

Mechanisms of Atherosclerotic Plaque Instability

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Abstract: Cardiovascular disease (CVD) is the leading cause of mortality in humans worldwide. The main cause of CVD is the formation of thrombi due to by unstable atherosclerotic plaque rupture on the arterial wall. Long-term accumulation of thrombi results in vascular remodeling, and subsequent-stenosis of the lumen obstructs the blood flow, thereby leading to myocardial tissue ischemia and hypoxia. Sustained ischemia and hypoxia lead to myocyte necrosis, resulting in irreversible myocardial injury. Many molecular and cellular mechanisms are associated with atherosclerotic plaque instability (API). For example, macrophages can produce various inflammatory factors, adhesion factors, chemokines and matrix metalloproteinases (MMPs), which play important roles in the pathophysiological mechanisms of API and in maintaining plaque stability. These molecules may help predict unstable atherosclerotic plaques. If the plaque is stable, it will not be prone to rupture or thrombosis. Accordingly, in this review, we will discuss the different pathophysiological mechanisms of API and the related roles of macrophages in the mechanisms of API mainly in animal models and humans. We believe this review will provide a theoretical basis for the development of treatments and diagnostic approaches for the management of API.

Keywords: Atherosclerotic Plaque Instability; Cardiovascular Disease; Mechanism; Plaque.

1. Introduction

Cardiovascular disease (CVD) is a chronic inflammatory disease associated with high morbidity and mortality. Atherosclerotic plaque rupture is a main cause of acute cardiovascular events, including heart attacks, acute coronary syndrome (ACS) and strokes [1]. Thus, stabilizing unstable atherosclerotic plaques is clinically significant for the treatment and prevention of CVD [2]. Unstable plaques rupture is typically characterized by large thrombogenic necrotic cores with lipids, intraplaque hemorrhage, a thinner fibrous cap, inflammatory cells infiltration, eventually resulting in platelet aggregation and thrombus formation. The long-term accumulation of thrombi results in vascular remodeling, and stenosis of the lumen obstructs the blood flow, causing infarction in relevant tissues [3]. Several factors contribute to API, including vascular wall thickening, endothelial dysfunction, vascular smooth muscle cell (VSMC) apoptosis, inflammation, neoangiogenesis, oxidative stress, extracellular matrix (ECM) degradation and decreased collagen content. The stability of atherosclerotic plaques is assessed by the area of the necrotic core, the thickness of the fibrous cap, the content of collagen, and the degrees of inflammation and intraplaque hemorrhage [4]. In this review, we will discuss the pathophysiological mechanisms of API.

2. Mechanisms of API

2.1. Inflammation

Atherosclerotic plaques convert into unstable plaques, the immune system is immediately activated. Many immune cells, including monocytes, macrophages, T lymphocytes, B lymphocytes, dendritic cells (DCs) and neutrophils have been detected at lesion sites, and inflammatory cytokines released these cells play critical roles in API. At atherosclerotic lesions, multiple kinds of activated endothelial cells (ECs), macrophages and surrounding immune cells may generate pro-inflammatory chemokines and cytokines that can

stimulate inflammatory responses and process of atherosclerotic lesions. CD3⁺T lymphocytes can be divided into T helper (Th) cells, which function in the development of atherosclerosis [5]. Th1 cells may recognize low density lipoprotein (LDL) then produce pro-inflammatory various cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-1 β and interferon (IFN)- γ . For example, IFN- γ inhibits collagen synthesis and promotes smooth muscle cell VSMC apoptosis, IFN- γ also induces IL-18 which accelerates inflammatory effects [6]. TNF- α is a multifunctional inflammatory factor that aggravates inflammatory effects, stimulates T-cell proliferation and promotes VSMC apoptosis. Excess IL-6 may enhance fatty streak lesions and increase the sizes of the plaques and cause a variety of immune effects, resulting in API. Upregulation of IL-6 also stimulates liver cells to produce C-reactive protein (CRP), which is a clinically important biomarker for predicting CVD risk [7].

2.2. Recruitment of Circulating Monocytes and the Generation of Macrophages with Differing Phenotypes

Hyperlipidemia and hypercholesterolemia can cause infiltration and accumulation of monocytes into the vascular wall. These cells are heterogeneous cells group that play multiple roles in the immune system and inflammatory diseases. Circulating monocytes are recruited to atherosclerotic lesion in the vascular wall. At the lesion site, recruited monocytes can differentiate into macrophages with different phenotypes, and these macrophages play vital roles in the progression of atherosclerotic lesions [8]. Monocyte chemoattractant protein (MCP)-1 is a chemokine produced mainly by ECs and inflammatory cells. MCP-1 stimulates monocyte recruitment to atherosclerotic lesions, results in inflammatory effects. Macrophages are also important biomarkers for measuring the stability of atherosclerotic plaques and can show varying phenotypes based on specific signaling mechanisms. Th1-related cytokines such as IL-1 β

and IFN- γ may selectively activate M1 macrophages, that selectively produce the pro-inflammatory cytokines IL-6, IL-12 and TNF- α . Pro-inflammatory macrophages also produce reactive oxygen species (ROS) and reactive nitrogen species by activation of NADPH oxidase, which aggravates inflammatory effects. Th2 cytokines, such as IL-13 and IL-4, can selectively activate M2 macrophages, anti-inflammatory cells that selectively secrete the anti-inflammatory factors IL-10 and transforming growth factor- β (TGF- β). These anti-inflammatory factors can reduce inflammation and protect against atherosclerotic plaque rupture. TGF- β can reduce the infiltration of immune cells into the plaques, increase collagen in the plaques, and inhibit the proliferation and differentiation of VSMCs to maintain the normal structure of the arterial wall [9]. Anti-inflammatory macrophages and anti-inflammatory cytokines lead to tissue repair and plaque stabilization. In advanced lesions, pro-inflammatory M1 macrophages account for a higher proportion than M2 macrophages, there are more M1 biomarkers, including TNF- α , IL-6 and IL-12 in the plaques [10]. Therefore, pro-inflammatory macrophage subtypes may reflect the occurrence of API. Growing studies have also indicated that M2-related biomarkers may act as predictors of plaque stability.

2.3. Macrophage Efferocytosis and Autophagy

Macrophage apoptosis usually occurs at all stages of atherosclerosis and affects the early formation of atherosclerotic lesions and the stability of atherosclerotic plaques. Macrophages can also efficiently clear apoptotic cells and dead cells in a process known as efferocytosis. Efficient efferocytosis can inhibit atherosclerotic progression and prevent secondary necrosis and protect against atherosclerotic plaque rupture. Defective efferocytosis may result in secondary necrosis through alterations to lipid metabolism and the formation of foam cells or necrotic cores [11]. Macrophage autophagy also shows a crucial role in API progression and may effectively clear cumulative lipid and cholesterol deposition in those macrophage-foam cells. Macrophage autophagy has been reported to suppress the formation of necrotic core and strengthen stability of plaques. Increased levels of LC3II and decreased level of SQSTM1/p62 are indicative of enhanced macrophage autophagy, suggesting that macrophage autophagy has cardioprotective effects [12].

2.4. Oxidative Phosphorylation and Oxidative Stress

Oxidative phosphorylation plays an important role in the normal energy metabolism of myocardium. Disorders of oxidative phosphorylation and increased levels of ROS and NADPH oxidase may lead to oxidative stress. Simultaneously, oxidative stress also promotes the formation of ROS and atherogenic oxLDL [13]. Oxidative stress can promote macrophage inflammatory phenotype and aggravate macrophage inflammatory effects. In the normal physiological state, vascular ECs have anti-oxidative effects; these cells also remove excessive oxygen free radicals and maintain homeostasis. Several studies have shown that oxidative stress can induce lipid peroxidation and DNA oxidative damage, increase expression levels of superoxide dismutase 1 and heme oxygenase 1 and decrease the activity of catalase and glutathione peroxidase. Additionally, the endothelial antioxidant defense system is damaged, thereby

aggravating API. Oxidative stress can stimulate the proliferation and migration of VSMCs, promoting the formation of VSMC-derived foam cells and participating in the chronic inflammatory reaction. During oxidative stress, activation of Nrf2 is beneficial for the survival of VSMCs, while inhibiting the proliferation of VSMCs and preventing plaques from continuing to grow and rupture [14].

2.5. Endoplasmic Reticulum Stress and the Unfolded Protein Response

ER stress can contribute to API, particularly ACS. Accumulation of cholesterol can cause ER stress, which is associated with multiple mechanisms of API. Activated ER stress in lesional resident cells may result in the formation of macrophage-foam cells and the expression of some inflammatory factors. Several studies have suggested that compared with stable atherosclerotic plaques, unstable or ruptured atherosclerotic plaques generated by ER stress show upregulation of PERK, p-eukaryotic initiation factor 2 α (eIF2 α), glucose-regulated protein 78 (GRP78), and CCAAT-enhancer-binding protein homologous protein [15]. ER stress is also associated with protein synthesis in SMCs, and activation of ER stress can stimulate the unfolded protein response, which results in SMC apoptosis and atherosclerotic plaque rupture [16].

2.6. Cell Apoptosis

SMC or macrophage apoptosis leads to API. Disruption of lipid or cholesterol metabolism results in excess lipid or cholesterol accumulation in macrophages and leads to the formation of foam cells or necrotic cores, thereby promoting API. Macrophage apoptosis is detected at all stages of atherosclerosis. SMC apoptosis can directly affect API and plays an important role in vascular remodeling. SMCs have a function of synthesizing interstitial collagen fibers, which protect from atherosclerotic plaque rupture. The c-Jun N-terminal kinase signaling pathway plays a vital role in VSMC apoptosis induced via oxidative stress in unstable or ruptured atherosclerotic plaques [17]. CD137, a member of the TNF receptor superfamily, induces API by increasing plaque necrosis, decreasing collagen content, and reducing VSMC content. Activation of CD137 signaling increases VSMC apoptosis by decreasing Bcl-2 expression and subsequently upregulating cleaved caspase-3, resulting in API [18].

2.7. Endothelial Dysfunction

Endothelial dysfunction is also an important cause of plaque instability. ECs are involved in several areas of vascular biology, including vasoconstriction, vasodilation, antithrombosis, anticoagulation, and cell adhesion. Endothelial dysfunction increases the permeability of the vascular intima, promotes the deposition of lipids and fibrin, and enhances the inflammation of various inflammatory cells in the intima. Vasodilators (such as nitric oxide [NO] and prostaglandin) and vasoconstrictors (such as endothelin-1 and angiotensin II) are also important indicators of vascular endothelial function. NO plays an important role in regulating blood vessel tension, lowering lipid levels, and inhibiting platelet aggregation [19]. At the site of endothelial dysfunction, recruited monocytes can differentiate into different phenotypic macrophages, which play vital roles at all stages of progression of atherosclerotic lesions. Macrophages participate in various mechanisms of API. Due to endothelial dysfunction, circulating monocytes emigrating

into atherosclerotic plaques are regulated by chemokine receptor-chemokine pairs (mainly CCR2/CCL2, CX3CR1/CX3CRL1, and CCR5/CCL5). Upregulation of cellular adhesion molecules, including VCAM-1, ICAM-1, and platelet EC adhesion molecule-1, is essential for recruitment of monocytes from the circulation and adherence to ECs [20]. Additionally, these molecules play important roles in endothelial dysfunction during the early development of API.

2.8. Degradation of the Extracellular Matrix

Pro-inflammatory macrophages may produce MMPs, including MMP-2 and MMP-9. And these MMPs may show proteolytic activity and may effectively degrade the ECM, resulting in thinning of the fibrous cap. CD147 is a transmembrane glycoprotein that can induce MMP expression in SMCs and monocytes. Additionally, CD147 is an upstream regulatory factor of MMPs that also can induce excess expression of MMPs, including MMP-1, MMP-2 and MMP-9. These MMPs degrade the ECM and promote cell emigration and inflammatory activity, thereby enhancing immune diseases. CD147 in plaques plays an important role in plaque stability by affecting the expression of MMPs [21]. Tissue metalloproteinase inhibitors (TIMPs) inhibit MMP-dependent degradation of the ECM and protect against atherosclerotic plaque rupture. When the balance of TIMPs and MMPs is altered, the stability of the plaques is affected. Growing evidence has suggested that cathepsins (Cats) and cystatin C (Cys C) are associated with atherosclerotic plaque instability by affecting the degradation of the ECM. Zhao et al. have demonstrated that the expression levels of Cat K were significantly higher in human unstable plaques, whereas the expression levels of Cys C were significantly lower than those in human stable plaques. Cys C is negatively correlated with Cat K, and Cat K plus collagenase and elastase activities can degrade the ECM [22].

2.9. Angiogenesis

In atherosclerotic lesions, abnormal angiogenesis in the plaque may promote plaque expansion, eventually resulting in intraplaque hemorrhage and rupture. Neoangiogenesis plays an important role in the progression of atherosclerotic lesion and the stability of plaques [23]. The angiogenic mechanisms are tightly related to hypoxia that promote angiogenic factor formation to induce neo-angiogenesis. Vascular endothelial growth factor (VEGF) is a common pro-angiogenic cytokine that may induce intraplaque hemorrhage. And its crucial receptors VEGFR-1 or 2 also play essential roles in modulating intraplaque angiogenesis. Meanwhile, VEGF-A is also an endothelial-related growth factor that can induce neoangiogenesis through activating EC transportation [24].

2.10. Plaque Erosion

ACS is a common cardiovascular disease. Pathology studies have identified plaque erosion as an important mechanism underlying ACS. Plaque erosion is characterized by an unbroken fibrous cap overlying a necrotic core full of lipid. Plaque erosion usually occurs over lesions rich in proteoglycans and SMCs. Thrombi are also accumulated in sites that lack the endothelium. Sugiyama et al. showed that endothelial loss occurred at sites of plaque erosion via EC apoptosis and desquamation resulting from exposure to myeloperoxidase. Moreover, macrophages may express myeloperoxidase in atherosclerotic plaques. Several studies

have also suggested that thrombi covering plaque erosions have higher levels of myeloperoxidase than those in ruptured plaques [25]. Quillard et al. showed that activated TLR-2 can contribute to EC denudation at the site of superficial plaque erosion. Eroded plaques also typically contain neutrophil extracellular traps, which can stimulate EC apoptosis and denudation. Thus, these studies have shown that plaque erosion is closely related to API [26].

3. Conclusion

Increasing our understanding of the pathophysiological mechanisms of API and the progression of atherosclerosis are the main goals of cardiovascular research. However, the complex pathophysiological mechanisms underlying API remain largely unclear. Researchers have shown that macrophages play a decisive role in API. Studies on the mechanisms of API have found that many compounds, factors, and receptors are closely related to API. Analysis of the expression levels of these factors, receptors, and other biomarkers may help to elucidate the relevant mechanisms of API. In recent years, multiple biomarkers have been found to serve as predictors of atherosclerotic plaques in animal models and humans. However, few biomarkers can be used for predicting the risk of CVD in the clinical setting. There are also significant differences between human and animal models, as well as between different animal models. Therefore, it is necessary to combine animal models with clinical trials to verify the relationships between biomarkers and plaque stability. Because the pathogenesis of API is extremely complex, there is no comprehensive approach to addressing all aspects of API. Therefore, further studies should seek to explore more of the mechanisms involved in API in order to establish additional biomarkers associated with API by combining different disciplines. Such analyses may facilitate the identification of comprehensive strategies and targets for clinical prediction, diagnosis, and treatment of CVD.

In this review, we summarized only some studies describing the different mechanisms of API in the mechanisms of API. The development of promising treatment strategies targeting mechanisms related to API to prevent plaque rupture may contribute to protection against CVD.

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