Death-Defying Pathways Linking Cell Cycle and Apoptosis

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One of the most intriguing and perplexing issues to impact contemporary cardiology to date is the concept of myocyte regeneration after injury. In contrast to other cells of the body that retain the ability to regenerate throughout life, cardiac muscle cells lose this inherent property soon after birth. Consequently, growth of the neonatal myocardium occurs by myocyte hypertrophy, as manifested by an increase in cell volume and myofibrillar protein content rather than by cell number. The molecular mechanisms that dissociate cardiac growth from hyperplasia remain unknown. This basic biological fact has significant clinical implications for patients, because loss of viable cardiac cells after acute or chronic myocardial injury or during conditions of increased hemodynamic loading, such as those imposed by uncontrolled hypertension, can have detrimental effects on ventricular performance. In these cases, adaptive cellular growth of adjacent myocytes compensates for the cellular loss and impending contractile load to normalize wall stress. However, in some patients, for reasons unknown, these adaptive physiological processes become inadequate and deteriorate into dilatation and overt heart failure. Despite the substantial progress that has been made during the last two decades of cardiac research, heart failure still remains a prominent cause of death in North America. In particular, chronic congestive heart failure represents a major financial and socioeconomic burden, because patients diagnosed with this form of heart disease require costly medical treatments and chronic long-term care.

At the cellular level, defects in contractile proteins and, more recently, loss of cells through an apoptotic process are considered independent contributing factors to heart failure. Although cell death can occur by apoptosis or necrosis, apoptosis is an energy-requiring process that is genetically regulated.1 Cell death by necrosis involves depletion of cellular ATP stores, cell swelling, and disruption of the cell membrane. In contrast, apoptotic cell death occurs in the absence of membrane rupture and is devoid of an inflammatory response. Therefore, inappropriate or excessive cell loss through an apoptotic program may underlie ventricular remodeling and ventricular dysfunction associated with end-stage heart failure, given the limited and meager ability of the adult myocardium for regeneration after injury. The fact that apoptosis is a genetically regulated event suggests that components of the apoptotic pathway may be targeted to modulate or prevent the inappropriate cell death in disease conditions.2

Because it is generally believed that the number of available ventricular myocytes can directly influence cardiac performance, the ultimate therapeutic objective in reducing morbidity and mortality in patients with diminished pump function would be to either prevent excessive cell loss from occurring or, alternatively, increase the number of functionally active myocytes through a regenerative process. Toward this goal, several innovative and genetic strategies have been devised to increase the number of functionally active ventricular myocytes in the myocardium. These include the phenotypic conversion of fibroblasts to cells of the muscle lineage,3 exogenous grafting of muscle cells directly into the myocardium,4 the generation of myocardium de novo with bone marrow stem cells,5 and manipulation of cellular factors to promote myocyte cell-cycle reentry and cell proliferation.6–8 The latter approach has received considerable attention over the last several years given the identification of key cell-cycle regulatory proteins and the ability to reactivate DNA synthesis in vivo and in vitro in postmitotic ventricular myocytes.6

Transition through the cell cycle is a tightly regulated event involving multiple checkpoint proteins that ensure genetic stability of the host-cell DNA. The retinoblastoma gene product Rb is the prototypic member of a family of cellular proteins that also includes p107 and p130. 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.9 These include retinoblastoma and prostate carcinomas as well as glioblastoma and leukemias. Equivalent human malignancies dependent on functional loss of p107 or p130 have not yet been identified. The biochemical mechanisms by which Rb family members suppress tumor growth are ascribed to their ability to interact with the E2F cellular factors through a binding domain that classically has been referred to as the large pocket that retains the cell in G0/G1 of the cell cycle.10 Several cellular genes that are required for cell-cycle progression and DNA synthesis contain consensus E2F binding sites within their promoters, supporting a role for E2F in transactivation of S phase–dependent genes.11 To date, at least five E2F homologues (E2F-1 to E2F-5) together with their dimerization partners DP1 and DP2 have been identified, each with preferential binding affinities for Rb family members. This may account for the differing abilities of E2F factors to regulate cell cycle and growth, because E2F-1, E2F-2, and E2F-3 can override...
G1-mediated growth arrest in quiescent cells whereas E2F-4 and E2F-5 only marginally promote S-phase entry.

Rb family members are postulated to be downstream targets for mitogen-activated G1 cyclins, which include cyclin D1, D2, D3, and E as well as the cyclin-dependent protein kinases (cdk2, cdk4, and cdk6). In response to positive growth signals, cyclin D-cdk complexes are activated by the cdk-activating kinase MO15 and cdk7-cyclin H, resulting in Rb phosphorylation and the release of E2F factors.12 Other cyclins include cyclin A and B, which are predominantly expressed in S and G2/M. Inappropriate cell-cycle activation leads to apoptosis largely through the activation of another tumor suppressor and G1 checkpoint protein, p53.13

Specific proteins of the DNA tumor viruses, including adenovirus early region 1 (E1A), human papilloma virus E6 protein, and simian virus 40 large T antigen (T-Ag) can drive quiescent nonproliferating cells into S phase.14 Ostensibly, viral oncoproteins physically interact with members of the Rb family and inactivate their function by displacing E2F factors from the pocket domain, promoting cell-cycle entry. Hence, the interaction of viral oncoproteins with negative regulators of cell-cycle provides an attractive mechanistic explanation for virally induced cellular transformation and a potential mode for reactivating cell-cycle progression in postmitotic ventricular myocytes. This is substantiated by transgenic studies in which atrial and ventricular muscle growth was augmented by expression of T-Ag.15 Furthermore, adenovirus-mediated delivery of E1A proteins to neonatal or adult ventricular myocytes in vivo and in vitro resulted in S-phase entry with the cells arresting in G2/M.6 Moreover, expression of E1A or the cellular transcription factor E2F-1, which is presumably liberated by Rb inactivation, was sufficient to provoke cell-cycle reentry, illustrating a key role for Rb-like molecules in cell-cycle control in cardiac tissue.6,7

However, one caveat to these experiments is that whereas E1A and E2F-1 proteins promoted cell-cycle reentry, an increased incidence of apoptosis was evident when either protein was expressed alone in ventricular myocytes. Apoptosis triggered by E1A or E2F-1 could readily be abrogated by coexpression of antiapoptotic proteins, adenovirus E1B, or Bcl-2.6,16 Whereas T-Ag and E1A functionally share the ability to bind to Rb, p107, and p130 equivalently, differences in cellular binding partners, such as p300 in the case of E1A and p53 in the case of T-Ag, clearly may account for the biochemical and functional differences in these viral proteins, such as the observed lack of apoptosis with T-Ag (Figure). Difference in cellular binding targets may provide important clues into the molecular mechanisms that govern cell-cycle progression and its relation to apoptosis.

In this issue of Circulation Research, Pasumarthi et al17 provide evidence for the existence of a novel proapoptotic protein p193, a member of the BH3-only family of proapoptotic Bcl-2 that binds to T-Ag, that may account for phenotypic differences observed with E1A and T-Ag binding targets. Structure-function analysis of p193 indicates that the full-length protein exhibits proapoptotic properties when overexpressed in cells. Importantly, a large C-terminal truncation mutation of the p193 protein was found to promote cell growth and protection from proapoptotic signals mediated through both p53-dependent and p53-independent pathways. The fact that T-Ag can evoke a proliferative response in transgenic mice without provoking apoptosis illustrates the existence of multiple points of regulation that must be overcome by T-Ag to provoke cell-cycle reentry without triggering apoptosis. This implies the existence of cellular proteins that coordinately link cell-cycle progression to apoptosis. In these elegant studies, Pasumarthi et al17 test the possibility that the function of proapoptotic proteins p53 and p193 must be inhibited to successfully promote cell proliferation in the absence of cell death. Using embryonic stem (ES) cell–derived cardiac myocytes and a genetic approach of dominant-negative mutants of p53 and p193, the authors demonstrate that expression of a mutant p53 alone is not sufficient to promote cell growth or overcome the apoptosis triggered by adenovirus E1A. Moreover, expression of the mutant p193 alone also failed to suppress E1A-mediated apoptosis. Interestingly, coinjection of mutant p53 and mutant p193 in cardiac ES cells was sufficient to support E1A-induced cell proliferation in the absence of apoptosis. These experiments illustrate that for viral protein–induced cell-cycle progression and proliferation to be successful, the proapoptotic pathways mediated through p53 and p193 must be compromised. The differential interaction of E1A and T-Ag with alternative cellular targets explains the apparent biochemical differences in the two proteins. In the case of T-Ag, the E1A protein has the ability to promote S-phase entry but provokes apoptosis because of an inability to override the p193 checkpoint. Whether ablation of these pathways alone will be sufficient to globally promote myocyte proliferation by mitogenic agents or is solely restricted to viral oncoproteins like E1A and T-Ag is undetermined and awaits additional investigation. Moreover, the role played by other death-promoting pathways, such as caspases and mitochondria, is equivalently unknown.

Although these elegant and important studies point toward potential cellular targets that could be genetically manipulated to overcome G1 exit, the experiments must be inter-
preted with caution. These studies were conducted in cardiac embryonic stem cells, which may have a greater propensity for cell-cycle exit and proliferation than neonatal or differentiated adult ventricular myocytes. Furthermore, whether equivalent biochemical pathways are functionally operational in normal or diseased adult myocardium will have to be addressed. Although there are some reports that adult ventricular myocytes may still retain the ability to reenter the cell cycle and proliferate under certain conditions,\textsuperscript{18–20} the proof of concept of reactivating cardiac myocyte proliferation by manipulating putative cellular regulators or conversion of pluripotent stem cells to cardiac myocytes\textsuperscript{5} holds promise for the design of novel therapeutic interventions to enhance ventricular performance during disease conditions.

References


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