

Review

Future perspectives and potential implications of cardiac myocyte apoptosis

Armin Haunstetter^a, Seigo Izumo^{b,*}

^aDepartment of Cardiology, University of Heidelberg, Heidelberg, Germany

^bCardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Received 7 June 1999; accepted 5 August 1999

Abstract

Recent advances in the understanding of the molecular mechanisms of apoptosis has gained increasing interest in the cardiovascular research community. Apoptotic myocyte loss has been detected in different cardiac disease states such as ischemic heart disease and congestive heart failure. In addition, some evidence for the molecular mechanisms in cardiac myocyte apoptosis has been evolving, although at present the implications thereof for clinical cardiac disease are not known in most of the cases. Based on these new insights, it is the intention of this article to highlight some topics in apoptosis research that might be of particular interest to define the future role and potentials of new therapeutic approaches aimed at preventing myocyte apoptosis. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Apoptosis; Myocytes; Growth factors; Mitochondria

1. Introduction

The pathophysiology of major cardiac disease states such as ischemic heart disease and congestive heart failure is multifactorial. At the level of the myocyte both dysfunction due to altered calcium handling, impaired excitation–contraction coupling and electrical instability on the one hand and irreversible myocyte loss on the other hand are believed to contribute to disease initiation and progression. The rationale of current therapy is mainly to modify myocyte dysfunction and to minimize the intensity of the lethal injury acting on the myocyte such as duration of ischemia or the degree of pressure overload. Preservation of cells subjected to a lethal injury remains an attractive, but elusive goal. This is mainly due to the notion that once the death-inducing stimulus has reached the myocyte, the cell can no longer evade its demise leaving no room for regulation or therapeutic intervention.

Recent progress in the study of myocyte apoptosis has once again increased interest in modifying myocyte death at the cellular level (for a detailed review on apoptosis in

cardiovascular disease see [1]). In contrast to necrotic cell death, initiation and regulation of apoptosis involves several checks and balances that allow for cell survival even though a death stimulus directly acts on the cell (e.g. Bcl-2 preventing cell death induced by staurosporine). In addition, cell death has been shown to be amenable to pharmacologic intervention (e.g. caspase inhibition with zVAD.fmk). However, although promising new results on myocyte apoptosis have been published in the last few years, several unresolved issues remain, of which the pathophysiologic significance of myocyte, the role of growth factors, mitochondrial mechanisms, death receptor pathways and cell cycle control appear to be of particular interest.

2. Significance of myocyte apoptosis

Increased interest in apoptosis research in cardiology mainly stems from the hope that understanding the mechanisms of apoptosis in cardiac myocytes may provide new strategies to prevent myocyte loss. A major determinant

*Corresponding author. Tel.: +1-617-667-4858; fax: +1-617-975-5268.

Time for primary review 30 days.

for the success of this novel approach is the degree to which apoptosis contributes to total myocyte loss and to which extent this additional loss of contractile mass can be prevented to improve functional deterioration and mortality in cardiac disease.

Current evidence for myocyte apoptosis has mostly been gathered in isolated cardiac myocytes (both neonatal and adult), isolated perfused hearts and in animal models of ischemia and pressure-overload [2–7]. Although isolated myocyte preparations are well suited to study the apoptotic potential of a given stimulus and the intracellular mechanisms involved, one has to be prudent in extrapolating quantitative analyses to the *in vivo* situation. Baseline rate of apoptosis is reported to be in the range of 5–10% in most studies of cultured myocytes, which exceeds the true baseline apoptotic rate in heart sections by approximately 1000-fold [8]. Likewise, some stimuli induce apoptosis in as many as 75% of the cells, which certainly overestimates the true incidence even under pathophysiological conditions [2,3].

Although studies in isolated perfused hearts, animal models of cardiovascular disease and post-mortem studies in patients allow for a better estimate of the importance of myocyte apoptosis in the setting of cardiac disease, the reported extent of myocyte apoptosis sometimes varies widely. For example, in congestive heart failure the apoptotic index has been reported to range from 0.2 to 30% [8,9]. One recent report even questioned the occurrence of apoptosis in heart failure, relating positive TUNEL staining to DNA repair and not to apoptotic DNA breakdown [10]. Part of this may be due to differences in the severity of the disease, heterogeneity in tissue sampling or experimental protocols, but further clarification will be of major importance. Furthermore, apoptosis as assessed by TUNEL staining which implies DNA nicking, may not necessarily represent the cell population that is amenable to rescue by anti-apoptotic therapy, as myocytes might have been damaged irreversibly and inhibition of apoptosis at this stage would just alter the modality of cell death, but would not prevent myocyte loss.

Only a few reports provide evidence for the potential of anti-apoptotic therapy to improve outcome in cardiac disease. In a rat model of ischemia and reperfusion, Yaoita et al. showed that caspase inhibition reduced infarct size and improved cardiac function [11]. However, the observation period of this study was limited to 24 h and it remains to be shown whether the beneficial effect observed will last for an extended follow-up period. One major focus of future research should therefore be directed not only at verifying apoptosis in certain disease conditions, but at providing evidence that inhibition of apoptotic myocyte loss translates into a long-term benefit in terms of cardiac function and/or survival. This will help to outline the treatment potential of anti-apoptotic therapy in heart disease.

3. Trophic signals and survival factors

Several cell types like lymphoid and neuronal cells critically depend on growth factors for survival in culture. Deprivation of cells of interleukin-3 or nerve growth factor in culture induces extensive apoptosis [12,13]. Although initially not appreciated, there is increasing evidence that survival of cardiac myocytes may also depend on the presence of survival signals. Cardiotrophin-1 is a member of the interleukin-6 family of cytokines and exerts a strong hypertrophic stimulus on isolated myocytes [14]. Cardiotrophin-1 has been shown to promote cell survival of isolated neonatal cardiac myocytes kept under serum-free conditions [15]. A similar effect was observed for leukemia inhibitory factor that signals through a receptor sharing the gp130 large receptor subunit with the CT-1 receptor [15]. The concept of gp130-dependent cardioprotection was recently further substantiated in a cardiac-specific knockout of gp130 [16]. While transverse aortic banding in wild-type mice induced concentric, compensated hypertrophy, gp130-deficient mice exhibited marked biventricular chamber dilation, impaired left ventricular function and reduced survival. Development of chamber dilation correlated with a marked increase in apoptosis, suggesting that under stress conditions a trophic signal may be essential to maintain myocyte viability.

Likewise, insulin-like growth factor I was shown to reduce myocyte apoptosis in response to myocardial ischemia, ischemia–reperfusion [17,18] and doxorubicin treatment [19]. However, although the number of apoptotic myocytes was reduced in the peri-infarct region of IGF-1 transgenic mice, cardiac functional parameters did not differ between transgenic and wild-type mice [18]. Recently, also the neuregulin group of growth and differentiation factors has been implicated in the protection of isolated cardiac myocytes from apoptosis [20], increasing the list of potential inputs that may contribute to the long-term survival of cardiac myocytes.

However, some questions still remain unanswered. Does signaling through gp130 and the receptors for IGF-1 and neuregulin converge on the same point of the apoptotic cascade or does it affect different targets? For example, different anti-apoptotic strategies of different trophic factors might include the possibility of an additive anti-apoptotic effect. Furthermore, if protection interferes with upstream events of apoptotic myocyte death and not with common downstream pathways (e.g. caspases), the protective effect of certain survival factors may depend on the stimulus that induces apoptosis.

Current evidence suggests that insulin-like growth factor I activates phosphatidylinositol-3 kinase (PI-3K) [21]. Through activation of the downstream kinase Akt (also known as protein kinase B) the pro-apoptotic regulator Bad is phosphorylated and neutralized through cytosolic sequestration [22]. Nevertheless, signaling through PI-3K

may be more complex and may involve additional anti-apoptotic mechanisms. In contrast, the anti-apoptotic signaling of gp130 was shown to involve the MEK1/ERK pathway of mitogen-activated protein kinases [15]. However, ERK activation may not be enough to determine the anti-apoptotic characteristic of cardiotrophin-1, as other receptor ligands that activate ERK (like angiotensin II) do not protect against apoptosis [23]. Concomitant activation of other signaling pathways or failure to do so may critically influence the role of ERK activation. In this regard, it will be of major interest to further define the role of other mitogen-activated protein kinase such as JNK and p38 which have been implicated in the regulation of apoptosis in isolated cardiac myocytes [24,25]. A better understanding of the downstream targets of MAPK cascades may elucidate additional regulatory mechanisms in myocyte apoptosis.

In addition, the role of intercellular contact and cell–matrix interaction in myocyte survival is not well understood. For myocyte isolation, culture dishes need to be coated either with gelatin or laminin to allow for cell adherence. Failure to adhere inevitably leads to cell death (anoikis) [26]. Multiple cell–matrix interactions involving focal adhesions or integrins are known in cardiac myocytes [27,28]. These are linked to intracellular signaling cascades and may thus be capable of regulating both pro-apoptotic and anti-apoptotic signals.

Initial attempts have been made to introduce growth factors as a new treatment modality into clinical practice. A preliminary study of human growth hormone, which increases serum levels of IGF-1, in patients with dilated cardiomyopathy showed increased cardiac performance and exercise capacity after 3 months of treatment, although it is not known whether apoptotic myocyte loss was reduced in these patients [29]. However, in a more recent study only myocardial hypertrophy was observed, but no functional improvement could be documented after 3 months [30]. Both studies included only a small number of patients and the observation period was short. Further animal and clinical studies are required to substantiate treatment benefits of this novel therapeutic approach in cardiac disease.

4. Mechanisms of myocyte apoptosis mediated by mitochondria

With the localization of the apoptotic regulatory proteins of the Bcl-2 family to mitochondria and the demonstration that isolated mitochondria can exert a pro-apoptotic effect in *in vitro* model systems, mitochondrial involvement in apoptosis has gained increasing interest. Currently, two mechanisms have been described relating mitochondria to the induction of apoptosis. One involves the release of cytochrome *c*, formation of a complex with apaf-1,

caspase-9 and (d)ATP that finally leads to the activation of caspase-9 and of the downstream caspase-3 [31]. The second pathway is initiated by the opening of the permeability transition pore, release of apoptosis-inducing factor (AIF) and the induction of apoptotic changes of the nucleus and the cell membrane [32]. At present, it is not known how these pathways are related to each other, and whether the apoptotic pathway is dependent on the type of death stimulus. Of importance, the AIF pathway is caspase-independent and thus can only be inhibited by Bcl-2, but not by caspase inhibition [32].

Little is known in how far this applies to apoptosis in cardiac myocytes. Myocytes are permanently performing a huge mechanical work load that is fueled by oxidative phosphorylation of glucose, fatty acids and ketone bodies. Consequently, mitochondria make up a substantial proportion of total cellular volume. This may put cardiac myocytes at increased risk of involuntary induction of apoptosis, as turnover of mitochondria may cause leakage of pro-apoptotic mitochondrial contents into the cytosol. In addition, short-term ischemia and cardioplegia during cardiac surgery can induce morphological alterations in myocyte mitochondria at an early stage [33]. To counteract this permanent threat myocytes might have evolved specialized anti-apoptotic strategies, either by rendering mitochondria less pro-apoptotic or by expressing powerful anti-apoptotic safeguards.

Myocytes are known to express Bcl-2 and Bcl-x and overexpression of Bcl-2 in isolated neonatal rat ventricular myocytes can inhibit apoptosis induced by overexpression of p53, which is believed to involve mitochondria [34]. However, given the abundance of mitochondria in cardiac myocytes, baseline expression of Bcl-2 is low compared to other cell lineages such as lymphoid cells. And, mice deficient in Bcl-2 were not reported to exhibit an increased rate of apoptosis of cardiac myocytes or cardiac failure [35,36]. Alternatively, endogenous inhibitors of caspases like IAP-1 or XIAP might neutralize low-level caspase activation and, unless overwhelmed by massive cytochrome *c* release from mitochondria, effectively prevent inadvertent apoptosis. It will be of interest to see whether inactivation of endogenous caspase inhibitors (IAP-1, XIAP) [37,38] will affect baseline apoptotic rate in cardiac myocytes and whether this will lead to deterioration of cardiac function.

A recent observation in primary cultures of sympathetic neurons adds to the complexity of regulatory mechanisms involved in apoptosis. Deprivation of these cells of nerve growth factor (NGF) induces delayed apoptosis (>18 h) that is dependent on Bax and involves protein synthesis and cytochrome *c* release from mitochondria [39]. Surprisingly, microinjection of cytochrome *c* into the cytosol did not induce apoptosis in cells kept with NGF. Only when cells were deprived of NGF (and kept alive by inhibition of protein synthesis or due to Bax deficiency) did they

undergo apoptosis in response to cytochrome c injection, indicating that NGF withdrawal induces apoptosis by affecting two checkpoints in one pathway: one regulating the barrier function of the outer mitochondrial membrane and an additional one that prevents apoptosis downstream of cytochrome c release, the nature of which is not well understood. The susceptibility of cardiac myocytes to microinjected cytochrome c and the concentration of cytochrome c necessary to induce myocyte apoptosis are not known, but it would be of major interest to detect additional anti-apoptotic mechanisms that lie downstream of mitochondrial damage and that are amenable to regulation.

5. Death receptors

Stimulation of a group of cell surface receptors of the tumor necrosis factor receptor family induces apoptosis in several cell types such as hepatocytes or the lymphoid Jurkat cell line. Death receptor/ligand combinations include Fas/Fas ligand, TNFR1/TNF- α , death receptor (DR) 3/DR3 ligand and the death receptors 4 and 5, which both bind the ligand TRAIL [40]. In the case of Fas, TNFR1 and DR3 downstream signaling involves the adaptor protein FADD and caspase-8, which activates the executioner caspases-3 and -7, while intracellular signaling of DR4 and DR5 has not yet been characterized in detail. At least expression of Fas, TNFR1, DR4 and DR5 has been documented either at the protein or mRNA level in cardiac myocytes [41–44]. During ischemia or hypoxia expression of Fas was increased and correlated with the induction of apoptosis [6,42,45]. In addition, myocardial concentrations of TNF- α , the ligand for TNFR1, were increased during ischemia and after stimulation with lipopolysaccharide [46,47].

However, despite all these observations suggestive for death receptor-mediated apoptosis of cardiac myocytes, direct evidence for apoptosis in response to death receptor activation is scant and limited to cell culture studies [48]. So far, most observations indicate a strong negative inotropic effect for TNF- α [49], while Fas stimulation appears to affect action potential and calcium handling [50]. One explanation for this might be that cardiac myocytes, in addition to the expression of pro-apoptotic constituents of the death receptor pathways, also show strong expression of the anti-apoptotic mechanisms that control apoptosis in response to death receptor agonists. This includes the expression of decoy receptors for TRAIL like TRID [43], which lacks the intracellular domains essential for signaling, and FLAME-1 and ARC [51,52], which interfere with caspase-8 activation and its proteolytic activity, respectively. The almost exclusive expression of ARC in skeletal and cardiac muscle further supports an important role of anti-apoptotic mechanisms in cells of the myocyte lineage that counterbalance the

activation of death receptors. Therefore, expression or stimulation of death receptors might not be sufficient to induce myocyte apoptosis as long as these anti-apoptotic mechanisms are still in place. A two-step hypothesis of myocyte apoptosis in response to death receptor stimulation may be postulated, that requires both death receptor activation and concomitant relief of the post-receptor block of apoptosis. This may be an important safeguard of a cell type that, on the one hand, has lost its regenerative capacity and, on the other hand, expresses efficient pro-apoptotic mechanisms. Future studies should therefore analyze both the pro-apoptotic and anti-apoptotic constituents of the death receptor pathway.

Additional and very intriguing evidence for the importance of death receptors and especially FADD and caspase-8 in the heart comes from observations in knockout mice. Mice deficient in either FADD or caspase-8 exhibit thinned myocardium, reduced myocardial trabeculations and intrauterine death at around day 10–13 [53,54]. Neither Fas deficiency, nor deficiency of TNFR1 induces a similar cardiac phenotype during development [55–57]. At present, it is not known which receptor pathway is upstream of FADD and caspase-8 activation during cardiac development. It also remains to be established whether the cardiac phenotype is due to FADD and caspase-8 deficiency in cardiac myocytes or another cell lineage such as endothelial cells. Furthermore, during development FADD and caspase-8 may not necessarily be involved in pro-apoptotic signaling, as at least FADD has a role in signal pathways not related to apoptosis [58]. In a recent study in mice, overexpressing a dominant-negative mutant of FADD under the control of a lymphocyte-specific promoter, both thymocytes and peripheral T cells exhibited an abnormal proliferative response to stimulation of CD3 and CD28 [58]. Clearly, further studies are required to delineate the role of death receptors, FADD and caspase-8 during cardiac development. Stimulation of death receptors might constitute an important mechanism that allows for the cross-talk between different cell lineages to adjust relative cell numbers and promote differentiation.

6. Cell cycle regulation and myocyte apoptosis

Acute or progressive myocyte loss leads to cardiac functional deterioration, once loss of contractile tissue is no longer compensated by functional reserve. As myocytes have no or at least an insufficient replicative capacity to replace cell losses [59,60], it has been a challenge to disentangle the mechanisms that keep myocytes arrested in the G0/G1 phase of the cell cycle. Cell proliferation encompasses replication of genomic DNA, mitotic nuclear division and cytokinesis, all of which are regulated by effective checkpoint controls. A major mechanism of G1/S phase control is based on the phosphorylation of the retinoblastoma gene product by a complex of cyclin-

dependent kinases and cyclin D, which results in the release and thus activation of the positive cell cycle regulator E2F. However, when E2F-1 or the adenoviral protein E1A are overexpressed in isolated neonatal rat cardiac myocytes or in adult mouse myocardial tissue, cell cycle progression is associated with myocyte apoptosis [61–63]. Are therefore cell cycle progression and apoptosis linked inextricably, providing a safeguard against uncontrolled proliferation? Not necessarily, as evidenced by the fact that overexpression of cyclin D1 in cardiac myocytes results in the generation of multinucleate cardiac myocytes, which must have past both the G1/S and G2/M checkpoints [64]. Furthermore, in a fibroblast cell line, contrary to E2F-1, overexpression of E2F-2 and E2F-3 allowed for the dissociation of cell proliferation from apoptosis [65]. Nevertheless, a better understanding of the relationship between cell cycle control and apoptosis in cardiac myocytes will be necessary for the pursuit of strategies to induce myocyte proliferation *in vitro* and *in vivo*.

7. Conclusion

Although evidence has been provided that myocyte apoptosis is a feature of several cardiac disease states such as myocardial ischemia and cardiomyopathy [1], there are still several unsolved issues that need to be addressed by future research. At the molecular level it still remains uncertain which mechanisms initiate the apoptotic process in cardiac myocytes in clinical cardiac disease. Although several interventions (e.g. catecholamines, atrial natriuretic peptide, angiotensin II or stretch) were shown to induce apoptosis in cultured myocytes, their role in clinical cardiac disease has not been proven. Therefore, it will be of major interest to discern the apoptotic pathways that lead to DNA fragmentation and positive TUNEL staining in clinically relevant models of heart disease (e.g. mitochondrion-dependent vs. receptor-mediated pathways, caspase-dependent vs. AIF-induced apoptosis, pro-apoptotic and anti-apoptotic signal pathways). This will also help to define potential targets for future intervention.

Another aspect of myocyte apoptosis that has not attracted enough attention, is the relation of apoptosis to pathologic alterations. In many published myocardial sections with positive TUNEL staining, apoptotic cells and nuclei exhibit a strikingly normal morphology and only few reports provide evidence for ultrastructural alterations consistent with apoptosis such as chromatin condensation, nuclear fragmentation, formation of apoptotic bodies and phagocytosis of cell remnants [5,66]. This has increased doubts that evidence for apoptosis solely based on TUNEL staining and even DNA laddering may not suffice to prove apoptotic cell death [10,67] and that morphological alterations of myocytes *in situ* are required to confirm apoptosis of myocytes.

Of even greater importance is to define the implications of myocyte apoptosis and its extent to the progression of cardiac disease, as this will delineate the potentials of an anti-apoptotic strategy in cardiac disease states. Almost all studies on apoptosis in patients and in animal models of cardiac disease are descriptive verifying the presence of apoptotic cells, while the relation of apoptosis of myocytes to functional outcome remains mostly unknown. Unfortunately, the number of studies using an interventional approach in *in vivo* models of cardiac disease are still limited [11,16].

In summary, substantial evidence has accumulated that apoptosis is a component of clinical cardiac disease states of major epidemiologic importance. However, future research will have to better define pathologic and molecular criteria to verify the occurrence and extent of apoptosis of cardiac myocytes with greater sensitivity and especially specificity. In addition, more studies are required to provide evidence for the pathophysiologic significance of apoptosis for organ dysfunction and clinical outcome, in order to further substantiate current hopes that apoptosis of myocytes will become a new target for future therapeutic intervention.

Acknowledgements

Armin Haunstetter was supported by the Deutsche Forschungsgemeinschaft (HA 2606/1-1) and Seigo Izumo by the NIH (grant AG17008).

References

- [1] Haunstetter A, Izumo S. Apoptosis. *Circ Res* 1998;82:1111–1129.
- [2] Aikawa R, Komuro I, Yamazaki T et al. Oxidative stress activates extracellular signal-regulated kinases through src and ras in cultured cardiac myocytes of neonatal rats. *J Clin Invest* 1997;100:1813–1821.
- [3] Long X, Boluyt MO, Hipolito ML et al. p53 and the hypoxia-induced apoptosis of cultured neonatal rat cardiac myocytes. *J Clin Invest* 1997;99:2635–2643.
- [4] Cheng W, Li B, Kajstura J, Li P et al. Stretch-induced programmed myocyte cell death. *J Clin Invest* 1995;96:2247–2259.
- [5] Gottlieb RA, Burlison KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest* 1994;94:1621–1628.
- [6] Kajstura J, Cheng W, Reiss K et al. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab Invest* 1996;74:86–107.
- [7] Teiger E, Than VD, Richard L et al. Apoptosis in pressure overload-induced heart hypertrophy in the rat. *J Clin Invest* 1996;97:2891–2897.
- [8] Olivetti G, Abbi R, Quaini F et al. Apoptosis in the failing human heart. *New Engl J Med* 1997;336:1131–1141.
- [9] Narula J, Haider N, Virmani R et al. Apoptosis in myocytes in end-stage heart. *New Engl J Med* 1996;335:1182–1189.
- [10] Kanoh M, Takemura G, Misao J et al. Significance of myocytes with positive DNA *in situ* nick end-labeling (TUNEL) in hearts with dilated cardiomyopathy. *Circulation* 1999;99:2757–2764.

- [11] Yaoita H, Ogawa K, Maehara K, Maruyama Y. Attenuation of ischemia–reperfusion injury in rats by a caspase inhibitor. *Circulation* 1998;97:276–281.
- [12] Rodriguez-Tarduchy G, Collins M, Lopez-Rivas A. Regulation of apoptosis in interleukin-3-dependent hemopoietic cells by interleukin-3 and calcium ionophores. *EMBO J* 1990;9:2997–3002.
- [13] Edwards SN, Tolkovsky AM. Characterization of apoptosis in cultured rat sympathetic neurons after nerve growth factor withdrawal. *J Cell Biol* 1994;124:537–546.
- [14] Pennica D, King KL, Shaw KJ et al. Expression cloning of cardiotrophin-1, a cytokine that induces cardiac myocyte hypertrophy. *Proc Natl Acad Sci* 1995;92:1142–1146.
- [15] Sheng Z, Knowlton K, Chen J et al. Cardiotrophin 1 (CT-1) inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway. Divergence from downstream CT-1 signals for myocardial cell hypertrophy. *J Biol Chem* 1997;272:5783–5791.
- [16] Hirota H, Chen J, Betz UAK et al. Loss of gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. *Cell* 1999;97:189–198.
- [17] Buerke M, Murohara T, Skurk C et al. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. *Proc Natl Acad Sci USA* 1995;92:8031–8035.
- [18] Li Q, Li B, Wang X et al. Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress, and cardiac hypertrophy. *J Clin Invest* 1997;100:1991–1999.
- [19] Wang L, Ma W, Markovich R, Chen J, Wnag PH. Regulation of cardiomyocyte apoptotic signaling by insulin-like growth factor I. *Circ Res* 1998;83:516–522.
- [20] Zhao Y, Sawyer DR, Baliga RR et al. Neuregulins promote survival and growth of cardiac myocytes. *J Biol Chem* 1998;273:10261–10269.
- [21] Dudek H, Datta SR, Franke TF et al. Regulation of neuronal survival by the serine–threonine protein kinase Akt. *Science* 1997;275:661–665.
- [22] Datta SR, Dudek H, Tao X et al. Akt phosphorylation of BAD couples survival signals to the cell — intrinsic death machinery. *Cell* 1997;91:231–241.
- [23] Kajstura J, Cigola E, Malhotra A et al. Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. *J Mol Cell Cardiol* 1997;29:859–870.
- [24] Zechner D, Craig R, Hanford DS et al. MKK6 activates myocardial cell NF- κ B and inhibits apoptosis in a p38 mitogen-activated protein kinase-dependent manner. *J Biol Chem* 1998;273:8232–8239.
- [25] Wang Y, Huang S, Sah VP et al. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 1998;273:2161–2168.
- [26] Frisch SM, Ruoslahti E. Integrins and anoikis. *Curr Opin Cell Biol* 1997;9:701–706.
- [27] Sharp WW, Simpson DG, Borg TK, Samarel AM, Terracio L. Mechanical forces regulate focal adhesion and costamere assembly in cardiac myocytes. *Am J Physiol* 1997;273:H546–H556.
- [28] Terracio L, Rubin K, Gullberg D et al. Expression of collagen binding integrins during cardiac development and hypertrophy. *Circ Res* 1991;68:734–744.
- [29] Fazio S, Sabatini D, Capaldo B et al. A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. *New Engl J Med* 1996;334:809–814.
- [30] Osterziel KJ, Strohm O, Schuler J et al. Randomised, double-blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy. *Lancet* 1998;351:1233–1237.
- [31] Li P, Nijhawan D, Budihardjo I et al. Cytochrome c and ATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997;91:479–489.
- [32] Susin SA, Lorenzo HK, Zamzami N et al. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 1999;397:441–446.
- [33] Schmedl A, Schnabel PA, Mall G et al. The surface to volume ratio of mitochondria, a suitable parameter for evaluating mitochondrial swelling. Correlations during the course of myocardial global ischemia. *Virch Arch A Pathol Anat Histopathol* 1990;416:305–315.
- [34] Kirshenbaum LA, de Moissac D. The bcl-2 gene product prevents programmed cell death of ventricular myocytes. *Circulation* 1997;96:1580–1585.
- [35] Nakayama K, Nakayama K, Negishi I et al. Targeted disruption of Bcl-2 alpha beta in mice: occurrence of gray hair, polycystic kidney disease, and lymphocytopenia. *Proc Natl Acad Sci USA* 1994;91:3700–3704.
- [36] Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 1993;75:229–240.
- [37] Devereaux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 1997;388:300–304.
- [38] Roy N, Devereaux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J* 1997;16:6914–6925.
- [39] Deshmukh M, Johnson EM. Evidence of a novel event during neuronal death: Development of competence-to-die in response to cytoplasmic cytochrome c. *Neuron* 1998;21:695–705.
- [40] Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science* 1998;281:1305–1308.
- [41] Torre-Amione G, Kapadia S, Lee J et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation* 1996;93:704–711.
- [42] Yue TL, Ma XL, Wang X et al. Possible involvement of stress-activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia–reperfusion-induced cardiomyocyte apoptosis by carvedilol. *Circ Res* 1998;82:166–174.
- [43] Pan G, Ni J, Wei YF et al. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* 1997;277:815–818.
- [44] Pan G, O'Rourke K, Chinnaiyan AM et al. The receptor for the cytotoxic ligand TRAIL. *Science* 1997;276:111–113.
- [45] Tanaka M, Ito H, Adachi S et al. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. *Circ Res* 1994;75:426–433.
- [46] Kapadia S, Lee J, Torre-Amione G et al. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. *J Clin Invest* 1995;96:1042–1052.
- [47] Meldrum DR, Meng X, Dinarello CA et al. Human myocardial tissue TNF α expression following acute global ischemia in vivo. *J Mol Cell Cardiol* 1998;30:1683–1689.
- [48] Krown KA, Page MT, Nguyen C et al. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. *J Clin Invest* 1996;98:2854–2865.
- [49] Kubota T, McTiernan CF, Frye CS et al. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. *Circ Res* 1997;81:627–635.
- [50] Felzen B, Shilkrot M, Less H et al. Fas (CD95/Apo-1)-mediated damage to ventricular myocytes induced by cytotoxic T lymphocytes from perforin-deficient mice. *Circ Res* 1998;82:438–450.
- [51] Srinivasula SM, Ahmad M, Otilie S et al. FLAME-1, a novel FADD-like anti-apoptotic molecule that regulates Fas/TNFR1-induced apoptosis. *J Biol Chem* 1997;272:18542–18545.
- [52] Koseki T, Inohara N, Chen S, Nunez G. ARC, an inhibitor of apoptosis expressed in skeletal muscle and heart that interacts selectively with caspases. *Proc Natl Acad Sci USA* 1998;95:5156–5160.
- [53] Yeh WC, Pompa JL, McCurrach ME et al. FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science* 1998;279:1954–1958.

- [54] Varfolomeev EE, Schuchmann M, Luria V et al. Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity* 1998;9:267–276.
- [55] Adachi M, Suematsu S, Kondo T et al. Targeted mutation in the Fas gene causes hyperplasia in peripheral lymphoid organs and liver. *Nat Genet* 1995;11:294–300.
- [56] Rothe J, Lesslauer W, Lotscher H et al. Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature* 1993;364:798–802.
- [57] Pfeffer K, Matsuyama T, Kundig TM et al. Mice deficient for the 55 kD tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell* 1993;73:457–467.
- [58] Walsh CM, Wen BG, Chinnaiyan AM et al. A role for FADD in T cell activation and development. *Immunity* 1998;8:439–449.
- [59] Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res* 1998;83:15–26.
- [60] Anversa P, Kajstura J. Ventricular myocytes are not terminally differentiated in the adult mammalian heart. *Circ Res* 1998;83:1–14.
- [61] Kirshenbaum LA, Schneider MD. Adenovirus E1A represses cardiac gene transcription and reactivates DNA synthesis in ventricular myocytes, via alternative pocket protein- and p300-binding domains. *J Biol Chem* 1995;270:7791–7794.
- [62] Kirshenbaum LA, Abdellatif M, Chakraborty S, Schneider MD. Human E2F-1 reactivates cell cycle progression in ventricular myocytes and represses cardiac gene transcription. *Dev Biol* 1996;179:402–411.
- [63] Agah R, Kirshenbaum LA, Abdellatif M et al. Adenoviral delivery of E2F-1 directs cell cycle reentry and p53- independent apoptosis in postmitotic adult myocardium in vivo. *J Clin Invest* 1997;100:2722–2728.
- [64] Soonpaa MH, Koh GY, Pajak L et al. Cyclin D1 overexpression promotes cardiomyocyte DNA synthesis and multinucleation in transgenic mice. *J Clin Invest* 1997;99:2644–2654.
- [65] DeGregori J, Leone G, Miron A, Jakoi L, Nevins JR. Distinct roles for E2F proteins in cell growth control and apoptosis. *Proc Natl Acad Sci USA* 1997;94:7245–7250.
- [66] Elsasser A, Schleppe M, Klovekorn WP et al. Hibernating myocardium: an incomplete adaptation to ischemia. *Circulation* 1997;96:2920–2931.
- [67] Ohno M, Takemura G, Ohno A et al. ‘Apoptotic’ myocytes in infarct area in rabbit hearts may be oncotic myocytes with DNA fragmentation. *Circulation* 1998;98:1422–1430.