Cell Death in Neuromuscular Diseases

Cell Death in the Cardiac Myocyte

Tetsuro Marunouchi* and Kouichi Tanonaka

Department of Molecular and Cellular Pharmacology, Tokyo University of Pharmacy and Life Sciences; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan.

Received March 30, 2015

Loss of cardiac myocytes plays a critical role in the pathogenesis of cardiovascular disorders. A decrease in the number of cardiac myocytes in cardiac diseases results in sustained, irreversible contractile failure of myocardium. Therefore prevention of cardiac cell death is a potential therapeutic strategy for various heart diseases. It is well accepted that three types of phenomena such as apoptosis, necrosis, and autophagy may be involved in myocardial cell death. Apoptosis is a highly regulated process that is promoted via death receptor pathway in the plasma membrane or via mitochondrial pathway. Necrosis is induced via mitochondrial swelling, cell rupture, and subsequent inflammation. Autophagy is a cell survival mechanism that involves degradation and recycling of cytoplasmic components. As compared with the other two mechanisms, autophagy may mediate cell death under specific conditions. These three types of cell death in the myocardium are discussed in this article.

Key words  cardiac myocyte; cell death; neuromuscular disease; apoptosis; necrosis

1. INTRODUCTION

Cell death is defined as an impairment of plasma membrane integrity.1 Several types of cell death have been distinguished in mammalian cells according to morphological criteria. Apoptosis, necrosis, and autophagy of cardiac myocytes are seen in various cardiac diseases including heart failure, myocardial infarction, and cardiac ischemia/reperfusion injury.2–5) Since cardiac myocytes are terminally differentiated cells, they have limited capacity for regeneration and repair in the damaged myocardium. Thus the affected heart exhibits more pronounced pathological features as a consequence of excessive cell death. Furthermore, the resultant decrease in the number of cardiac myocytes confers cardiac dysfunction. We summarize the recent findings for cell death in the heart and provide an overview of part of the literature on cardiac cell death in neuromuscular disease.

2. APOPTOSIS

Apoptotic cells undergo structural changes including cell shrinkage, nuclear condensation, and nucleus fragmentation.6,7) For instance, apoptosis has been detected in myocardium obtained from patients with end-stage congestive heart failure, arrhythmogenic right ventricular dysplasia, and myocardial infarction.5,8,9) In addition, apoptosis is induced in cardiac myocytes during hypoxia/reoxygenation, mechanical stretching, and exposure to doxorubicin.9–11) Apoptosis is mediated by two well-characterized pathways, the extrinsic and the intrinsic pathways, and both seem activated in cardiac myocytes under pathophysiological conditions12) (Fig. 1A).

In the extrinsic pathway, cell death is induced via activation of a death domain-containing receptor located at the plasma membrane. It can be triggered by Fas ligand or tumor necrosis factor (TNF)-α.13) Both Fas receptors and TNF receptors are expressed in cardiac myocytes and their stimulation has been implicated in cardiovascular pathology. Moreover, an increased Fas expression was associated with increased apoptosis of cardiac myocytes in various experimental heart disease models using hypoxic, ischemic/reperfused, and overstretched myocardium.11,14) Increased levels of TNF-α were observed in the congestive failing heart and the ischemic/reperfused myocardium.15) Cardiac myocytes express functional TNF-α receptors, which may undergo apoptosis after binding with TNF-α. Both Fas and TNF receptors have an intracellular death domain that may recruit and activate caspase-8 at the inside of sarcolemma. The recruitment of caspase-8 subsequently activates an effector for downstream caspases including caspase-3 without involvement of the B-cell lymphoma 2 (Bcl-2) family.16) Therefore several studies have shown that death receptor ligands and subsequent activation of their receptors may be involved in the development of heart failure.

The intrinsic pathway is activated by intracellular stress signals such as hypoxia, oxidative stress, and DNA damage.5,7) The intrinsic pathway is regulated by anti- and pro-apoptotic Bcl-2 family proteins.16) Once activated, the pro-apoptotic Bcl-2 family proteins including BH3-only proteins generate and/or transduce stress signals by translocating from the cytosol to mitochondria, where they neutralize the anti-apoptotic Bcl-2 family proteins or directly activate pro-apoptotic Bcl-2 family proteins such as Bax and Bak. Bax and Bak oligomerize to form pores on the mitochondrial outer membrane that allow the release of cytoxic proteins such as cytochrome c. Cytochrome c activates procaspase-9; active caspase-9 subsequently cleaves and activates the effector for caspase-3 and caspase-7.16) Cardiac myocytes have a very large energy requirement and their mitochondria share approximately 30% of the total intracellular volume.17) In activation of the mitochondrial pathway of apoptotic cell death, release of cytochrome c in cardiac myocytes has been reported using

* To whom correspondence should be addressed. e-mail: tetsurom@toyaku.ac.jp

© 2015 The Pharmaceutical Society of Japan
Various experimental models. Activation of the mitochondrial pathway has also been implicated in ischemia/reperfusion injury. For instance, exposure of isolated perfused hearts to ischemia/reperfusion resulted in the release of cytochrome c from mitochondria followed by activation of caspase-9 in the myocardium. 26

Apoptosis is rare in the normal mammalian myocardium; the prevalence terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL)-positive cardiac myocytes is approximately 0.01%. In the failing heart, however, apoptotic cells are increased to approximately 1%. 19 Because of the limited ability of cardiac myocytes for proliferation, a very low rate of apoptosis in cardiac myocytes is an important factor to prevent contractile dysfunction in the heart.

3. NECROSIS

Recent evidence suggests that necrotic cell death is also regulated by serial signaling events in a well-controlled manner. Necrosis is marked by distinct morphological changes, including plasma membrane derangement, loss of high-energy phosphates, and mitochondrial swelling. 27 Opening of the mitochondrial permeability transition pore (mPTP) is a cardinal factor to necrotic cell death. mPTP is mainly constituted with the voltage-dependent anion channel (VDAC), the adenine nucleotide translocator (ANT), cyclophilin D (CypD), and other molecules. 21 This complex contributes to an increase in the permeability of the mitochondrial membrane, which may especially induce Ca2+-release from mitochondria into cytosol. 21 Therefore mPTP opening leads to mitochondrial swelling. Ca2+ is an important second messenger in various physiological processes. In this case, changes in intracellular Ca2+ levels may activate pathways that lead to cell death. Furthermore, mPTP opening results in disappearance of the proton gradient responsible for oxidative phosphorylation associated with ATP synthesis in mitochondria. 22 Activation of anaerobic glycolysis to provide ATP leads to accumulation of H+, that is, acidosis in cardiac myocytes under ischemic conditions (Fig. 1B). Since excess H+ is removed via Na+/H+ exchanger (NHE) on the sarcolemma, stimulation of NHE results in an increase in Na+ flux across the sarcolemma into cytosol in ischemic cardiac myocytes (Na+ overload in cardiac myocytes). 23 In the phase of reperfusion, increase in intracellular Na+ levels stimulates the Na+/Ca2+ exchanger in reverse mode, followed by augmentation of intracellular Ca2+, so-called Ca2+ overload. Then, increased Ca2+ in cytosol is uptaken via Ca2+ uniporter into myocardial mitochondria. 24 An increase in Ca2+ in mitochondria activates Ca2+-dependent dehydrogenase, declines in reduced nicotinamide adenine dinucleotide (NADH) and electron flux from the electron transport chain, and then decreases ATP production. These changes in mitochondrial parameters are associated with an increased reactive oxygen species (ROS). 23 Furthermore, myocardial Na+ accumulation under ischemic conditions directly leads to mitochondrial Na+ overload and impairment of mitochondrial energy-producing ability. 24 These findings suggest that accumulation of tissue Na+ and Ca2+ contents plays key roles in cardiac cell death during myocardial ischemia/reperfusion.

4. AUTOPHAGY

Autophagy is seen in both hypertrophic and failing hearts following ischemic heart disease, dilated cardiomyopathy, and valvular cardiac disease. 25–27 Autophagy is considered to be a conserved process for bulk degradation and recycling of cytoplasmic components. Autophagy is an intracellular lysosomal-mediated catabolic process. After senescent and/or damaged proteins aggregate, organelles are sequestered by double membrane-limited vesicles called autophagosomes and then the vesicles are degraded by lysosomes. 28 Autophagosome formation is controlled by autophagy-related genes (Atg) such as Beclin-1 and microtubule light chain 3 (LC3) (Fig. 1C). Although autophagy is essential for tissue homeostasis, several investigations suggest that de-regulated autophagy beyond a certain threshold can be detrimental and promote cell death. Autophagy may be activated by stresses and several factors that regulate cell death have overlapping roles with autophagy. 29 Thus autophagy may misleadingly suggest a form of cell death caused by apoptosis. However, this process often promotes cell survival, as many cytotoxic compounds induce autophagy. Therefore it is important to determine whether autophagy can be induced independently of cell death.

5. CARDIAC CELL DEATH IN NEUROMUSCULAR DISEASE

Myofibrillar myopathies are a heterogeneous group of neuromuscular disorders characterized by disintegration of myofibrils. 30,31 In myofibrillar myopathies, morphologic changes in skeletal and cardiac muscles result from disintegration of the sarcomeric Z disc and the myofibrils, followed by abnormal ectopic accumulation of multiple proteins. Desmin-related cardiomyopathy belongs to a genetically heterogeneous group of myofibrillar myopathies that are caused by mutations in desmin, αB-crystallin (CryAB), filamin C, myotilin, ZASP, BAG3, FHL1, VCP, and plectin, characterized by desmin-positive sarcoplasmic protein aggregates. 31 In the experimental animal model, the CryAB R120G transgenic (TG) mouse exhibits accumulation of CryAB aggregates including desmin, and sarcomeric disorganization in the heart. 32 Recent studies suggest that the cellular toxicity induced by CryAB R120G aggregates (amyloid oligomer) is associated with mitochondrial dysfunction and induction of apoptotic cell death via the release of cytochrome c from mitochondria.33 A recent study investigated whether inhibition of apoptotic cell death resulted in therapeutic effects for desmin-related cardiomyopathy.34 The results showed that sustained Bcl-2 overexpression in CryAB R120G TG mouse hearts prolonged survival rate and was associated with mitochondrial restoration for an improvement of cardiac function and attenuation of apoptosis. However, necrotic cell death was increased in CryAB R120G TG mouse hearts, when apoptotic signaling was inhibited. These findings imply that inhibition of apoptotic signaling activates autophagy and the increment of necrotic cell death. Thus although inhibition of apoptotic cell death prolonged survival time of CryAB R120G TG mouse, in the absence of apoptosis, it is possible that another death pathway could be activated. Further investigations are required to elucidate the importance of cell death and the mechanisms that control activation of the machinery in cardiac diseases.
6. CONCLUSION

Evidence from in vivo and in vitro studies suggests that cardiac cell death plays important roles in the pathogenesis of cardiovascular diseases. Thus inhibition of cardiac myocytes' death may be a novel approach as a possible treatment for cardiac disease. Although studies in several experimental animal models have revealed that inhibition of apoptosis and/or necrosis is a valuable therapeutic strategy, a number of problems still remain. For instance, it is unclear whether proteins involved in regulating cell death have physiological roles beyond apoptosis or necrosis. In addition, crosstalk between apoptosis, necrosis, and autophagy is not yet fully understood. Further investigations are required to determine the key factors in cell death and the mechanisms underlying activation of the machinery in cardiac diseases.

Conflict of Interest  The authors declare no conflict of interest.

REFERENCES


