Apoptotic and non-apoptotic programmed cardiomyocyte death in ventricular remodelling

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1. Introduction
Ventricular mass, function, and geometry evolve in a predictable manner after myocardial injury or in response to chronically increased haemodynamic load. Cardiac mass increases as a result of cardiomyocyte hypertrophy and ventricular wall thickening, which help to maintain ejection performance. With continued haemodynamic overload the heart dilates and cavity walls thin, resulting in a geometry that contributes to systolic dysfunction by increasing wall stress. At the cellular level, cardiomyocytes lengthen, rearrange within the myocardial matrix (‘cell slippage’), and die, to be replaced by fibrous tissue. These changes are collectively referred to as ‘remodelling’.1,2

Because ventricular remodelling is an active process that contributes to physiological deterioration long after the primary cardiac insult, it is an attractive therapeutic target. Simply enforcing a more normal geometry on remodelled ventricles using mechanical devices is sufficient to improve ventricular function in heart failure and mitral regurgitation.3–5 Efforts are also underway to address cardiomyocyte slippage at the molecular and biochemical level by targeting the extracellular matrix. Collagen secreted by myocardial fibroblasts is a major determinant of myocardial architecture by stabilizing cardiomyocytes in their proper three-dimensional alignment, in parallel, in series, and in layers. With cardiac stress, collagen cross-links degenerate and myocytes lengthen and slip past one another, which produces ventricular dilatation. Protecting the matrix by inhibiting metalloproteinases that are responsible for its degradation is being studied to correct or prevent cardiac remodelling.6,7 Finally, modulation of myocardial substrate metabolism has reversed remodelling in experimental models.8

Treatment of remodelling by regenerating lost myocardium is perhaps the most spectacular of the various investigational approaches. Here, the promise is that cardiomyocyte drop-out and replacement fibrosis can be addressed by stimulating resident or itinerant stem cells to differentiate into cardiomyocytes and functionally integrate into the failing myocardium.9,10 This approach largely bypasses the processes that engender remodelling by generating new myocardium to replace that which was lost. The hurdles to be overcome before myocardial regeneration is clinically applicable are substantial, and there is another way of potentially achieving much the same end: preventing programmed loss of cardiac myocytes. This review examines the recent experimental developments relating apoptotic and non-apoptotic programmed cardiomyocyte death to ventricular remodelling and development of heart failure,11 and explores the therapeutic potential of inhibiting programmed cell death in cardiac disease.

2. Mechanisms of apoptosis in heart failure
Apoptosis, or cell suicide, is a highly conserved and tightly regulated inducible cell response. Apoptosis has no known physiological role in cardiomyocytes of the normal adult heart, but is essential to form the cardiac valves and outflow tract during cardiac development.12 In many adult tissues, apoptosis restrains proliferation of abnormal cells that could otherwise undergo neoplastic transformation. Accordingly, mutations in apoptosis-regulating genes can be oncogenic.13
Apoptosis is characterized by protein and chromatin fragmentation into small packages that can be engulfed by scavenger cells without provoking a potentially injurious inflammatory response. Nuclear chromatin condensation and nuclear fragmentation form dense ‘apoptotic bodies’ that are pathognomonic of apoptosis, and form the basis for standard, but imperfect, laboratory assays (DNA laddering) and histological techniques (TUNEL staining) used to differentiate it from other forms of cell death.\(^{14-16}\) Complementary techniques measure cytoplasmic release of the key mitochondrial apoptosis intermediate, cytochrome c, activation of the key terminal effector of apoptosis, caspase 3, and cleavage of the caspase substrate and key mediator of cell death, PARP.\(^{17}\)

Signalling pathways that regulate apoptosis in the heart and elsewhere have been reviewed in detail.\(^{18-20}\) Briefly, apoptosis is mediated by cascade activation of the caspase family of cysteine proteases. Caspases exist as zymogens (procaspases) that are activated in sequence by cleavage of their pro-domains, ending with activation of the terminal effector caspase, caspase 3. When apoptosis is stimulated by extrinsic factors, such as tumor necrosis factor (TNF)-α or other cytokines, binding to their respective death receptors stimulates receptor oligomerization and recruitment of FADD (Fas Associated Death Domain-containing protein) and the initiator caspase, caspase 8, to a ‘death-inducible signalling complex’ (DISC). Caspase activation then initiates systematic proteolytic degradation of intracellular proteins and oligonucleosomal cleavage of nuclear DNA (Figure 1).

Complementing extrinsic activation of apoptosis in chronic heart failure is intrinsic stimulation of apoptosis through transcriptional upregulation and post-translational modification of pro-apoptotic genes. Of special importance in cardiac hypertrophy and acute ischaemic injury is increased expression of genes encoding pro-apoptotic Bcl-2 family members that activate the caspase cascade by selectively permeabilizing mitochondrial outer membranes, thus permitting cytochrome c to diffuse from the mitochondrial inter-membranous space into the cytoplasm and catalyze the formation of the apoptosome complex with Apaf-1 (apoptotic protease activating factor-1), dATP, and the initiator caspase, caspase 9 (Figure 1).

Apoptosis is rare in normal human hearts, with a reported prevalence of \(~1\) TUNEL positive cardiomyocyte in \(10^4\).\(^{21}\) The rate of cardiac myocyte apoptosis can increase several hundred fold in dilated and ischaemic cardiomyopathies,\(^{22,23}\) hypertrophic heart disease,\(^{11}\) and arrhythmogenic right ventricular dysplasia,\(^{24}\) but even in these diseases the absolute prevalence is typically \(<1\%\). Thus, the significance of apoptosis to human ventricular remodelling continues to be debated, although an association between apoptosis, cardiac myocyte drop-out, ventricular remodelling, and deterioration of systolic performance exists in multiple experimental models.\(^{25-30}\) The potential also exists for activation of apoptotic pathways to contribute to cardiac dysfunction in heart failure independent of cell loss: proximal apoptotic signals are activated, but apoptosis signalling is terminated downstream prior to irreversible protein degradation and DNA cleavage by concomitant upregulation of endogenous caspase inhibitors, X-linked inhibitor of apoptosis protein (XIAP)\(^{31,32}\) and apoptosis repressor with caspase recruitment domain.\(^{33,34}\) Such cells exhibit an intermediate phenotype of ‘apoptosis interruptus’.\(^{35-37}\)

Histological evidence for incomplete apoptosis in heart failure consists of cytosolic localization of mitochondrial cytochrome c and/or caspase 3 cleavage without the

![Figure 1](image-url)
hallmark condensation and oligonucleosomal cleavage of nuclear DNA. Cardiac myocytes that exhibit these features have been labelled as ‘zombie myocytes’, and it is likely that defects in respiration and ATP production by their cytochrome c-leaking mitochondria confer upon the cells the ‘zombie’ phenotype of being lifeless, but not completely dead. This condition impairs cardiac contractile performance without causing cardiac myocyte drop-out. However, it is also possible that interrupting apoptosis downstream of mitochondrial outer membrane permeabilization, as with XIAP induction in apoptosis interruptus or by pharmacological caspase inhibition, simply commits the cell to death by another means. Since apoptosis is a highly ordered and ATP-consuming process, energy deprivation in zombie cardiomyocytes could inhibit the terminal events in apoptosis, but lead to calcium release down its diffusion gradient from sarcoplasmic reticulum (SR) and the calcium-dependent opening of mitochondrial permeability transition pores (MPTPs). These events could lead to programmed cell necrosis (see below).

Another possible outcome of apoptosis interruptus is ‘caspase-independent apoptosis’, characterized by DNA cleavage into ~50 000 bp fragments that are much larger than the 200 bp fragments and multiples thereof seen after conventional apoptosis. The critical mediators of caspase-independent apoptosis are apoptosis-inducing factor (AIF) and endonuclease G (Endo G). Like cytochrome c, AIF and Endo G are normally localized in the mitochondrial intermembranous space, and released by permeabilization of outer mitochondrial membranes. These two proteins directly cause apoptosis by translocating from the cytosol to the nucleus where they cause DNA fragmentation without activation of the caspase cascade (Figure 1). Caspase-independent apoptosis mediated by AIF is thought to play significant roles in programmed cell death seen in ischaemia-reperfusion and oxidant injury, and may involve processing of pro-AIF to the mature enzyme by calpains, cysteine proteases that are related to caspases and activated by increases in calcium. Thus, mitochondrial outer membrane permeabilization by Bcl2-family proteins or other means releases intermembranous cytochrome c, AIF, and Endo G into the cytosol where, having been displaced from their normal subcellular locale, their functions transform from those that are essential to cell survival to those that mediate programmed cell death.

3. Bcl2 family proteins and cardiac remodelling

The Bcl2 proteins comprise a family of largely mitochondrial-targeted pro- and anti-apoptotic proteins that play key roles as the initiators, regulators, and effectors of intrinsic pathway apoptosis. Cardiac myocytes express a number of Bcl2 family members, several of which are under transcriptional control in heart disease, including anti-apoptotic Bcl2 and Bcl-xL and pro-apoptotic Bnip3 and Nix (Bnip3L). Pro-apoptotic Bcl2 proteins are classified according to structural features that reflect different functions. The ‘multi-domain’ proteins, of which Bax and Bak are the prototypes, have three Bcl2-homology (BH) domains and are the essential pore-forming proteins that lead to mitochondrial outer membrane permeabilization, cytochrome c release, and induction of the intrinsic apoptosis signalling cascade. The ‘BH3 domain-only’ proteins are represented by Bnip3 and Bad and have only a single BH-3 domain. BH3-only proteins are diverse in their structural features, levels of expression in various tissues, and in their transcriptional or post-translational control mechanisms. The principal role of BH3 domain-only proteins is sensing stress signals and promoting mitochondrial translocation of Bax or activation of mitochondrial-localized Bak. The anti-apoptotic Bcl2-proteins inhibit Bax and Bak-mediated pore formation by hetero-dimerizing with their pro-apoptotic relatives. Thus, the multidomain Bcl2 proteins are the effectors, the BH3-only proteins the sensors, and the anti-apoptotic Bcl2 proteins the negative regulators, of mitochondrial pathway apoptosis (Figure 1). Cell fate is determined in large part by the relative expression levels of these pro- and anti-apoptotic factors.

Even though the essential function of apoptosis in adults is to restrain proliferation of abnormal cells, and cardiac myocytes are incapable of self-regeneration, cardiac myocytes nevertheless upregulate programmed cell death genes under conditions of stress. Bax expression increases and Bcl2 expression decreases during chronic experimental cardiac pressure overloading. Nix is upregulated in human and experimental cardiac hypertrophy, and Bnip3 expression increases in ischaemic cardiac models. Recent details have emerged supporting a particularly prominent role for the BH3-domain only proteins Nix and Bnip3 in cardiac remodelling subsequent to pressure overload hypertrophy and cardiac ischaemic injury, respectively, so these factors are discussed in greater detail.

4. Nix and Bnip3 in remodelling of hypertrophied and ischaemic hearts

Nix was first identified as a potential mediator of cardiac remodelling in a mouse genetic model of load-independent cardiac hypertrophy that uniquely undergoes apoptotic decompensation under conditions of physiological stress, the Gq transgenic mouse. Gq transduces signals from epinephrine, angiotensin II, and endothelin receptors that mediate pressure overload hypertrophy through autocrine and paracrine effects. Thus, the hypertrophy that develops as a result of increased Gq signalling has many of the molecular and structural features of pressure overload. Hearts from these mice are mildly diluted, but do not fail. However, with increased Gq transgene ‘dose’ or the haemodynamic stressors of pressure overload or pregnancy, apoptosis is reliably provoked. In the case of the Gq peripartum cardiomyopathy, apoptosis is aggressive and half of the mice die of heart failure within two weeks. In the case of pressure overloaded Gq mice, apoptosis and cardiac myocyte drop-out are more modest, and the ventricles dilate over several months. Thus, the rate and consequences of cardiomyocyte apoptosis in this model system can be tailored to the particular needs of a given experiment, and the peripartum Gq mouse has been useful for molecular dissections of neurohormone-stimulated intrinsic pathway cardiac apoptosis.

Nix was detected in non-failing Gq transgenic hearts by transcriptome analysis that revealed several components of an inducible cardiomyocyte death gene programme. Nix is also increased in experimental pressure overload hypertrophy and human hypertensive heart disease. Analysis of
the transcriptional events leading to Nix induction has revealed a role for PKC.

Recombinant Nix localizes to mitochondria in cultured cells and forced Nix expression causes apoptosis associated with appearance of cytosolic cytochrome c and activation of caspase 3. Likewise, forced Nix expression in hearts of transgenic mice produces an apoptotic cardiomyopathy that is lethal in neonatal mice, but that is milder and can be better tolerated in adult hearts. The different effects of forced Nix expression in growing neonatal hearts vs. non-growing adult hearts suggests a complementary role for cardiotoxic stimuli in cardiomyocyte apoptosis mediated by this factor. Based on these findings we hypothesized that cardiomyocyte apoptosis after systemic pressure overload might be an important mechanism for ventricular remodelling and the transition to failure, and that transcriptional induction of Nix could mediate some of these events.

To test this hypothesis we employed loss-of-function. Since germ-line ablation of the Nix gene created haematological abnormalities that could confound the results of long-term cardiac studies, we used Cre-lox technology in combination with Nkx-2.5 driven Cre to selectively delete Nix from cardiac myocytes of the in vivo mouse. Nix deleted hearts were histologically and structurally normal. Cardiomyocyte-specific Nix ablation did not alter the extent or time course of reactive pressure overload hypertrophy, but left ventricular remodelling and the deterioration in ejection performance were markedly abrogated, and cardiomyocyte apoptosis was diminished. Together, these results demonstrated that reactive apoptosis is an important mechanism for cardiac remodelling in pressure overload hypertrophy, and that induction of Nix plays a key role mediating this adverse response.

A similar effect on apoptotic ventricular remodelling after ischaemia–reperfusion injury is observed with the Nix relative, Bnip3. Bnip3 is transcriptionally upregulated in ischaemic tissues, including cardiac myocytes, through the actions of hypoxia-inducible factor (HIF)-1α and nuclear factor (NF)-κB. Bnip3 ablation has no detectable effects at baseline in any organ system, and so germ-line Bnip3 knockout mice were examined after left anterior descending coronary artery ligation/reperfusion. In contrast to transgenic overexpression of anti-apoptotic Bcl2, which decreases infract size in a similar model, genetic ablation of Bnip3 had no effect on acute infract size measured as the area of gadolinium-enhanced left ventricular myocardium detected by magnetic resonance imaging (MRI). However, post-infarction left ventricular remodelling (measured as the increase in MRI-determined diastolic chamber volume) was strikingly diminished, and left ventricular ejection performance (measured as MRI-determined ejection fraction) was preserved in Bnip3 knockout mice. These functional and structural benefits were associated with decreased apoptosis, measured as TUNEL positivity and caspase 3 activation, in the peri-infarct and non-infarcted left ventricular walls. Together with the findings from cardiac-specific Bnip3 overexpression studies and correlative studies of Bnip3 gene expression in ischaemic hearts, these findings demonstrate that upregulation of Bnip3 in the myocardium that survives an ischaemic insult produces apoptotic cardiac myocyte death in the days and weeks following myocardial infarction that contribute to ventricular remodelling. The similar effects on remodelling between Bnip3 in cardiac ischaemia and Nix in cardiac hypertrophy suggest that stimulus-specific apoptotic responses might be susceptible to individual targeting in order to minimize the programmed cardiomyocyte drop-out.

5. Mechanisms of non-apoptotic programmed cell death

The above findings reveal essential functions for mitochondria as the source of critical mediators for caspase-dependent and -independent apoptosis. Mitochondria are also central to programmed necrotic death mediated through the opening of PTPs. Mitochondria are normally the major source of cellular ATP stores created through F1F0-ATP synthesis, which both requires and sustains the electrochemical gradient (Δψm) across normally impermeable mitochondrial inner membranes. Under conditions triggered by external calcium overload, calcium-sensitive mitochondrial matrix dehydrogenases are stimulated and NADH production within the respiratory chain is impaired. As a consequence, the inner mitochondrial membrane becomes more permeable to ions and small solutes, and the electrochemical gradient is lost (called the mitochondrial permeability transition, MPT). The resulting influx of water driven by oncotic pressure causes a characteristic swelling and deformation of the mitochondrial matrix. Because ATP production stops with mitochondrial depolarization, and ATP is rapidly consumed trying to re-establish Δψm, mitochondria become net consumers rather than producers of cell energy, and the cell can become doomed to a necrotic death from suspension of minimal essential homeostatic functions. In addition, mitochondrial matrix swelling can lead to physical rupture of the outer mitochondrial membrane and release of normally sequestered intermembranous proteins, cytochrome c, AIF, and EndoG, with the potential to cause apoptotic cell death as described above. However, since apoptosis is an energy-consuming process, the role played by apoptotic effectors released from irreversibly damaged mitochondria in ATP-starved cells is unclear. Indeed, the cellular level of ATP may be the critical determinant of whether a cell with suicidal tendencies will die by necrosis or apoptosis.

As noted above, calcium is an important stimulus for MPT, and therefore for programmed necrotic death. Calcium is taken up by mitochondria through a poorly characterized energy-dependent calcium uniport transporter. Although it has a low affinity for calcium, and therefore plays a modest role in minute-by-minute calcium homeostasis, the net effect of the calcium uniporter is increased under pathological conditions such as ischaemia–reperfusion injury and chronic heart failure, in which cytosolic calcium levels are abnormally high. Physical proximity between mitochondria and the major intracellular storage depot for calcium in cardiac myocytes, SR, can create junctional calcium 'hotspots' at which local calcium concentrations can be very high. Calcium liberated into these hotspots from SR calcium release channels can therefore be immediately and specifically taken up by the mitochondrial uniporter.
Because cardiomyocyte free calcium concentrations increase in response to cellular stress, this SR-mitochondrial calcium cross-talk may provide for an additional input reflecting the level of cellular stress in programmed cell death responses. Indeed, SR calcium overload produced by cardiomyocyte death in vitro and in vivo.

The recent observation that Bcl2-family proteins can modulate mitochondrial compartmentalization in endoplasmic reticulum (and by extrapolation, cardiac SR), in addition to regulating mitochondrial outer membrane permeabilization, suggests that these factors have multiple modulatory functions in programmed cell death (Figure 1).

6. Programmed cell death and protein degradation through the ER stress response and autophagy

A major function of ER/SR, in addition to being the major intracellular site for calcium storage and release, is the folding of proteins newly synthesized on adjacent ribosomes. These two functions are inter-related, and disturbances in cell calcium (either calcium overload or ER calcium depletion) can result in abnormal protein folding and sorting, the so-called ER stress response. One of the consequences of ER stress is activation of a unique, ER-localized caspase, caspase 12, by calcium-dependent calpain 2. Caspase 12 can then initiate the caspase cascade and induce apoptosis.

Another link between ER stress and apoptosis occurs as a direct consequence of activating the unfolded protein and ER overload responses involving the ubiquitin proteasome system. As recently reviewed, this is the major mechanism for protein quality control in which damaged, senescent, or misfolded proteins undergo chaperone-dependent degradation. Misfolded proteins are identified by molecular chaperones and, if they cannot be re-folded or repaired, are targeted for polyubiquitination by ubiquitin ligases, resulting in their transfer to the proteasome for proteolytic degradation. The ubiquitin/proteasome system therefore constitutes a mechanistic 'off-switch' for critical cell death proteins that is complementary to the 'on-switches' of transcriptional upregulation and post-translational activation. Apoptotic proteins that are regulated by the ubiquitin–proteasome system include members of the Bcl2 family, NF-κB, and anti-apoptotic Smac/DIABLO.

Indeed, proteasome inhibitors can activate the caspase cascade, and several conditions in which proteasome dysfunction is causative or contributory to myocardial disease are associated with apoptosis and myocardial remodelling.

Whereas the ubiquitin/proteasome system is the major mechanism for non-lysosomal degradation of misfolded proteins and for normal turnover of shorter lived and/or regulatory proteins, autophagy is the primary mechanism for lysosomal degradation of large organelles and long-lived proteins. Since protein renewal is continuous, autophagy is also normally a continuous process essential to maintaining cellular homeostasis. However, autophagy can be stimulated by extrinsic stressors (classically starvation) and intrinsic factors. When this occurs and autophagy increases beyond normal physiological limits, it can produce a form of programmed cell death characterized by physical destruction of critical cellular organelles and massive cellular dysfunction. Autophagy is distinguished from apoptosis by absence of caspase activation or nuclearosomal DNA degradation, and by the presence of characteristic double-membrane delimited autophagosomes that enclose proteins destined for degradation and transport them to lysosomes.

The initial report of autophagy in dying cardiomyocytes described organelle degeneration and the presence of autophagosomes in cultured foetal mouse hearts. Dying myocytes containing autophagosomes have since been described in dilated failing hearts, hypertrophied hearts, and chronically ischaemic hearts. Some studies have suggested that autophagy may have a greater contribution to cardiac myocyte drop-out in heart failure because histological markers for the two processes have shown a greater prevalence of autophagic than apoptotic cardiomyocytes. Caution is warranted before fully accepting this conclusion, however, because assessments of autophagy based on the presence of granular-ubiquitin inclusions may not be entirely specific, and because the time from 'snap-shot' detection of a histological marker for one or the other process to the point of terminal cell dysfunction may be longer for autophagy than for apoptosis. If this is the case, then instantaneous prevalence does not equate to relative pathophysiological impact.

Autophagic markers are observed in clinical and experimental models of heart failure, but is it not known whether autophagosome formation in cardiomyopathy is predominantly protective or detrimental. With nutrient deprivation, the activation of autophagic pathways provides additional cellular energy sources as unnecessary proteins are degraded and their constituent amino acids are released. Thus, intracellular ATP depletion, as has been reported in heart failure, can conceivably be rectified by autophagy. However, when the net effect of autophagy is the destruction of ER and mitochondria that are necessary for maintaining cellular homeostasis, the cell is committed to death (Figure 1).

Inappropriate autophagy can be the consequence of cross-talk between signals typically associated with apoptosis or the MPT. Both the pro-apoptotic Bcl2-family protein, Bnip3, and the activation of the MPTP are associated with autophagy in cell culture models. Recent work has demonstrated that the Bnip3-related protein, Nix, specifically targets mitochondria for autophagic destruction in maturing erythroblasts. It is therefore likely that either mitochondrial-targeting of Bnip3 or Nix after transcriptional upregulation, or direct mitochondrial damage caused by calcium overload and the MPT, can lead to autophagic consumption of mitochondria in cardiac tissue. Taken together, available data suggest that the pathological consequences of autophagy are determined both by the nature of the stimulus and the context of the response.

7. Cardiac remodelling due to non-programmed (but entirely predictable) cardiomyocyte death

The preceding discussion has focused upon apoptotic, autophagic, and programmed necrotic (MPT) cardiomyocyte death in ventricular remodelling. Cardiac myocyte drop-out that may
contribute to remodelling can also be the result of conventional cardiac myocyte necrosis that is not programmed, but rather is a collateral effect of the hypertrophic process itself. The hypothesis linking cardiomyocyte hypertrophy to non-programmed necrotic death postulates the existence of an 'ischaemic core' in hypertrophied myocytes. The notion is that increased cardiomyocyte cross-sectional area exceeds the physical limits of oxygen delivery to the centre (core) of the myocyte. Since the rate of oxygen diffusion through water (cell cytoplasm) is constant, and the range of oxygen tension that forms the diffusion gradient across the cell is narrowly constrained, mitochondria at the core of the cardiomyocyte can become ischaemic when their cross-sectional area exceeds the distance across which oxygen can diffuse from adjacent capillaries. The resulting decrease in ATP generation across the hypertrophied cardiac myocyte. There is also accumulating evidence that cardiac hypertrophy leads to decreased oxygen delivery to the cardiomyocyte because capillary angiogenesis does not increase in parallel with hypertrophying myocytes, and the relative decrease in myocardial capillary density results in an absolute reduction in myocardial oxygen delivery per unit of myocardium. The relative contribution of capillary/hypertrophy mismatch to cardiomyocyte dropout and ventricular remodelling has been difficult to establish, although experimental support has been provided by a mouse model of cardiac inducible activated Akt/Protein Kinase B. The Akt-signalling pathway mediates physiological myocardial growth, as in highly trained athletes. However, forced, sustained activation of this signalling pathway results in progressive left ventricular remodelling and development of heart failure. Remodelling should not have been observed if Akt-induced hypertrophy is truly 'physiologic', unless the absolute change in cardiomyocyte size itself creates a secondary pathology, such as core ischaemia. Indeed, conditional myocardial expression of activated Akt causes reversible hypertrophy at 2 weeks, but a progressive and irreversible cardiomyopathy associated with decreased capillary density and myocardial fibrosis after 6 weeks. Notably, coronary angiogenesis was enhanced during the compensated acute hypertrophic phase, but was reduced during the period of pathological remodelling and hypertrophy decompensation. Thus, myocardial remodelling was temporally associated with the development of capillary/hypertrophy mismatch. Likewise, angiogenesis mediated by a cardiac p53/HIF-1 pathway is essential for normal hypertrophy and maintenance of cardiac function in experimental pressure overload hypertrophy. In these studies, inhibition of angiogenesis using a decoy VEGF receptor in the acute phase led to decreased capillary density,contractile dysfunction, and impaired cardiac growth, whereas forced angiogenesis by adenoviral-mediated expression of VEGF or angiopoietin 1 enhanced hypertrophy and preserved contractile function. The association of decreased vascularity and progression to decompensated cardiomyopathy in a model of excessive 'physiological' hypertrophy supports the necrotic core hypothesis. These findings also show that the extent of angiogenesis can be a critical determinant of whether cardiomyocytes can be spared, such that hypertrophy will remain compensated, or whether they succumb, the ventricle remodels, and the heart fails.

8. Clinical applications

Programmed cardiomyocyte death is observed in virtually every myocardial disease. Because it tends to be a chronic process with subtle but insidious effects on cardiac structure and function (as opposed to the acute and dramatic effects of direct myocardial injury), it receives little attention from the clinical community. Notwithstanding clinical studies suggesting that benefits of pharmacological ACE inhibition and β-adrenergic receptor blockade in human heart failure are partly attributable to interruption of apoptosis, and compelling experimental data showing that programmed cell death after myocardial injury contributes in a major way to ventricular remodelling and the development of heart failure, the imperative for 'myocardial salvage' is currently focused almost exclusively upon minimizing acute injury, with only the promise of myocardial regeneration to replace lost cardiac myocytes. Yet, there is a favourable therapeutic window for interrupting programmed cardiomyocyte death (days and weeks after injury) and, like accumulation of compound interest, the long-term benefits of early and ongoing interventions to preventing the drip, drip, drip of myocyte drop-out may be great. Indeed, apoptosis inhibition is likely to be a necessary adjunct to cardiac myocyte regeneration by preventing the programmed death of transplanted cardiac myocyte progenitor cells, thereby enhancing engraftment.

Our growing understanding of the multiple forms of programmed cell death and their overlapping death signalling pathways highlights what are likely to be two major challenges in developing clinically applicable therapeutics: (i) How do you target the suicidal proclivities of cardiac myocytes without producing untoward effects on them (they 'choose' another way to die) or on non-myocytes with greater regenerative potential (unrestrained proliferation)? (ii) How do you minimize the potential to activate programmed cell death pathways by therapeutics directed at other targets? This second issue is one with which cardiovascular medicine has a history, as past clinical use of catecholamines and phosphodiesterase inhibitors (known now to stimulate cardiomyocyte apoptosis) adversely impacted outcomes in heart failure. A new generation of pharmaceuticals and gene therapies is currently under development to treat cardiac myocyte contractile dysfunction in heart failure by increasing cardiac SR calcium stores. The evidence for a connection between SR calcium, activation of the MPTP, and programmed apoptotic and necrotic cardiomyocyte death was described above, and is strengthening. Will enhanced contractility afforded by increased calcium stores be associated with a lower threshold for myocyte death in treated human hearts? If so, is combining calcium augmentation with anti-programmed death therapies a possible solution? Answers to these questions may be forthcoming with the results of ongoing clinical trials.

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