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Growth Factor Enhancement of Cardiac Regeneration

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The potential for endogenous or supplementary stem cells to restore the form and function of damaged tissues is particularly promising for overcoming the restricted regenerative capacity of the mammalian heart. To maintain blood circulation, this essential organ needs to launch a rapid response to repair damage of the muscle wall and to prevent muscle loss. The capacity of growth factors to supplement the repair process has been successfully applied to restore the integrity of damaged skeletal muscle, reducing the fibrotic response to injury, and recruiting local populations of self-renewing precursor cells and circulating stem cells. We review the recent evidence that extension of growth factor supplementation to the heart may overcome its inherent regenerative impediments through improvement of the local tissue environment and stimulation of cell replacement, and we speculate on future research directions for treatment of myocardial damage.

Key words: Muscle; Heart; Stem cells; Regeneration

INTRODUCTION

The mammalian heart must maintain its structural and functional integrity for decades, yet the response to damage in this vital organ is remarkably inadequate and often results in heart failure, an increasingly prevalent cause of morbidity and mortality in the industrialized world. The impediments to cardiac repair have been attributed to the distinct embryonic history of the heart, which is the first fully differentiated structure to form and function in development. During fetal stages, actively contracting cardiomyocytes undergo rapid cell division to ensure further growth of the heart. This phase of proliferation ends soon after birth, when the capacity for cardiomyocytes to reenter the cell cycle appears to be largely lost, and increases in myocardial mass are achieved largely through cellular hypertrophy. In the adult, the relative rarity of progenitor cells residing within the heart may impose severe limits on tissue repair. The injured heart needs a rapid response to repair damage to the muscle wall and maintain adequate blood flow to the rest of the body, but too often cannot restore the muscle loss that accompanies myocardial infarction and ischemia-reperfusion injury. Interruption of the coronary blood supply results in further cell death and fibrotic scar formation at the cost of functional muscle. Remaining cardiomyocytes respond by cellular hypertrophy, leading to decompensated function and congestive heart failure.

Although cardiac regeneration has been presented as an evolutionary variable (6) illustrated by the robust proliferative capacity of the injured heart in other vertebrate such as newt and zebrafish (20,21), the mammalian heart is capable of homeostasis, occurring throughout life by cell renewal in the myocardium (18,19). However, the complete regenerative program in case of extended injury is precluded in mammalian heart by fibrotic tissue formation and consequent cardiac functional impairment. It is likely that the restricted cardiac repair program in mammals is presumably due to missing signals that render the damaged myocardium of lower vertebrates a permissive environment for regenerative activity.

Given these limitations, strategies for repair of the damaged heart have followed two general approaches: injection or implantation of autologous or heterologous regeneration-competent progenitor cells, or induction of endogenous regenerative mechanisms in the damaged myocardium. Although the first approach has been moderately successful at improving function through as yet undetermined mechanisms (24), it is increasingly clear that the appropriate tissue environment is critical for progenitor cells to be productively recruited and reconstitute injured tissue. Indeed, improving the tissue envi-

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ronment may broaden the scope of adult cell types that can participate in functional restoration of the myocardium.

One strategy to provide a regenerative environment in the damaged heart involves the use of insulin-like growth factor-1 (IGF-1), implicated in the control of cardiac growth in vitro and in vivo (9). Injury of mammalian tissues induces transient production of locally acting IGF-1 isoforms that control growth, survival, and differentiation (10,26). By contrast, circulating IGF-1 isoforms have been implicated in the restriction of life span (1,14) and have contrasting effects on the heart when expressed as transgenes, variously promoting cell survival, or inducing prolonged hypertrophy with pathological consequences (7,23). The diverse forms of IGF-1 are encoded in a single Igf-1 gene with complex structure and regulation, as well as posttranslational modifications that remodel IGF-1 during protein biogenesis (26).

LESSONS FROM SKELETAL MUSCLE

The traditional paradigm of the heart as an organ incapable of regeneration has been put into question by recent descriptions of a compartment of multipotent cardiac stem cells in the adult heart, capable of regenerating myocytes and coronary vessels throughout life (10). These studies provide a plausible explanation for the inherent self-renewing capacity of the adult organ in an unchallenged state, but major questions regarding cardiac physiology remain to be resolved. If cardiac muscle possesses a stem cell compartment it is not clear why the heart fails to regenerate under pathological conditions. Either the resident cardiac stem cells are too rare or intrinsically incapable of repairing major damage, or perhaps the damaged myocardium is a prohibitive environment for activation. Although we lack definitive answers to the questions, we can speculate on the basis of the processes involved in the regeneration of skeletal muscle that the mere presence of endogenous stem cells may not be sufficient to guarantee cardiac regeneration, and that the presence of appropriate stimuli and factors is necessary to provide a permissive environment that allows stem cell-mediated cardiac regeneration and repair.

The molecular mechanisms governing skeletal muscle regeneration are likely to be informative if not paradigmatic in the activation of cardiac regenerative processes. Skeletal muscle regeneration is a coordinate process in which several factors are sequentially activated to maintain and preserve muscle structure and function upon injured stimuli.

The major role in the growth, remodeling, and regeneration is played by satellite cells, a quiescent population of myogenic cells that reside between the basal lamina and plasmalemma and are rapidly activated in response to appropriate stimuli. Among these, IGF-1 has been implicated in many anabolic pathways in skeletal muscle and plays a central role during muscle regeneration (26). We have previously documented the regenerative properties of a locally acting isoform, mIGF-1 in skeletal muscle and its dramatic promotion of cell survival and renewal in senescent muscle (16). Expressed as a muscle-specific transgene or on a viral vector, mIGF-1 elicits a striking increase in skeletal muscle mass and strength, a rapid restoration of injured muscle, reducing scar formation. Enhanced mobilization and homing of bone marrow-derived cells by local mIGF-1 is triggered by damage, and presumably contributes to increases in muscle mass, strength, and resistance in age-related atrophy and degenerative disease (17).

In mIGF-1 transgenic muscle, the dramatic increase in the bone marrow stem cell pool immediately after local muscle injury suggested a potential feedback mechanism whereby this rich source of stem cells is mobilized in response to distal trauma. After lethal irradiation of mIGF-1 mice to ablate native bone marrow, exploitation of donor bone marrow carrying a GFP transgene driven by the c-kit promoter allowed the tracking of transplanted bone marrow-derived cells to skeletal muscle early in the regeneration process. The fourfold increase in GFP-positive cells migrating to the injured muscle of mIGF-1 transgenic mice compared to wild-type littermates suggests that local mIGF-1 induces the production of signals to increase the mobilization of uncommitted cell subsets in the bone marrow, which migrate to sites of tissue damage and participate either directly or indirectly in the regeneration process. The enhancement of circulating cells drawn to regenerating mIGF-1 muscle tissue was not dependent upon lethal irradiation, as confirmed by the increase in c-kit/GFP+ cells after muscle injury of nonirradiated double transgenic mice. However, the dramatic increase in progenitor cells in mIGF-1 transgenic injured muscles was ablated by administration of 5-FU, a potent repressor of the cell cycle, which did not affect the abundance of nonproliferative cells at the site of injury. Thus, regenerative cell populations increased by the presence of mIGF-1 rely on proliferative capacity (16).

In primary cultures, mIGF-1 transgene expression expanded the myogenic population in response to muscle damage, containing 50% more myoblasts than did wild-type control cultures. When cocultured with bone marrow subpopulations that are not inherently myogenic, skeletal myoblasts isolated from mIGF-1 transgenic muscle stimulated the conversion of bone marrow cells to a myogenic lineage. In the skeletal muscle context, the mIGF-1 isoform is a powerful enhancer of local re-

generation, mediating the recruitment of bone marrow cells to sites of tissue damage and their contribution to muscle repair (16). Taken together, these data emphasize the importance of inductive signals, which increase the efficiency of stem cell action and improve tissue regeneration.

FROM SKELETAL TO CARDIAC MUSCLE

Recent application of IGF-1 to the setting of myocardial infarction has demonstrated that this growth factor can also contribute to an improved tissue environment in the damaged heart. Cardiac stem cells and early committed cells express IGF-1 receptors and synthesize and secrete IGF-1 (25). When fully processed IGF-1 was injected together with hepatocyte growth factor (HGF) into infarcted mouse hearts, this combination of growth factors induced the formation of new myocardium that contained arterioles, capillaries, and functionally competent myocytes that gradually increased in size, improving ventricular performance. This improvement in myocardial regeneration was achieved through stimulation of an endogenous reserve of progenitor cells that can be activated to reconstitute dead myocardium and recover cardiac function. This exciting study suggests that the heart can be transformed into a regeneration-competent organ through the combined actions of growth factors that improve the local environment and activate endogenous stem cell reserves (25).

To determine the extent to which supplemental expression of the mIGF-1 isoform on its own was capable of promoting regeneration of cardiac tissue, we generated transgenic mice in which the mIGF-1 transgene was driven by a cardiac gene promoter, to restrict expression of mIGF-1 to the mouse myocardium and exclude possible endocrine effects on other tissues. Transgenic mIGF-1 expression levels increased with age exclusively in the heart, which precociously attained adult size without perturbing function. In contrast to the characteristic progression of scar formation in wild-type hearts after myocardial infarction, mIGF-1 transgene expression induced repair of the injured tissue, with minimal scar formation after 1 month and integrity of the posterior wall and normal echocardiographic profiles. Functional parameters of the recovered mIGF-1 hearts were also significantly improved when compared to infracted control animals. This functional improvement was due in part to an amelioration of the cellular environment of the post-infarct heart, with marked reduction in inflammatory markers and increased peri-infarct proliferative activity. These preliminary results suggest that in the mIGF-1 transgenic hearts, cardiac regeneration involves early resolution of inflammation at the site of injury to prevent scar formation, making way for the subsequent tissue replacement that restores form and function (M.P.S. and N.R., manuscript in preparation).

The origin and identity of cells that are stimulated by IGF-1 to promote cardiac regeneration is still under debate. Some of these cells may correspond to a population of rare small cycling cardiomyocytes that retain the capacity to proliferate in response to damage (5,8,12). Cells capable of differentiating into a myocyte in the adult heart (15,22) could also originate through the commitment of precursor cells to the myocyte lineage. Indeed, undifferentiated precursor cells in the adult heart (2,11) have been identified through cell surface proteins that mark stem cell populations in other tissues. In some cases these cells give rise to clones that express biochemical markers of myocytes, smooth muscle, and endothelial cells in vitro, underscoring their stem cell-like nature. It is also possible that endothelial precursors are stimulated by mIGF-1 to contribute directly to regenerating myocardial tissue. Indeed, adult human endothelial progenitor cells, derived from peripheral blood mononuclear cells, or hematopoietic progenitor cells can convert into cardiomyocytes when cocultured with rat cardiomyocytes (3). In these studies, cell–cell contact or an extracellular matrix-associated signaling appeared to be critical, because conditioned media from cardiocyte cultures was not sufficient for conversion to a cardiomyocyte phenotype. Thus, it is possible that supplemental mIGF-1 improves the tissue environment by reducing the fibrotic response through specific cell signaling pathways.

Cycling myocardial cells induced by growth factor administration may also derive from recently characterized cells isolated from the adult rat heart that retain stem cell characteristics (4). These cells are self-renewing, clonogenic, and multipotent in vitro and in vivo and give rise to myocytes and smooth muscle and endothelial vascular cells. When injected into an ischemic rat heart, a population of these cells or their clonal progeny reconstitute up to 70% of the injured myocardial wall. This endogenous pool of primitive multipotent cells can be recruited into the regenerative process by the intramyocardial injection of a growth factor cocktail (IGF-1 and HGF) to promote their translocation to the damaged area and activate their growth and differentiation, resulting in the formation of functionally competent myocardium. In this study, the blunted response to HGF or IGF-1 reported for alone has been attributed to the need for improvement of survival and promotion growth provided by IGF-1 together with the chemotactic effects of HGF. However it is possible that fully processed IGF-1 may not maintain the full spectrum of activities seen for the endogenous forms such as mIGF-1 (16). However, the possibility that endogenous cardiac stem cells can be mobilized by growth factors to migrate from their niche

within the healthy heart to support regeneration of diseased myocardium has exciting implications for therapeutic intervention (13).

FUTURE PROSPECTS

The repair of the mammalian heart is fast becoming a feasible goal of regenerative medicine. In the near future, cardiac restoration is likely to be augmented through a number of avenues (Fig. 1). The appropriate source of cells for these therapeutic applications is hotly debated, but the technical feasibility of using stem cell therapy to aid in replacement of cardiac tissues is well within realistic projections. Recent advances in our understanding have uncovered an unexpected dynamism in cardiac homeostasis and highlight the heterogeneous proliferative potential of resident cardiomyocytes. Although resident cardiac progenitor cell populations have now been identified, novel approaches are required to overcome the insufficiencies of endogenous stem cells to alleviate acute and chronic damage to mammalian cardiac tissue. Because supplementary growth factors such as IGF-1 enhance myocardial regeneration following injury by enhancing endogenous repair mechanisms without affecting long-term postnatal organ function, they may constitute clinically feasible therapeutic reagents to bypass the normal restrictions on mammalian cardiac repair. Although the evidence to date for growth factor supplementation is encouraging, it remains to be determined which IGF-1 isoforms or combination of growth factors elicit the most effective response. These prelimi-

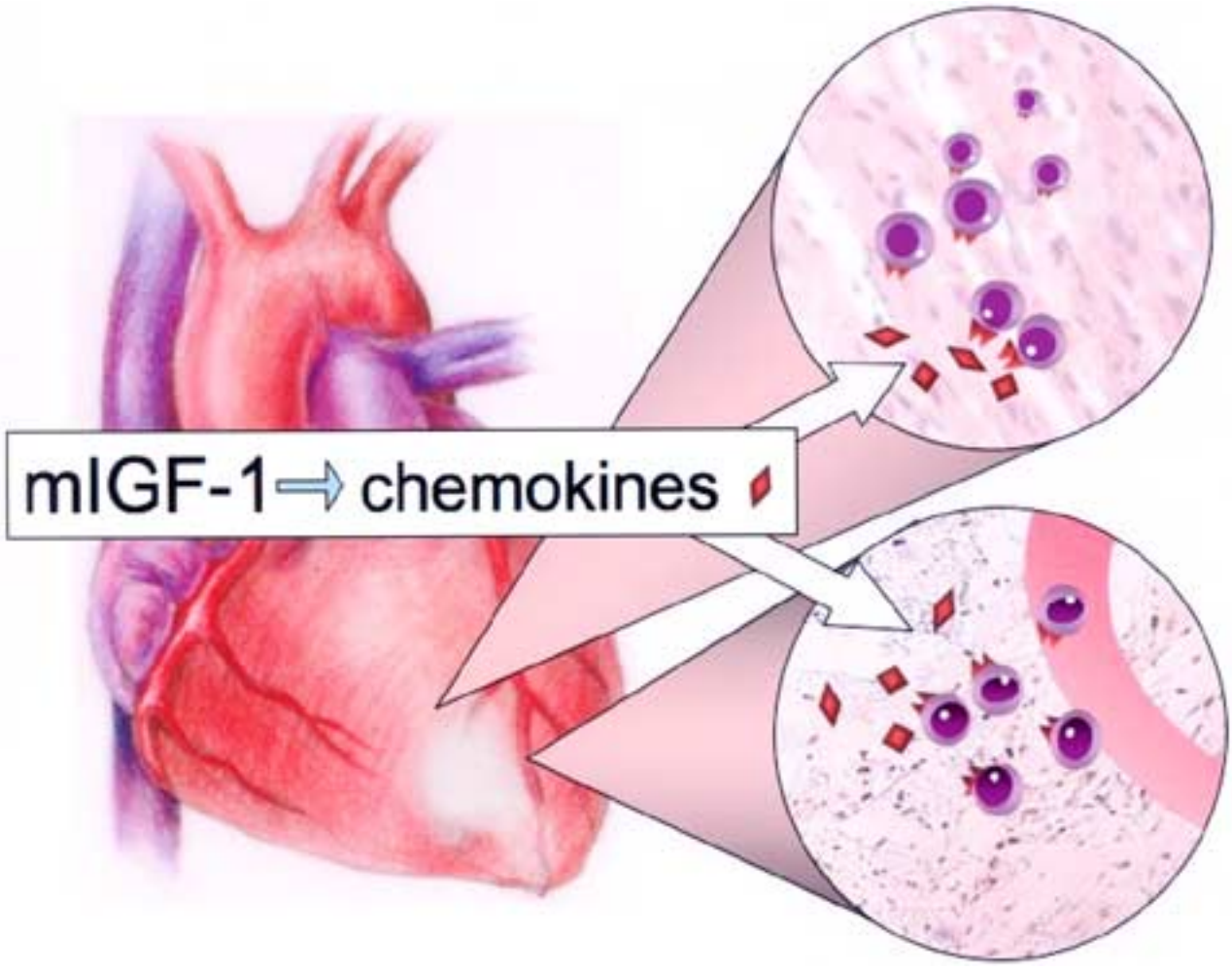

Figure 1. Modes of regeneration enhanced by mIGF-1. In the wake of a myocardial infarction, the action of IGF-1 stabilizes the necrotic zone and restricts fibrosis by activation of survival signals, secreting anti-inflammatory cytokines and chemoattractive molecules that promote contribution of progenitor cells to the areas of injury. In the top inset, endogenous precursor cell pools are activated to proliferate, and are recruited to the damage tissues. In the bottom inset, progenitor cell pools emanating from the bone marrow are attracted from the circulation to participate in the repair process. The precise identities of the cells in each scenario are not yet known, nor are the two scenarios seen to be mutually exclusive.

nary studies provide exciting avenues for future discovery; however, true innovation in this field will undoubtedly derive from the integration of our insights with other key advances in regenerative research, to achieve precise modulation of the regenerative response, and to form a cohesive and coherent strategy that addresses the short-, medium-, and long-term aspects of the therapeutic process.

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