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Review

The role of ferroptosis and pyroptosis in atherosclerosis development: a comprehensive exploration

Programmed cell death in atherosclerosis

Ertugrul Yigit, Orhan Deger
Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Türkiye

Abstract

This review comprehensively explores the interplay between ferroptosis, pyroptosis, and the pathogenesis of atherosclerosis, a chronic vascular inflammatory disorder. It offers an in-depth analysis of atherosclerosis, emphasizing its status as a prevalent and fatal group of cardiovascular diseases. The etiology of cardiovascular diseases is discussed, with a particular emphasis on atherosclerosis as a chronic process characterized by the accumulation of lipid and fibrous elements in the arteries. The pathogenesis of atherosclerosis, from the formation of plaque/atheroma to the progression of atherosclerotic lesions, is detailed, shedding light on the critical role of foam cells, endothelial dysfunction, and inflammatory mediators in this process. Furthermore, the article thoroughly examines the relatively recent concepts of ferroptosis and pyroptosis and their implications in atherosclerosis. Ferroptosis, described as a form of programmed cell death characterized by iron accumulation and reactive oxygen species, is scrutinized in the context of atherosclerosis. The molecular mechanisms underlying ferroptosis, including oxidative membrane damage and iron metabolism, are elucidated.

Similarly, pyroptosis, a regulated cell death process involving the release of proinflammatory cytokines and pore formation in the cytoplasmic membrane, is meticulously discussed in atherosclerosis. The involvement of vascular endothelial cells, vascular smooth muscle cells, and macrophages in the pathophysiology of ferroptosis and pyroptosis during atherosclerosis is also thoroughly explored. Overall, the article offers valuable insights into the potential therapeutic targets presented by ferroptosis and pyroptosis in the treatment of atherosclerosis, paving the way for further research and clinical implications in this field.

Keywords

atherosclerosis, ferroptosis, hyperlipidemia, macrophage, pyroptosis

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Corresponding Author: Ertugrul Yigit, Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Türkiye.
E-mail: ertugrulyigit@ktu.edu.tr P: +90 553 304 45 46
Corresponding Author ORCID ID: https://orcid.org/0000-0001-9780-2808
Other Author ORCID ID: Orhan Deger, https://orcid.org/0000-0003-3584-6324

Introduction

Cardiovascular diseases, particularly atherosclerosis, are significant global health concerns and a leading cause of mortality. Atherosclerosis is a complex condition characterized by the accumulation of lipid and fibrous elements in the arteries, leading to chronic vascular inflammation and subsequent cardiovascular complications. Understanding atherosclerosis's intricate mechanisms is crucial for developing effective therapeutic strategies [1].

This comprehensive review aims to explore the interplay between ferroptosis, pyroptosis, and the pathogenesis of atherosclerosis.It provides an in-depth analysis of the etiology of atherosclerosis etiology, its mechanisms, and implications, emphasizing its status as a prevalent and fatal group of cardiovascular diseases. Additionally, the review delves into the relatively recent concepts of ferroptosis and pyroptosis, shedding light on their potential implications in atherosclerosis and paving the way for further research in this field.

Pathophysiology of Atherosclerosis and Emerging Cell Death Mechanisms

Cardiovascular diseases are the most common, increasingly common, and most fatal disease group in the world. According to a report published by the World Health Organization, approximately 32% of the people who died in the world in 2019 (~17.9 million) were due to cardiovascular diseases. Approximately 85% of deaths from those diseases were reported to be due to coronary heart disease and stroke [2]. The etiology of cardiovascular diseases includes cardiometabolic risk factors, mainly atherosclerosis, dyslipidemia, higher blood pressure, higher hyperglycemia, excessive inflammatory response, and prothrombotic state [3]. The most studied of these risk factors is undoubtedly the most common atherosclerosis (AS) progress. AS is a chronic pathology initiated by the accumulation of atherogenic lipoproteins in the intima of the arteries. It is characterized as a vascular inflammatory impaired by lipid peroxidation, endothelial dysfunction, and inflammatory mediators mediated by vascular smooth muscle cells (VSMC), macrophage (M), and platelets that narrow the vessel lumen [4]. In the pathogenesis of AS, plaque/atheroma is formed from cholesterol-rich macrophages following endothelial damage. It progresses with the participation of platelets and leukocytes in the process, resulting in atherosclerotic lesion formation [5]. It has been reported that AS begins with the transformation of macrophages into foam cells due to disorders in lipoprotein metabolism [6]. The most important theory proposed for the onset of atherosclerosis is the transition of circulating low-density lipoprotein (LDL) to the subendothelial space and oxidation. When oxidized LDL (OxLDL) levels exceed the clearance capacity of macrophages, lipid droplets (LD) are formed, and macrophages become foam-like, called foam cells, with these LDs in their structure. These foam cells have been reported to be critical in atherosclerotic plaque formation and increased plaque burden [7]. Subendothelial accumulation of foam cells is followed by endothelial dysfunction with dysfunction or abnormal death of vascular endothelial cells (VEC) and VSMC. Adhesion molecules, vascular cell adhesion molecule (VCAM-1), intracellular adhesion molecule (ICAM-1)], monocyte chemoattractant protein 1 (MCP1), tumor necrosis factor-alpha (TNF-α) and some interleukins release from VEC, VSMC and Ms, lead to infiltration of monocytes into the neointima layer. Then, with the activity of all these molecules, the atherosclerotic lesion grows due to the accumulation of platelets and other leukocytes in the atheroma region. As a result, the narrowing of the lumen, especially in small and medium-sized arteries, has been reported to increase blood pressure and circulatory disorders [8,9]. It has been revealed that the atherosclerotic plaques progress silently until thrombus formation, which can cause ischemic damage in surrounding tissues by rupture or erosion (Figure 1) [10].

Recent studies have shown that ferroptosis and pyroptosis, two of the cell death types identified in recent years, play an important role in developing AS and may be new therapeutic targets [11]. Ferroptosis, a newly identified form of programmed cell death (PCD) as described by Dixon and colleagues in 2012, is characterized by the accumulation of iron, reactive oxygen species (ROS), and reactive lipid peroxides (LPO) [12]. Pyroptosis is a form of programmed cell death characterized by the release of proinflammatory intracellular cytokines, including interleukin (IL)-1β, IL-18, and MCP-1. These cytokines bind to the gasdermin protein family, leading to pore formation in the cytoplasmic membrane and the activation of caspases. [13]. The pathophysiology of ferroptosis and pyroptosis during AS has been elucidated through VECs, VSMCs, and macrophages.

Ferroptosis

Understanding the Molecular Mechanisms and Characteristics of Ferroptosis

The fundamental molecular process of ferroptosis involves an imbalance between oxidative harm and the body's natural antioxidant defense system. The excessive buildup of iron and lipid peroxidation are the primary factors that trigger oxidative damage to cell membranes during ferroptosis [14]. Iron enters the systemic circulation in most mammals as Fe^{3+} [15]. Iron binds to its carrier apo-transferrin (Apo-TF) in the blood and forms holo-transferrin (Holo-TF). Holo-TF binds to Transferrin Receptor Protein-1 (TFR1) in the membrane to form the Holo-TF/TFR1 complex [16]. While this complex is taken from the membrane into the cell by endocytosis, Apo-TF is released back into the circulation. Fe^{3+} that enters the cell is reduced to Fe^{2+} via six-transmembrane epithelial antigen prostate 3 (STEAP3). Fe^{2+} is then transported to the cytosolic labile iron pool (LIP) by divalent metal transporter 1 (DMT1) and bound to ferritin [17]. In addition, Fe^{+2} in the heme ring structure formed by hemoglobin degradation in this region also binds to ferritin and accumulates in LIP [18]. With the decrease of Fe^{2+} levels in circulation, Fe^{2+} in the ferritin structure is released via ferroportin [19]. However, if increased Fe^{2+} levels in LIP exceed the binding capacity of ferritin, Fe^{2+} is oxidized to Fe^{3+} by Fenton reactions, while hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH) are produced [20].

Reactive oxygen species (ROS) are produced due to excessive accumulation of Fe^{2+} targets, especially lipids in the cell and organelle membrane. Among the lipids in the membrane, phospholipids containing polyunsaturated fatty acids (PUFA-PLs) have been shown to undergo oxidation the most [21]. Acyl CoA Synthetase Long Chain Family-4 (ACSL4), which catalyzes the formation of adrenic acid or arachidonic acid in PUFA-PLs synthesis, and lysophosphatidylcholine acyl transferase (LPCAT3), which catalyzes the formation of phosphatidylethanolamine (PE) and phosphatidylcholine (PC), play an important role in the ferroptosis process [22]. In addition, ACSL3 from the ACSL family has been reported to show protective activity against ferroptosis by playing a role

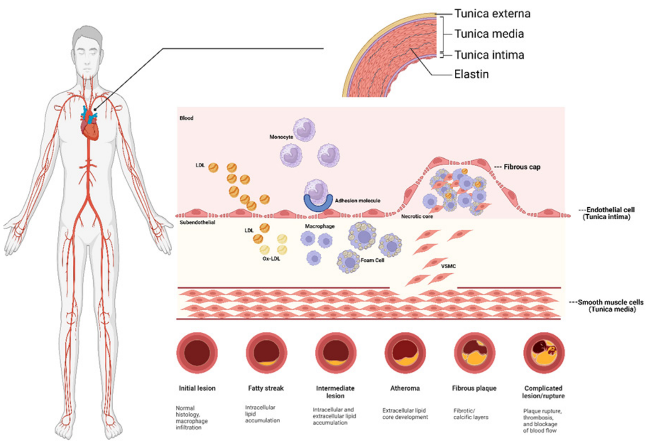

Figure 1. Initiation and progression of atherosclerosis. Created in BioRender. Yigit, E. (2024) BioRender.com/q35j271

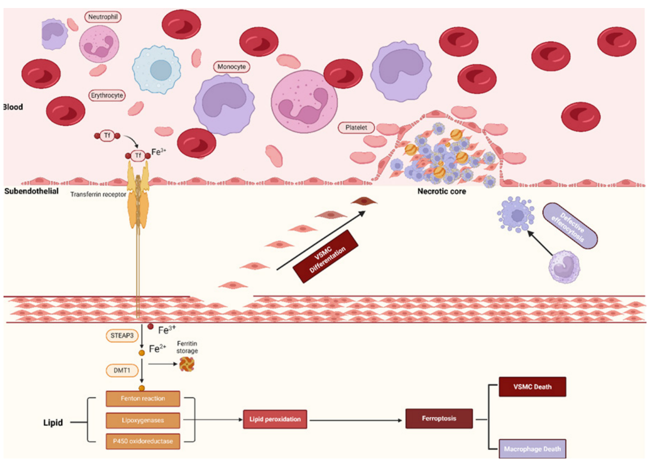

Figure 2. The role of ferroptosis in the development of atherosclerosis.Created in BioRender. Yigit, E. (2024) BioRender.com/l66h617

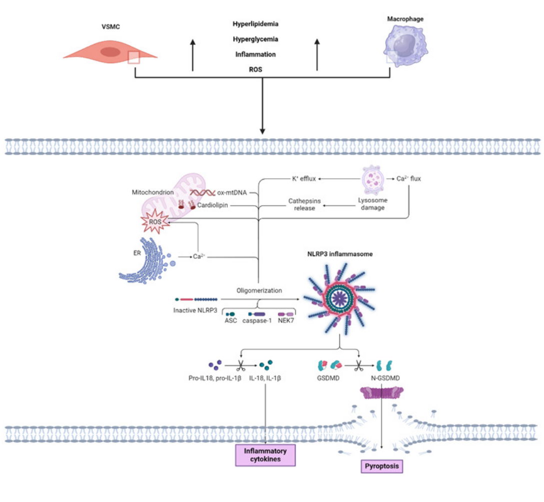

Figure 3. The role of pyroptosis in the development of atherosclerosis.Created in BioRender. Yigit, E. (2024) BioRender.com/x58x919

in the synthesis of monounsaturated fatty acid-containing phospholipids (MUFA-PLs), which are more protected against oxidation than PUFA-PLs [23]. Disorders occur in NADPH oxidase (NOX) and mitochondrial respiratory chain with increasing ROS due to iron accumulation in the cell [24]. As a result, superoxide radical ($O_2^{\cdot-}$) is formed. $O_2^{\cdot-}$ radical is converted into H_2O_2 by superoxide dismutase (SOD) and H_2O_2 into •OH by catalase (Cat). Increased •OH radical nonenzymatically takes the bisallic H atom from PUFA-PLs and forms PL• radical. PL• radical reacts with O_2 to form PLOO• radical [25,26]. PLOO• radical forms PLOOH, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE) from PLs. As a result, cell and organelle membrane integrity is disrupted, and ferroptosis occurs [27].

Furthermore, increased iron in the cell can lead to the induction of ferroptosis through the actions of lipoxygenases independent of Fenton reactions, prostaglandin synthase 2/cyclooxygenase 2, and cytochrome P450 reductase enzymes, which can form PLOOH directly from PUFA-PLs [28]. Moreover, the roles of selective autophagic pathways in lipid peroxidation-induced ferroptosis have been elucidated. Lipophagy is an autophagic pathway activated by directing LDs in the cytosol to lysosomes for degradation via autophagosomes [29].

Ferroptosis is a form of programmed cell death characterized by distinct morphological, biochemical, genetic, and metabolic features. Unlike other programmed cell death mechanisms such as apoptosis, pyroptosis, necroptosis, and autophagy, ferroptosis displays unique traits at the cellular level. These unique characteristics make ferroptosis a distinct and important process to study in the context of cell biology and disease mechanisms. Regarding cellular morphology, when cells undergo ferroptosis, they exhibit significant mitochondrial abnormalities. Those abnormalities include reduced mitochondrial cristae, increased mitochondrial membrane densities, and outer membrane rupture. From a biochemical perspective, ferroptosis is characterized by the excessive accumulation of iron within the cellular environment, the depletion of glutathione (GSH), the inactivation of glutathione peroxidase 4 (GPx4), and the peroxidation of membrane PUFA-PLs [30,31].

Endogenous Defense Mechanisms in Ferroptosis

It shows endogenous defense activity against pathophysiological signals in the living system in ferroptosis, as in other cell deaths. Studies on ferroptosis identified the Xc-/GSH/GPx4 pathway as the first antioxidant response pathway [32]. GPx4 efficiently transforms the PLOOH radical into phospholipid alcohol (PLOH) using GSH, showcasing its remarkable efficacy [33]. GSH acts as a cofactor in this reaction. GSH is a tripeptide composed of the amino acids glycine, cysteine, and glutamate. GSH synthesis is controlled by the concentration of cysteine in the cell. Although it can be synthesized from methionine in the cell under normal conditions, many cells take cysteine into the cell as its oxidized form, cystine, through the Xc- transmembrane system [34]. The Xc- transmembrane system consists of SLC7A11(XcT) with cystine transport activity and SLC3A2 subunits with chaperone activity [35]. The Xc- system takes cystine into the cell and removes glutamate. Cystine is converted to cysteine by thioredoxin reductase 1 (TrxR1) inside the cell [36]. Another endogenous defense mechanism is ferroptosis suppressor protein-1, which provides NADPH-mediated conversion of extramitochondrial ubiquinone to ubiquinol. Ubiquinol can directly scavenge lipid radicals as a lipophilic radical scavenger [37].

The Role of Ferroptosis in Atherosclerosis: Mechanisms and Therapeutic Implications

Recent studies have observed excessive iron accumulation, decreased GPx4 levels, and increased ROS levels in AS [38]. It has also been shown that apolipoprotein E knockout (ApoE$^{-/-}$FPNwt/C326S) mice have excessive iron accumulation in VECs and VSMCs, which perpetuates endothelial dysfunction, accelerates macrophage foam cell formation, increases dyslipidemia in plasma, causes more inflammation and leads to plaque destabilization. Iron chelator administration also decreased iron accumulation, anti-inflammatory activity, and atherosclerotic plaque burden [39]. Marques et al. observed that iron-dextran loading significantly induced iron accumulation in atheroma plaques and accelerated plaque formation with increased endothelial dysfunction and oxidative stress in a model of atherosclerosis induced in ApoE$^{-/-}$ mice [40]. In a study using mouse aortic endothelial cells from isolated hGPx4Tg/ApoE$^{-/-}$ mice, researchers observed that the overexpression of GPx4 led to a reduction in monocyte adhesion, lipid peroxidation and the development of atherosclerotic lesions. The finding suggests that GPx4 may play a role in mitigating atherosclerosis-related processes at the cellular level [41]. Monocytes undergo differentiation through various signaling pathways, giving rise to two distinct phenotypes: M1 macrophages, which possess pro-inflammatory properties, and M2 macrophages, known for their anti-inflammatory properties. Notably, an elevation in M1 macrophage levels has been linked to an upsurge in ROS production, which plays a significant role in the pathology of atherosclerosis [42]. Knockdown of LDL receptor (LDLR$^{-/-}$) and hamp$^{-/-}$ has been demonstrated to decrease macrophage iron levels and the quantity of M1 macrophages, resulting in reduced lipid accumulation in plaques [43]. Hepcidin is a peptide hormone that regulates iron metabolism. One of its key functions is to suppress the expression of ferroportin (FPN), a transmembrane protein responsible for allowing the export of intracellular iron. This regulation by hepcidin ultimately influences the body's iron levels and distribution [44]. Cai et al. showed that FPN deficiency in ApoE$^{-/-}$FPN1LysM/LysM and ApoE$^{-/-}$ mice in a high-fat diet (HFD)-induced atherosclerosis model caused an increase in iron accumulation, especially in macrophages. However, it did not affect serum total cholesterol and triglyceride levels. They reported that increased iron accumulation may play a role in foam cell formation, oxidative stress, systemic inflammation, and larger atherosclerotic lesions [45]. The available data strongly suggests a noteworthy correlation between the development of AS and the process of ferroptosis. This finding could have important implications for understanding the pathophysiology of atherosclerosis and developing potential therapeutic interventions. VEC dysfunction is recognized for its role in speeding up the development of AS plaques and triggering the formation of blood clots by enhancing the permeability of the inner arterial wall and promoting the adhesion of white blood cells [46]. Bai et al. reported that ApoE$^{-/-}$ mice fed an HFD had more iron accumulation in the atherosclerotic lesion area, increased NOX and lipid peroxide levels, and decreased Xct and GSH levels compared to wild-type (WT) mice. In contrast, administration of ferrostatin-1, a ferroptosis inhibitor, decreased atherosclerotic parameters. In addition, they reported that oxidized LDL, a pro-atherosclerotic factor, caused an increase in the amount of iron and ROS and decreased Xct and GPx4 levels in mouse endothelial cell lines [47]. Li et al. injected endothelial progenitor cell (EPC)-derived extracellular vesicles (EVs) (miR-199a-3p) into ApoE$^{-/-}$ mice fed HFD. They revealed that EPC-EVs inhibited cell death, GSH depletion, ROS production, lipid peroxidation, and iron accumulation in endothelial cells, thereby suppressing endothelial cell ferroptosis. They also observed decreased atherosclerotic plaque burden, serum levels of TNF-α, and interleukin 6 (IL-6) [48]. In addition to VEC death, macrophage death plays a significant role in the AS progress. A decrease in metalloprotease synthesis at the beginning of AS can result from macrophage death. Macrophage death may be a protective immune response against AS at physiologic levels. However, an increase in macrophage death can lead to the observed formation of a necrotic core in the morphology of atherosclerotic plaques [49]. In their study, Bao et al. found that exposure to cigarette smoke activates the nuclear factor kappa-B (NF-Kb) signaling pathway, reducing the expression of FPN and Xct in THP-1 macrophages from atherosclerotic mice. This decrease in FPN and Xct expression promotes ferroptosis through the hepcidin/FPN/Xct axis. These findings underscore the detrimental impact of macrophage ferroptosis on the development of atherosclerosis [50]. Hu et al. found that GPx4 expression was mainly localized in the CD68-positive area and showed a negative correlation with the progression of atherosclerotic plaques, suggesting that ferroptosis occurs predominantly in macrophages during the development of atherosclerosis. Further investigations revealed that ox-LDL promotes macrophage ferroptosis by upregulating hypoxia-inducible factor-1α expression and suppressing autophagy (Figure 2) [51].

Pyroptosis

NLRP3 Inflammasome Activation and Pyroptosis: Mechanisms and Implications

The activation of inflammasomes marks the onset of pyroptosis. Pattern recognition receptors (PRRs) are crucial components of the inflammatory complex and can be categorized into membrane-bound toll-like receptors (TLR) and cytosolic nod-like receptors (NLR). The innate immune system detects pathogen-associated molecules (PAMPs) and damage-associated molecules (DAMPs) through PRRs. While TLRs are believed only to recognize PAMPs, NLRs can identify both PAMPs and DAMPs, allowing them to recognize a wide range of substances from pathogens, host cells, and the environment [52]. NLRs are a class of intracellular proteins that play a crucial role in the innate immune system. These receptors are divided into two main categories: NLRPs, which form inflammasomes, and NLRCs, which are involved in other signaling pathways. Among these, the NLRP3 inflammasome has been the focus of numerous studies due to its significant role in mediating immune responses and its implications in various diseases [53]. The NLRP3 protein, which is involved in forming inflammasomes, typically exists at a baseline level that is insufficient for assembling normal inflammasomes. Additionally, it is usually ubiquitinated in this state. Therefore, before the assembly of inflammasomes, it is necessary to activate the NLRP3 protein [54]. Three critical events that trigger the activation of NLRP3 have been identified in scientific studies. Firstly, the increased ROS induced by mitochondria has been shown to play a key role [55]. Secondly, the disruption of lysosomal membranes and lysosomes, often caused by pathogenic particles such as cholesterol crystals, has been identified as another crucial factor [56]. Lastly, abnormal ion flux, specifically involving the influx and efflux of potassium and calcium ions, significantly contributes to NLRP3 activation [57]. Furthermore, the NLRP3 inflammasome can be activated by various inflammatory cytokines, intracellular signaling through TLRs, and the NF-κB pathway [58]. The activation and assembly of NLRP3, a key inflammasome component, are regulated by various molecules, cellular processes, and post-translational modifications. NLRP3 undergoes conformational changes upon activation, allowing it to associate with apoptosis-associated speck-like proteins (ASCs) via the pyrin domain (PYD). Additionally, NLRP3 can interact with other NLR family members or the absence of melanoma 2 (AIM2) signaling pathway. This interaction results in the forming of a large signaling complex known as the ASC speck, which subsequently recruits and activates pro-caspase-1. The activated caspase-1 then cleaves pro-inflammatory cytokines pro-IL-1β and pro-IL-18 into their active forms and initiates the cleavage of gasdermin D (GSDMD), leading to the formation of membrane pores and, ultimately, pyroptotic cell death [59]. IL-1β and IL-18 in the bloodstream stimulate the production of other inflammatory cytokines, chemokines, and adhesion factors. When GSDMD cleaves, its N-terminus becomes lipophilic and can move into cell membranes, forming pore channels by spontaneously polymerizing. This pore formation leads to an imbalance in osmotic pressure, causing rapid swelling and the release of cellular contents into the extracellular space [60]. The components within a cell that are released into the extracellular space, such as ATP, DNA, and other molecules, can act as DAMPs when detected by neighboring cells. Additionally, when NLRP3 and ASC are released from the cell, they can form complexes outside the cell, leading to the continuous activation of caspase-1 and sustained release of the pro-inflammatory cytokine IL-1β. This process is known as the formation of the NLRP3 inflammasome. This mechanism can also occur within immune cells, particularly macrophages, after phagocytosing foreign particles or pathogens [61]. Therefore, pyroptosis causes localized inflammation and amplifies the inflammatory response.

Understanding the Role of Pyroptosis in Atherosclerosis Progression

The progression of plaque instability exacerbates the risk of plaque rupture and subsequent formation of blood clots. This process can ultimately lead to cardiovascular events, such as heart attacks or strokes [62]. A study focused on vascular endothelial cells (VECs) discovered that when IL-1β and IL-18 stimulate these cells, they exhibit an overproduction of inflammatory molecules such as ICAM-1, VCAM-1, IL-6, and C-reactive protein. This excessive production of inflammatory proteins exacerbates the inflammatory response within the body [63]. VECs exhibit a high sensitivity to conditions such as hyperlipidemia and hyperglycemia. In the initial stages, hyperlipidemia can trigger the activation of endothelial cells through the caspase-1-sirtuin-1-activating protein-1 pathway, subsequently leading to the recruitment of macrophages. This process underscores the intricate interplay between lipid metabolism, cellular signaling, and immune response within the vascular endothelium [64]. Hyperglycemia, which refers to high levels of glucose in the blood, has been shown to potentially activate the NLRP3 inflammasome through a series of molecular mechanisms. This includes a decrease in the expression of histone methyltransferase and an increase in the activity of SET8 and MARK4 promoters. These changes at the molecular level could contribute to the activation of the NLRP3 inflammasome, which is involved in the regulation of inflammatory responses [65]. Smoking can promote inflammasome production. Nicotine and acrolein in tobacco induce NLRP3 inflammasome formation. Nicotine triggers pyroptosis and enhances ability of macrophages to absorb lipids, generate ROS, and activate the TXNIP/NLRP3 pathway in VECs [66, 67]. Jiang et al. observed that when human umbilical vein endothelial cells (HUVECs) are exposed to acrolein, there is an increase in the production of ROS. Additionally, exposure to acrolein leads to the activation of autophagy and pyroptosis pathways within the cells. Furthermore, it has been found that acrolein treatment reduces the migration of these cells [68]. Following VEC dysfunction, monocytes undergo a process of differentiation into macrophages at the site of the lesion. These macrophages then ingest lipoproteins, ultimately transforming into foam cells [69]. In advanced AS plaques, it has been observed that most of the cells that have undergone cell death are macrophages [70]. The death of these macrophages has been demonstrated to trigger the formation of inflammasomes, mainly by inducing pyroptosis in nearby macrophages. This mechanism can lead to the inflammatory response linked to the progression of AS. In more developed atherosclerotic plaques, dying macrophages release cellular contents, cytokines, and proteinases into the surrounding extracellular space [71]. As a result, the necrotic core within atherosclerotic plaques undergo enlargement, contributing to the worsening instability of the plaques [72,73]. Cholesterol crystals, along with fatty acids and their metabolites associated with hyperlipidemia, including ceramide and triacylglycerol, have been identified as significant risk factors contributing to the development of atherosclerosis [74, 75]. Numerous studies have discovered that the above substances can further provoke pyroptosis by activating inflammasomes in various ways. When cholesterol crystals come into contact with macrophages, they activate NLRP3 inflammasomes. The activation occurs due to the rupture of lysosomes, which are cellular organelles responsible for digestion and waste removal, leading to the release of cathepsin proteins. The process plays a significant role in the inflammatory response within the body, highlighting the intricate interplay between cholesterol metabolism and immune system regulation [76]. Palmitic acid can trigger the activation of NLRP3 inflammasomes by inducing impairments in mitochondrial autophagy. The process results in an excessive production of mitochondrial ROS [77].

Oxidized LDL can stimulate the synthesis of reactive oxygen species in macrophages, activate NLRP3 inflammasomes, and lead to cell lysis and release of cytokines [78]. The substances generated during pyroptosis promote macrophage migration, lipoprotein phagocytosis, and foam cell formation. Specifically, IL-1 activated by NLRP3 inflammasomes can enhance the expression of sterol regulatory element binding protein 1 in monocytes and macrophages. The process facilitates ox-LDL-mediated lipid accumulation and foam cell formation [79]. Nogiec et al. reported that macrophages exposed to Ox-LDL for prolonged periods are less sensitive to foam cells, TLR ligands, and inactivated bacteria. In addition, some surface receptors that may function as accessories in recognizing particulate antigens (CD47, CD81, and CD11b) were significantly reduced. In contrast, the long-term response to inflammasome activation caused by LPS, resulting in extensive, necrotic-type cell death, was found to be partially independent of caspase-1. Those findings suggest that mature foam cells may significantly contribute to the inflammatory state by intensifying the burden of immunogenic cell death. The observed interaction between foam cell death pathways could potentially serve as a groundbreaking marker for determining the severity of atherosclerosis disease [80]. Activated monocytes containing ox-LDL have been discovered to promote pyroptosis, a type of programmed cell death, in VECs. This process is facilitated by the activation of caspase-1 and the subsequent release of IL-1β [81]. Recent studies indicate that IL-1β can be secreted through a macrophage-induced process, leading to functional changes in VSMCs related to adhesion, inflammation, and apoptosis, which can be reversed through STAT3 activation. Inflammatory macrophages can also stimulate the early-stage proliferation, migration, and trans-differentiation of VSMCs [82]. Studies have shown that pyroptosis of VSMCs is linked to destroying fibrous cap in necrotic core and activating inflammatory responses [83]. Pan and colleagues showed that AIM2 plays an important role not only in inflammasome activation but also in different processes of atherosclerosis in the HFD-induced atherosclerosis model in ApoE$^{-/-}$ mice and in VSMCs [84]. The involvement of monocytes in the activation of VECs and the subsequent transformation of VSMCs through the NLRP3 inflammasome pathway is a significant aspect of cardiovascular pathophysiology [85] (Figure 3).

Conclusion

The primary cause of cardiovascular disease is atherosclerosis. This process initiates with hyperlipidemia, oxidative stress, and inflammation in the inner layer of the arteries and advances silently until the formation of blood clots. The presence of deficiencies in diagnostic and therapeutic methods indicates that there are numerous unknown pathways in the pathophysiology of atherosclerosis. In recent years, there has been a growing interest in the role of oxidative stress-related ferroptosis and inflammation-related pyroptosis processes in the pathology of atherosclerosis. It is anticipated that conducting experimental in vivo studies on ferroptosis and pyroptosis in the atherosclerosis process may yield significant findings and contribute to developing solutions that can enhance the lives of individuals affected by cardiovascular diseases.

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The authors declare that they are responsible for the article's scientific content, including study design, data collection, analysis and interpretation, writing, and some of the main line, or all of the preparation and scientific review of the contents, and approval of the final version of the article.

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All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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