Transgenic Expression of A20 Prevents Cardiac Cell Death and Myocardial Dysfunction After Myocardial Infarction

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Notwithstanding the significant advances in cardiovascular medicine over the last 2 decades, cardiovascular disease is responsible for >45% of all deaths in North America and is reaching pandemic proportions worldwide. In particular, heart failure that results from ischemic injury represents a major clinical challenge because individuals diagnosed with this form of heart disease require costly medical treatments and long-term care. Though the exact cellular defects that ultimately contribute to ventricular dysfunction remain unknown, a unifying theme is that functional loss of cardiac myocytes through an apoptotic process contributes to ventricular remodeling and a decline in ventricular performance in patients post–myocardial infarction (MI).

Article p 1885

Historically, the adult myocardium has been viewed as nonproliferative with a limited capacity for de novo myocyte self-renewal after injury. However, the recent discovery of resident cardiac progenitors coupled with the acknowledged ability of adult cardiac myocytes to actively synthesize DNA has challenged the current dogma. Despite these seminal observations, the infrequency of synthetic events together with the limited numbers of cardiac progenitors appear inadequate to functionally restore ventricular function in patients with heart failure after MI. Given that cardiac output is directly influenced by the number of functional cardiac myocytes, the ultimate therapeutic goal in the reduction of morbidity and mortality in patients with heart failure post-MI would be to preserve the number of existing myocytes by self-renewal after injury. However, the recent discovery of nonproliferative with a limited capacity for de novo myocyte self-renewal after injury.

Apoptosis has received considerable attention over the recent years by virtue of the fact that the events that lead to cell death occur in a highly ordered and genetically regulated process. The very nature of the cells’ demise by this process allows for versatility in the design of genetic therapies against cellular targets known to activate or repress cell death. It is appreciated that 2 distinct but overlapping pathways can initiate the apoptotic process. The first involves the receptor-mediated process or “extrinsic pathway,” which involves death receptors (DR4, DR5), Fas, CD95, and members of the tumor necrosis factor-α (TNF-α) super family. In this paradigm, engagement of ligand with receptor promotes assembly of the death-inducing signaling complex, which recruits death domain, death effector domain, and other coupling proteins to the cytoplasmic face of the receptor, which thereby facilitates the proteolytic activation of “initiator” caspases 8 and 10. Distal targets of initiator caspases include apoptotic proteins such as Bid, which becomes proteolytically cleaved to t-Bid by caspase 8, and the “death effector” caspases (caspase 3, caspase 6, and caspase 7).

The second and perhaps most intriguing pathway is the “intrinsic” or mitochondrial regulated death pathway. In this model, perturbations to mitochondria that result in permeability transition pore opening and loss of mitochondrial membrane potential are requisite features of the intrinsic death pathway. Moreover, permeability transition pore opening is believed to facilitate release of proapoptotic factors by mitochondria, such as second mitochondrial activator of caspases (Smac), cytochrome c, apoptosis-inducing factor, Htr2A/Omi, endonuclease G, and others (reviewed by Regula and Kirshenbaum). Members of the Bcl-2 gene family play a central role in regulation of the intrinsic death pathway. Survival factors such as Bcl-2 gene family play a central role in regulation of the intrinsic death pathway. Survival factors such as Bcl-2 inhibit the intrinsic death pathway in that they antagonize caspase activation and/or the actions of death proteins Bax, Bad, Bak, Bin, and others at the level of the mitochondrion (reviewed by Regula and Kirshenbaum).

Nuclear factor-κB (NF-κB) was first identified as a key regulatory molecule required for B-lymphocyte proliferation and maturation. Since these original observations, NF-κB is now appreciated as an ubiquitously expressed dimeric transcription factor involved in a number of biological processes such as inflammation, cell adhesion, and cell survival (reviewed by Bauerle and Baltimore). NF-κB belongs to a family of highly conserved transcription factors that include Rel-A, c-Rel, v-Rel, and Drosophila dorsal proteins. The major form of NF-κB in cells is a dimeric complex composed of p50 and p65 (p50/p65) kDa protein subunits, and minor complexes of p50/p50 homodimers have also been reported. In unstimulated cells, NF-κB is retained as an inactive complex bound to the cytoplasmic inhibitor protein 1κB. Classically, inflammatory cytokines, which include members of the TNF-α super family, are known to activate NF-κB by initiation of the phosphorylation and subsequent proteasomal
degradation of IκBα. Notably, the IκB kinase signaling complex (IKK), composed of IKKα, IKKβ, and IKKγ (NEMO), are necessary and sufficient to activate NF-κB. Ostensibly, the phosphorylation-dependent loss of IκBα permits NF-κB to translocate to the nucleus and affect gene transcription.14

Recently, a survival role for NF-κB has been described.16,17 This has largely been substantiated by studies in cells in which the p65 gene had been deleted or functionally inactivated, which then increased apoptosis. The fact that deletion of the p65 NF-κB subunit is embryonically lethal18 supports a crucial role for NF-κB in cell survival during development. Moreover, the inability of the p50 subunit of NF-κB to prevent apoptosis or rescue the embryonic lethality of the p65−/− mice illustrates the lack of functional redundancy between the p50 and p65 subunits. A cytoprotective role for the NF-κB p65 subunit is further illustrated by the fact that mouse embryonic fibroblasts derived from p65 knockout embryos were sensitized to death signals imposed by TNF-α and ultraviolet irradiation.17 The fact that mammalian cells such as cardiac myocytes do not undergo apoptosis when treated with TNF-α, yet readily succumb to death when NF-κB or components of the NF-κB signaling pathway are blocked, further supports a survival role for NF-κB. Perhaps the most compelling argument to support a survival role for NF-κB in the heart stems from studies in which the TNF-α R1 gene had been ablated.19 These mice displayed poor recovery of ventricular function and larger infarcts than wild-type (WT) control mice. Furthermore, NF-κB has recently been shown to avert hypoxia-induced mitochondrial perturbations and cell death of ventricular myocytes.20,21 Despite the overwhelming evidence to support a survival role for NF-κB, this feature may not be universally conserved because under certain instances NF-κB can reportedly promote rather than prevent cell death.22 In fact, NF-κB is crucial for Simbus virus replication in permissive cells, and prolonged or protracted NF-κB activation underlies the cytokine-induced apoptosis of endothelial cells. Hence, the ability of NF-κB to provoke or alternatively prevent cell death appears to be cell- and context-specific.

Though the mode by which NF-κB influences cell survival is poorly understood, it has been postulated that it may influence the expression of certain genes in the apoptotic pathway. For example several antiapoptotic factors, such as cellular inhibitors of apoptosis (c-IAP1 and c-IAP2), TRAF2, IEX-1L, Bcl-2, and the zinc finger protein A20 are known transcriptional targets of NF-κB.23,24 Presumably, these factors block activation of caspase 8 and death signals propagated through the death-inducing signaling complex.25

In this regard, the cellular factor A20 was first identified as a TNF-α-inducible factor that was crucial for cell survival in endothelial cells. In fact, genetic deletion or functional ablation of A20 in mice protracted the inflammatory response after LPS or TNF-α treatment. Presumably, the E3-ubiquitin ligase activity of A20 can terminate NF-κB activation by targeting the degradation of the NF-κB signaling proteins RIP, NEMO, and TRAF6. Collectively, these observations identify A20 as a putative antiapoptotic factor and potential therapeutic modulator of cardiac cell death.26

In this issue of Circulation, Li et al27 test the possibility that cardiac restricted overexpression of A20 in mice can protect against apoptosis, ventricular remodeling, and inflammation post-MI. With the use of a well established coronary artery ligation model, the authors examine the effects of A20 expression in WT and transgenic (TG) mice that overexpress A20 on cardiac performance, inflammatory markers, NF-κB activity, apoptosis, fibrosis, and mouse survival. In short, the authors find that the presence of the A20 transgene remarkably protected hearts across all end points studied, with the notable exception of mouse survival.

Indeed, A20 improved post-MI cardiac performance and reduced the numbers of infiltrating neutrophils, monocytes, and macrophages, as well as cardiac myeloperoxidase activity. Moreover, A20 animals also exhibited lower levels of proinflammatory cytokines such as TNF-α and IL-6 compared with WT animals. Interestingly, the apoptotic index in noninfarcted tissue of the A20 hearts was markedly reduced compared with WT controls. Although not directly studied, the authors attribute the reduction in apoptosis in the A20 mice to the inhibition of the mitochondrial death pathway. Presumably this was caused by the enhanced expression of the anti-death genes survivin, Bcl-2, XIAP, and c-FLIP, in concert with the downregulation of the death genes Fas, FasL, and Bax. Furthermore, markers of cardiac hypertrophy atrial natriuretic peptide, brain natriuretic peptide, and heart weight/body weight ratio were found to be significantly reduced in the A20 mice compared with WT controls. Though collagen deposition was not significantly altered in the infarct region of the A20 hearts, a reduction in collagen and fibrosis in noninfarcted myocardium was observed. The authors ascribe this to the activation of profibrotic factors matrix metalloproteinase 9, transforming growth factor-β1, collagen I, and collagen III in WT mice versus the A20 TG mice. In contrast to WT littermates, A20 TG mice displayed lower NF-κB levels and IKKβ activity, a finding consistent with the impaired NF-κB activation in the A20 mice. Surprisingly, despite the apparent benefits of the A20 transgene on the end points studied, similar survival rates were observed at 7 days post-MI between the WT and A20 TG mice.

On the basis of these findings, Li et al purport A20 as a potential candidate for improvement of cardiac outcome post-MI. Although the study provides compelling evidence to support a protective role for A20 in the heart post-MI, the underlying mechanisms by which A20 confers cardioprotection were not fully addressed. The conclusion that A20-mediated inhibition of NF-κB accounts for the reduction in infarct size and the sequelae of events that follow infarction is interesting; however, it remains unclear whether the reduction in infarct size by A20 is a direct consequence of NF-κB inhibition or is somehow related to the impact of A20 on other cellular targets. The fact that survival rates were not different between the WT and A20 TG mice post-MI, despite the obvious repression of inflammatory cytokines and apoptosis, raises the possibility that A20 likely impinges on cellular targets other than NF-κB.

Furthermore, several of the inflammatory cytokines and antiapoptotic genes, such as A20 itself, are regulated by NF-κB. This raises the question as to how inhibition of
NF-κB by A20 could suppress inflammation on the one hand, yet on the other hand activate survival pathways to avert cell death, as inferred by the Li et al study. One possibility that could explain this apparent paradox may reside in the differences in spatial and temporal expression of NF-κB, NF-κB-regulated cytokines, and survival factors post-MI. This could explain why NF-κB can promote rather than prevent cell death under certain circumstances. Because only late NF-κB activation or repression. Because the A20 transgene was expressed constitutively, it could have masked any dynamic relationship between NF-κB and A20 pre- and post-MI in terms of the activation or repression of survival factors. This could potentially account for the reported dichotomous actions of NF-κB on cell survival and cell death, respectively.

To resolve this conundrum, one could test whether inducible cardiac expression of A20 pre- and post-MI would have any benefit regarding infarct expansion and recovery of function. As reported in the present study, endogenous A20 levels increased at 6 hours post-MI, but fell by 24 hours. However, in lieu of constitutive expression of A20 by the α-myosin heavy chain promoter, there may be an optimal window of opportunity for which the A20 transgene confers protection. In this regard, the counterintuitive decline of the endogenous A20 gene post-MI may be viewed as desirable, which would allow for early ventricular compensation and infarct healing. Late induction of the A20 transgene post-MI could confer resistance against heart failure by activation or repression of signaling pathways linked to NF-κB. Given that different death signals converge on distinct death effectors within the cell, it is unknown whether transgenic overexpression of A20 would confer protection from apoptosis induced by death signals other than MI.

Nevertheless, the studies described herein provide a novel and interesting role for the zinc finger protein A20 as a putative regulator of cell survival and ventricular function post-MI. The data provide promising glimpses into the application of survival factors for future genetic interventions for suppression of ventricular remodeling and heart failure.

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Disclosures

None.

References


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