Current perspectives

Plasticity of the pathologic heart

Piero Anversa

Department of Medicine, New York Medical College, Valhalla, NY, USA

Key words:
Apoptosis; Necrosis; Myocyte division; Ventricular remodeling.

This review addresses two relevant issues concerning the adaptation of the failing heart: myocyte growth and myocyte death. Recent results are summarized to support the notion that adult ventricular myocytes are not terminally differentiated cells and myocyte replication occurs in the normal heart and is potentiated by overloads. On this basis, myocyte hypertrophy and proliferation both contribute to the remodeling of the pathologic heart in animals and humans. Additionally, the controversy regarding the activation of apoptosis in the stressed myocardium is emphasized and published results are discussed. Available information demonstrates unequivocally that cell death by this mechanism takes place in the diseased heart and may have significant implications in the progression of ventricular dysfunction to end-stage failure. The importance of recognizing that electron microscopy is inappropriate for the identification and quantification of myocyte apoptosis is strongly indicated. Moreover, myocyte necrosis is presented as a relevant component of the decompensated heart. In summary, the dogma that myocytes cannot reenter the cell cycle and undergo mitotic division is proven to be obsolete and invalid. Similarly, the dogma that myocytes can die only by necrosis is contrary to any objective interpretation of published findings. Myocyte necrosis and apoptosis, and myocyte hypertrophy and proliferation are major elements of the plasticity of the heart.

(Ital Heart J 2000; 1 (2): 91-95)

Supported by National Institutes of Health grants HL-38132, HL-39902, HL-43023, AG-15756 and AG-17042.

Received February 3, 2000; accepted February 7, 2000.

Address:

Piero Anversa, MD

Department of Medicine
Vosburgh Pavilion
Room 302
New York Medical College
Valhalla, NY 10595, USA
E-mail:
piero_anversa@nymc.edu

Myocyte regeneration

More than 10 years ago the term plasticity of the myocardium was introduced to emphasize the ability of cardiac myocytes to reexpress fetal proteins when challenged by a sudden abnormal elevation in ventricular loading[1]. The induction of a fetal program was interpreted as a molecular marker of cellular and organ hypertrophy, but the physiologic implications of this response were largely unexplained. A myriad of studies on the regulation of these gene products were performed but a link between the accumulation of one or more fetal proteins and the onset of ventricular dysfunction and its progression to terminal failure was never proven. With the exception of a small percentage of individuals with genetic defects leading to a decompensated dilated myopathy, the mechanisms responsible for the development of cardiac failure following an increase in pressure and/or volume load or ischemic myocardial damage were and remain obscure[2]. On the premise that the number of myocytes is established at birth, that the same cells contract for the entire life of the organ and that they die only with the death of the organism[3,4], the focus has been understanding the molecular control of myocyte hypertrophy[3-6]. This line of investigation reflected the notion that the expansion in myocyte mass with overloads occurs exclusively through enlargement of the existing cells. Myocyte death and regeneration were not considered relevant components of the remodeling process of the stressed myocardium. This belief largely persists today.

For decades, the general contention has been that endothelial cells, smooth muscle cells and fibroblasts are the only cardiac cells capable of reentering the cell cycle and undergoing mitotic division[7,8]. The postulated inability of myocytes to replicate has led to the conclusion that defects in muscle mass of the pathologic heart are the cause of the onset and evolution of ventricular failure. On this basis, attempts have been made to overcome the block present in terminally differentiated myocytes in order to promote additional growth[9,10]. The failing heart is typically a hypertrophied organ in which the augmentation of the muscle compartment can exceed by 2-3-fold that of a nor-

mal heart[11-16]. The increase in the coronary vasculature, microvasculature and capillary network is inadequate, failing to match the growth of myocytes[17]. Although growth limitations affect more proliferating endothelial and smooth muscle cells of coronary vessels than post-mitotic myocytes, the dogma concerning the inability of myocytes to divide continues, adding confusion to the confusion. The paradox between opinions and facts is ignored; in the absence of any real proof, myocytes continue to be seen as cells unable to experience cytokinesis. Conversely, several studies in animals and humans have demonstrated unequivocally that cardiac myocytes divide at low rate in the normal adult myocardium and at much higher levels in the diseased decompensated heart[18-26]. Myocyte proliferation participates in the adaptation of the overloaded ventricle as much as nonmyocytes[21]. In end-stage cardiac failure of humans, the myocyte mitotic index is higher than that of interstitial cells[20], suggesting a growth limitation of the coronary bed.

The post-infarcted heart is frequently provided as an example of the inability of myocytes to regenerate and replace necrotic myocardium[10]. Myocytes in the infarcted area are all dead by 6 hours after coronary artery occlusion[27,28]. Since ischemic damage involves the vasculature and nonvascular compartments of the interstitium, the formation of new myocardium in the infarcted region through myocyte growth only is impossible. Cell proliferation occurs in the viable tissue of the border zone and in the more distant myocardium where tissue oxygenation is maintained[20,29,30]. It is sad that the scientific community at large ignores that whether an organ is composed of parenchymal cells that possess or lack the capacity to proliferate, the consequences of a sudden interruption of blood supply to the tissue do not vary. The kidney is made of cells that can reenter the cell cycle and actively proliferate. However, occlusion of a renal artery branch results in cell death of the ischemic region, loss of tissue and scar formation. There is no difference in the response of the kidney and the heart to ischemic injury. The intestine possesses stem cells but this does not help to rapidly repair the necrotic segment when blood supply is interrupted by thrombotic or embolic occlusion of a mesenteric artery.

To the best of our knowledge, there is no single published study proving that myocytes are terminally differentiated cells and that this condition is established at birth. Moreover, there is no information documenting a defect in the molecular components regulating the cell cycle in adult myocytes. The entire machinery controlling the reentry of cells into the cell cycle is present in normal nonstressed myocytes and is upregulated in the failing heart[31,32]. Telomeric shortening increases[33] and telomerase activity[34] decreases in myocytes of male rats from adulthood to senescence, further indicating that a fraction of these cells undergo multiple divisions throughout life in the absence of a superimposed pathologic load. Myocyte cellular hyperplasia participates in the restructuring of the heart with aging[18], hypertensive hypertrophy[35] and ischemic cardiomyopathy[21,29]. However, this form of myocyte growth cannot be necessarily interpreted as a good adaptive response helpful to the heart, contrasting the maladaptive nature of cellular hypertrophy.

The process of cell proliferation may have a positive or negative impact on ventricular anatomy and cardiac performance. When myocyte replication is characterized by the parallel addition of newly formed cells, mural thickening takes place and cavitary volume is reduced[36]. On the other hand, the in-series insertion of new myocytes leads to chamber dilation without a proportional increase in wall thickness[21]. In the first case, the changes in the ratio of mural thickness-to-chamber radius decrease wall stress and oxygen consumption, ameliorating ventricular hemodynamics. In the second, the opposite occurs, leading to a progressive deterioration of cardiac pump function. Similarly, myocyte hypertrophy due to an increase in cell cross-sectional area, or transverse diameter, produces wall thickening and a reduction in mural stress. Conversely, myocyte lengthening expands cavitary volume, resulting in a decrease in relative wall thickness and an increase in ventricular loading. Importantly, the larger are the cells, the greater is the depression in mechanical behavior[37,38]. Myofilament Ca^{2+} sensitivity is also impaired in hypertrophied cells[39]. Myocyte multiplication, however, generates new cells of small size that can be expected to possess an enhanced contractile performance. Thus, a difference may exist between the functional properties of hypertrophied and proliferating cells.

Myocyte death

In the last 5 years, numerous studies have documented that cell death by apoptosis affects the human heart, defeating the dogma that the cell necrosis is the exclusive way by which ventricular myocytes die[40-44]. These results created great excitement because they uncovered a potential new mechanism implicated in the pathophysiology of heart failure. After an initial enthusiasm, several questions were raised concerning not only the role of apoptosis in cardiac diseases, but also the actual existence of this form of myocyte death. Rather unexpectedly, myocyte death by apoptosis was accepted as *in vitro* phenomenon, but whether it could occur *in vivo* became a matter of controversy. This confusion was prompted by a few studies published in *Circulation*[45,46] and an editorial in *Circulation Research*[47]. It is curious that myocyte proliferation has been challenged for decades on the basis that cytokinesis was never shown *in vitro*, while the opposite argument has been advanced for apoptosis. Once again, the heart had to be regarded as a unique organ different from all others including the brain. Neurons can die by apoptosis[48,49] and regenerate[50,51], but not cardiac myocytes.

The problem originates from misinterpretation of the original and subsequent descriptions of the ultrastructural characteristics of cells undergoing apoptosis under a variety of conditions in several organs. These observations were made nearly two decades ago and were obtained by morphologic techniques available at that time[52-54]. However, no actual quantitative information was collected by electron microscopy[52-54]. Without a clear understanding of the limitations inherent in electron microscopy and the complexity of deriving quantitative data from the heart with this methodology[55], the same approach was applied by some investigators to the analysis of the myocardium and strong criticisms were made of published results in animals and humans[45-47]. For reasons without any obvious rational basis, *in vitro* data were not challenged. The sad part is that electron microscopy was performed utilizing fragments of myocardium which were sampled from beating hearts and prepared by immersion fixation[45,46,56-60]. It was shown more than 25 years ago that this methodology is inappropriate for the ultrastructural analysis of the myocardium; artifacts are diffuse and involve the myofibrillar and mitochondrial compartments as well as the sarcoplasmic reticulum and T-system[26,55,61]. Real findings cannot be distinguished from artifacts introduced by the preparation. Hearts have to be arrested in diastole and the tissue has to be fixed by perfusion of the coronary vasculature to examine the ultrastructural properties of the myocardium correctly. The same approach was recently emphasized for the analysis of apoptosis in all organs[54]. So far, the critics of cardiac apoptosis have used a wrong technique and a magnitude of sampling which does not permit to collect any meaningful quantitative result[55].

The recognition that myocyte death by apoptosis may play a role in ventricular dysfunction and failure of humans is significant[40-43], but apoptosis affects only 0.18% and 0.08% of myocytes in men and women, respectively[44]. These values may suggest a limited impact of apoptosis on the depression of ventricular hemodynamics with time. However, myocyte necrosis comprises 1.2% of myocytes in men and 0.5% in women, and exceeds apoptosis in both sexes. Although the number of necrotic myocytes is several-fold greater than apoptotic myocytes in the male and female myocardium, the time required for the completion of each form of cell death is unknown. *In vitro* studies in various model systems have shown that apoptosis may be completed in a period ranging from 30 min to 2 hours[62]. Whether these timing parameters can be applied to myocytes remains to be established. An identical limitation exists for myocyte necrosis. This form of cell death has been claimed to reach its final stage in 1 to 2 days in infarcted rats[27]; this period is necessary for the cell to be engulfed by surrounding macrophages. Apoptosis may be much faster than necrosis, suggesting that the higher value of myocyte necrosis in the failing heart may not reflect a relevant difference in the number of cells dying by these two distinct mechanisms[63,64].

If the assumption is made that at any time nearly 1.5% of myocytes are experiencing cell death, the heart should rapidly disappear. This contention does not consider two critical points: myocyte proliferation does occur in the failing heart and these hearts are in the terminal phases of decompensation[20,24,25,30,63]. The cause of myocyte death with cardiac failure remains to be identified. Additionally, it is not obvious why apoptosis and necrosis occur simultaneously in ischemic and dilated cardiomyopathy[44]. Alterations in coronary blood flow are severe in the decompensated heart[65] and these defects in coronary perfusion and tissue oxygenation may trigger necrotic and apoptotic myocyte death. Transient ischemia activates myocyte apoptosis, but sustained reductions in coronary blood flow result in myocyte necrosis, which exceeds apoptosis[63]. Formation of reactive oxygen species is most likely a phenomenon that complicates further the performance and viability of myocytes in the severely depressed heart. By single electron additions, molecular oxygen sequentially generates superoxide anion, hydrogen peroxide and hydroxyl radical; the first two are only moderately reactive with other molecules, but the third one is highly reactive and causes extensive oxidative damage to macromolecules[66,67]. Oxidant challenge to aerobic cells may promote apoptotic cell death and high levels of oxygen toxicity induce cell necrosis[68]. Whether these factors are all implicated in the initiation of cell necrosis and/or apoptosis remains to be determined.

The primary event differs in ischemic and dilated cardiomyopathy, but foci of replacement fibrosis and collagen accumulation are present in the noninfarcted myocardium and throughout the ventricular wall of the dilated myopathy[15,16]. These findings point to the relevant role played by necrosis in the chronic remodeling of the diseased heart[62,63]. Such form of cell death is characterized by stimulation of an inflammatory response, fibroblast proliferation, collagen deposition and scar formation. Conversely, apoptosis does not trigger a tissue reaction and apoptotic bodies are engulfed by neighboring cells[52-54,62,63]. Additionally, myocyte apoptosis is a rapid event that is operative during acute changes in cardiac anatomy associated with sudden increases in ventricular loading[69]. Mechanical deformation of myocytes and sarcomere elongation *in vitro* lead to the release of angiotensin II that may phosphorylate the transcription factor p53[70]; p53, in turn, may upregulate the local renin-angiotensin system which may sustain p53 function and the generation of angiotensin II[70,71]. The continuous synthesis of this hormone, in combination with p53-mediated downregulation of the antiapoptotic gene bcl-2 and upregulation of the proapoptotic gene bax, may activate the endogenous cell death pathway and apoptosis. Although the extrapolation of *in vitro* observations to the *in vivo* state requires considerable caution, the recognition that stretch may be connected to cell death is critical for the heart. Diastolic loads are abnormal in all forms of cardiac failure and myocyte death facilitates ventricular dilation, counteracts compensatory hyper-

trophy and exacerbates the magnitude of stress on the remaining viable cells. A link may exist between angiotensin II formation and oxidative stress[72,73]; this possibility would suggest that a relationship may be present between mechanical deformation, angiotensin II and reactive oxygen, on the one hand, and myocyte apoptosis and necrosis, on the other.

Conclusions

In summary, effort has been made in the last 10 years to identify the mechanisms of myocyte growth and myocyte death which constitute fundamental elements of the plasticity of the heart. Improvement in the methodological approach to the analysis of the myocardium has defeated the dogma introduced more than 60 years ago that myocytes are terminally differentiated cells. This cell population expresses all the molecular components regulating the entry and progression through the cell cycle, karyokinesis and cytokinesis. Moreover, the dogma that myocytes die only by necrosis has been similarly defeated and unequivocal documentation of cell apoptosis has been obtained. Thus, the recognition that myocyte hypertrophy and replication, and myocyte necrosis and apoptosis do occur in the pathologic heart, has contributed to enhance significantly our understanding of the plasticity of the myocardium. Whether an imbalance between cell death and cell growth reflects the etiologic factor responsible for the evolution of ventricular dysfunction to terminal failure remains a critical unanswered question.

References

1. Nadal-Ginard B, Mahdavi V. Molecular basis of cardiac performance. Plasticity of the myocardium generated through protein isoform switches. J Clin Invest 1989; 84: 1693-700.
2. Cohn JN, Bristow MR, Chien KR, et al. Report of the National Heart, Lung, and Blood Institute Special Emphasis Panel on Heart Failure Research. Circulation 1997; 95: 766-70.
3. Parker TG, Schneider MD. Growth factors, proto-oncogenes, and plasticity of cardiac phenotype. Annu Rev Physiol 1991; 53: 179-200.
4. Kirshenbaum LA, Schneider MD. The cardiac cell cycle, pocket proteins and p300. Trends in Cardiovascular Medicine 1995; 5: 230-5.
5. Simpson PC. Proto-oncogenes and cardiac hypertrophy. Annu Rev Physiol 1989; 51: 189-202.
6. Chien KR, Knowlton KU, Zhu H, Chien S. Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. FASEB J 1991; 55: 3037-46.
7. Morkin E, Ashford TP. Myocardial DNA synthesis in experimental cardiac hypertrophy. Am J Physiol 1968; 215: 1409-13.
8. Zak R. Development and proliferative capacity of cardiac muscle cells. Circ Res 1974; 35 (Suppl 2): 17-26.
9. 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: 2644-54.
10. Agah R, Kirshenbaum LA, Abdellatif M, et al. Adnoviral delivery of E2F-1 directs cell cycle reentry and p53-independent apoptosis in postmitotic adult myocardium in vivo. J Clin Invest 1997; 100: 27228.
11. Linzbach AJ. Heart failure from the point of view of quantitative anatomy. Am J Cardiol 1960; 5: 370-82.
12. Astorri E, Chizzola A, Visioli O, Anversa P, Olivetti G, Vitali-Mazza L. Right ventricular hypertrophy: a cytometric study on 55 human hearts. J Mol Cell Cardiol 1971; 2: 99-110.
13. Astorri E, Bolognesi R, Colla B, Chizzola A, Visioli O. Left ventricular hypertrophy: a cytometric study on 42 human hearts. J Mol Cell Cardiol 1997; 9: 763-75.
14. Grajek S, Lesiak M, Pyda M, Zajac M, Paradowski S, Kaczmarek E. Hypertrophy or hyperplasia in cardiac muscle: postmortem human morphometric study. Eur Heart J 1993; 14: 40-7.
15. Beltrami CA, Finato N, Rocco M, et al. The cellular basis of dilated cardiomyopathy in humans. J Mol Cell Cardiol 1995; 27: 291-305.
16. Beltrami CA, Finato N, Rocco M, et al. Structural basis of end-stage failure in ischemic cardiomyopathy in humans. Circulation 1994; 89: 151-63.
17. Rakusan K. Cardiac growth maturation, and aging. In: Zak R, ed. Growth of the heart in health and disease. New York, NY: Raven Press, 1984: 131-64.
18. Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Meggs LG, Capasso JM. Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. Circ Res 1990; 67: 871-85.
19. Olivetti G, Melissari M, Balbi T, Quaini F, Sonnenblick EH, Anversa P. Myocyte nuclear and possible cellular hyperplasia contribute to ventricular remodeling in the hypertrophic senescent heart in humans. J Am Coll Cardiol 1994; 24: 140-9.
20. Quaini F, Cigola E, Lagrasta C, et al. End-stage cardiac failure in humans is coupled with the induction of proliferating cell nuclear antigen and nuclear mitotic division in ventricular myocytes. Circ Res 1994; 75: 1050-63.
21. Kajstura J, Zhang X, Reiss K, et al. Myocyte cellular hyperplasia and myocyte cellular hypertrophy contribute to chronic ventricular remodeling in coronary artery narrowing-induced cardiomyopathy in rats. Circ Res 1994; 74: 383-400.
22. Reiss K, Cheng W, Ferber A, et al. Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice. Proc Natl Acad Sci USA 1996; 93: 8630-5.
23. Beltrami CA, Di Loreto C, Finato N, et al. Proliferating cell nuclear antigen (PCNA), DNA synthesis and mitosis in myocytes following cardiac transplantation in man. J Mol Cell Cardiol 1997; 29: 2789-802.
24. Anversa P, Kajstura J. Ventricular myocytes are not terminally differentiated in the adult mammalian heart. Circ Res 1998; 83: 1-14.
25. Kajstura J, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P. Myocyte proliferation in end-stage cardiac failure in humans. Proc Natl Acad Sci USA 1998; 95: 8801-5.
26. Anversa P, Olivetti G. Cellular basis of physiologic and pathologic myocardial growth. In: Page E, Fozzard HA, Solaro RJ, eds. Handbook of physiology. Section 2: The cardiovascular system: the heart. Vol 1. Oxford: Oxford University Press, in press.
27. Kajstura J, Cheng W, Reiss K, et al. Apoptotic and necrotic myocyte cell death are independent contributing variables of infarct size in rats. Lab Invest 1996; 74: 86-107.
28. Bardales RH, Hailey S, Xie SS, Schaefer RF, Hsu S-M. In situ apoptosis assay for the detection of early acute myocardial infarction. Am J Pathol 1996; 149: 821-9.
29. Reiss K, Kajstura J, Zhang X, et al. Acute myocardial in-

farction leads to upregulation of the IGF-1 autocrine system, DNA replication, and nuclear mitotic division in the remaining viable cardiac myocytes. Exp Cell Res 1994; 213: 463-72.
30. Anversa P, Kajstura J, Cheng W, Reiss K, Cigola E, Olivetti G. Insulin-like growth factor-1 and myocyte growth: the danger of a dogma. Part 11. Induced myocardial growth: pathologic hypertrophy. Cardiovasc Res 1996; 32: 484-95.
31. Reiss K, Cheng W, Giordano A, et al. Myocardial infarction is coupled with the activation of cyclins and cyclin-dependent kinases in myocytes. Exp Cell Res 1996; 225: 44-54.
32. Setoguchi M, Leri A, Wang S, et al. Activation of cyclins and cyclin-dependent kinases, DNA synthesis, and myocyte mitotic division in pacing-induced heart failure in dogs. Lab Invest 1999; 79: 1545-58.
33. Kajstura J, Pertoldi B, Leri A, et al. Telomere shortening is an in vivo marker of myocyte replication and aging. Am J Pathol 2000, in press.
34. Leri A, Malhotra A, Liew C-C, Kajstura J, Anversa P. Telomerase activity in rat cardiac myocytes is age and gender dependent. J Mol Cell Cardiol 2000, in press.
35. Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Capasso JM. Hypertensive cardiomyopathy: myocyte nuclei hyperplasia in the mammalian rat heart. J Clin Invest 1990; 85: 994-7.
36. Olivetti G, Ricci R, Lagrasta C, Maniga E, Sonnenblick EH, Anversa P. Cellular basis of wall remodeling in long-term pressure overload-induced right ventricular hypertrophy in rats. Circ Res 1988; 63: 648-57.
37. Capasso JM, Anversa P. Mechanical performance and morphometric characteristics of spared myocytes after acute myocardial infarction in rats: effects of captopril treatment. Am J Physiol 1992; 263: H841-H849.
38. Capasso JM, Li P, Anversa P. Cytosolic calcium transients in myocytes isolated from rats with ischemic heart failure. Am J Physiol 1993; 265: H1953-H1964.
39. Li P, Park C, Micheletti R, et al. Myocyte performance during evolution of myocardial infarction in rats: effects of propionyl-L-carnitine. Am J Physiol 1995; 268: H1702-H1713.
40. Narula J, Haider N, Virmani R, et al. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996; 335: 1182-9.
41. Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. N Engl J Med 1996; 335: 1190-6.
42. Olivetti G, Abbi R, Quaini F, et al. Apoptosis in the failing human heart. N Engl J Med 1997; 336: 1131-41.
43. Frustaci A, Chimenti C, Setoguchi M, et al. Cell death in acromegalic cardiomyopathy. Circulation 1999; 99: 1426-34.
44. Guerra S, Leri A, Wang X, et al. Myocyte death in the failing human heart is gender dependent. Circ Res 1999; 85: 856-66.
45. 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: 1422-30.
46. 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: 2757-64.
47. Schaper J, Els sser A, Kostin S. The role of cell death in heart failure. Circ Res 1999; 85: 867-9.
48. Gould E, Cameron HA, Daniels DC, et al. Adrenal hormones suppress cell division in the adult rat dentate gyrus. J Neurosci 1992; 12: 3642-50.
49. Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997; 286: 493-5.
50. Gould E, Reeves AJ, Graziano MSA, Gross CG. Neurogenesis in the neocortex of adult primates. Science 1999; 286: 548-52.
51. Zindy F, Cunningham JJ, Sherr CJ, Jogal S, Smeyne RJ, Roussel MF. Postnatal neuronal proliferation in mice lacking Ink4d and Kip1 inhibitors of cyclin-dependent kinases. Proc Natl Acad Sci USA 1999; 96: 13462-7.
52. Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. Int Rev Cytol 1980; 68: 251-306.
53. Majno G, Joris I. Apoptosis, oncosis, and necrosis: an overview of cell death. Am J Pathol 1995; 146: 3-15.
54. Kerr JFR, GobØ GC, Winterford CM, Harmon BV. Anatomical methods in cell death. In: Schwartz LM, Osborne BA, eds. Methods in cell biology. San Diego, CA: Academic Press, 1995: 1-27.
55. Anversa P. Myocyte death in the pathological heart. Circ Res 2000, in press.
56. Schaper J. Effects of multiple ischaemic events on human myocardium - an ultrastructural study. Eur Heart J 1988; 9 (Suppl A): 141-9.
57. Schaper J, Froede R, Hein S, et al. Impairment of the myocardial ultrastructure and changes of the cytoskeleton in dilated cardiomyopathy. Circulation 1991; 83: 504-14.
58. Els sser A, Schlepper M, Kl vekorn W-P, et al. Hibernating myocardium: an incomplete adaption to ischemia. Circulation 1997; 96: 2920-31.
59. Takemura G, Ohno M, Hayakawa Y, et al. Role of apoptosis in the disappearance of infiltrated and proliferated interstitial cells after myocardial infarction. Circ Res 1998; 82: 1130-8.
60. Matsumura K, Jeremy RW, Schaper J, Becker JC. Progression of myocardial necrosis during reperfusion of ischemic myocardium. Circulation 1998; 97: 795-804.
61. Loud AV, Anversa P. Morphometric analysis of biologic processes. Lab Invest 1984; 50: 250-61.
62. Anversa P, Olivetti G, Leri A, Liu Y, Kajstura J. Myocyte cell death and ventricular remodeling. Curr Opin Nephrol Hypertens 1997; 6: 169-76.
63. Anversa P, Leri A, Beltrami CA, Guerra S, Kajstura J. Myocyte death and growth in the failing heart. Lab Invest 1998; 78: 767-86.
64. Anversa P, Kajstura J. Myocyte cell death in the diseased heart. Circ Res 1998; 82: 1231-3.
65. Parodi O, De Maria R, Oltrona L, et al. Myocardial blood flow distribution in patients with ischemic heart disease or dilated cardiomyopathy undergoing heart transplantation. Circulation 1993; 88: 509-22.
66. Janssen YMW, Van Houten B, Borm PJA, Mossman BT. Cell and tissue responses to oxidative damage. Lab Invest 1993; 69: 261-74.
67. Davies KJA. Oxidative stress: the paradox of aerobic life. Biochem Soc Symp 1995; 61: 1-31.
68. Fiers W, Beyaert R, Declercq W, Vandenabeele P. More than one way to die: apoptosis, necrosis and reactive oxygen damage. Oncogene 1999; 18: 7719-30.
69. Cheng W, Li B, Kajstura J, et al. Stretch induced programmed myocyte cell death. J Clin Invest 1995; 96: 2247-59.
70. Leri A, Claudio PP, Li Q, et al. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2 to Bax protein ratio in the cell. J Clin Invest 1998; 101: 1326-42.
71. Leri A, Liu Y, Wang X, et al. Overexpression of insulin-like growth factor-1 attenuates the myocyte renin-angiotensin system in transgenic mice. Circ Res 1999; 84: 752-62.
72. Rajagopalan S, Kurz S, M nzel T, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996; 97: 1916-23.
73. Nakamura K, Fushimi K, Kouchi H, et al. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. Circulation 1998; 98: 794-9.