting for 31% of all deaths [1]. Of note, over three quarters of CVD deaths take place in low- and middle-income countries [2]. Of all CVDs deaths, 46% are due to ischemic heart disease (IHD), and this is so in spite of all the significant advances in early reperfusion strategies and medical treatment occurred over the past decades. The most severe complication of IHD is acute myocardial infarction (AMI), after which cardiac remodeling takes place [3,4]. This process comprises an early phase occurring in the first 3 days after AMI, during which the size of the infarct expands, and a late phase in which the remaining viable tissue undergoes hypertrophy, myocyte death, defective regeneration and progressive replacement of contractile myocytes by fibrotic tissue [4]. This progressive loss of contractile cells and their replacement by non contractile tissue leads to heart failure, a condition displaying about 40% one-year mortality, unless heart transplant is performed [5]. Diseases other than ischemic heart disease, such as dilated cardiomyopathy, chronic myocarditis, arterial hypertension and aortic valve stenosis, lead to remodeling and subsequent heart failure. According to recent estimations, heart failure prevalence is expected to increase, achieving approximately 46% by 2030 [6].

Unlike lower vertebrates (as for example the zebrafish and the salamander), the heart of adult mammals, including man, has very low regenerative potential, this determining that lost contractile tissue eventually results in heart failure. This reality has fostered, over the past 25 years, intense research aimed at regenerating the heart.

Several strategies have been tested in animal models of IHD, including protein injection, gene transfer, stem cell therapy and implant of bioresorbable scaffolds seeded with cells (Figure 1). Of them, those that have awoken the largest interest and research are gene and stem cell therapies. In the vast majority, these studies have conveyed positive results, basically consisting of infarct size reduction and improvement in left ventricular function. However, these results have not been observed in clinical trials, in which the beneficial effects, if any, have been minimal and devoid of clinical relevance.

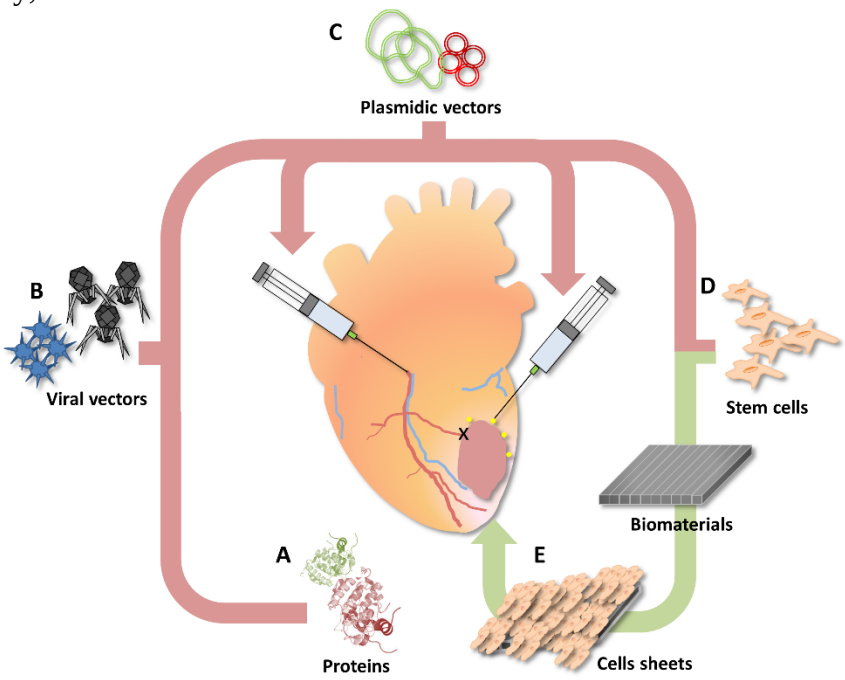

Figure 1. Cardiac regeneration strategies in a heart with a myocardial infarction resulting from coronary artery occlusion (cross indicates site of occlusion). Angiogenic proteins (A), gene transfer in viral (B) or plasmidic (C) vectors, and stem cells from diverse origins (D) can be delivered using the intracoronary (left syringe) of direct intramyocardial (right syringe) injection routes. (E) Stem cells seeded on bioresorbable scaffolds (cell sheets) can be implanted on epicardial surface of the infarct.

The present mini-review intends to summarize the principal strategies tested, point out some pitfalls obscuring their translational impact and refer to a couple of new approaches that may help to revive our hope in cardiac regeneration.

Gene therapy

Gene therapy is defined as the transfer of nucleic acids to somatic cells with a resulting therapeutic effect [7]. In the case of IHD, genes encoding for angiogenic growth factors and mitotic cytokines have been transferred using plasmidic and viral vectors in small and large mammalian models of chronic myocardial ischemia (CMI) and AMI. In pigs with Ameroid-induced CMI, we observed that the intramyocardial injection of a plasmid encoding human vascular endothelial growth factor-165 (VEGF165) induced, in addition to its classic angiogenic action, a cardiomyogenic effect consisting in a several-fold increase in cardiomyocyte mitotic index and cardiomyocyte hyperplasia [8,9]. These results, along with an attenuation of LV remodeling and improved cardiac function, were later confirmed in sheep with AMI at short- and long-term follow up periods [10,11]. VEGF165 gene therapy has been used in placebo-controlled clinical trials. The two largest ones were the EUROINJECT and the NORTHERN trials, including 80 and 93 patients with angina functional class 3 to 4, respectively [12,13]. None of them resulted in significantly improved stress-induced myocardial perfusion compared with placebo at 3 and 6 months follow up.

In the placebo-controlled AGENT-2 trial, adenoviral-fibroblast growth factor-4 (AdFGF4) or placebo administered to 52 patients with chronic angina pectoris induced a reduction in the LV ischemic burden [14]. On this basis, 2 new trials (AGENT 3 and 4) started but were discontinued when an interim analysis anticipated that the primary end point [a change in exercise treadmill time (ETT) duration at 12 weeks] would not be achieved. Later, the authors performed a pooled analysis of the 532 patients that had been enrolled in those 2 trials and found no differences in the same end point between groups. Surprisingly, when analyzing men and women separately, the differences achieved significance in the latter because, unlike in men, in women the placebo effect was negligible [15].

Other genes (or their encoded proteins) that, on the basis of positive results in animal studies, have been tested in controlled clinical trials include VEGF121 [16], granulocyte colony-stimulating factor (G-CSF) [17], GM-CSF (granulocyte-macrophage colony stimulating factor [18] and FGF2 combined with VEGF165 [19]. Overall, and even in the few cases in which some of the end points were met, the results were disappointing in terms of clinical relevance.

Stem Cell therapy

Currently, the most investigated strategy for heart regeneration is the myocardial implantation of stem cells of diverse origins and differentiation potential. As occurs with gene therapy, the most habitual routes of administration are intracoronary instillation and direct intramyocardial injection. The cells types that after undergoing preclinical evaluation in animal models have been tested in randomized, placebo-controlled clinical trials include skeletal myoblasts (SM), bone marrow mononuclear cells (BMMC), mesenchymal stromal cells (MSC), and cardiac stem cells (CSC).

When administered to patients with IHD, SM not only failed in differentiating into cardiomyocytes but also induced life-threatening arrhythmias [20].

As for BMMC, more than 3000 patients have been included in over 50 clinical trials of IHD [21]. Of the few that were designed in a randomized, double-blind, placebo-controlled fashion, only the REPAIR-AMI trial showed significant, though clinically

modest, benefit [22]. In fact, a meta-analysis of 2625 patients enrolled in 50 published studies [23], as well as more recent reviews [24], concluded that 1) the treatment is safe, and 2) patients receiving BMMC displayed only moderate improvements in LV function parameters. Furthermore, to date, true myocardial regeneration with new cardiomyocytes or vessels emerging from the transplanted cells has not been shown in any clinical study. This is not surprising, because although the therapy approach was originally founded on the potential ability of bone marrow-derived stem cells to differentiate into cardiomyocytes, this phenomenon was later shown to be extremely rare or inexistent [25-27]. It is now proposed (yet not studied in depth) that the benefits, if any, of these cells are due to paracrine effects of the multiple growth factors and cytokines that they secrete during the very few days that they stay viable in the injected site [28].

As in the case of BMMC, the controlled clinical trials carried out with mesenchymal stromal cells harvested from the bone marrow [29,30] demonstrated a good safety profile but modest or no benefit.

In 2003 Beltrami and colleagues from the Piero Anversa's group, using immunohistochemistry, reported that the adult heart contains a population of c-kit+ cells responsible for the normal, physiological turnover of cardiomyocytes in humans [31], and named them cardiac stem cells (CSC). On the basis of later studies reporting that CSC, or clusters of CSC termed cardiospheres, induced myocardial regeneration in animal models, the phase I CADUCEUS trial was conducted. In this study, intracoronary delivery of cardiospheres in patients with recent myocardial infarction resulted in infarct size reduction but no improvement in LV function [32]. Recently, studies using laboratory methods much more rigorous and sensitive than immunohistochemistry have severely and repeatedly challenged the regenerative role and even the existence of CSC in the adult heart [33-35]. As a consequence, several papers of the Anversa's group have been retracted [36].

Over the past 10 years, the advent of induced pluripotent stem cells (iPSC) has casted a grim of light to the otherwise gloomy scenario of stem cell-induced cardiac regeneration. Since the pioneering work of Takahashi and colleagues [37], generating a pluripotent stem cell from a terminally differentiated cell has become a reality in many laboratories worldwide. By means of the appropriate differentiating protocol, practically any cardiac cell type can be obtained from iPSC. Many pitfalls, including the risk of teratoma formation, have to be overcome before clinical testing, but there is no doubt that this is a very promising field in heart regeneration. In fact, it has been recently shown that iPSC restore cardiac function in primates with myocardial infarction [38].

Discussion and perspectives

The hope of repairing a failing heart, initially by transferring genes and later by implanting stem cells, has evolved from the enthusiasm generated by positive, promising results in animal models to the frustration emerging from the disappointing outcomes in patients.

In a recent superb critical analysis of stem cell-mediated cardiac regeneration, Chien et al. have qualified this regrettable process as a scientific and clinical tragedy, and point out a number of reasons for it, including a tendency of the scientific community to ignore troubling signals, deficiencies in the peer review systems that regulates scientific publication, a strong tendency of the journals to privilege positive results or breakthroughs, lack of skepticism or self-criticism, and widespread scientific misconduct [39].

One of the problems that have not been rigorously taken into account is the fact that the myocardium is an electromechanical syncytium with a complex, precise physiology.

Therefore, repopulating the heart with new cells does not suffice to mend an injured heart and recover its pump function. The implanted cells, even if they are shown to be contractile prior to implantation, should establish the correct, physiological connections with the resident cells. Otherwise, the pumping ability will fail to improve. So far, electromechanical coupling of implanted cells has not been convincingly demonstrated in vivo with any stem cell type, except iPSC [40]. Hence, it is not surprising that in the few controlled clinical trials showing positive results (note that no controlled trials using iPSC have been conducted) the benefit consisted in infarct size limitation but not in improved LV function.

A compelling approach to overcome this limitation would be to induce the adult, resident cardiomyocytes to re-enter the cell cycle and divide into daughter cells through interventions targeting the cell cycle regulators (Figure 2). In this way the electromechanical coupling indispensable for an adequate functioning of the myocardial syncytium is more likely to be preserved, thus representing a more physiological approach to cardiomyogenesis [41].

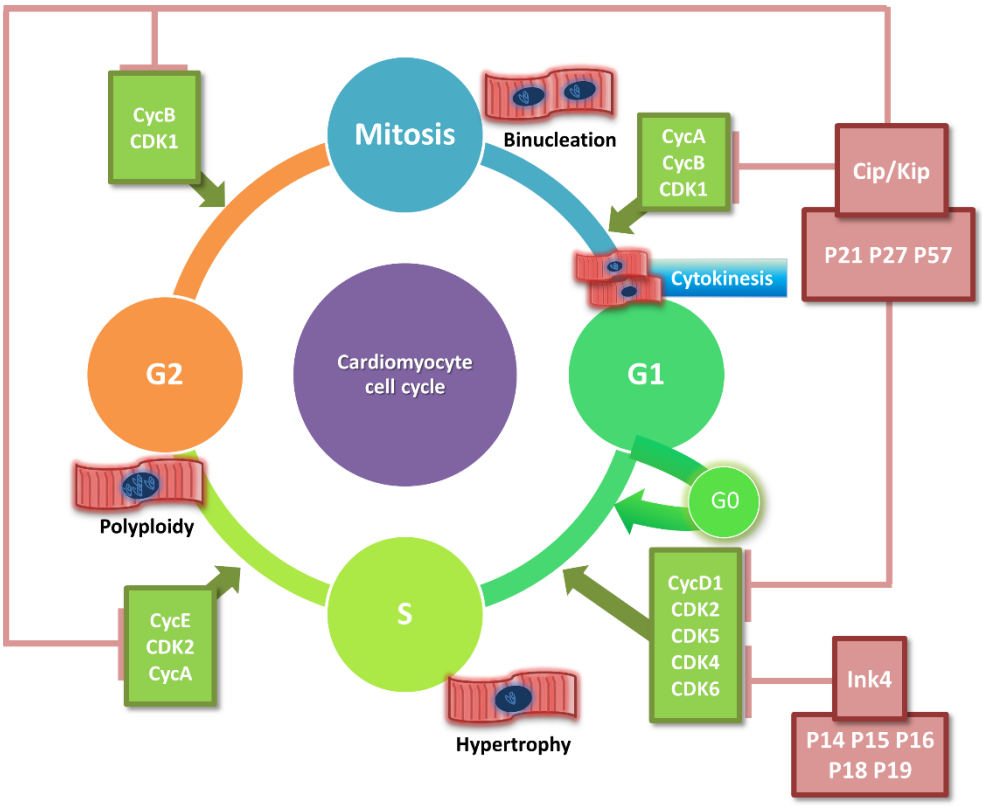

Figure 2. Schematic representation of the cardiomyocyte cell cycle. In each phase, cell cycle promoters are shown in green and their corresponding inhibitors in red. Transition from G1 to S allows for cardiomyocyte hypertrophy and the S/G2 transition results in polyploydization. Further advancement into the M phase to allow for mitosis and eventual cytokinesis are very infrequent, this explaining the extremely limited regenerative capacity of the adult myocardium. Abbreviations: Cyc: cyclin; CDK: cyclin-dependent kinase; Ink4 and Cip/Kip: CDKs-inhibitors families; P: pocket proteins.

The classical dogma of cardiomyocytes being a totally post-mitotic cell is no longer valid. Adult cardiomyocytes can reenter the cell cycle and even advance into mitosis. In necropsies of patients dying of AMI mitotic cardiomyocytes may be found (Figure 3). Unfortunately, this is an infrequent event, and not at all sufficient to replace the lost contractile tissue. Overall, the regenerative capacity of human cardiomyocytes is very

poor, its annual turnover rates being 1% at age 20 and 0.5% at age 50 [42]. However, since cardiomyocytes display cell cycle activity without cytokinesis in several processes such as cell growth, polyploidization and binuleation, there is a growing interest in disclosing cell-cycle modulators that could eventually be targeted to encourage the adult cardiomyocyte to divide into daughter cells. In murine cardiomyocytes there are at least three different levels of regulation that limit proliferation: 1) epigenetic regulation involving pre-transcriptional heterochromatin-mediated gene silencing of positive cell cycle regulators [43], 2) transcriptional activation of negative cell cycle regulators [44], and 3) post-transcriptional regulation through microRNAs (miRNAs) [45]. Strategies to intervene over these 3 levels using genes encoding proteins that stimulate cell cycle progression and/or microRNA that inhibit cell cycle negative regulators have been made in animal models, and are being intensively investigated at present [46,47].

While it is now too early to conclude that cardiac regeneration based on resident cardiomyocyte proliferation is safe, effective and feasible, it is worth continuing and intensifying its research, given that it is founded on a sound, physiological rationale that takes into account not only cardiomyogenesis but also preservation of the normal syncytial function. Meanwhile, basic research disclosing the multiple still unknown molecular mechanisms governing the cardiomyocyte cell cycle in large mammals are indispensable, should this compelling approach to cardiac generation be successful.

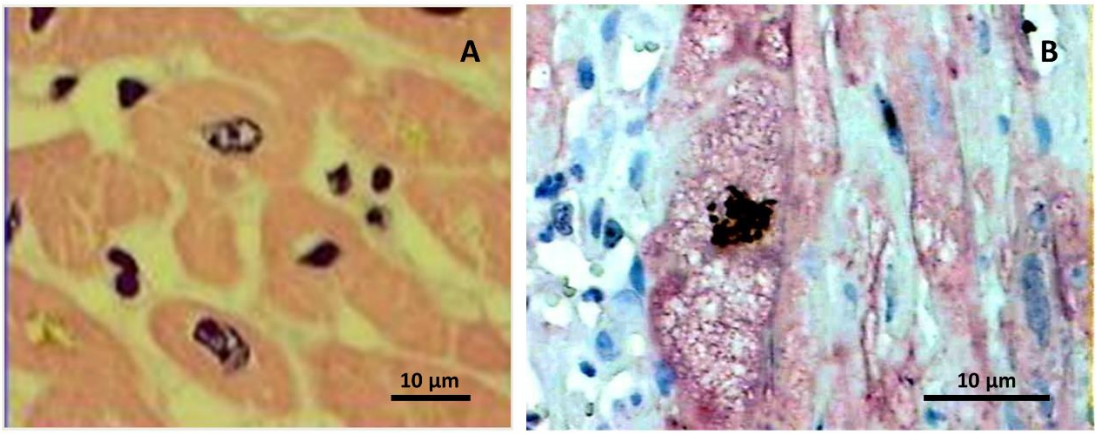

Figure 3. Cell cycle activity in human adult cardiomyocyes as indicated by positive reaction against de Ki67 antigen. (A) Confocal microscopy image of polyploid cardomyocytes in a necropsy sample of a patient that had suffered arterial hypertension. The red staining corresponds to immunohistochemistry against sarcomeric α-actin. (B) Mitotic image of a cardiomyocyte in the border of a myocardial infarction (necropsy sample). Anti-sarcomeric α -actin immunohistochemistry and hematoxylin counterstain.

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Dr. Carlos Sebastián Giménez graduated in Biotechnology and Molecular Biology at La Plata University. In 2013 he started his PhD studies at the Department of Physiology, Favaloro University, directed by Dr. Alberto Crottogini, as a doctoral fellow of the National Agency for the Promotion of Science and Technology and later of CONICET. In 2015 he received training in Gene Therapy at Sao Paulo University, Brasil, as a fellow of the Brasilian-Argentine Council of Biology (CABBIO). In March 2018 he received the PhD degree at University of Buenos Aires. Between 2012 and 2018 he was teaching assistant in General Chemistry at the Faculty of Exact Sciences, La Plata University. Since March 2018 he is a post-doctoral fellow of CONICET at the Institute of Translational Medicine, Transplant and Biomedical Engineering (CONICET-Favaloro University) under the direction of Dr. Crottogini, and senior teaching assistant at the Department of Physiology of the same University. He is author of 7 Pubmed-indexed papers and of several oral and poster communications in national and international scientific meetings. He has been awarded the Camilión de Hurtado Award of the Argentine Society of Physiology in 2016 and the Dr. Bernardo Houssay Award of the Argentine Society of cardiology in 2017. His research interest is cardiovascular regeneration using stem cells, genetic engineering and biomaterials.

Dr. Alberto José Crottogini received his MD, PhD and specialist in cardiology degrees from University of Buenos Aires. He was resident physician in internal medicine at Rawson Hospital, Buenos Aires, staff physician at the critical care unit of the Buenos Aires British Hospital and chief of the Intensive and Coronary Care Unit at Diego Thompson Hospital, San Martín, Buenos Aires Province. After completing a 2 years clinical and research fellowship at Copenhagen University, Denmark, granted by the Danish International Development Agency, he joined the Basic Science Research Institute of the Favaloro Foundation, and received further training in chronically instrumented large mammals for cardiac mechanics research at Dr. John Ross's Seaweed Canyon Laboratory, University of California, San Diego. Dr. Crottogini entered CONICET's Clinical Investigation Career in 2004. At present, he is Superior Investigator of CONICET, chairman and full professor of the Department of Physiology at Favaloro University in Buenos Aires, former Director of the Institute for Translational Medicine, Transplant and Biomedical Engineering (IMETTYB- CONICET-Favaloro University) and former president of the Argentine Society of Physiology (SAFIS). He has published 52 scientific papers indexed in Pubmed, has acted as invited speaker at more than 100 scientific meetings in Argentina and abroad, and has received 19 awards along his career. Currently, Dr. Crottogini is a member of the Inter-Ministry Advisory Committee on Cell Therapies and Regenerative Medicine (Ministry of Science and Ministry of Health) and of the Translational