Breaking Down Cell-Cycle Barriers in the Adult Heart

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Over the past several years, there has been considerable interest in the ability of the adult myocardium to re-enter the cell cycle. From a historical perspective, the adult heart has generally been viewed as a nonproliferative organ with a limited and meager capacity for de novo myocyte regeneration and/or self-renewal after injury. After birth, cardiac myocytes are believed to irreversibly exit from the cell cycle. As a result, growth of the postnatal heart occurs by hypertrophic rather than hyperplastic processes. The loss of functional cardiac myocytes through apoptotic/necrotic processes during ischemia or traumatic injury in the absence of de novo cell regeneration has been postulated to be an underlying cause of ventricular remodeling and diminished ventricular pump function. However, the recent discovery of cardiac progenitors within the myocardium itself, coupled with reports documenting the presence of adult myocytes synthesizing DNA in the nondiseased heart, has challenged the current dogma and has led to a re-evaluation of the true proliferative capacity of adult myocardium.

Although it is generally accepted that adult ventricular myocytes do possess some capacity for DNA synthesis, there remains considerable debate as to whether differentiated postmitotic ventricular myocytes readily traverse the cell cycle, the frequency of synthetic events, and whether DNA synthesis coincides with a concomitant increase in cell number. Notwithstanding the acknowledged ability of adult myocytes to synthesize DNA, these limited events alone do not appear to be adequate to functionally restore diminished ventricular performance in patients with heart failure post-myocardial infarction. Given that myocyte number can directly influence cardiac pump function, the ultimate therapeutic goal in reducing morbidity and mortality in patients with heart failure would be either to preserve the number of functionally active myocytes by overcoming these initial barriers hold promise for repopulating the diseased myocardium with cells capable of differentiating into functionally active ventricular myocytes. Alternative approaches for repopulating the adult myocardium with functionally active ventricular myocytes include strategies that manipulate cell-cycle progression.

Transition through the mammalian cell cycle is a tightly regulated event involving the sequential and coordinated activation of checkpoint proteins to ensure genomic stability of the host cell DNA. Cell cycle is controlled by a complex system of proteins that coordinate the biochemical events within the cell necessary for cell division. These proteins include cyclins, cyclin-dependent protein kinases (cdk), cdk-activating kinases (Cak), Cdk inhibitors, and members of the retinoblastoma (Rb) family, which function at different stages of the cell cycle.

Cyclins are the regulatory subunit for the activity of Cdns. Cdns in complex with cyclins phosphorylate key checkpoint proteins to drive otherwise quiescent cells into S phase. The G1 cyclins include cyclin D1, D2, and D3 and cyclin E. These are expressed consecutively throughout G1 phase and are required for entry into S phase. D-type cyclins with Cdk 4 and Cdk 6 play a critical role in the transition of a cell from G1 to G1 by facilitating the phosphorylation of Rb, p107, and p130. Other cyclins, including cyclins A and B, are expressed during S phase and G2/M. The assembly, activation, and disassembly of cyclin B/cdc2 complexes modulate entry and exit from mitosis.

The retinoblastoma gene product, Rb, was first identified as a putative negative regulator of cell growth by the observation that functional loss or inactivation of Rb correlated with the development of a variety of human malignancies. The biochemical mechanisms by which Rb suppresses growth have largely been attributed to its ability to negatively regulate the cell cycle by sequestering the transcription factor E2F-1. The ability of Rb to interact with E2F-1 represents a crucial step in the regulation of cellular E2F-1 activity.
E2F-1 is required for the activation of genes required for DNA synthesis.12 Certain DNA tumor viruses encode transforming proteins such as adenovirus E1A, human papilloma virus E6, and SV 40 large T antigen (SV40TAg). These proteins physically interact with Rb and p107 and promote G1 exit of nonproliferating cells presumably by displacing E2F proteins.13 Expression of E1A proteins was found to reactivate cell-cycle progression and DNA synthesis in postnatal ventricular myocytes.9 Similarly, expression of E2F-1 proteins in cardiac muscle was sufficient to promote DNA synthesis in adult ventricular myocytes in vitro and in vivo.10,16 supporting the role of Rb and related family members as key regulators of cell-cycle control in the heart. However, although these exciting early studies hinted toward the possibility of manipulating cell-cycle constituents as a means to reactivate DNA synthesis in the adult myocardium, one impediment to either E1A or E2F-1 expression was an increased incidence of apoptosis. This was attributed to the activation of the tumor suppressor p53, a surveillance protein that protects against aberrant or inappropriate DNA synthesis by initiating the cell death program. Importantly, the cell death triggered by either E1A or E2F-1 could be abrogated by Bcl-2 and related adenovirus E1B proteins.9 Although E1A and the SV40TAg share similarities in their ability to effectively interact with Rb and p107, differences in the affinity for other cellular factors, such as p300 as in the case of E1A and p53 in the case of SV40TAg, may functionally account for biological differences of these oncoproteins. This point is perhaps best highlighted by the fact that in contrast to E1A, SV40TAg can promote G1 exit and augment growth of atrial and ventricular myocytes without provoking apoptosis.17,18 This suggests that alternative cellular binding partners (E1A versus SV 40 TAg) may underlie differing abilities of either of the 2 proteins to promote complete cell-cycle progression in the absence of cell death.

As a step toward identifying putative cellular targets of the SV40TAg that mechanistically underlie the ability of the SV40TAg to override growth inhibitory signals by p53 to promote G1 exit, studies by Loren J. Field’s laboratory (Indiana University School of Medicine, Indianapolis, Ind.) have identified a novel SV40TAg interacting protein in cardiac muscle with a molecular weight of 193 kDa.19 The exact function of p193 is undetermined; however, early structure function studies revealed that full-length p193 protein is proapoptotic and provokes cell death when expressed in cells. Interestingly, deletion of the carboxyl terminus of p193 was found sufficient to support cell-cycle re-entry and protect against p53-dependent and independent apoptotic signals.19 In the same study, the authors demonstrated that inactivation of both p53 and p193 pathways in cardiac-derived embryonic stem cells was required for E1A-induced cell-cycle progression without cell death. These studies position p193 as a key SV40TAg cellular target and cell-cycle checkpoint protein that together with p53 coordinates signals that impinge on cell-cycle and cell death pathways mediated by viral proteins. It further illustrates the exciting possibility that functional inactivation of p193 may be required to overcome growth-arresting signals without provoking cell death in the adult myocardium.

In this issue of Circulation Research, Nakajima et al20 extend these studies by testing the impact of dominant-interfering mutants of p193 and p53, respectively, on DNA synthesis in the context of the normal and infarcted adult myocardium. The authors demonstrate that cardiac restricted expression of either transgene alone or in combination had no apparent effect on DNA synthesis in the normal noninjured myocardium. In contrast, however, a notable increase in myocyte DNA synthesis was observed in the border zone of infarcted hearts expressing either transgene. Interestingly, despite the apparent increase in DNA synthesis by mutant transgenes, cell-cycle reactivation was only observed in the interventricular septa of transgenic mice expressing the p193 mutant. Moreover, a reduction in ventricular remodeling and septal hypertrophy was observed, but only in mice expressing the dominant-negative p193. No apparent difference in the apoptotic index or caspase 3 activity was observed in the septa of either p193 or p53 transgenic hearts after injury. Detailed microscopic analysis revealed that minimal cardiac fiber diameter was markedly reduced in the mutant p193 hearts in comparison to either mutant p53 or Lac Z control hearts.

The studies presented are intriguing and indicate that complete or partial inactivation of p193 and p53 activities may be required to reactivate DNA synthesis in adult ventricular myocytes after myocardial injury. Perhaps most compelling is the observed increase in DNA synthesis in the interventricular septa of mutant p193 mice compared with mutant p53 hearts or Lac Z controls. These differential effects suggest that the septum must be subjected to different physiologic signals (loads) after injury compared with the infarcted border zone. The findings also highlight the possibility for regional heterogeneity among cells within the myocardium itself (septum versus ventricular free wall), which may be differentially coupled to different subsets of cell-cycle checkpoint proteins and regulated in a stimulus-dependent manner. Alternatively, the preferential increase DNA synthesis in the septum of the mutant p193 mice compared with p53 mutant mice despite the abilities of both to reactive DNA synthesis in the border zone may reflect partial or incomplete unmasking of overlapping pathways that regulate cell cycle.

Further, the data presented suggest that myocardial injury likely activates conflicting but overlapping signals in attempts to limit or salvage damaged myocardium (Figure). One pathway involved reinitiating cell cycle and repair, whereas the other involved initiating cell death. The fact that blockade of p53 function was sufficient to reactivate DNA synthesis in the infarcted border zone without apoptosis supports this contention. This raises the interesting possibility that targeted inactivation of p193 and p53 cell-cycle checkpoints after myocardial infarction may permit G1 exit and reactivation of the cell-cycle program without provoking cell death.

The data presented provide new important insight into the molecular switches that govern cell-cycle control and cell death pathways in the adult myocardium, and support a
paradigm shift toward the regenerative capacity of the adult myocardium. The question that arises is whether p193 or p53 regulates equivalent pathways in other models of cardiac injury or cardiomyopathies, or whether inducible expression or conditional ablation of p193 would have alternative effects on DNA synthesis and mitosis in the nonischemic or infarcted adult heart. Also, it is not clear whether expression of the p193 mutant alone would be sufficient to prevent progression to heart failure, given that only 1 time point was examined. Nevertheless, the data presented are intriguing and support the exciting possibility of manipulating cellular factors involved in cell-cycle control for reactivating DNA synthesis in the adult heart. The data further suggest that genetic manipulation of p193 or related cell-cycle regulators may have therapeutic advantage in modulating ventricular remodeling and improve ventricular performance by enhancing regenerative cardiac growth.

References


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