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## FOCUS: *Apoptosis*

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### BRIEF REVIEW

## Apoptosis and the Cardiovascular System

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**A**poptosis (from the Greek “falling off,” as in petals of a flower) is an essential process contributing to normal fetal development and the remodeling of adult tissues in both health and disease. Apoptosis, or programmed cell death, is the regulated deletion of cells to establish and maintain normal organ architecture and cell mass. In the embryo, organ precursor cells expand their numbers prior to the sculpting (remodeling) of distinct architectural features through apoptosis, as in the central nervous system and the distal limbs. In the adult, apoptosis is a consequence of cellular senescence and contributes to normal turnover. Organs comprised of rapidly renewing epithelia are characterized by high rates of both proliferation and apoptosis. In the immune system, apoptosis is employed to delete T cell clones in the process of negative selection. Errors in this process can result in autoimmune phenomena or defects in appropriate immune responses. Apoptosis may occur in response to pathological as well as physiological influences, but whether it occurs as a consequence or as an adaptive response in the latter context is not always clear. Documenting the location, identity and proportion of apoptotic cells and the circumstances leading to their programmed deletion is required in order to understand the role that apoptosis serves in the particular context of interest. Such studies have formed the basis for investigating apoptosis in general, and provide a focal point for beginning to understand the role of apoptosis in the cardiovascular system.

### Apoptosis: Defining Cellular Suicide

Morphological and biochemical criteria have been established to distinguish apoptosis from necrosis (accidental cell death). These include a reduction in cell volume (as opposed to cell swelling), condensation of nuclear chromatin, blebbing of the plasma membrane, nuclear fragmentation, and cleavage of chromosomal DNA into oligonucleosomal fragments. Apoptotic cells are identified by histopathological or ultrastructural documentation of these morphological changes, histochemical identification of nuclear DNA fragmentation by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL),

and the “laddering” of DNA fragments displayed by agarose gel electrophoresis. Using such techniques, investigators have established the occurrence and begun to explore the role of apoptosis in the cardiovascular system. Although still a relatively new area of cardiovascular investigation, apoptosis occurs under a variety of circumstances that may well provide unique diagnostic and therapeutic opportunities to the cardiovascular researcher and clinician.

### Apoptosis in the Heart

Apoptosis occurs during normal embryonic development of the cardiovascular system and in the adult heart and vasculature. During cardiac organogenesis, apoptosis within the conotruncal cushions contributes to the remodeling that occurs during formation of the bulbus cordis (1). Postnatally, apoptosis of cardiomyocytes within the right ventricle leads to an appropriate reduction in muscle mass during the transition from a fetal to an adult circulation (2). Apoptosis in the mature cardiovascular system has been observed in the settings of chronic heart failure, arrhythmogenic right ventricular dysplasia, acute myocardial infarction, normal cardiac aging and in cardiomyopathy induced by rapid ventricular pacing. In acute myocardial infarction, apoptosis predominates within the first few hours of ischemia followed by massive necrosis later in time. A second wave of apoptosis about 1 week later likely contributes to post-infarction ventricular remodeling (3). Two recent studies demonstrating apoptosis in chronic heart failure (4) and arrhythmogenic right ventricular dysplasia (5) suggest that apoptosis may contribute to remodeling in the non-infarcted human heart. These observational studies have stimulated efforts to establish the precise role of apoptosis in these conditions, reflecting growing interest in the mechanisms and consequences of myocardial remodeling in general.

### Apoptosis in the Vasculature

Extensive apoptosis occurs in the medial smooth muscle of the lower abdominal aorta and umbilical vessels during postnatal remodeling as a part of the normal adaptation from fetal to adult circulatory hemodynamics (6). In the embryo, the resorption of paired great vessels and multiple aortic arches that results in the adult pattern of the great vessels likely requires extensive apoptosis as well. Errors in this process are likely responsible for benign or hemodynamically significant vascular anomalies. Apoptosis has been observed following acute arterial balloon injury, in the smooth muscle cells of restenotic lesions, in advanced atherosclerotic plaques, in saphenous vein grafts, and in the setting of spontaneous hypertension. In all of these pathological settings, it is thought that apoptosis of vascular smooth muscle cells may play an important role in the vascular remodeling now considered an essential feature of these disparate processes. In atherosclerotic lesions apopto-

sis is most prevalent in the layer of smooth muscle cells nearest the vessel lumen. The regulated deletion of smooth muscle cells in atherosclerosis might represent a beneficial adaptive response, resulting in smaller, less cellular and less obstructive lesions. It almost surely plays a role in the remodeling resulting in overall vessel enlargement observed during this disease process. On the other hand, loss of cells could result in less elaboration and maintenance of extracellular matrix proteins such as interstitial collagen, suggesting a role for apoptosis in determining overall plaque stability. These examples of apoptosis in the vasculature suggest that in addition to proliferation, migration, and extracellular matrix metabolism, apoptosis may be an important feature of vascular remodeling under a variety of circumstances. The body of evidence documenting the specific circumstances under which apoptosis occurs continues to grow, but such observational studies provide little information regarding the initiation, regulation, or consequences of apoptosis in the heart and vasculature. However, much progress has been made in the study of apoptosis at the cellular and molecular levels that provides the basis for more mechanistic studies in the cardiovascular system.

### Molecular Triggers of Apoptosis

Apoptosis requires specific triggers to initiate a highly orchestrated and genetically defined chain of events. The specific physiological and pathological triggers are still being defined for particular tissues and cell types but include ionizing radiation or other DNA damaging agents, inflammatory and immunoregulatory cytokines, oxidative stress and changes in redox potential, growth factor or other trophic factor deprivation, and mechanical stress.

The identification of reactive oxygen species as pathological mediators in heart failure and myocardial ischemia is well established. There is experimental evidence that apoptosis occurs in reperfusion injury as well as acute myocardial infarction. It has been postulated that oxygen-containing free radicals induce DNA damage and subsequent apoptosis in the same manner as other DNA-damaging agents, though this remains to be firmly established.

Inflammatory cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ) can induce apoptosis, depending on the cell type. Serum TNF- $\alpha$  is elevated in cardiac diseases including heart failure and acute myocardial infarction, and is produced within the heart by cardiac myocytes. It has been postulated that local myocardial levels of TNF- $\alpha$  may be elevated under certain circumstances, promoting an increased susceptibility of diseased cardiomyocytes to apoptosis. TNF- $\alpha$  has been shown experimentally to induce apoptosis in isolated rat cardiomyocytes. The two major subtypes of TNF- $\alpha$  receptors (TNFR-1 and TNFR-2) are expressed by cardiac myocytes and apoptosis is specifically induced by TNFR-1 agonists (7). Fas, a transmembrane

protein belonging to the TNF receptor superfamily, is responsible for regulating T cell apoptosis and is expressed in cardiac myocytes. Fas and Fas-ligand interact to trigger apoptosis, and their expression has also been detected in atherosclerotic plaques. Thus the Fas/Fas-ligand system as well as TNF- $\alpha$  may be involved in the development or progression of cardiac or vascular lesions by mediating apoptosis.

In contrast to apoptosis that occurs in response to TNF- $\alpha$  and other pro-inflammatory cytokines, the withdrawal of trophic factors such as basic fibroblast growth factor can also induce apoptosis in smooth muscle (8) and endothelial cells. Apparently the absence of specific growth- or differentiation-related signals is sufficient to trigger the cell death program, and may be related to the normal process of cellular senescence. Conversely, the unregulated expression of certain proliferation-related transcription factors (c-myc, E2F) and viral proteins (adenovirus E1A, HPV E6) can also induce cell cycle abnormalities that result in apoptosis. For example, deliberate efforts have been made to override normal cell cycle constraints associated with terminal differentiation in ventricular myocytes by ectopically expressing the adenoviral protein E1A, which is known to interfere with the cell cycle regulator Rb (the retinoblastoma gene product). Such unregulated expression of proteins altering cell cycle control forces the cells into S-phase (the cell cycle phase of DNA replication) but is insufficient to permit the cells to progress through the normal cycle to cell division, resulting in subsequent death by apoptosis (9). These studies point out that in the cardiovascular system the regulation of proliferation, differentiation, senescence and survival is complex and that apoptosis occurs as a common default response when one or more signals is absent or unregulated.

### Molecular Regulators of Apoptosis

The molecular and biochemical pathways regulating apoptosis have been intensively investigated using biochemical and genetic means. Studies indicate that the caspase cascade and the bcl-2 family of proteins are central regulators of apoptosis under most if not all circumstances. The caspase family of proteases are so named for their unusual substrate specificity, cleaving peptides between cysteinyl and aspartyl residues. They are also known as ICE-like proteases after the prototype interleukin-1 $\beta$ -converting enzyme (ICE). The caspases are synthesized and stored in the cell as zymogens that are activated in response to diverse apoptotic signals. The cellular targets for these proteases include other inactive caspase zymogens, cytoskeletal proteins, nuclease precursors, and nuclear envelope proteins. A detailed discussion of the molecular regulation of the caspase cascade is beyond the scope of this brief review, but the interaction between the caspases and cytochrome c, an important determinant of mitochondrial function, has recently assumed central im-

portance. The bcl-2 family of apoptosis regulators might function in part by modulating the activity of caspases toward their targets, especially through interactions with mitochondria that alter the distribution of cytochrome c.

The bcl-2 family includes both anti-apoptotic (e.g. bcl-2, Bcl-X<sub>L</sub>) and pro-apoptotic members (e.g. Bax, Bad), and they function in part by forming dimers with each other. The prototype bcl-2 is overexpressed in certain B-cell lymphomas where it inhibits apoptosis and thus contributes to uncontrolled tumor expansion. The members of the bcl-2 family can form ion channels in lipid bilayers and contain lipophilic domains targeting them to the outer mitochondrial membrane. Key pieces in the puzzle relating caspase activation, bcl-2 family members and mitochondrial release of cytochrome c have recently been provided (10). Induction of apoptosis is accompanied by rapid mitochondrial cytochrome c release, which is antagonized by bcl-2 and Bcl-X<sub>L</sub>. Bax may function not only by binding and antagonizing bcl-2 but by directly promoting cytochrome c release. Conversely, bcl-2 and Bcl-X<sub>L</sub> may function in part by preventing Bax-induced cytochrome c release. Cytochrome c can directly activate certain caspases, especially the important effector caspase-3 (CPP32, YAMA), and is sufficient to trigger apoptosis when injected into cells or added to cell-free extracts. Cytochrome c release may in fact be required for caspase activation and apoptosis. One important exception is if Fas or TNFR-1 have been engaged, since these receptors directly activate the caspase cascade. Even in the case of Fas/TNFR, cytochrome c release may act to amplify the apoptotic signal as caspase activity can also promote cytochrome c release. Thus there appears to be a key role for the mitochondrion as a central target of the apoptosis regulatory machinery, although how specific changes in the structure and function of this organelle are regulated to result in either apoptosis or necrosis remains controversial. The resolution of these issues should yield new opportunities for establishing tissue- and disease-specific relationships and developing novel therapies.

#### Future Directions

That apoptosis occurs in the human cardiovascular system is well established, yet the exact role of this process in health and disease are still poorly understood. Research in this area is expanding and contributing to our knowledge of the cellular and molecular mechanisms underlying cardiovascular disease. A greater understanding of how apoptosis is

regulated will undoubtedly provide new therapeutic opportunities including gene therapy to influence cardiac and vascular remodeling. Obvious targets include the caspases and the anti-apoptotic bcl-2 family members but other rational targets such as subdomains of cytochrome c will undoubtedly be identified. The challenge to the cardiovascular research community is to bring our knowledge in this area to the point where new therapeutic approaches can be developed and added to the armamentarium of cardiovascular practitioners for the benefit of their patients.

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