Am J Cardiovasc Dis 2016;6(4):163-170
www.AJCD.us /ISSN:2160-200X/AJCD0039010

Original Article

The function of cathepsins B, D, and X in atherosclerosis

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Received August 29, 2016; Accepted November 7, 2016; Epub November 30, 2016; Published December 15, 2016

Abstract: Cathepsins are proteolytic enzymes typically located within the lysosomes of macrophages. Once released, they can enhance the inflammatory process in atherosclerosis. Cathepsin X aids in the migration of T-lymphocytes and the release of cytokines. Cathepsin D modifies low-density lipoprotein to promote its uptake by macrophages and its subsequent foam cell formation. Furthermore, cathepsin D regulates apoptosis. Cathepsin B degrades the extracellular matrix within the arterial intima. Together, they increase plaque vulnerability. This evidence suggests that cathepsins play an important role in the pathogenesis of atherosclerosis.

Keywords: Atherosclerosis, cathepsin, TNF-alpha, low density lipoprotein, apoptosis, vulnerable plaque

Introduction

Atherosclerosis and vascular disease have remained a major cause of morbidity and mortality throughout most of the developed world [1]. Despite a decrease in the mortality rate from coronary disease and the wealth of knowledge about its pathogenesis, there are still areas of uncertainty and limitations in our understanding of how best to prevent or treat this disease. Emerging evidence has shown that several members of the family of cathepsin proteases may be involved in the pathogenesis of atherosclerosis. This paper will discuss the stages of atherosclerosis and summarize the current knowledge about cathepsins and their contribution to the development and progression of atherosclerotic vascular disease.

Overview of the pathogenesis of atherosclerosis

Atherosclerosis is characterized by plaque buildup in the arterial wall. Plaques are composed of cholesterol and other lipids, calcium, and abnormal collections of inflammatory and smooth muscle cells (SMCs). Plaques cause arterial wall stiffness, narrowing, and, in some cases, rupture of the intimal surface resulting in sudden thrombotic occlusion-limiting flow of oxygenated blood to downstream vital organs, leading to ischemia, myocardial infarction, stroke, and possible death. Although the initiation of atherosclerosis is principally attributed to high plasma concentrations of cholesterol, particularly low density lipoprotein (LDL) cholesterol [2], many other factors play important roles in determining the onset, severity, and progression of the disease.

Cathepsins, enzymes typically localized in the lysosomes and endosomes of macrophages, are proteases that degrade unwanted endocytosed or intracellular proteins. However, emerging research has shown that cathepsins, specifically cathepsins B and X, cysteine proteases, and cathepsin D, an aspartic protease, are upregulated in atherosclerotic lesion [3]. It now appears that in addition to the conventional role as lysosomal enzymes, activated cathepsins may also play important roles in several key steps in the pathogenesis and progression of atherosclerosis, including modification and accumulation of LDL cholesterol, cellular targeting of inflammatory cells, and extracellular matrix (ECM) remodeling. This review aims to summarize currently available data concerning the potential mechanisms whereby cathepsins B, D, and X, and, by extension, a key upstream master regulator of cathepsins-TNF-alpha-may contribute to the development of atherosclerosis.

Preclinical atherosclerosis

Initial inflammatory response: It has previously been accepted that increased presence of LDL is a factor in the development of atherosclerosis. Plasma LDL may then relocate to the arterial intima where it is modified. Modified LDL and its subsequent unregulated uptake by monocyte-derived-macrophages induce an inflammatory response that attracts additional monocytes and converts the macrophages into foam cells. An accumulation of foam cells leads to the development of a fatty streak [4]. The monocytes are attracted due to the endothelial dysfunction that occurs when high levels of modified LDL is present, as well as in reaction to macrophage secretion of chemokines, thus following the response-to-injury hypothesis [1]. Monocytes are attracted to the area through chemotaxis and adhere to and migrate along the arterial endothelium through adhesion and rolling.

The activation and release of cathepsins

Human cysteine cathepsins are a group of eleven proteases that include cathepsins B and X. The majority of them, including these two cathepsins, are expressed in human tissue and implicated in cellular protein degradation and turnover [5]. Aspartic proteases, which include cathepsin D, are known to cleave dipeptide bonds in a single step, rather than forming an intermediate [6]. Cathepsin D is specifically distributed in lysosomes where it functions as a degrader of proteins and activator of proteins in pre-lysosomal compartments [7].

Cathepsins are typically localized within lysosomes and endosomes of macrophages, thus requiring stimulation to be released. It has been previously shown that treatment with TNF-alpha and IFN-gamma both result in cathepsin B secretion, while other stimulants did not [8]. Macrophages are known to produce the cytokine TNF-alpha [9]. Stimulation of the macrophages with TNF-alpha may induce the secretion of cathepsin B from macrophages, allowing it to enter the arterial intima. Little is known about the secretion of cathepsin D from macrophages, but in breast cancer it has been shown that an alteration in pro-cathepsin D sorting is altered in cancerous cell lines compared with normal mammary cells [10]. The same review also proposed that mannose-6-phosphate receptor saturation is responsible for this hypersecretion of cathepsin D. This is in line with further research that shows that when the cation-independent mannose-6-phosphate receptor is aberrantly distributed, it accumulates in lysosomes, which results in defective acidification, leading to aberrant secretion of cathepsin D in cancer cells [11].

Cathepsins have been shown to be active at low pHs [5, 12]. It has been previously suggested that atherosclerotic lesions have an acidic pH [13, 14], potentially facilitating the activation of secreted cathepsin in the arterial intima [12, 15]. However, pH across atherosclerotic lesions has been shown to be heterogeneous, and thus the pH may not be acidic enough to activate cathepsins [16]. If cathepsins are not activated due to the acidic pH, there are other known activators, such as ceramide.

Ceramide, which is known to be formed through the hydrolysis of sphingomyelin, catalyzed by sphingomyelinase, or formed from the release of monosaccharide unites from the ends oligosaccharide chains of sphingolipids and glycosphingolipids [17, 18]. The production of acid sphingomyelinase, and thus ceramide, is possibly increased by the presence of TNF-alpha, a cytokine produced by macrophages as a result of oLDL stimulation [19, 20]. Ceramide can bind to and activate cathepsin D, which in turn has been shown to activate cathepsin B [21].

Cathepsin D and proteolytic modification of LDL

Cathepsin D, previously activated by ceramide or by an acidic environment within the atherosclerotic lesion, has been shown to play a role in the proteolytic modification of LDL. Cathepsin D, when released from macrophages, modifies LDL particles, which have been implicated in the generation of extracellular and intracellular accumulation of lipids in the arterial intima [20]. Cathepsin D has specifically been implicated in the partial proteolysis of apolipoprotein B-100 (apoB), which results in the aggregation of LDL particles and their subsequent uptake by macrophages, thus enhancing foam cell formation [22]. Specifically, cathepsin D induces the hydrolytic modification of apoB, rendering the LDL particles unstable and thus inducing their extracellular accumulation in the

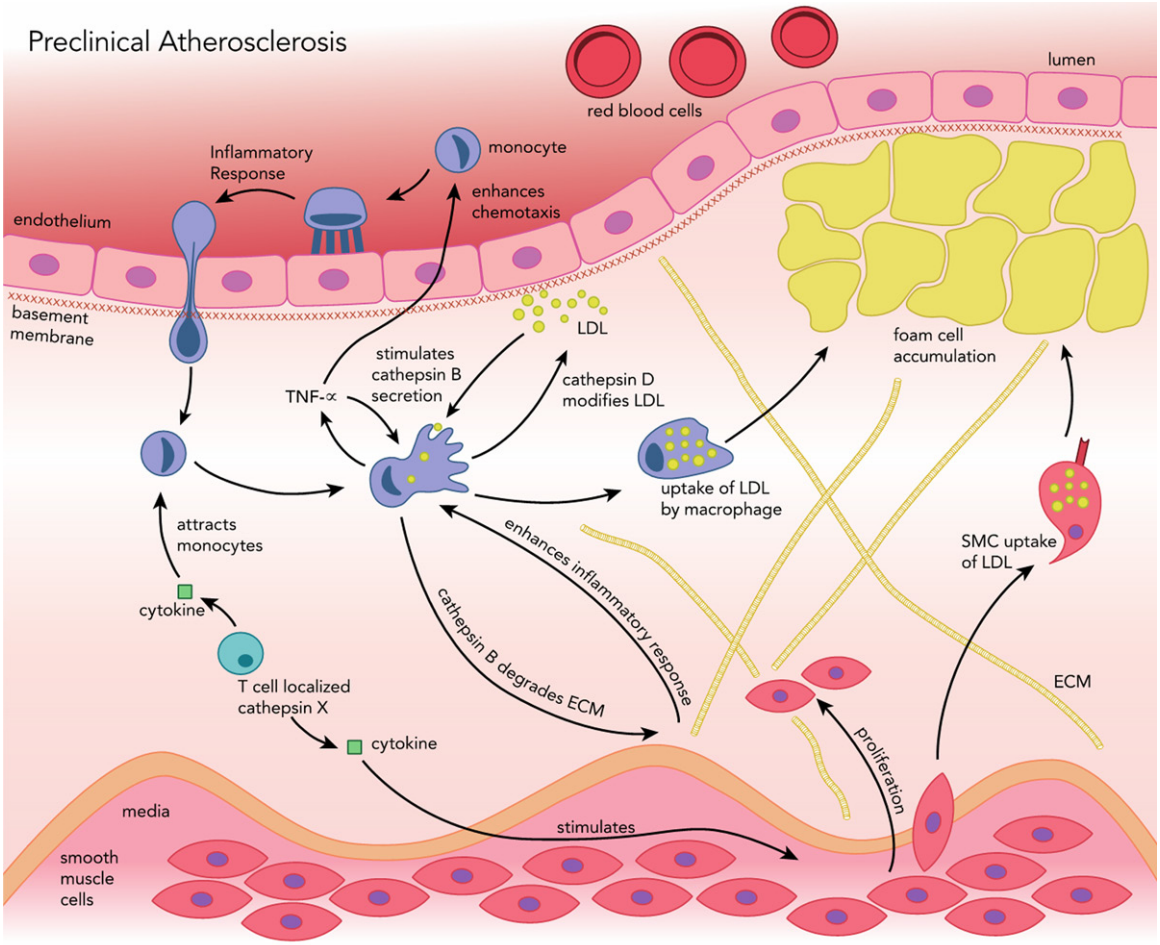

Figure 1. The Role of Cathepsins in Preclinical Atherosclerosis. A model of cathepsin functions in preclinical atherosclerosis. The functions are as follows: cathepsin D modifies of low density lipoproteins (LDLs) allowing for uptake by macrophages, cathepsin B degrades the extracellular matrix (ECM) which amplifies the inflammatory response, and cathepsin X aids in migration of T-cells and their secretion of cytokines. (Adapted from http://sphweb.bumc.bu.edu/otlt/MPH-Modules/PH/PH709_Heart/PH709_Heart3.html).

arterial intima [23]. This process of hydrolysis also initiates both degradation and fusion of apoB [23]. The degradation of this protein results in the rapid accumulation of a limited number of smaller fragments, thus also contributing to the accumulation of LDL particles within the arterial intima [24].

LDL, when oxidized, also enhances the inflammatory response [4]. This potentially leads to a positive feedback loop in which inflammation induces cathepsin release and activation which in turn exacerbates the inflammatory response, amount of lipid accumulation, and further degradation of the arterial wall (**Figure 1**). This also promotes SMC adverse remodeling, which contributes to SMC proliferation and migration into the arterial intima [25].

Inflammatory response and cathepsin X

The deposition of LDL particles promotes TNF-alpha production, which in turn recruits more inflammatory cells and enhances the expression of cathepsin X [26, 27].

The inflammatory response that is characteristic of preclinical atherosclerosis is enhanced by TNF-alpha. One of the results is the upregulation of cathepsin X, which is produced only in immune cells [28]. While little is known about the mechanisms of cathepsin X in cardiovascular disease, research exists on its mechanisms in cancer. In tumor progression, cathepsin X's interactions with integrin receptors changed cell adhesion to the proteins of the ECM, which affected the migration of tumor cells through

the ECM [29]. Cathepsin X has also been implicated in the migration of immune cells, specifically aiding in the migration, adhesion, and activation of T-lymphocytes [30, 31]. It is possible that, in atherosclerosis, cathepsin X also affects the adhesion of proteins to the ECM, thus reducing ECM stability, enhancing chemotaxis, and allowing for cells to further accumulate within the arterial intima [1]. It also enhances inflammatory cell chemotaxis, thus also increasing mLDL uptake by monocyte derived macrophages. The continuation of the inflammatory response, amplified by cathepsin B, indicates that the site of injury is not neutralized, thus the response continues. However, this may cause these cells to overexpress cathepsin X. Cathepsin X overexpression causes inflammatory cells to become invasive [32].

As both existing and inflammatory cells die in the now developing lesion, it is possible that they are not being cleared out through efferocytosis. It has been shown that in atherosclerotic lesions, efferocytosis is not functional or is not adequate enough, contributing to a buildup of extracellular debris within the lesion [33].

The T-lymphocytes also secrete cytokines that induce smooth muscle cells to migrate from the media into the intima [1]. The SMCs then proliferate and contribute to accumulation in the arterial intima by thickening and remodeling the artery wall [1, 34].

As the inflammation continues to be amplified, the macrophages, which contain more cathepsins B and D, and lymphocytes accumulate within the lesion. As the macrophages produce TNF-alpha and induce the release of active cathepsins into the intima, a positive feedback cycle emerges as cathepsins continue amplifying the inflammatory response, in turn increasing the cathepsin levels in the atherosclerotic lesion. This eventually leads to the formation of a fibrous cap over the necrotic core of lipid, a sign of an advanced atherosclerotic lesion [1].

Degradation of the arterial wall and cathepsin B

Within a macrophage's lysosomes, acidic pH values have increased the secretion of lysosomal proteases [35], including cathepsin B. Cathepsin B's pericellular mobilization by macrophages has been shown to be facilitated by the presence of elastin-containing ECM, which may increase the pathophysiological remodeling of the ECM [35]. In the arterial intima, cathepsin B then functions to degrade the extracellular matrix in the arterial intima, including laminin, fibronectin, elastin, and collagen IV [5, 35, 36]. This degradation enhances inflammatory cell chemotaxis and amplifies the inflammatory response that is already in effect (**Figure 1**) [1]. This amplification also increases mLDL uptake by monocyte-derived macrophages, implicating cathepsin B in the formation of foam cells and TNF-alpha upregulation [37].

Clinical atherosclerotic vascular events

Role of cathepsins in apoptosis: As the atherosclerotic lesion develops, some involved cells undergo apoptosis. TNF-alpha has been shown to promote the expression of pro-apoptotic genes, while cathepsin B has been shown to mediate and enhance apoptosis, and cathepsin D has been shown to regulate apoptosis [21, 38]. When macrophages that have become foam cells undergo apoptosis, the lipids that were within that foam cell are disgorged resulting in free cholesterol-ester in the intimal space. This results in a soft lipid core that will eventually encroach on and narrow the arterial lumen [39]. As the plaque accumulates, the severity of the lesion increases. Furthermore, when SMCs undergo apoptosis, which is shown to be related to ligand binding of TNF Receptor 1 (TNF-R1), caspase 8 is cleaved and apoptosis is induced [40]. The specific functions of cathepsins B and D in apoptosis have not been thoroughly reviewed, but it has been shown that intracellular cathepsins generally induce cell apoptosis through the activation of the Bid/Bax pathway [41]. Specifically, cathepsin D cleaves Bid to form truncated Bid, which triggers the insertion of Bax into the mitochondrial membrane and contributes to the triggering of apoptosis [42].

Role of cathepsins in formation of vulnerable plaques and plaque rupture

When plaque accumulates in the arterial wall, it can become unstable and rupture. When SMCs undergo apoptosis, plaque stability is compromised because the amount of collagen fibers produced by SMCs is decreased. The collagen fibers secreted by SMCs serve to stabilize the

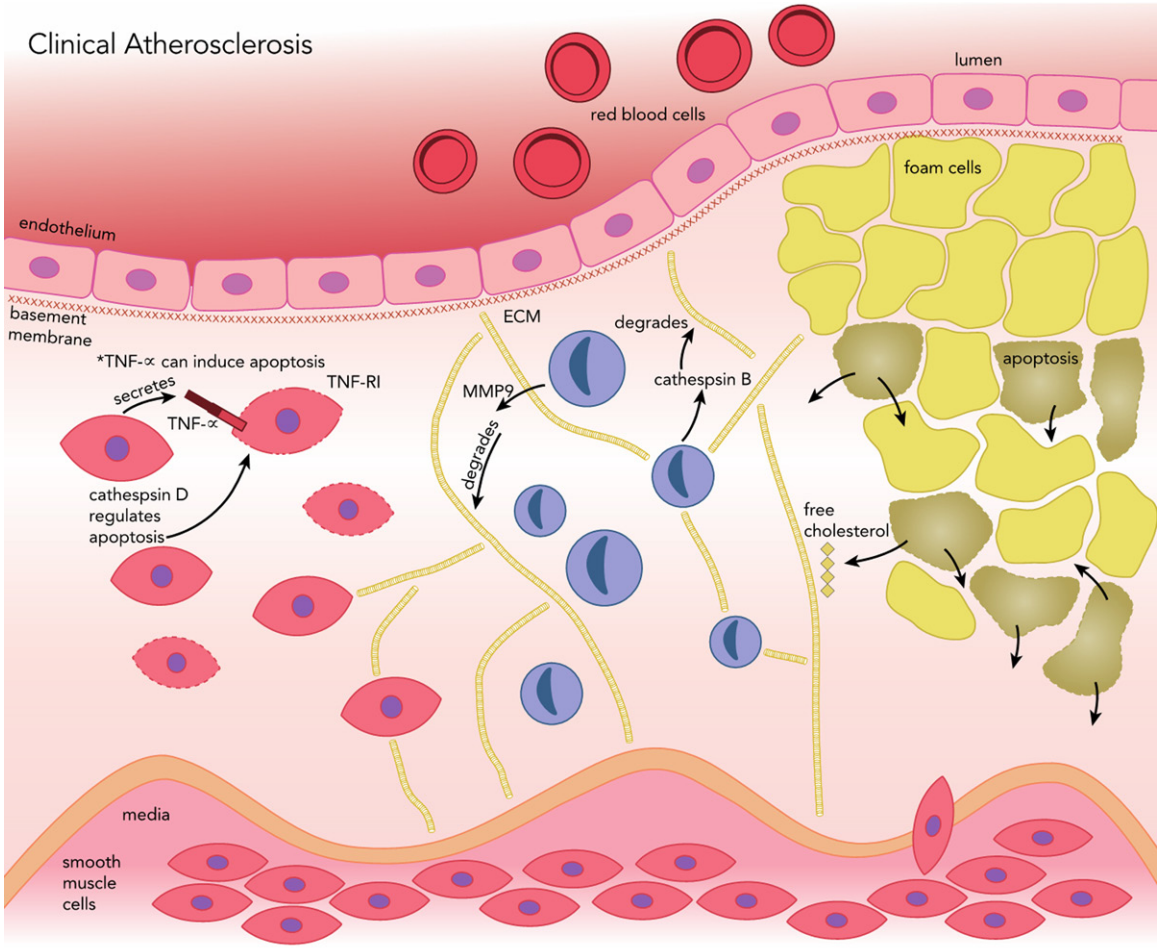

Figure 2. The role of cathepsins in clinical atherosclerosis. A model of cathepsin functions in clinical atherosclerosis. Cathepsin B degradation of the arterial ECM affects the structural integrity within the atherosclerotic lesion and may lead to plaque destabilization. Cathepsin D and Tumor Necrosis Factor-alpha (TNF-alpha) regulate the apoptosis of smooth muscle cells (SMCs). Since SMCs produce collagen, a vital component for a stable plaque, their apoptosis may also be identified as a contributing factor to plaque destabilization and possible subsequent rupture.

plaque; a decrease in their production would increase the chance of plaque rupture, as the fibrous cap would be thinner, reducing the ratio of fibrous tissue to lipids in the plaque (**Figure 2**) [43]. The effect of macrophage apoptosis on plaque stability is less clear. Macrophage debris from apoptosis promotes inflammation (due to release of cathepsins) and plaque instability (addition of lipids to the plaque and contributing to the necrotic core of the lesion). However, activated macrophages also kill SMCs, so macrophage apoptosis may actually be beneficial because fewer SMCs would die, thus the amount of collagen fibers would not be decreased [43].

It is also possible that cathepsins play a role in plaque stability. Lipid-rich lesions, which have been shown to be more vulnerable, contain the largest concentrations of inflammatory cells compared to other more stable plaques [44]. These cells occupy the fibrous cap and the shoulder of the lesions, which are more prone to rupture, and inflammation has been associated with the initiation of plaque rupture in the past [44]. Studies have already shown that the lesions of patients with unstable coronary syndromes have larger amounts of inflammatory cells [44]. As discussed earlier, inflammatory cell migration is mediated by cathepsin X. It would be helpful to determine if cathepsin X is also more abundant in the shoulder regions of vulnerable plaques. If cathepsin X is more abundant in these regions, this may suggest that it plays a role in the pathway leading to plaque rupture. These inflammatory cells are

T-cells, macrophages, and mast cells that secrete TNF, MMP9, IL-2R, and thrombosis initiator tissue factor [44]. This enhances the apoptosis of SMCs and the degradation of the fibrous cap thereby increasing the likelihood of plaque rupture.

Smooth muscle cells are surrounded by type IV collagen, which has a vital role in stabilizing plaque and contributes to the fibrous cap [45]. While this type of collagen has shown resistance to some matrix-degrading enzymes, cathepsin B has demonstrated an ability to degrade collagen IV [22]. Elastin, which can also be degraded by cathepsin B, is also a known component of the fibrous cap. This may implicate cathepsin B in destabilization of atherosclerotic plaque (**Figure 2**). It has been previously hypothesized that, once cathepsins are released, they may degrade the ECM and weaken the atheroma [46]. TNF-alpha has also been shown to suppress the synthesis of collagen types I, III, IV, and V [46] as well as stimulate the production of MMPs, which are also known to degrade the arterial ECM [47]. Lipids contained within the lesion exert pressure on the arterial wall and fibrous cap, which may lead to rupture as the ECM is degraded in part by cathepsins.

Summary

In the pathogenesis of atherosclerosis, it has been shown that the accumulation of modified LDL in the arterial intima leads to chemotaxis and the attraction of monocytes to the arterial intima. The monocytes then differentiate into macrophages and take up LDL, forming foam cells. The accumulation of foam cells results in fatty streak formation.

Macrophages contain cathepsins B and D, which, once outside the cell, may become active through being in an acidic environment or possibly through the binding of ceramide to cathepsin D and subsequent activation of cathepsin B by cathepsin D.

Once active, cathepsin B serves to amplify the in-progress inflammatory response through the degradation of the arterial intima. Active cathepsin D has been implicated in proteolytic modification of lipids, from which intracellular and extracellular lipid droplets may be produced. Cathepsin X may aid in the migration of immune cells within the lesion and is predominately implicated in T-lymphocyte signaling and secretion of cytokines that stimulate the proliferation of SMCs. It may also play a role in localizing inflammatory cells to the shoulder of unstable plaques.

In clinical atherosclerosis, cathepsin D has been implicated in the induction of apoptosis of macrophages and SMCs, thus affecting plaque stabilization, while cathepsin B may have a more direct role in plaque destabilization through degradation of the fibrous cap. However the effect of macrophage apoptosis may be beneficial or detrimental, thus cathepsin D may harbor beneficial effects. Further research is necessary to determine why cathepsins are often located in the shoulders of plaques.

Current data raises the question of whether inhibitors of these cathepsins would have clinical applicability. There is emerging evidence on the biologic effects of cathepsin inhibitors, but it is not yet known if there is a clinical use for these as preventative therapies to slow or prevent the progression of atherosclerosis [47-49]. Thus far, published research on these inhibitors has focused on inhibiting cathepsins in cancer [47]. More information is needed on the function of cathepsin inhibitors, specifically in the pathogenesis of vascular disease, to determine whether or not a preventative therapy can be developed.

Acknowledgements

Research reported in this publication was supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number R01HL111362. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosure of conflict of interest

None.

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