Review

Granzyme B as a novel factor involved in cardiovascular diseases

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Summary Apoptosis plays an important role in cardiovascular diseases such as atherosclerosis, ischemic heart disease, and congestive heart failure. Previous studies have demonstrated that oxidative stress, physiological stress, and inflammatory cytokines such as tumor necrosis factor and Fas ligand are involved in apoptosis of cardiovascular system. We demonstrate that another apoptosis-related pathway, i.e. granzyme B/perforin system is involved in cardiovascular diseases. Expression of granzyme B, a member of serine protease family is increased in acute coronary syndrome, coronary artery disease with end-stage renal disease, and subacute stage of acute myocardial infarction. Although granzyme B is extensively researched in immunological disorders, the role of granzyme B/perforin system was not clear in the cardiovascular field. In addition, little is known regarding the inhibition of granzyme B system in the clinical situation. In this review we demonstrate recent findings of granzyme B in cardiovascular diseases and possible therapeutic applications of inhibiting the granzyme B/perforin system.

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Introduction

Atherosclerosis has been found to be induced by complex processes including lipid accumulation, cellular migration, inflammation, matrix remodeling, and apoptosis. Apoptosis is supposed to play important roles in cardiovascular diseases [1]. It has been reported that apoptosis is involved in progression of atherosclerosis, plaque rupture, and extracellular matrix remodeling [2—4]. Apoptosis has been found to be induced by a number of mechanisms, including oxidative stress, serum deprivation, chemical inducers, ultraviolet exposure, thermal stress, and cytokines. Several cytokines are found to induce apoptosis by binding to their specific receptors. Most of these “death-ligands” belong to the tumor necrosis factor (TNF) family, including TNF-α, lymphotoxin α, Fas ligand, and TRAIL/Apo2L.

There has been a report of the critical role of cytotoxic immune responses as a mediator of apoptotic cell death in atherosclerotic diseases [5]. Cytotoxic T lymphocytes (CTLs) exert critical protection against viral infection and tumor cells, however they can also induce harmful reactions such as autoimmune disease, graft rejection, and graft versus host disease (GVHD). Granzyme B/perforin system is mainly exerted by circulating white blood cells such as CTLs (adaptive immune system) and natural killer (NK) cells (innate immune system). Granzyme B and perforin are stored in secretory granules inside the leukocytes. These molecules are released outside of the cells by cell to cell contact, integrin engagement with extracellular matrix proteins [6,7], receptor activation by cytokotyks (interleukin-2 and TNF) [8], chemokines (macrophage inflammatory protein-1 and monocyte chemoattractant protein-1) [9,10], and stimulation by bacteria or lipopolysaccharide. Perforin polymerizes to form transmembrane channels and allows granzyme B access into target cells (Fig. 1). Granzyme B is a member of the serine protease family and induces cell death by mechanisms such as the activation of caspases, degradation of structural proteins and directing the proapoptotic molecule Bid to the mitochondrial compartment.

Atherosclerotic disease and granzyme B

Activation of the immune response is essential to eliminate infected cells and tumor cells in humans. However, excess immune response contributes to various pathological processes, i.e. autoimmune disease, chronic obstructive pulmonary disease, graft versus host reaction, and atherosclerosis. Endothelium has a critical role to prevent atherosclerosis by releasing nitric oxide, producing anticoagulant factors, and regulating permeability of circulating substances into vessel walls. Endothelial apoptosis leads to atherosclerotic plaque formation by increasing smooth muscle cell proliferation, leukocyte migration into subendothelial layers, thrombus formation, and accumulation of circulating lipids to vessel walls. Clément et al. have reported that granzyme B can be a predictive marker for acute GVHD or cardiac rejection after bone marrow or heart transplantation [23]. Acceleration of coronary atherosclerosis is well-known in patients receiving heart transplantation. Choy et al. have shown the critical role of granzyme B in cardiovascular disease in patients receiving heart transplantation [24].

Accumulation of T lymphocytes is reported in atherosclerotic lesions, in particular, shoulder lesion of the plaques
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Figure 2  The relationship between levels of granzyme B in the supernatant of cultured peripheral blood mononuclear cells and TIMI risk score in patients with unstable angina pectoris. Patients with unstable angina were classified into five groups according to the Thrombolysis In Myocardial Infarction (TIMI) risk score (TIMI 1, 2, 3, 4, and more than 5). The levels of granzyme B in the supernatant of cultured peripheral blood mononuclear cells were elevated when the TIMI risk score increased (TIMI 1, 14.8 ± 3.1 pg/mL; TIMI 2, 15.2 ± 3.4 pg/mL; TIMI 3, 25.2 ± 5.1 pg/mL; TIMI 4, 71.7 ± 22.3 pg/mL; TIMI 5 and 6, 132 ± 31.0 pg/mL). *p < 0.05 versus TIMI1 and 3; **p < 0.01 versus TIMI 2; †p < 0.05 versus TIMI 2.

[25–27]. Activated T lymphocytes are supposed to play an important role in vulnerability of plaques. It has been reported that expression of matrix degrading enzymes was increased both in systemic circulation and in vulnerable atheromatous plaques in patients with acute coronary syndrome. We have observed the increased production of granzyme B from peripheral blood mononuclear cells (PBMCs) in patients with unstable angina pectoris [28] (Fig. 2). We have also found granzyme B expression in mononuclear cells in ruptured coronary plaques. Importantly, the granzyme B production from PBMCs was related to Thrombolysis in Myocardial Infarction (TIMI) risk score of acute coronary syndrome. Interestingly, studies have clarified that granzyme B can cleave extracellular matrix proteins including fibronectin [29], vitronectin, and laminin [30]. The primary cleavage site of vitronectin was demonstrated as the Arg-Gly-Asp (RGD) integrin site, which is consistent with Asp-ase activity of granzyme B. Thus granzyme B not only induces apoptotic cell death but also degrades extracellular matrix, leading to weakening of the fibrous cap of atheromatous plaques. Previous studies have reported that a number of proteases such as matrix metalloproteinases (MMPs), elastases, myeloperoxidase, neutrophil proteinase 3, and neutral serine proteases (chymase and tryptase) are supposed to play important roles in degradation of the extracellular matrix, leading to the thinning and weakening of the fibrous cap [27,31,32]. Among these enzymes, MMPs have been extensively investigated. Our results suggest that extracellular granzyme B released from PBMCs might be another candidate that induces atherosclerosis and weakening of the fibrous cap [27,31,32].

Figure 3  Levels of plasma granzyme B in patients with coronary artery and chronic kidney disease. The Kruskal–Wallis test revealed that plasma levels of granzyme B in CAD complicated CKD patients were significantly higher than those in patients with CAD or patients with CKD (CAD, 10.6 ± 2.8; CKD, 27.3 ± 3.3; CAD/CKD, 35.5 ± 8.1 pg/mL, p < 0.05). *p < 0.05; **p < 0.001. CAD, coronary artery disease; CKD, chronic kidney disease; CAD/CKD, patients with both coronary artery and chronic kidney disease.

Acute myocardial infarction and granzyme B

Left ventricular (LV) remodeling after acute myocardial infarction (AMI) is a critical complication that leads to thrombosis, heart failure, and ventricular arrhythmia, lead-
The size of infarction determined within several hours after the attack is the most critical determinant of subsequent heart failure. However, mechanical factors, medical interventions, and humoral factors such as vasoactive substances, growth factors, and cytokines have been reported to be associated with LV remodeling in the chronic phase after AMI [39–42]. In particular, apoptotic cell death in infarcted tissue is reported to play an important role in the progression of LV remodeling after AMI [43,44]. Hayakawa et al. reported that inhibition of granulation tissue cell apoptosis by a pancaspase inhibitor significantly improved LV remodeling and heart failure in the chronic stage after myocardial infarction [45].

We hypothesized that granzyme B is involved in LV remodeling through induction of apoptotic cell death and extracellular matrix degradation activity. We have measured plasma levels of granzyme B in patients with AMI and found increased plasma levels of granzyme B after AMI [46]. The plasma levels of granzyme B peaked on day 7 after AMI with a 4.6-fold increase compared to that on the day of the admission. Interestingly, plasma granzyme B level on day 14 after AMI was correlated with increase of LV end-diastolic volume 6 months after the onset (Fig. 4). TNFα and Fas ligand also increased after AMI, however we did not observe a significant relationship between these markers and LV remodeling. In the rat model of AMI, we found that protein expression of granzyme B was increased at the infarcted myocardium from day 3 to 17 after AMI (unpublished observation). The finding is quite interesting to prove increased local expression of granzyme B in infarcted myocardium.

Inhibition of granzyme B

Normally, activity of proteases is tightly regulated to avoid unfavorable damage of tissues in vivo. For example, MMPs are inhibited by binding of their internal inhibitors: tissue inhibitors of MMPs (TIMPs) in a 1 to 1 molar stoichiometric fashion. It has been reported that granzyme B is efficiently inhibited by α1-antitrypsin and to a lesser extent by α2-macroglobulin [47]. However, other reports showed that the inhibitory action of α1-antitrypsin for granzyme B is still controversial [48,49].

Serpins are a group of proteins with similar structures that inhibit proteases by forming complexes with their proteases. The first members of the serpin superfamily extensively studied were antithrombin and antitrypsin, which play key roles in controlling blood coagulation and inflammation [50]. Nowadays, more than 1000 serpins have now been identified. Human protease inhibitor-9 (PI-9, serpinB9) is a member of the serpin family that has been found to be an intrinsic inhibitor for granzyme B. PI-9 was originally found in T cells to protect themselves against apoptosis by secreted granzyme B. It has been reported that PI-9 forms a complex with granzyme B and inhibits its activity [51,52]. PI-9 is predominately distributed in cytosol and nucleus because it lacks a peptide sequence for secretion. The presence of circulating PI-9 was proved by Rowshani et al. [53], however it is still not clear that circulating PI-9 can inhibit extracellular granzyme B in vivo. Kurschus et al. have shown activity of granzyme B is not inhibited by co-incubation with human plasma in vitro [49]. However, a protective role of PI-9 and SPI-6, a murine orthologue of human PI-9 against granzyme B-induced apoptosis has been reported in both in vitro and in vivo models [54–56]. Sipione et al. reported that Sertoli cells secret another serpin, serpinA3n that can bind and inhibit activity of granzyme B [57]. The regulation mechanism of PI-9 is still not clear. Lazarczyk et al. have reported that pentoxyfilline upregulated PI-9 mRNA expression in human erythroleukemia cell lines [58]. Jiang et al. have demonstrated that estrogen induces PI-9 expression in hepatoblastoma cells, HEPG2ER7 cells [59]. Induction of PI-9 by estrogen might account for a novel mechanism for estrogen-mediated increase in tumor incidence and gender difference of prevalence in cardiovascular diseases. Interestingly, Young et al. have demonstrated increased expression of interleukin-1β and decreased expression of PI-9 in unstable human atherosclerotic plaques [60]. They have demonstrated that PI-9 was an endogenous inhibitor for interleukin-1β (IL-1β)-converting enzyme. Thus administration of PI-9 inhibits chronic inflammation and could be a novel therapeutic tool to treat and prevent cardiovascular diseases.

Our data suggest that PI-9 can be applicable for prevention of acute coronary syndrome and atherosclerotic plaque formation in patients with CKD and left ventricular remodeling after AMI. In our preliminary experiments, marked elevation of granzyme B was found, however no apparent expression of PI-9 was observed in the infarcted myocardium of the rat model of AMI. Thus inhibition of granzyme B might prevent cardiac rupture and progression of ventricular remodeling after AMI. Because PI-9 is predominantly expressed in cytosol, administration of PI-9 can block extracellular granzyme B more efficiently. Interestingly, a recent study demonstrated inhibition of IL-1 by a recombinant human IL-1 receptor antagonist (anakinra) reduced left ventricular remodeling [61]. It is possible that PI-9 prevents ventricular remodeling after AMI by inhib-
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Conclusions

Granzyme B could be a novel molecule that plays an important role in cardiovascular diseases. It is suggested that granzyme B is involved in chronic as well as acute inflammation in atherosclerotic coronary artery diseases. Inhibition of granzyme B could be a novel therapeutic approach to treating cardiovascular diseases such as prevention of progression of atherosclerosis, plaque rupture, and ventricular remodeling after AMI.

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