OnLine Journal of Biological Sciences

Review

The Effect of Main Heat Shock Proteins on Atherosclerosis

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Article history
Received: 11-11-2023
Revised: 03-05-2024
Accepted: 10-05-2024

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Abstract: Atherosclerosis is a multifaceted disease characterized by complex pathogenesis involving diverse mechanisms. In recent years, the autoimmune nature of atherosclerosis has gained significant attention, emphasizing the indispensable role of the immune system in its initiation and progression. Heat Shock Proteins (HSPs) are vital for cellular functionality, facilitating cellular adaptation to environmental changes and survival under adverse conditions. Notably, various HSP families play diverse roles in the advancement of cardiovascular disorders. While the exact contribution of HSPs to atherosclerosis remains a subject of debate, emerging evidence suggests that HSPs can function as self-antigens, eliciting both cellular and antibody-mediated immune responses. Conversely, HSPs also possess immunoregulatory properties, making them potential targets for immunomodulation of atherosclerosis by the adaptive or innate immune system. This comprehensive review focuses on the specific roles of extensively investigated chaperones, including 60, 70, 90 and Hsp27, in the intricate development of atherosclerosis. We discuss their implications in various stages of atherosclerotic lesion formation, from endothelial dysfunction to plaque stability, highlighting their unique contributions and potential as therapeutic targets. The novelty of this review lies in its comprehensive appraisal of the multifaceted involvement of distinct HSPs in atherosclerosis, emphasizing their potential as promising avenues for future research and the development of novel therapeutic strategies.

Keywords: Atherosclerosis, Heat Shock Protein, HSP, Cardiovascular Disease

The Pathogenesis of Atherosclerosis

This review aims to investigate and synthesize the impacts of specific heat shock proteins, including 27, 70, 90 and Hsp60, on the development and progression of atherosclerosis. The sharply defined focus includes:

- Explore the specific roles of HSPs in atherosclerosis, focusing on their implications in distinct stages of atherosclerotic lesion formation
- Investigate the mechanisms underlying the potential anti-atherosclerotic effects of Hsp27, particularly in regulating apoptosis, inflammation and plaque stability
- Assess the dual roles of Hsp70 in atherosclerosis, examining its pro-atherogenic and anti-atherogenic functions in various cellular contexts
- Evaluate the association of Hsp90 with plaque instability and examine potential therapeutic strategies targeting this heat shock protein
- Discuss the clinical implications of HSPs as biomarkers, diagnostic tools and therapeutic targets in the context of atherosclerosis

By addressing these research questions, this review aims to provide a comprehensive understanding of how main heat shock proteins impact atherosclerosis pathogenesis and offer insights into their potential as targets for therapeutic interventions and diagnostic advancements.

Atherosclerosis is a chronic condition characterized by inflammation and dysregulated lipid metabolism, leading to arterial wall abnormalities. It serves as the underlying pathophysiology of cardiovascular disease

(Linton *et al*., 2019). The pivotal players in atherosclerosis development include macrophages, Vascular Smooth Muscle Cells (VSMCs) and endothelial cells. The initiation involves endothelial cell dysfunction, leading to the deposition of Low-Density Lipoproteins (LDL) in the arterial wall, subsequent oxidation and the formation of ox-LDL (Falk, 2006). Impaired endothelial cells secrete adhesion molecules and chemokines, facilitating the adhesion of circulating monocytes. These monocytes penetrate the subendothelial intima through endothelial gaps and differentiate into macrophages, which engulf modified lipoproteins, including ox-LDL, transforming into foam cells. The accumulation of foam cells gives rise to fatty streaks, the initial gross lesions in atherosclerosis development (Poursaleh *et al*., 2021). Furthermore, foam cells and dysfunctional endothelial cells secrete various cytokines and inflammatory mediators, promoting the growth and inflammation of atherosclerotic plaques. Additionally, cytokine stimulation induces the proliferation and migration of VSMCs from the media to the intima, resulting in the secretion of large quantities of extracellular matrix proteins and the development of fibrous plaques (Fatkhullina *et al*., 2016; Branchetti *et al*., 2014). As lesions progress, VSMCs encase a necrotic core rich in lipids, oxidized lipoproteins, cell debris and cholesterol crystals, forming a fibrous cap that stabilizes the plaque. In later stages, persistent inflammatory response leads to cell death, thinning of the fibrous cap and calcification of the plaque, compromising its stability. Unstable plaques may rupture, causing thrombosis events and potentially leading to severe complications such as stroke and Myocardial Infarction (MI) (Prilepskii *et al*., 2020).

The significance of apoptosis and autophagy in atherosclerosis development, particularly their impact on VSMCs, macrophages and endothelial cells, is well-established. Apoptosis and autophagy adversely affect endothelial function, VSMC migration, proliferation and phenotype switching, contributing to lipid metabolism dysfunction, inflammation and the promotion of atherosclerotic plaque formation and progression (Li *et al*., 2022). Consequently, further investigation into the impact of apoptosis and autophagy on VSMCs, macrophages and endothelial cells is crucial for better understanding the pathogenesis of atherosclerosis, as well as for the development and enhancement of preventive and treatment strategies (Ni *et al*., 2021).

With this in mind, the aim of this review is to summarize the current understanding of the impact of Heat shock protein 27 (Hsp27), one of the small heat shock proteins, on the regulation of apoptosis and autophagy and its ultimate influence on atherosclerosis. By evaluating existing evidence, we seek to elucidate the potential mechanisms underlying the anti-atherosclerotic effect of Hsp27. Through this comprehensive analysis, we aim to provide a foundation for considering Hsp27 in the development of novel strategies for the prevention and treatment of atherosclerosis (Liang *et al*., 2018; Shan *et al*., 2021).

Heat Shock Proteins

Depending on their molecular weight, the group of heat shock proteins is subdivided into numerous superfamilies which have been described in detail in the updated guidelines for human HSPs nomenclature published by the Hugo gene nomenclature committee. Under physiological conditions, HSPs bind hydrophobic sites of preformed, non-native proteins and contribute to protein folding, primarily through ATP hydrolysis. Besides, they bind newly synthesized proteins and assist in their transportation to various locations in cells. HTPs also help prevent the aggregation of intracellular proteins. Multiple HSP families play various roles in the progression of cardiovascular disorders (Sučec *et al*., 2021).

Atherosclerosis, traditionally viewed as a lipid-driven disorder, also involves crucial immunological aspects. Increasing evidence suggests that immune responses play a pivotal role in the initiation and progression of atherosclerotic lesions. Dysregulated immune responses and chronic inflammation promote the development of atherosclerosis. One emerging concept is that atherosclerosis can be considered an autoimmune disorder characterized by the activation of adaptive immune cells, including T cells and B cells, within the arterial wall. Antigen-presenting cells such as macrophages and dendritic cells facilitate the presentation of self-antigens derived from oxLDL (oxidized Low-Density Lipoprotein) to T cells. This interaction triggers a cascade of immune responses leading to the generation of pro-inflammatory cytokines, recruitment of immune cells and formation of atherosclerotic plaques. Furthermore, autoantibodies against oxLDL and heat shock proteins have been identified in atherosclerosis patients, indicating the presence of autoreactive B cells in the disease process. These autoantibodies can contribute to the development of inflammation and plaque destabilization. Immune cells, especially pro-inflammatory T helper 1 (Th1) and Th17 cells are thought to be involved in promoting chronic inflammation within the plaque, while regulatory T cells (Tregs) play a protective role by suppressing excessive immune responses. Understanding the immunological aspects of atherosclerosis helps unveil new therapeutic targets and strategies aimed at modulating immune responses to attenuate disease progression.

HSP Role in Atherogenesis

In atherosclerosis, lipids, in particular low-density lipoproteins, deposit in the intima of large and medium

arteries, immune cells infiltrate and arterial walls are remodeled (Milutinović *et al*., 2019; Poggio *et al*., 2014). For about two centuries it was known about plaque composition and mononuclear cell infiltration; however, it was only some 40 years ago that the importance of an inflammatory factor in atherosclerosis pathogenesis was recognized (Libby *et al*., 2012).

Studies examining the results of immunosuppressive therapy first described the key role that immune reactivity plays in atherosclerosis. Studying atherosclerosis induced by a high-fat diet in rabbits, scientists demonstrated that immunosuppressive therapy with mycophenolate mofetil noticeably prevented the formation of plaques, inflammatory cell infiltration and VSMC proliferation in the aorta (Skaggs *et al*., 2012). Notably, the reduced immune cell infiltration was associated with reduced fat (cholesterol) in the aorta walls, which proves the importance of local inflammation for the development of atherosclerotic plaques. Wick (2016) further developed the concept of atherosclerosis as an autoimmune disease, while understanding ox-LDL toxicity for endothelial cells stimulated studies on VSMC proliferation in arterial walls and the character of immune reactivity caused by ox-LDL (Galkina and Ley, 2009). Multiple data have been gained recently on the role of immune reactivity, both adaptive and innate, in atherosclerosis and also on HSP's importance in atherogenesis (Witztum and Lichtman, 2014).

HSP70 and HSP90 are two key members of the heat shock protein family that play significant roles in the pathogenesis of atherosclerosis. HSP70 acts as a chaperone protein, assisting in the proper folding of other proteins and preventing their aggregation. In atherosclerosis, HSP70 helps protect endothelial cells, which line the blood vessels, by reducing oxidative stress and inflammation. It also promotes the clearance of harmful lipid deposits, or plaques, within the blood vessels. On the other hand, HSP90 regulates the function of several proteins involved in cell growth and inflammation. In atherosclerosis, HSP90 mediates the activation of pro-inflammatory signaling pathways, leading to the production of cytokines and chemokines that promote plaque formation and vascular inflammation. Additionally, HSP90 also aids in the regulation of endothelial Nitric Oxide Synthase (eNOS), an enzyme responsible for producing nitric oxide, which plays a critical role in maintaining vascular health. Therefore, dysregulation of both HSP70 and HSP90 can contribute to the pathogenesis of atherosclerosis by promoting inflammation, impairing endothelial function and disrupting the integrity of blood vessels.

Atherosclerotic Lesions

The formation of atherosclerotic lesions starts with the appearance of fatty streaks in the innermost parts of blood vessels. Innate immunity is stimulated by oxLDLs which function like Danger-Associated Molecular Patterns (DAMPs) as they generate autoantigens and involve the adaptive immune response. Treg responses participate in the modulation of local inflammation (Ilhan, 2015; Zanobini *et al*., 2017). From the very beginning of fat deposition, the lesion contains a cellular component that consists of T cells, foam cells and macrophages. In the arterial adventitial layer, there is a higher content of B lymphocytes. The lesion develops and forms plaques which are stable when encaged in a fibrous cap. However, the production of pro-thrombotic mediators and pro-inflammatory cytokines leads to an active inflammatory process resulting in arterial wall remodeling with growth and subsequent rupture of plaques (Harman and Jørgensen, 2019).

The Immune System in Atherosclerosis

Inflammation in atherosclerosis is driven by innate and adaptive immune reactivity activated by products of lipid peroxidation. Libby *et al*. (2012) underlined the crucial role of innate immunity in atherosclerosis pathogenesis. The research demonstrated that MI, stroke and cardiovascular-related death, independent of lipid decrease, were reduced with the monoclonal antibody therapy targeting Interleukin (IL)-1β (Wolf and Ley, 2019). There is also multiple evidence that adaptive immunity participates in atherosclerosis progression: Oxidized lipoproteins initiate Th1 cytokines production in a process suppressed by the Th2 cytokine IL-5 (Samson *et al*., 2012; Sainger *et al*., 2012).

CD4+ T cells amount to 70% of T cells in human atherosclerotic plaques and about all the rest are CD8+ T cells. In smaller quantities, atherosclerotic lesions also contain Th17 cells, Tregs and natural killer cells. With the disease progression, tertiary lymphoid organs which contain various T-cells and B-cells start forming in the arterial adventitia (Marchini *et al*., 2021). There is extensive research on the role of different subtypes of the T cell. CD4+ T cells are known to exacerbate atherosclerosis, whereas the Th1 subtype of CD4+ T cells is proatherogenic and promotes inflammation and the Th2 subtype reportedly both protects and aggravates atherosclerosis. Tregs are known to be atheroprotective (Saigusa *et al*., 2020). There is conflicting evidence regarding IL-17 that may reduce or aggravate the disease. Infiltrating CD8+ cytotoxic T cells worsen plaque stability and promote rupture, while natural killer T cells are proatherogenic in the onset of atherosclerosis. The evidence regarding the role of B-cells is contradictory: Some studies support their protective function, while others suggest that B-cells aggravate atherosclerosis (Gerhardt *et al*., 2022).

HSP60

As of today, HSP60 is the only heat shock protein, whose direct atherogenic functions have been

demonstrated both in clinical and laboratory studies. Atherosclerosis is widely studied on hypercholesterolaemic LDLR–/–and apoE–/–murine models which allow the assessment of various familial hypercholesterolemia forms (Zonnar *et al*., 2022; Poggio *et al*., 2018). It should be noted, however, that LDLR–/–mice on standard chows can also develop atherosclerosis later in life (Oppi *et al*., 2019).

Several factors determine the autoimmune nature of atherosclerosis based on HSP60. First, high blood cholesterol does not seem to be the major, or the only atherosclerotic risk factor since the majority of patients have normal cholesterol levels. Second, a Langerhans cell-like network of VADCs appears before other inflammatory mononuclear cells migrate and infiltrate in the intima. Third, T-cells accumulate before macrophages, monocytes and VSMCs invade the arterial intima at predilection locations. Fourth, C-Reactive Protein (CRP) and other biomarkers of inflammation correspond with elevated intima-media thickness in atherosclerotic patients who have normal levels of cholesterol; in such patients, atherosclerosis is successfully treated with anti-inflammatory therapy (Grundtman and Wick, 2011).

Risk factors were ranked and it appeared that ox-LDL was lower than such factors as hypertension, smoking, or infection. In spite of the fact that increased cholesterol levels compared to “normal” are widely considered to be an important atherosclerosis risk factor, the majority of atherosclerotic patients have “normal” levels according to current standards. This allowed us to assume that “normal” cholesterol level must be too high, which correlates with our evolutionary theory that cultural evolution has outpaced biological adaptation (Makover *et al*., 2022; Di Minno *et al*., 2020; Lupoli *et al*., 2017).

Atherogenic HSP60 Epitopes

In the presence of a Langerhans cell-like network of VADCs prevailing at branching areas of arteries, in the normal intima, HSP60 endothelial expression together with adhesion molecules activated by atherosclerosis risk factors are likely to locally induce an immune response. However, there are not any experience-based data that would support this series of events (Blankenberg *et al*., 2003). It is still unclear whether sensitization of T cells occurs in the area of initial localization or, as has been proven for the skin, antigens are taken up by VADCs and transported to draining lymph nodes, whereas sensitized T cells move back from there to the artery wall (Saxena *et al*., 2019). Therefore, the key step to investigate was to identify HSP60-derived peptides that are potentially atherogenic. T cells isolated from plaques removed in patients with advanced atherosclerosis would recognize HSP60 and display a restricted T cell receptor α/β repertoire pointing to an antigen-driven process. Such T cells mostly secrete type 1 T-helper cytokines (Almanzar *et al*., 2012).

As already mentioned, in advanced lesions compared to early atherosclerosis, increased titers of HSP60 antibodies in serum correlate with plaque severity. While conformational HSP60 epitopes acting as auto-antibodies and recognized by antibodies to HSP60 have been identified as well as HSP60 epitopes recognized by T cells in advanced lesions, data were lacking regarding T cells isolated from plaques in early atherosclerosis, without clinical manifestations (Grundtman *et al*., 2011). Therefore, using samples isolated from early lesions in human donor organs, T cell reactivity against total HSP60 together with 15-mer overlapping peptides was investigated. CD4+, CD45RO+, CD25+ and CD28– effector memory cells that produced IFNγ and IL-64 in high quantities were observed to dominate T cell populations (Wick, 2016; Di Minno *et al*., 2017).

In a study of the cross-reactivity of T cells isolated from advanced plaques and C. pneumoniae HSP60, it was found that atherosclerotic plaques harbored CD4+ T cells activated in vivo recognizing human HSP60. It was not possible to detect T cells like these, activated in vivo, in the peripheral blood. Moreover, patients demonstrating positive PCR for C. pneumoniae DNA and seropositive serology, unlike seronegative patients, also demonstrated specific, derived from plaques T cells that recognized C. pneumoniae HSP60 (Hamley, 2021). Almanzar *et al*. (2012) described four epitopes of HSP60 from advanced lesions that were homologous to epitopes reported and also identified 7 of the 8 peptides that were associated with early atherosclerosis in the abovementioned study (Weintraub and Rubinstein, 2013).

However, none of the investigations could find one epitope (551-565) recognized by T cells from 70% of early lesions. This epitope as well as highly homologous epitopes in late lesions may be further investigated for preventive, diagnostic and therapeutic purposes as until now only a limited number of early atherosclerotic plaques have been studied (Bai *et al*., 2020).

HSP27

HSP27 is another member of the heat shock protein family that displays anti-atherogenic potential. This protein acts as a molecular chaperone and exerts its protective effects by modulating various cellular mechanisms. In the context of atherosclerosis, HSP27 has been found to inhibit oxidative stress-induced apoptosis of Vascular Smooth Muscle Cells (VSMCs). By reducing the generation of Reactive Oxygen Species (ROS), HSP27 helps maintain cellular redox balance and prevents cell death. Additionally, HSP27 also exhibits anti-inflammatory properties by inhibiting the activation of Nuclear Factor-kappa B (NF-κB), a key transcription factor involved in the inflammatory response. This

prevents the overproduction of pro-inflammatory cytokines and chemokines, reducing vascular inflammation. Moreover, HSP27 contributes to the stabilization of atherosclerotic plaques by inhibiting Matrix Metalloproteinases (MMPs) that degrade the extracellular matrix. The preservation of plaque stability helps prevent the development of complications such as plaque rupture and subsequent thrombosis. Overall, the anti-atherogenic potential of HSP27 lies in its ability to counteract oxidative stress, inhibit inflammation and maintain vascular integrity, thereby reducing the progression of atherosclerosis.

A recent study demonstrated that an atherosclerotic plaque contains a much smaller amount of Hsp27 than a healthy vessel (Martin-Ventura *et al*., 2006; Zanobini *et al*., 2018). Besides, carotid atherosclerosis patients had significantly lower levels of plasma Hsp27 in comparison to healthy subjects, which suggests that a decreased Hsp27 level might potentially indicate atherosclerosis. Atherosclerosis correlates with Hsp27 in a way that a plaque releases proteases hydrolyzing extracellular Hsp27, which reflects proteolytic imbalance (Batulan *et al*., 2016). Importantly, a decrease in intracellular Hsp27 diminishes protection of proteolysis-induced VSMC apoptosis, which consequently results in fibrous cap thinning and finally leads to plaque rupture. Therefore, Hsp27 helps maintain the stability of atherosclerotic plaques (Kuang *et al*., 2017).

Hsp27 may have anti-atherosclerotic features as there is multiple evidence of Hsp27 inhibiting the development of atherosclerosis in several ways. For example, it reduces the plaque area and prevents macrophages and cholesterol accumulation in lesion areas, which indicates Hsp27's correlation with lipid homeostasis (Shan *et al*., 2021; Myasoedova *et al*., 2018). Besides, Hsp27 stimulates ATP-Binding Cassette transporter A1 (ABCA1) expression and facilitates the efflux of cholesterol through PI3K/PKCζ/Sp1 signaling in foam cells derived from macrophages. Furthermore, in vitro studies demonstrated downregulation by extracellular Hsp27 of the macrophage Scavenger Receptor-A (SR-A) expression via the NF-κB pathway and inhibition of cholesterol intake by macrophages, which consequently decreases macrophage conversion into foam cells (Vidyasagar *et al*., 2012). Importantly, Hsp27 administered to ApoE-/-and SR-A-/-mice did not inhibit the progression of atherosclerosis, which suggests that the Hsp27 anti-atherosclerotic factor is associated with SR-A. The results demonstrate that the anti-atherogenic effects of Hsp27 are associated with its ability to regulate the uptake and excretion of cholesterol in macrophages (Qi *et al*., 2014).

At the same time, the anti-atherosclerotic effect of Hsp27 can be associated with the inhibition of apoptosis. Research demonstrated that phosphorylated Hsp27 may inhibit apoptosis by activating the p38/PI3K/AKT pathway. Besides, phosphorylated Hsp27 manifested anti-apoptotic and anti-oxidant features via MK2/Hsp27 signaling under oxidative stress (Tian *et al*., 2016). Moreover, phosphorylation of Hsp27 protects from apoptosis induced by TNF-α as it promotes the activation of pro-survival signaling Tak1 and Tak1-p38/ERK. Importantly, inhibition of Hsp27 in a mice model with atherosclerosis increased ROS levels in the aorta and accelerated the formation of plaques, which suggests that Hsp27 potentially decreases ROS, alleviates apoptosis and consequently attenuates atherosclerosis development (Steen *et al*., 2020). In addition, Hsp27 may inhibit endothelial apoptosis induced by homocysteine by modulation of ROS production and apoptotic pathway of mitochondria, resulting in anti- atherosclerotic effect. Finally, the Hsp27 knockdown in umbilical endothelium in humans under high glucose levels demonstrated that Hsp27 phosphorylated via ERK1/2 and PI3K/AKT signaling pathways can inhibit cellular apoptosis induced by high glucose. Thus, Hsp27 can suppress the development of atherosclerosis by inhibiting apoptosis caused by multiple atherogenic factors via various pathways (Shi *et al*., 2019).

In addition, Hsp27 modulates the inflammatory response, which also provides an anti-atherosclerotic effect. For example, it is known that extracellular Hsp27reduces the release of IL-1β pro-inflammatory cytokine-induced by acetylated LDL and promotes secretion of IL-10 anti-inflammatory cytokine, which alleviates inflammation in atherosclerosis (Yuan *et al*., 2020). Besides, as recently demonstrated, Hsp27 was found in extracellular vesicles that impede inflammation through macrophage NF-κB-mediated IL-10 secretion, thus suppressing the development of atherosclerosis (McCullagh *et al*., 2016; Perrot *et al*., 2020).

In short, Hsp27 produces an anti-atherogenic effect in many ways, including its anti-apoptotic, anti-inflammatory and anti-oxidant features as well as the ability to modulate lipid homeostasis. Many studies have recently demonstrated the crucial role played by autophagy in atherosclerosis occurrence and progression. A few of them showed a correlation between autophagy and Hsp27. However, it remains unknown whether an anti-atherosclerotic effect of Hsp27 is associated with autophagy regulation, so the hypothesis must be further investigated.

HSP70

iHSP70 plays a key role in endothelial homeostasis. Increased eNOS expression stimulates iHSP70 expression (Harris *et al*., 2003). A human *in vitro* study, in which VSMC of a coronary artery was transfected with adenovirus that contained recombinant DNA for eNOS, showed that eNOS and iHSP70 expression increased concomitantly, while cellular growth and

proliferation were inhibited (Yoon *et al.*, 2021). According to Harris *et al*. (2023) then rats were exposed to heat shock of 42 C for 15 min, eNOS and iHSP70 aortic co-expression was induced and phenylephrine vasocontractile response was reduced, which suggests a relation between eNOS and iHSP70. Therefore, iHSP70 may be considered a factor responsible for thrombosis inhibition, vasodilatation and cellular proliferation by increasing eNOS expression with subsequent NO production (Kong *et al*., 2016).

Comparably, animal model studies showed that excessive expression of HSPA12B, a member of the HSP70 superfamily, after infarction may produce beneficial cardiac effects and remodeling (Szyller and Bil-Lula, 2021; Parolari *et al.*, 2016). Specific HSPA12B expression was found in vascular cells in endothelium. HSPA12B overexpression was associated with a reduced apoptosis of cardiomyocytes and cardiac angiogenesis improvement. The beneficial effects could be explained by an increase in eNOS expression as its inhibition reduces the effects of the excessive expression of intracellular HSPA12B (Savoia *et al*., 2011). Animal model studies also demonstrated that Geranyl-Geranyl-Acetone (GGA) diminishes myocardial ischemia and reperfusion injury as it increases iHSP70 expression for GGA-positive results. The relationship brought about a hypothesis that eNOS might be considered a stress or heat shock protein (Hsieh *et al*., 2014).

Dysfunction of endothelium is usually accompanied by morphological changes in vascular walls and together with transient episodes of hypertension this may result in blood flow turbulence. Compared to the normal, or laminar blood flow, the turbulent blood flow is characterized by excessive blood velocity (Park and Park, 2015). Under normal conditions, there is eNOS dependent No production induced by the laminar flow. On the contrary, in cardiovascular patients, whose vascular walls are seriously damaged, the turbulent flow brings about shear stress and aggravates the process through an increased O_2 production and oxidative environment (Chen *et al*., 2018).

Aortic impairment is sometimes characterized by the turbulent blood flow and dysfunction of endothelium leading to an increased secretion of Endothelin-1 (ET-1), an atherogenic factor and vasoconstrictor. In these circumstances, iHSP70 acts as an inhibitor of ET-1 and thrombomodulin production. Therefore, decreased expression of iHSP70 is crucial for oxidative and inflammatory processes triggering vascular diseases (Rosa *et al*., 2021).

ROS production, contributing to no neutralization and preventing over-vasodilatation, is very important for endothelial homeostasis. Still, excessive activity of enzymes producing ROS leads to inflammatory response. Inflammatory cytokines in high concentrations stimulate NOX activity, which leads to an increased production of hydrogen peroxide (H_2O_2) and superoxide anion (O_2-). Then comes a reaction between O_2- and NO resulting in a harmful and highly reactive peroxynitrite (ONOO-) that increases oxidative stress and decreases NO bioavailability, eventually promoting atherogenesis (Tarafdar and Pula, 2018).

There exist three Superoxide Dismutase (SOD) isoforms preventing over-neutralization of NO and neutralizing O_2- production. MnSOD, produced in the cytosol, is important as it maintains mitochondria redox homeostasis. Mitochondrial processing peptidases (MPP) cleave MnSOD and move it to the mitochondrial matrix; there it receives an Mnþ$_2$ ion and activates. iHSP70 plays a key role in the process; Afterward, Kinase B (AKT) phosphorylates iHSP70, which induces MnSOD migration from the cytosol to the mitochondrial matrix. Thus, due to iHSP70's ability to mediate MnSOD activation, it can be a protective factor against the dysfunction of the endothelium (Man *et al*., 2019).

iHSP70 is also responsible for NOX activity reduction as it inhibits NFjB or ubiquitinates NOX_2 and NOX5 subunits. It is known that NOX activity and expression can be increased by suppression of iHSP70 expression in VSMC. It may be a possible mechanism of iHSP70 functioning as an antioxidant (Szyller and Bil-Lula, 2021). Thus, by stimulating iHSP70 aortic and renal expression renal atherosclerosis can be ameliorated and leukocyte infiltration significantly reduced by TNF-a and NFjB inhibition. Endothelial function can be notably improved by increasing iHSP70 expression in the aorta via the Sirtuin-1 (SIRT-1)-HSF-1- HSP70 pathway induced by heat shock. Atherosclerosis suppresses this anti-inflammatory pathway, whereas SIRT-1 deacetylates and activates eNOS (Moore *et al*., 2013).

In a positive feedback stage, inflammation and oxidative stress create an imbalance between tissue expression and exportation of HSP70 to the extracellular medium in favor of the latter. Reduced Glutathione (GSH) levels are reduced by excessive ROS production resulting in HSF-1 activation and HSP70 expression and release. Whereas vaso-and cytoprotective functions of iHSP70 are well-known, the role of eHSP70 in atherosclerosis is unclear (Jiang *et al*., 2022). Both eHSP70 and iHSP70 alter in stages that precede vascular diseases and/or occur during them. Neutrophils, monocytes, lymphocytes, as well as oxLDL-exposed endothelial cells export eHSP70 under stress. Secretory vesicles in lymphocytes contain HSP70 which can be released in exocytosis by increasing intracellular Caþ$_2$ or by adrenaline/noradrenaline stimulation. At the same time, SIRT-1 can both increase the expression of iHSP70 and inhibit the release of eHSP70 (Kattoor *et al*., 2019).

Activating NOX, eHSP70 propagates oxidative stress that may lead to oxidation of LDL making it extremely highly atherogenic. Caveolin-1 mediates LDL circulation and migration beyond the endothelial barrier. In sub-endothelium, LDL is oxidized and retained, which increases the synthesis of caveolin and, consequently, inhibits NO production and eNOS activity (Xie *et al*., 2016).

Lectin-like Ox-LDL receptor-1 (LOX-1) recognizes ox-LDL, whereas macrophages phagocytize it, morph into foam cells and start attracting immune cells forming the inflammatory focus. In an inflammatory setting, eHSP70 is also recognized by LOX-1 which contributes to the antigen presentation and apoptosis via Ca_2+-independent pathways. IL-8, IL-1, Monocyte Chemoattractant Protein (MCP-1) and eHSP70, together with caveolin-1 overexpression stimulate excessive expression of adhesive molecules. Caveolin-1 promotes the expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) and controls NFjB activation and TNF-a inflammatory pathway (González-Ramos *et al*., 2016).

eHSP70 influences endothelial cells and monocytes stimulates the synthesis of IL-6 and expression of Intercellular Adhesion Molecule-1 (ICAM-1) and regulates their interaction. *In vitro* research demonstrated that incubation of HSP70 with monocytes (cellular line PBMC) induced the production of IL-6 and endothelial cells (cellular line RAEC) adhesion to monocytes. At that, IL-6 was mediating the mutual adhesion of endothelial cells and leukocytes as it stimulated the expression of ICAM-1 by endothelium. IL-6 promotes adhesion of cells because it reduces NO bioavailability and inhibits eNOS activity (Kowalsky *et al*., 2008).

eHSP70 is a mediator of Transforming Growth Factor-1 (TGF-1) synthesis and release, as it binds Toll-Like Receptor-4 (TLR-4) and activates Activator Protein-1 (AP-1). TGF-1 activates VSMC growth and proliferation of VSMC and stimulates the secretion of fibronectin and collagen which form an extra-cellular matrix. Through binding to LOX-1membrane, eHSP70 induces inflammatory responses releasing IL-1b and IL-6, which leads to increased formation of plaques and vascular lumen reduction (Costa-Beber *et al*., 2022).

In the stages preceding and/or occurring during the progression of atherosclerosis, oxLDL induces an increase of eHSP70 exportation by endothelium. An animal model study of diet-induced disease demonstrated an increase in ox-LDL concentration after two weeks and an increase in eHSP70 levels and monocyte adhesion to endothelial cells during the fourth week. HSP70 induction may be connected with oxLDL cytotoxicity, as detection of HSP70 in atherosclerosis may indicate oxLDL presence. In another similar study, atherosclerotic plaques appeared in animals after four weeks of a high-fat diet, whereas iHSP70 content in the aorta increased only after twelve weeks and HSP70 content in the plaques after 24 weeks (Araujo *et al*., 2019).

These studies demonstrated that just an excess of cholesterol in the bloodstream is not a sufficient factor to modulate the expression and exportation of HSP70, which, however, can be caused by inflammation that follows hypercholesterolemia. An increase in iHSP70 expression brings about a response of endothelium-derived foam cells.

iHSP70 and adhesion molecules (VCAM-1 and VLA-4 (very late antigen-4)) express simultaneously, which suggests that the chaperone's expression and exportation can be an attempt of the cells to ameliorate the inflammation. In animals with hypercholesterolemia and atherosclerosis, translocation of HSP70 to the extracellular membrane face of the aortic endothelium is associated with the concentration of oxLDL (Leng *et al*., 2013). If this is the case, the resulting endothelium-derived HSP70 (esmHSP70– endothelial surface membrane HSP70) can potentially cause inflammation because of antibody reactions. Besides, esmHSP70 is associated with thrombomodulin and inhibits its activity under shear stress, causing atherosclerosis (Wang *et al*., 2021).

Endothelial dysfunction is marked with anti-HSP70 antibodies. They are correlated with the development of plaques in animal models with atherosclerosis, suggesting increased concentration of eHSP70 in the animals. At the same time, the autoimmune reaction to eHSP70 might mediate endothelial injury, which is confirmed by the Von Willebrand factor and might be adverse in different circumstances (Liu *et al*., 2015). That is why eHSP70 is supposed to induce atherosclerosis and not just mark dysfunction or disease. It was found that endothelial cells export HSP70 through exosomes to the extracellular medium and the process aggravates when treated with ox-LDL compared to basal conditions. In chronic kidney patients, eHSP70 is associated with atherosclerosis progression and general worsening. eHSP60 and eHSP70 react with peripheral blood-isolated lymphocytes T CD4þCD28null, which increases the expression of interferon-gamma (INF-c) (Dabravolski *et al*., 2022).

Patients with severe calcification of the arteries demonstrated eHSP70 in high concentrations which is a risk factor for the development of acute coronary syndrome and arterial calcification. While iHSP70 is considered to be a vasoprotector, there is conflicting evidence as regards eHSP70 function in the endothelium. In a study comparing healthy subjects and cerebrovascular atherosclerotic patients, lower eHSP70 values were revealed in patients with atherosclerotic lesions, which suggests that eHSP70 may also play a protective role against atherosclerosis development (Collins *et al*., 2013).

The contradictory findings surrounding the role of Hsp70 in atherosclerosis reflect the complexity of this heat shock protein's functions within the context of the

disease. Understanding and reconciling these contradictions are essential for elucidating the precise mechanisms through which Hsp70 influences atherosclerosis progression.

Pro-atherogenic role of Hsp70: Studies suggesting a pro-atherogenic role of Hsp70 point to its upregulation in atherosclerotic plaques, particularly in macrophages and smooth muscle cells. The increased expression of Hsp70 in these cells has been associated with plaque progression and destabilization. This upregulation may contribute to the promotion of inflammatory responses, cholesterol trafficking abnormalities and foam cell formation, all of which are key processes in atherosclerosis development. The pro-atherogenic effects of Hsp70 may involve the facilitation of plaque vulnerability and inflammation, leading to the progression of atherosclerosis.

Anti-atherogenic role of Hsp70: Conversely, contrasting evidence indicates a potential anti-atherogenic role of Hsp70 in atherosclerosis. Hsp70 has been found to inhibit apoptosis and inflammation in vascular cells, thereby promoting cell survival and maintaining plaque stability. Additionally, Hsp70 is involved in the cellular stress response, aiding in protein folding and preventing protein aggregation. Experimental studies demonstrating the overexpression of Hsp70 have shown attenuation of atherosclerotic lesion development and reduction of plaque burden. These observations suggest that Hsp70 possesses protective, anti-atherogenic properties that may help mitigate atherosclerosis progression.

Reconciling contradictory findings: Resolving the discrepancies surrounding the role of Hsp70 in atherosclerosis requires a nuanced approach, taking into account various factors that may influence its functional outcomes. Factors such as the specific cellular context, the stage of plaque development and the interplay with other molecular pathways can impact the divergent roles of Hsp70 observed in different studies.

Future directions for research: To reconcile the conflicting findings, future research could focus on utilizing more standardized experimental approaches that consider the diverse stages of atherosclerosis and analyze the effects of Hsp70 in a cell-specific manner. Employing genetically modified animal models and advanced imaging techniques may provide deeper insights into the role of Hsp70 in atherosclerosis progression. Additionally, comprehensive molecular profiling and functional studies are necessary to unravel the intricate mechanisms by which Hsp70 influences atherosclerosis pathogenesis.

In conclusion, understanding the contrasting roles of Hsp70 in atherosclerosis necessitates meticulous investigation and interpretation of the existing evidence. By addressing these contradictions, researchers can gain a more comprehensive understanding of how Hsp70 impacts atherosclerosis and pave the way for potential therapeutic interventions targeting this heat shock protein.

HSP90

The majority of research is devoted to the role of HSP90 in cancer. Some studies have found out that excessive HSP90 expression in atherosclerosis is associated with plaque instability. HSP90 inhibition reduces oxidative stress and inflammation due to the reduction of transcription factors activation (activators of transcription and NFκB and signal transducers) and pro-inflammatory cytokines suppression (Collins *et al*., 2013). Notably, the favorable effect of HSP90 inhibition is associated with excessive HSP70 expression and is considered to contribute to the general atheroprotective and anti-inflammatory effect of suppressing the activation of HSP90 (Sainger *et al*., 2012).

Discussion

Comparing the evidence across different HSP families in relation to their pro- and anti-atherogenic mechanisms, it is apparent that HSP60 exhibits direct atherogenic functions, while HSP27 shows potential anti-atherosclerotic effects. HSP60, found in atherosclerotic plaques, stimulates an autoimmune response with T-cell activation and antibody production. Its epitopes have been identified in advanced and early lesions and it plays a role in local inflammation, arterial wall remodeling and plaque severity. On the other hand, HSP27 is found in lower amounts in atherosclerotic plaques and decreased levels of plasma HSP27 are associated with atherosclerosis. HSP27 helps maintain plaque stability by protecting against VSMC apoptosis and fibrous cap thinning. It also correlates with lipid homeostasis, reduces plaque area, prevents macrophage accumulation and stimulates cholesterol efflux. Additionally, HSP27 inhibits apoptosis via the p38/PI3K/AKT pathway. Thus, while HSP60 is associated with a pro-atherogenic role, HSP27 exhibits potential anti-atherosclerotic mechanisms.

Discussing the contradictory evidence regarding the role of Hsp70 in atherogenesis can indeed shed light on the complexity of its functions in this context. Though it is implicated in both pro-atherogenic and anti-atherogenic mechanisms, reconciling these conflicting findings requires careful analysis.

On one hand, studies have suggested that Hsp70 exerts a pro-atherogenic effect. It has been observed that Hsp70 expression is upregulated in atherosclerotic plaques, particularly in macrophages and smooth muscle cells. This upregulation has been linked to plaque progression and destabilization. Hsp70's pro-atherogenic potential is thought to involve the promotion of inflammatory responses, cholesterol trafficking and foam cell formation. These findings suggest that Hsp70 may contribute to atherosclerosis development and plaque vulnerability.

On the other hand, contrasting evidence indicates that Hsp70 may have protective effects against

atherosclerosis. Hsp70 has been found to inhibit apoptosis and inflammation in vascular cells, promoting cell survival and maintaining plaque stability. It also participates in the cellular response to stress, facilitating protein folding and preventing protein aggregation. In experimental studies, overexpression of Hsp70 has been shown to attenuate atherosclerotic lesion formation and reduce plaque burden. These observations suggest that Hsp70 possesses anti-atherogenic properties.

Resolving these contradictions is crucial to gaining a comprehensive understanding of Hsp70's role in atherogenesis. Further research is needed to investigate and reconcile the divergent findings. Factors such as the specific cellular context, the stage of plaque development and the interplay with other molecular pathways may influence Hsp70's functional outcomes.

To address these contradictions, future studies could employ more standardized experimental approaches, considering the various stages of atherosclerosis and analyzing Hsp70's effects in a cell-specific manner. Utilizing genetically modified animal models and advanced imaging techniques could provide additional insights. Additionally, comprehensive molecular profiling and functional studies are required to unravel the intricate mechanisms through which Hsp70 acts in atherosclerosis.

Taking into account the contradictory findings surrounding Hsp70's role in atherosclerosis, it is essential to approach the subject with an open mind, acknowledging the complexity of the disease and the intricacies of protein function. By combining robust scientific methods, considering multiple factors and collaboratively analyzing the data gathered, researchers can work towards resolving these contradictions and gaining a clearer understanding of Hsp70's contributions to atherosclerosis.

The reliance on rodent models in research investigating Heat Shock Proteins (HSPs) and atherosclerosis is a notable aspect that warrants further discussion due to its implications for translating findings to human cardiovascular health. While rodent models have provided valuable insights into the roles of HSPs in atherosclerosis, several limitations and considerations should be taken into account when interpreting and extrapolating data from these studies.

Advantages of rodent models in HSP research:

1. Genetic manipulation: Rodent models allow researchers to manipulate specific genes encoding HSPs or their regulatory factors, enabling the study of their precise functions in atherosclerosis. This genetic control provides insights into the causal relationships between HSP expression levels and disease phenotypes, aiding in the identification of therapeutic targets
2. Experimental control: Rodent models afford researchers a high level of experimental control over environmental factors, diet and other variables influencing atherosclerosis development. This control enhances the reproducibility and reliability of study outcomes, facilitating the identification of molecular mechanisms underlying HSP-mediated effects on disease progression
3. Feasibility of longitudinal studies: Rodent models offer the opportunity to conduct longitudinal studies tracking disease progression over time, which is essential for investigating the dynamic changes in atherosclerotic lesions and understanding the impact of HSP interventions at different stages of disease development

Limitations of rodent models in HSP research:

1. Species-specific differences: Rodents and humans exhibit significant differences in cardiovascular physiology, lipid metabolism and immune responses. These interspecies variations may limit the translatability of findings from rodent models to human atherosclerosis, emphasizing the need for caution when extrapolating preclinical data to clinical applications
2. Atherosclerosis models: While rodent models have been instrumental in studying atherosclerosis, they often fail to fully recapitulate the complexity and heterogeneous nature of human atherosclerotic lesions. The simplification of disease pathophysiology in these models may overlook critical aspects relevant to human disease progression, raising questions about the generalizability of findings
3. Therapeutic efficacy: The efficacy of HSP-targeted therapies tested in rodent models may not always translate to human clinical settings due to species-specific drug responses and pharmacokinetic differences. The extrapolation of treatment outcomes from rodent studies to human patients requires rigorous validation in translational research pipelines
4. Ethical and practical considerations: The use of rodent models in research raises ethical considerations related to animal welfare and resource allocation. Researchers must adhere to stringent ethical guidelines and ensure that animal studies are justified and conducted in a manner that minimizes harm to the subjects

When interpreting the findings of studies that rely heavily on *in vitro* experiments and rodent models, it is crucial to consider the limitations of extrapolating these results to human atherosclerosis. While these models provide valuable insights, there are several inherent differences and complexities that must be taken into account.

Firstly, *in vitro* studies often utilize simplified systems that fail to capture the intricate cellular and molecular interactions present in the human body. Human atherosclerosis is a multifaceted disease influenced by various factors, including genetics, environmental factors and complex cell-to-cell communication. Therefore, the direct translation of observations made in isolated cells or cell lines to the complex human system may oversimplify the disease process.

Secondly, rodent models, although widely used in atherosclerosis research, differ from humans in important aspects. There are inherent biological variations between species, including disparities in the metabolism of lipids, immune response and plaque composition. These discrepancies can limit the direct relevance and translatability of rodent findings to human pathophysiology.

Additionally, it is important to consider the variations in atherosclerotic plaque progression and composition between humans and rodents. Differences may arise in terms of the dominant cell types involved, the distribution and characteristics of lipid deposition and the dynamics of plaque rupture. These dissimilarities can impact the interpretation and generalization of findings obtained from rodent models to human atherosclerosis.

Moreover, atherosclerosis is a chronic and progressive disease that develops over many years in humans, whereas experimental animal models often induce accelerated and exaggerated disease progression within a relatively short period. This discrepancy in the timeline and disease progression dynamics may hinder the direct translation of experimental observations to the clinical setting.

To overcome these limitations and improve the translational value of preclinical studies, researchers can consider utilizing more sophisticated animal models that better mimic human atherosclerosis and include larger and more diverse cohorts. Furthermore, conducting comparative studies involving both animal models and human samples can provide valuable insights into the similarities and differences in disease mechanisms.

In conclusion, while in vitro studies and rodent models contribute significantly to our understanding of atherosclerosis, the limitations of extrapolating these findings to human disease should be acknowledged. Careful consideration of these limitations and the application of complementary approaches, such as human-based research and improved animal models, can enhance the translational value of preclinical studies and help bridge the gap between experimental findings and human atherosclerosis.

In conclusion, while rodent models have been invaluable tools for studying the roles of HSPs in atherosclerosis, their limitations underscore the need for complementary approaches, including human tissue studies, *in vitro* models and clinical investigations. Integrating data from diverse sources can enhance our understanding of HSP functions in cardiovascular disease and facilitate the development of more effective diagnostic and therapeutic strategies tailored to human patients. Researchers should be mindful of the intricacies of rodent models and the challenges associated with translating preclinical findings to clinical applications in the context of HSP research in atherosclerosis.

The implications of Heat Shock Proteins (HSPs) in the clinical and therapeutic context of atherosclerosis are significant. HSPs are a group of protective proteins that aid cells in responding to stress. Emerging research suggests that they hold potential as therapeutic targets for atherosclerosis.

One important implication is their potential as biomarkers. HSPs can be utilized as indicators to assess the severity and progression of atherosclerosis. By monitoring the levels of specific HSPs in blood or tissues, valuable information about the disease's activity and response to treatment can be obtained.

HSPs may also play a role in the diagnosis of atherosclerosis. Detecting specific HSPs, such as HSP60 or HSP70, in blood samples or vulnerable plaques can aid in identifying individuals at risk or facilitating early disease detection.

Therapeutically targeting HSPs has shown promise in preclinical studies. Methods such as enhancing HSP expression or activity, using HSP-derived peptides or proteins as therapy, or stimulating the endogenous HSP system have the potential to provide protective effects against atherosclerosis. Additionally, delivering exogenous HSPs through targeted drug delivery systems can be explored.

HSPs possess immunomodulatory properties that influence the immune response associated with atherosclerosis progression. They can regulate the activity of immune cells and promote anti-inflammatory effects, potentially reducing plaque formation and stabilizing existing plaques.

Furthermore, HSPs have been associated with stabilized atherosclerotic plaques. Increasing HSP expression in vulnerable plaques may enhance plaque stability, thereby preventing rupture and subsequent cardiovascular events.

Understanding the role of HSPs in atherosclerosis has implications for the development and evaluation of therapeutic interventions. HSPs can serve as targets for novel drugs and as markers to assess treatment efficacy and patient response.

It is important to note that while preliminary evidence indicates the potential of HSPs in the context of atherosclerosis, further research, including clinical trials, is necessary to establish their therapeutic value and optimize their usage in clinical practice.

Conclusion

In conclusion, this review has shed light on the involvement of various heat shock proteins in the pathogenesis of atherosclerosis. Among these proteins, Hsp60 has demonstrated clear anti-atherosclerotic properties both in laboratory and clinical settings. On the other hand, the role of other heat shock proteins remains less defined:

1. Overlapping findings: Immunomodulatory role: Various Heat Shock Protein (HSP) families, including Hsp27, Hsp70 and Hsp90, exhibit immunomodulatory properties in atherosclerosis. They regulate immune responses and can impact inflammation associated with disease progression. Despite differences in their specific mechanisms, all HSP families contribute to immune regulation within the atherosclerotic plaque microenvironment
2. Protective effects: Different HSP families, such as Hsp27 and Hsp70, demonstrate protective effects against key processes in atherosclerosis, including apoptosis, inflammation and plaque stability. By inhibiting apoptotic pathways, reducing inflammatory responses and aiding in the preservation of plaque integrity, these HSPs play crucial roles in mitigating atherosclerosis progression
3. Contradictory findings: Dual roles in atherosclerosis progression: While some HSP families exhibit predominantly anti-atherogenic effects, such as Hsp27, others like Hsp70 may display both pro- and anti-atherogenic properties. This dual nature of Hsp70 can contribute to conflicting findings regarding its overall impact on atherosclerosis development, showcasing the complexity of heat shock protein functions in the disease
4. Influence on plaque stability: Hsp27 is associated with maintaining plaque stability by inhibiting VSMC apoptosis and reducing fibrous cap thinning. In contrast, Hsp90 inhibition is linked to enhanced plaque instability due to its effects on oxidative stress and inflammation. These contradictory effects on plaque stability highlight the divergent roles of different HSP families in atherosclerosis progression
5. Relationship to inflammation: While Hsp70 has been implicated in promoting inflammation and plaque progression through its upregulation in atherosclerotic plaques, Hsp27 and other HSP families may exert anti-inflammatory effects that contribute to plaque stabilization. These conflicting roles in modulating inflammation underscore the need for further research to elucidate the precise mechanisms through which HSPs influence immune responses in atherosclerosis
6. Diagnostic and therapeutic potential: The utility of HSPs as biomarkers and therapeutic targets in atherosclerosis may vary between different families. While Hsp27 shows potential as a diagnostic marker and therapeutic agent, conflicting evidence regarding the roles of Hsp70 and Hsp90 in disease progression complicates their translational applications. Resolving these contradictions is crucial for harnessing the full diagnostic and therapeutic potential of HSPs in managing atherosclerosis

Overall, the overlapping and contradictory findings between different HSP families in atherosclerosis underscore the intricate and multifaceted nature of these proteins' roles in the disease. Further research is needed to clarify the specific contributions of each HSP family to atherosclerosis pathogenesis, paving the way for more targeted diagnostic and therapeutic strategies tailored to modulating heat shock protein functions in the context of cardiovascular health.

Notably, Hsp27 has shown potential as an anti-atherogenic protein through its influence on lipid homeostasis and inflammation, as supported by several studies. The contrasting functions of the two isoforms of Hsp70, iHsp70 and eHsp70, add complexity to understanding their roles in atherosclerosis. While iHsp70 is considered protective for blood vessels, the function of eHsp70 in the endothelium remains a topic of debate. Conversely, overexpression of Hsp90 has been linked to plaque instability, highlighting its association with disease progression.

Overall, the involvement of heat shock proteins in key processes of atherosclerosis is evident, presenting valuable opportunities for the development of therapeutic and diagnostic strategies. Further research into the precise mechanisms by which these proteins influence atherosclerosis will be instrumental in advancing our understanding and potentially identifying novel targets for intervention.

In summary, targeting heat shock proteins holds significant clinical and therapeutic implications for the management of atherosclerosis. By harnessing the anti-atherogenic potential of specific heat shock proteins like Hsp60 and Hsp27, it may be possible to develop innovative approaches to prevent, diagnose and treat this prevalent cardiovascular disease. These findings underscore the importance of continued research in this field and reinforce the potential of heat shock proteins as valuable targets for improving patient outcomes in atherosclerosis.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the

resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the effort.

Funding Information

This research was funded by the Russian Science Foundation, grant number 23-65-10014.

Author's Contributions

Anastasia Vladimirovna Poznyak: Draft preparation. **Anastasia Vladimirovna Poznyak, Alexey V. Churov, Vasily Nikolaevich Sukhorukov, Alexandra Alexandrovna Melnichenko, Tatiana Ivanovna Kovyanova and Alexander Nikolaevich Orekhov:** Draft review and editing.

Ethics

This article is original and contains unpublished material. The corresponding author certifies that all co-authors have read and approved the manuscript, and confirms that there are no ethical issues involved.

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