Review

Role of Lysosomal Cathepsins in Post-Myocardial Infarction Remodeling

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Left ventricular remodeling after myocardial infarction (MI) includes extensive cardiac cell death, inflammatory cell infiltration, cell differentiation, and scar formation. Lysosomal proteases cathepsins participate in all these events during post-MI cardiac repair. These cathepsins cleave Bcl-2 interacting protein Bid, and degrade the anti-apoptotic members Bcl-2, Bcl-xL and Mcl-1, thereby triggering a mitochondrial pathway of apoptosis. Cathepsins also contribute to monocyte and macrophage differentiation and migration. Monocytes, macrophages, and neutrophils are recruited to the site of infarction, where they also release lysosomal cathepsins as inflammatory mediators to regulate post-MI inflammatory responses. Cathepsins also regulate fibroblast trans-differentiation and further affect collagen or other matrix protein synthesis during post-MI extracellular matrix remodeling.
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INTRODUCTION

Although great progress has been made in prevention and treatment during the past few decades, coronary heart diseases remain one of the leading causes of mortality in the United States.[1] The global medical burden of ischemic heart disease, especially new and recurrent myocardial infarction (MI), is still heavy.[2]

The post-MI cardiac healing process can be grouped into four phases: (1) the death of cardiomyocytes, including necrosis, apoptosis, and autophagy; (2) the inflammatory phase, which features monocyte and lymphocyte migration into the necrotic myocardium for the removal of dead cardiomyocytes; (3) granulation tissue formation, characterized by the presence of fibroblasts, macrophages, myofibroblasts, new blood vessels, and extracellular matrix (ECM) proteins; and (4) scar formation, characterized by acellular and cross-linked collagen-rich regions.[3] After MI, the left ventricle (LV) undergoes a remodeling process including molecular, cellular, and extracellular responses, which result in structural, physiological, and neurohormonal changes in the LV wall. Adequate remodeling helps to preserve cardiac functions, while adverse remodeling usually associates with a poor prognosis such as cardiac rupture or further LV dilatation and dysfunction[4]. Many factors participate in LV remodeling, including inflammatory responses, hemodynamic loads, neurohormonal activation, extracellular responses such as cytokine production, and fibrosis and protease activation.[5]

Cathepsins refer to the papain family of cysteine proteases in general, which has 12 members. A few other cathepsins, however, are not cysteine proteases — for example, cathepsins A and G are serine proteases, and cathepsins D and E are aspartic proteases. These cathepsins are primarily localized in the lysosomes, with diverse but important functions in cancer, stroke, arthritis, and neurological diseases. Cathepsins mainly function intracellularly to degrade unwanted proteins,[6] but we have demonstrated that cysteinyl cathepsins play important roles in cardiovascular diseases, such as atherosclerosis and abdominal aortic aneurysms, through degradation of ECM proteins — including collagen, elastin, fibronectin, and laminin.[7-12] These lysosomal proteases therefore may act in the cytosol and extracellularly to produce bioactive fragments. For example, cathepsin S degrades lamin-5 and generates 100 kDa γ2' and 80 kDa γ2χ pro-angiogenic fragments.[12] Recent work has uncovered the functions of cathepsins in the myocardium.[13-15] In this short review, we will focus on our current understanding of the biological roles and molecular mechanisms of cathepsins in post-MI cardiac remodeling.

CATHEPSINS AND POST-MI APOPTOSIS

Various cell death stimuli could induce lysosomes to destabilize and subsequently lead to lysosomal membrane permeabilization (LMP), which releases cathepsins from the lysosomes into the cytosol and participates in apoptosis signaling (**Figure 1**). Cathepsin D was the first lysosomal protease identified as a positive mediator of apoptosis in human cervical cancer HeLa cells and human monocytic U937 cells.[16] Suppression of cathepsin B protected murine embryonic fibroblasts (MEF) from tumor necrosis factor-α (TNF-α)–induced apoptosis.[17] Cathepsin G can induce

neonatal rat cardiomyocyte detachment and apoptosis by anoikis through a caspase-3–dependent pathway.[18] The underlying mechanisms, however, were not fully understood in those early studies.
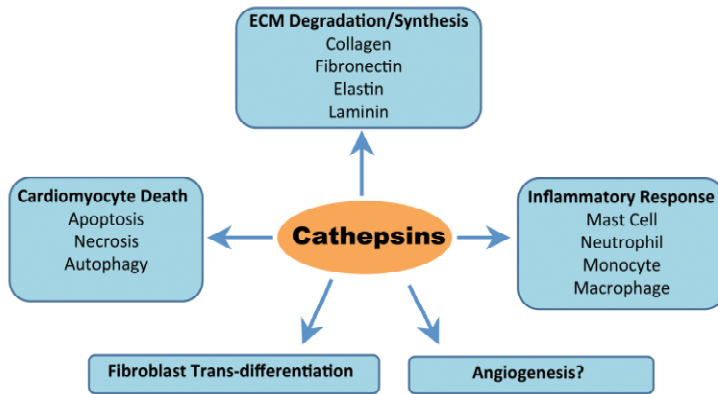

Figure 1. Possible role of lysosomal protease cathepsins in post-MI remodeling.

Recently, it became clear that cathepsins can mediate caspase-dependent apoptosis. In several human cancer or fibroblast cell lines, LMP induced by the lysosomotropic agent LeuLeuOMe released cathepsins from lysosomes to cytosol to cleave Bid, a Bcl-2 interacting protein that mediates cytochrome c release from mitochondria,[19] and to degrade the anti-apoptotic family members Bcl-2, Bcl-xL, and Mcl-1. Together, these proteolytic cathepsin activities trigger the mitochondrial pathway of apoptosis. Treatment with E64d, a broad-spectrum inhibitor of cysteinyl cathepsins that inhibits the activities of several well-known cathepsins - including cathepsins S, K, B, and L - successfully prevented LeuLeuOMe-induced apoptosis in a series of cell lines such as human neuroblastoma cell line SH-SY5Y, human immortalized keratinocytes HaCaT, human adenocarcinoma cell line HeLa, human hepatoma cell line HepG2, human colon carcinoma cell line CaCo-2, human embryonic kidney fibroblasts HEK293, human breast carcinoma cell line MCF-7 and normal human dermal fibroblasts.[20] In vitro data showed that cathepsins B, L, S, K, and H could cleave Bcl-2, Bcl-xL, Mcl-1, Bak, and BimEL, whereas no Bax cleavage was observed.[20] E64d also inhibited reactive oxidative species–induced cardiomyocyte apoptosis.[14]

Cathepsins also may participate in other types of cell death, including necrosis and autophagy (**Figure 1**).[21,22] But whether cathepsins mediate cell survival or cell death is still controversial in different tissue and cell types. For example, cathepsin B and L dual-knockout mice showed massive apoptosis of selected neurons in the cerebral cortex and in the cerebellar Purkinje and granule cell layers,[23] suggesting that cathepsins B and L play an anti-apoptotic role in the central nervous system. Cathepsin L induces apoptosis but inhibits autophagy in human monocyte-derived macrophages.[24]

CATHEPSINS AND POST-MI INFLAMMATORY RESPONSES

Dying cardiomyocytes elicit inflammatory responses for the clearance of dead cells and matrix debris, and activate reparative pathways leading to scar formation. Infarct healing can be divided into three phases: the inflammatory phase, the proliferative phase, and the maturation phase.[25] The triggering of inflammatory responses associates with activation of chemokine and cytokine cascades, resulting in recruitment of inflammatory leukocytes, including mast cells, neutrophils, monocytes and macrophages, into the infarcted tissues (**Figure 1**). Lysosomal enzymes are important mediators of acute myocardial infarction, and their release into the cytoplasm stimulates the formation of inflammatory mediators.[26] Neutrophils and macrophages clear dead cells and matrix debris from the infarcted myocardium,[27] whereas mast cells appear in infarcted myocardium as early as 1 day post-MI to release growth factors, angiogenic mediators,[28,29] and proteases, including cathepsins.[30,31] Post-surgery (e.g. coronary bypass) inflammatory responses associate with increased cathepsin B and decreased cathepsin S concentrations in the circulation.[32]

Cardiac mast cells increased by 15-fold and reached maximum at 21 days post-MI in rat left anterior descending coronary artery ligation-induced experimental MI.[33] In a canine model of myocardial ischemia and reperfusion, mast cells started to increase 72 hours after reperfusion and accumulated in areas of collagen deposition.[34] In rat coronary artery occlusion and reperfusion-induced experimental MI, mast cells peaked at both 1 day and 21 days post-MI.[28] Following infiltration to the myocardium, mast cells release cytokines, chemokines, histamine, growth factors, and proteases to induce cardiomyocyte death, inflammatory cell recruitment, fibroblast proliferation and differentiation, and protease activation, leading to impaired cardiac functions (**Figure 1**).

In vitro, treatment of rat cardiac fibroblasts with mast cell tryptase induced fibroblast proliferation and collagen synthesis.[35] Mast cells also release histamine and tryptase to differentiate fibroblasts into α-smooth muscle actin-positive myofibroblasts.[36] Mast cell proteases, such as chymase, tryptase, and cathepsins are capable of activating collagenase, gelatinase, and stromelysin.[30] In normal rat heart, although in low numbers, mast cells induced matrix metalloproteinase (MMP) activation and collagen degradation,[37] likely via both mast cell-specific proteases[30] as well as cathepsins.[31] Increased mast cell contents in infarcted hearts associated with concurrent increase in MMP activities.[29] In spontaneous hypertensive rats, mast cell stabilizer nedocromil prevented LV fibrosis and macrophage infiltration to the ventricle and normalized cytokine profile (IL4, IL6, IL10, and IFN-γ) in hypertensive hearts.[35] In canine hearts, mast cells were the predominant source of TNF-α in the first few hours following ischaemia-reperfusion,[38] and histamine levels increased by 2-fold following ischaemia-reperfusion. Blockade of histamine receptor H2 with antagonists decreased infarct sizes, although did not change cardiac functions.[39] In canine MI, many degranulated mast cells were clustered with newly

recruited macrophages and neutrophils.[40] In rat MI, injection of in vitro prepared mast cell granules to the infarct plate increased macrophage infiltration and angiogenesis, and decreased cardiomyocyte apoptosis and MI progression, thereby preserving post-MI LV function.[28] Mast cell stabilization with ketotifen reduced mast cell degranulation and myocardial injury. Degranulation of mast cells with compound 48/80 before rats undergoing ischaemia-reperfusion also attenuated myocardial injury.[41] In mouse model of ischaemia-reperfusion, mast cell-deficiency (W/W^v strain) increased cardiomyocyte viability,[42] reduced ventricular chamber dilatation, and increased cardiac wall thinning and collagen deposition in the viable myocardium.[29]

Neutrophils are mainly recruited 6~72 hours after MI (**Figure 1**).[43] Infiltrated neutrophils generate free radicals and release enzymes to clean dead cells and matrix debris. Moreover, they express mediators or chemokines, such as transforming growth factor-β (TGF-β), monocyte chemoattractant protein-1 (MCP-1) and interleukin-10 (IL-10), to amplify further cell recruitment. Although neutrophil activation may be beneficial early in post-MI remodeling, proteolytic enzymes or reactive oxygen species released by neutrophils can harm surrounding cardiomyocytes - a process called neutrophil-mediated cardiomyocyte injury.[25] Cathepsin G is mainly released by activated neutrophils, and plays a critical role in cardiomyocyte anoikis.[43] In vivo administration of a recombinant neutrophil serine protease inhibitor, LEX032, attenuated rat ischemia/reperfusion (I/R) injury by inhibiting neutrophil accumulation in the ischemic-reperfused myocardium and by inactivating cytotoxic metabolites (serine proteases and superoxide radicals) released from neutrophils.

Recruited by chemokines and cytokines of mast cells and neutrophils, monocytes from peripheral blood infiltrate the infarcted myocardium and differentiate into mature macrophages (**Figure 1**). Differentiated macrophages phagocytize necrotic and apoptotic cells, and secrete cytokines and growth factors such as TNF-α and TGF-β to regulate fibrosis and angiogenesis. Nucleophosmin (NPM1) cleaved by caspase is one of the mechanisms during macrophage colony stimulating factor (M-CSF)–induced monocyte differentiation. Caspases cleave NPM1 into a 30-kDa N-terminal fragment, which is subsequently cleaved by cathepsin B into a 20-kDa fragment. Together these fragments contribute to decrease in migration, motility, and phagocytosis capabilities of the resting macrophages. Furthermore, the cathepsin-mediated fragment cleavage may occur either in lysosomes, phagolysosomes, or cytosol.[44] Cathepsins therefore participate in protease-dependent macrophage migration. Cathepsins S, B, and L play roles in macrophage migration into the infarct area.[45-47] Visualized by multichannel fluorescent molecular tomography (FMT), active recruitment of macrophages and neutrophils, along with increased cathepsin activities in healing zones, were found 4 days post-MI, as compared to healthy myocardium.[48] Cathepsin L deficiency is associated with significantly impaired recruitment of monocytes, natural killer cells, fibrocytes, and c-kit positive cells in the peripheral circulation.[15]

CATHEPSINS AND POST-MI ECM REMODELING

ECM of the heart is a well-organized three-dimensional non-cellular network comprised of interstitial collagens (types I, III, IV, V, and VI); glycoproteins (fibronectin, laminin, periostin, fibromodulin, and vitronectin); proteoglycans (versican, lumican, and biglycan); glycosaminoglycans (hyaluronic acid and dermatan sulfate); and matricellular proteins (secreted protein, acidic and rich in cysteine, thrombospondins, and tenascins).[49] Collagens type I and type III, produced mainly by cardiac fibroblasts under physiological conditions, are the most abundant ECM proteins among all components.[50] After MI, many of these cardiomyocytes undergo apoptosis and necrosis and are replaced by a fibrous scar primarily composed of collagen, which preserves ventricular integrity and prevents cardiac rupture.[51]

Our group demonstrated that cysteinyl cathepsins play important roles in vascular ECM metabolism.[52] Cathepsins K and L are potent collagenases and elastases,[53] while cathepsin S can degrade elastin, laminin, and fibronectin[12,54] - suggesting a role of these proteases in post-MI LV remodeling. Cathepsins may also affect ECM metabolism through other mechanisms. Cathepsin L-deficient mice showed impaired collagen and elastin deposition in the infarct scar, diminished myofibroblast population and proliferation, and reduced blood vessel formation in their infarcted scars.[15]

Collagen turnover, predominantly regulated by myofibroblasts, occurs in both infarct and remote areas during post-MI stages. Although the origins of myofibroblasts are controversial, trans-differentiation from local cardiac fibroblasts is a major source.[55] Mechanical tension, TGF-β, and a splice variant of cellular fibronectin called ED-A, are three important factors that mediate cardiac fibroblast transdifferentiation.[56] E64d administration impaired cardiac fibroblast trans-differentiation into myofibroblasts, leading to decreased collagen synthesis and scar instability, and showing adverse remodeling (Shi, unpublished data). Cathepsin S can digest fibronectin network in vitro;[55] thus, it may participate in myofibroblast trans-differentiation (**Figure 1**). As expected, cathepsin S-deficient mice mimicked the function of E64d administration (Shi, unpublished data), suggesting that cathepsin S is crucial for fibroblast trans-differentiation.

Myocardial angiogenesis plays important role in cardiac regeneration and in improving post-MI cardiac functions.[57,58] Although a direct role of cathepsins in myocardial angiogenesis has not been tested, cathepsin function in angiogenesis in general has been thoroughly studied in tumor models[59,60] or in arterial diseases.[61] Several studies revealed controversial roles of MMPs in post-MI myocardial angiogenesis. In a rat model of MI, doxycycline inhibition reduced MMP-2 and MMP-9 activities, and therefore reduced vessel density in infarcted myocardium.[62] In rabbit experimental MI, however, inhibition of MMP with a

selective MMP inhibitor increased infarct subendocardial layer vessel numbers.[63] In mice, absence of MMP-9 increased vessel density in infarcted myocardium.[64] Inflammatory infiltrates in infarcted myocardium, such as macrophages, mast cells, and neutrophils, all produce cathepsins, which may also play critical roles in myocardial angiogenesis (**Figure 1**), a hypothesis remains to be tested.

PERSPECTIVE

As summarized in **Figure 1**, cathepsins participate in all steps of the post-MI healing process. Release of cathepsins from lysosomes regulates the apoptosis pathway, modulates inflammatory responses through proteolytic activities, and plays a pivotal role in ECM turnover after MI. Serum levels of cathepsins appear to be promising biomarkers in the diagnosis of coronary heart disease.[65-67] Although more extensive studies are required, and detailed mechanisms are still unknown, current available experimental evidence suggests that cathepsins are important in post-MI remodeling. Regulation of cathepsin activities, either biologically or pharmacologically, may have therapeutic potential in post-MI repair.

CONFLICT OF INTEREST
None.

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