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Review

Biochemistry and biology: Heart-to-heart to investigate cardiac progenitor cells ☆

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ABSTRACT

Background: Cardiac regenerative medicine is a rapidly evolving field, with promising future developments for effective personalized treatments. Several stem/progenitor cells are candidates for cardiac cell therapy, and emerging evidence suggests how multiple metabolic and biochemical pathways strictly regulate their fate and renewal.
Scope of review: In this review, we will explore a selection of areas of common interest for biology and biochemistry concerning stem/progenitor cells, and in particular cardiac progenitor cells. Numerous regulatory mechanisms have been identified that link stem cell signaling and functions to the modulation of metabolic pathways, and vice versa. Pharmacological treatments and culture requirements may be exploited to modulate stem cell pluripotency and self-renewal, possibly boosting their regenerative potential for cell therapy.
Major conclusions: Mitochondria and their many related metabolites and messengers, such as oxygen, ROS, calcium and glucose, have a crucial role in regulating stem cell fate and the balance of their functions, together with many metabolic enzymes. Furthermore, protein biochemistry and proteomics can provide precious clues on the definition of different progenitor cell populations, their physiology and their autocrine/paracrine regulatory/signaling networks.
General significance: Interdisciplinary approaches between biology and biochemistry can provide productive insights on stem/progenitor cells, allowing the development of novel strategies and protocols for effective cardiac cell therapy clinical translation. This article is part of a Special Issue entitled Biochemistry of Stem Cells.

1. Introduction

As a leading cause of worldwide morbidity and mortality, heart failure (HF) has pushed basic, pharmacological and clinical research efforts to develop novel effective therapeutic treatments, particularly in the last two/three decades, also for the rising number of affected individuals, parallel to the progressive aging of the global population. The result is that, while seen in the past as an untreatable condition, HF is now considered a chronic disease, nevertheless with a highly demanding human and social cost.

Pharmacological therapies and primary/secondary prevention have traditionally targeted the heart's pump function and the quality of life for end-stage HF patients, without leading to actual replacement of diseased tissue and, thus, without stopping or reversing the progression of adverse left ventricular (LV) remodeling [1]. The use of stem/progenitor cell-based therapy is becoming increasingly important as a powerful strategy to recover damaged myocardium and to promote endogenous repair of cardiac tissue [2,3]. The final aim is obviously the regeneration of functional, vascularized and integrated contractile tissue to compensate for the functional loss of the organ. Although the available data in this area is highly debatable, the potential of cell-based therapy for the treatment of HF remains an alternative option, with the result that widespread laboratory and clinical studies on their use for cardiac repair are ongoing, raising great expectations, as well as controversies. In fact, examining the basic and clinical studies concerning cardiogenic cells used for therapeutic purposes, major efforts have been spent to compare different kinds of possible adult cell sources and candidates (autologous/heterologous, bone marrow/skeletal/cardiac resident biopsy-derived), the delivery methods (intracoronary/direct injection, tissue-engineering combined) and the timing of sampling and intervention. Thus far, cell therapy with ectopic cells has reduced LV end-systolic volume (LVESV), a result that is consistent with a systolic functional benefit, but different from what has been obtained by other therapies. In fact, treatments based on pharmacological intervention or assist devices are able to reverse cardiac remodeling, but still cannot impart long-term benefits for patients.

The mechanism by which cell therapy reduces LVESV is most likely linked to paracrine signaling and the possible cross-talk with the surrounding environment, inducing a more or less prolonged angiovasculogenesis [4].

After the introduction of the first method for the isolation and expansion of cardiac progenitor cells (CPCs) from human heart biopsies

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[5] (Patent number: WO2005012510), multiple basic and preclinical studies have rapidly brought this technology to clinical application, with at least three phase I/II clinical trials almost completed (SCIPIO [6], CADUCEUS [7], ALCADIA [8]; see www.clinicaltrials.gov for details). Better clinical results, in terms of cardiac regeneration, have been reported by Makkar RR et al. [7] (CADUCEUS), who have isolated and expanded CPCs from endomyocardial bioptic samples obtained from the diseased human heart of patients with recent myocardial infarction (MI), followed by autologous retransplantation via intracoronary injection, thus suggesting that this therapeutic approach is feasible and has the potential to provide a treatment strategy for cardiac regeneration after MI. However, improvement in ejection fraction (EF) is not consistent with what was expected from preclinical studies [9,10]. Thus, other factors, such as the beneficial cell paracrine activity, the host tissue or even its cross-talk with the bone marrow, as well as other (still) unpredictable variables, represent open questions. Moreover, methodological bias would be introduced if the individual patient's specific features were not taken into account.

Over the past decade, the shifting of research interest from reversing the remodeling process to cell-based therapies has promoted the rationale of the two approaches being adequately combined together. This conceptual hole needs to be filled by figuring out how patients' specific cardiac remodeling (involving structural changes, such as hypertrophy, fibrosis, and dilation, and multiple abnormalities of cellular and molecular function, as well as inflammatory cytokines and growth factors) should be challenged and integrated with the individual molecular, biochemical and functional modifications occurring to cells during sampling, in vitro growth and in vivo interaction with the evolving microenvironment. These concepts are attracting particular interest from the scientific community, while acquiring potent tools with the integration of "omics" strategies with personalized medicine. The availability of genomic, proteomic, transcriptomic and metabolomic data combined, allows us to revisit the scientific basic and translational potential of these individual tools, which are expected to provide a much extended impact on regenerative medicine, and in other medical and biotechnological fields, as well.

Cardiac regenerative medicine is becoming oriented toward biochemistry, metabolism and related functions, in a more integrated and personalized approach. In order to use stem/progenitor cells for therapeutic purposes, it is important to control their differentiation and regulate their pluripotency and self-renewal. So far most of identified regulators of stem cell fate are growth factors, transcription factors, cell cycle regulators, as well as their associated downstream signaling pathways [11]. Recent evidences suggest that also mitochondria have a crucial role in regulating stem cell fate, both as the center of cellular respiration and as a central platform in the regulation of diverse cellular events [12].

Several regulatory mechanisms are well known that either link cell signaling to the modulation of metabolic pathways or enable cells to sense fuel availability and regulate signaling networks accordingly [13], overall influencing metabolism and gene expression. In the field of regenerative medicine, the importance of these networks is obviously gaining particular interest, to discover and exploit new metabolic key regulators of stem/progenitor cell proliferation/differentiation, in the contest of each individual diseased tissue.

In this regard, taking advantage of the extensive scientific knowledge on embryonic development and cancer biology, as a paradigmatic example of how a shift in metabolic pathways can strongly influence cell biology and functions, a fundamental question can be raised, that is why experts in the field of stem cells biology and biochemists should be mutually interested in their respective expertise, and should share their specialized knowledge. In this review we will attempt to provide some perspectives on this, particularly in the field of cardiac regenerative medicine or under a more general light for topics that have not been thoroughly investigated in cardiovascular biology, by focusing and discussing the following issues:

- stem cells and energy metabolism: external versus internal signaling networks and possible targets for preconditioning pharmacological interventions,
- stem cells and in vitro culture conditions: physical (oxygen), chemical (calcium) and nutrient (glucose) requirements,
- control of regulatory pathways and metabolic changes induced by different environmental conditions, stimulatory factors and small molecules,
- proteomic perspectives to discover novel networks and markers as diagnostic, tracing and therapeutic tools for clinical applications.

2. A brief overview: resident cardiac progenitor cells

The difficulty of regenerating damaged myocardial tissue has led researchers to test different stem/progenitor cell types as possible sources for cell therapy, including embryonic stem cells (ESCs), myoblasts (muscle stem cells), adult bone marrow-derived cells, mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), umbilical cord blood cells and cardiac progenitor cells (CPCs) that naturally reside within the heart. All have been tested in mouse or rat models, while some of them in large animal as well, such as pigs, and in human clinical trials [14–18].

The most appropriate cells for replacing dead cardiomyocytes appear to be cardiomyocytes of fetal or embryonic origin, since they can functionally integrate with the host tissue [19,20]. The ideal cell to be transplanted for cardiac regeneration, though, should probably be in between a highly undifferentiated phenotype (e.g. ESCs) and terminal differentiation (cardiomyocyte). It should be characterized by defined proliferative potential in the host without induction of immune reaction; cardiac commitment and capacity to develop gap-junctions with the host cells, and should preferably be resistant to ischemia, in order to avoid massive cell death and apoptosis, that currently are the biggest hurdles for cell therapy clinical translation.

With these premises, it seems obvious that the best cells to replace lost cardiomyocytes may be cells derived from the heart itself. Emerging evidence suggests that several populations of CPCs are present in the heart, and extensive basic research still needs to be performed to better understand the relationship among these different populations. CPCs are positive for various stem/progenitor cell markers (c-Kit, Sca-1, Isl-1) and have Side Population (SP) properties. In fact, their presence into the heart, the frequent co-expression of early cardiac progenitor transcription factors, and the capability for ex vivo and in vivo differentiation toward cardiac lineages offer the promise of enhanced cardiogenicity compared to other non-cardiac cell sources.

Different methods have been used to isolate CPCs from the heart, based on multiple criteria, and that have been characterized in vitro and tested in vivo in animal models:

- Ability to efflux Hoescht dye (side population, SP).
  SP cells, which have the ability to efflux Hoechst dye (a process dependent on the expression of MDR1, Abcg2 or similar ABC membrane transporters), have been identified not only in the developing, but also in the adult heart of mice [21,22]. These cells are rare and their ability to differentiate into contracting cardiac myocytes or to contribute to functional repair of damaged heart muscle has not been extensively evaluated yet.
- Expression of cell-surface stemness markers (c-kit or Sca-1).
  In the adult heart a distinct population of c-kit+ CPCs has been isolated. These relatively small and primitive cells are negative for blood lineage markers and positive for c-kit, the receptor for the stem cell factor. These cells are self-renewing, clonogenic and multipotent, giving rise to cardiomyocytes, smooth muscle and endothelial cells. When injected into the infarct border zone in adult rats, these CPCs differentiated into newly formed myocardium, including cardiomyocytes, capillaries and arterioles in the infarcted area [23]. C-kit positive cells have been tested in the SCIPIO clinical trial [6].

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