Review

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Review

Heat Shock Protein 60 as an Immunomodulator in Heart Diseases

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Abstract: The Characteristics of the cardiac muscle cell are described as a rod-shaped structure, single nucleus, and intercalated discs which make the heart's action practical for enhancing contraction efficiency. Mitochondria within these cells are vital for ATP synthesis, redox balance, calcium homeostasis, and lipid synthesis. The crucial role of Hsp60 is in protein folding and immune responses; Hsp60 is a mitochondrial chaperonin, and its expression rises when the heart is under a stress condition like myocardial infarction and heart failure. This review has tried to deeply understand the structural resemblance between cardiac and skeletal muscle cells, the amplitude number of mitochondria, its roles in cardiomyocytes, and the polyhedral functions of Hsp60. In this review, by investigating various articles, we brighten up Hsp60's distribution within cells, its association with apoptosis, and its action as a Damage Associated Molecular Pattern (DAMP), interacting with Toll-like receptors to modulate immune responses. Cardiac remodeling and failure are linked to cardiac-specific micro-RNAs whose expression may be affected by dysregulation of Hsp60. In conclusion, targeting mitochondrial function and Hsp60 expression may be of therapeutic potential for heart diseases.

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1. Introduction

The ability of mitochondria to generate large amounts of ATP from the breakdown of glucose by oxidative phosphorylation (OXPHOS) is the role that is typically given to them. The mitochondrial electron transport chain (ETC), which is made up of organic molecules and protein complexes, facilitates OXPHOS-dependent ATP production by transferring electrons to molecular oxygen and establishing the electrochemical gradient required for ATP synthase activity [1].

Apart from their functions in generating energy and building blocks, mitochondria also serve as signaling organelles that control vital biological processes including cell division, growth, and death, in addition to maintaining Ca2+ and redox balance. Thus, mitochondria play a crucial role in both physiological and pathological processes such as inflammation, cancer, and tissue repair [2–5]. In order to produce ATP from a variety of accessible substrates and respond to external stimuli, mitochondria must convert various energy sources into a steady flow of energy. This substrate flexibility allows for quick variations in workload, such those seen during exercise, as well as changes in the environment, including the amount of oxygen and nutrients in the blood. The preferred substrate is fatty acids, which give 60–90% of the energy needed by the myocardium [6].

In addition to being necessary for the differentiation and maturation of cardiomyocytes, mitochondria also undergo maturation. In the developing heart, they gain bulk, undergo structural development, and become functionally specialized [7]. In a study which scientist performed, they found that Hsp60 expression is strongly associated with heat-stressed Hsp60 is found in unstressed

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cells in the heart, and liver, mainly in the cytoplasm of myocardial fibers, hepatocytes, and renal tubular epithelial cells. In an experiment in the study, scientists figured out that ELISA levels were detected, and the induction of myocardial Hsp60 was accompanied by upregulation in response to high temperatures. This suggests Hsp60's importance in myocardial cell protection [8]. Not only are mitochondria necessary for the differentiation and maturation of cardiomyocytes, but they also undergo maturation of their own, gaining bulk, changing architecturally, and becoming functionally specialized in the developing heart [9,10].

The objective of this review is to investigate the complex interrelationships among the structure and function of cardiac muscle cells, the critical functions of mitochondria, and the growing importance of Hsp60 in heart health and illness.

2. Structure of the Cardiac Muscle Cell and Role of Mitochondria

The cardiac muscle cell shares similar characteristics with the skeletal muscle fiber cell in both structure and function. The cardiac muscle cell has a single nucleus, is rod-shaped, and is connected to other cells through intercalated discs, which serve as crucial structural connections. These protein discs enhance the effectiveness of cardiac contraction by aligning the cells with each other, thus creating the force lines of the cardiac wall. The cardiac cell contains myofibrils that are structurally like those in the skeletal muscle cell, though the proteins, despite having similar structures, have different amino acid sequences, allowing antibodies to distinguish between cardiac and skeletal proteins. The names of the sarcomere proteins are the same, the structure observed under the electron microscope is the same, and mitochondria are abundant in both types of cells.

Mitochondria in cardiomyocytes are in a space parallel to the myofibrils. Cardiomyocytes contain about 5,000 to 8,000 mitochondria per cell [11]. More than 95% of the ATP in the myocardium is synthesized by mitochondria. Additionally, mitochondria play important roles in regulating redox status, calcium homeostasis, and lipid synthesis [12].

Mitochondria transform fatty acids taken from the bloodstream into ATP, providing 60-90% of the myocardium's energy supply. They also counteract the accumulation of reactive oxygen species (ROS) through detoxification proteins and enzymes, such as the mitochondrial antioxidant manganese superoxide dismutase (MnSOD, SOD2) [13]. Many biochemical processes and pathways in mitochondria require high levels of calcium (Ca^{2+}) and other ions like Na^{+}, H^{+}, and K^{+}. Therefore, the mitochondrial membranes are rich in ion channels selective for these ions, which help preserve mitochondrial membrane potential [14].

Mitochondria also play a crucial role in lipid homeostasis. While most lipids are synthesized in the endoplasmic reticulum (ER), some components are synthesized in the inner mitochondrial membrane (IMM). One of the most important components of the IMM is cardiolipin, an abundant phospholipid first isolated from animal hearts, which constitutes about 20% of the total lipid composition of the IMM [15].

Given the abundance of mitochondria in cardiomyocytes and their important roles in energy supply and lipid homeostasis, it is not surprising that mitochondrial dysfunction is strongly linked to the development of cardiomyopathy and an increased risk of heart failure [16].

3. Hsp60 Expression and Localization in the Healthy Heart Tissue

The localization of heat shock proteins in cardiac tissue, such as Hsp60 and the associated Hsp10, is likely very similar to their localization in other tissues (Figure 1). Hsp60 has been found inside mitochondria, in the cytoplasm of cardiac cells [17], on the outer membrane, and within exosomes [18]—small vesicles released by cardiomyocytes—and finally in the blood [19]. In its cytosolic localization, Hsp60 is frequently associated with Bax and apoptosis. The expression levels of Hsp60 are typically so low that researchers have used heat shock to detect the localization of this protein. For example, in studies involving rats exposed to 42±1°C for 0 (control), 20, 80, and 100 minutes, researchers detected high levels of both Hsp60 and Hsp10 after 100 minutes, exhibiting a punctate distribution typical of mitochondrial localization [20].

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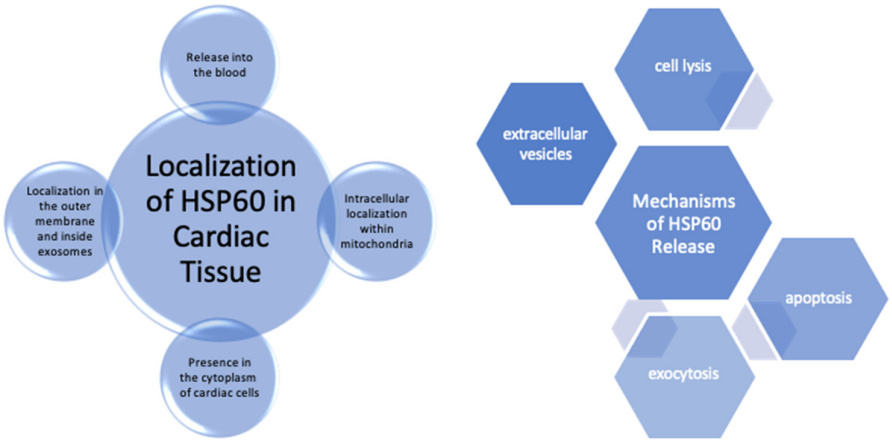

Figure 1. Localization and release of Hsp60.

The mechanism through which Hsp60 is released into the extracellular space, either inside extracellular vesicles or freely in the interstitial space, is not well understood and is widely debated. Hsp60 may be released as a free protein because of cell lysis or apoptosis, but transport mediated by exocytosis cannot be excluded. Recently, we demonstrated the release of Hsp60 inside small and large vesicles by immortalized muscle cell lines (C2C12 mouse myoblasts). We also showed that induced expression of high levels of Hsp60 can enhance its localization inside extracellular vesicles [21]. Immunolocalization studies using Transmission Electron Microscopy revealed either a sub-membrane or an intramembrane localization. In both cases, overexpression of Hsp60 increased its presence in extracellular vesicles, with higher levels inside large vesicles compared to small extracellular vesicles. Although we did not use cardiomyocytes, we can hypothesize that overexpression of Hsp60 using the same expression plasmid would induce a similar effect in isolated cardiomyocytes, leading to the induced and controlled release of Hsp60. The induced release of the protein inside extracellular vesicles or freely in the medium may explain the role of extracellular Hsp60 as an immune system modulator.

Hsp60 may play both pro-inflammatory and anti-inflammatory roles depending on its interactions with cell-surface receptors, including Toll-like receptors (TLRs). It may also bind to other proteins during an immune response to assist in their presentation to lymphocytes [22].

Human HSP60, acting as a Damage Associated Molecular Pattern (DAMP), elicits a rapid release of nitric oxide (NO), TNF-α, interleukin (IL)-1β, IL-6, IL-12, and IL-15 from macrophages. It also can upregulate costimulatory molecules of major histocompatibility complex class I (MHC-I) and II (MHC-II), CD86, and CD40, promoting the maturation of dendritic cells (DCs) and enhancing the antigen-presenting capacity of antigen-presenting cells (APCs) [23].

Recently, it has been suggested that Hsp60 may act as a ligand for TLR 2 and 4, modulating the immune response and inducing the release of TNF-α, IL-6, and IL-8 [24]. Activation of TLR-4 and the NF-κB pathway in injured cardiac tissue leads to apoptosis and impaired contractility of the cardiac tissue [25].

4. HSP60 and Cardiac Diseases

Cardiac diseases include unstable angina, heart attack, heart failure, arrhythmia (abnormal heart rhythms), valve disease, high blood pressure, congenital heart conditions, and inherited heart conditions. While there are no published papers on the expression and role of Hsp60 in unstable angina, there are a few studies on the role of this protein in heart attacks, heart failure and myocardial infarction (MI). Figure 2 is showing the expression levels of Hsp60 in cardiac diseases.

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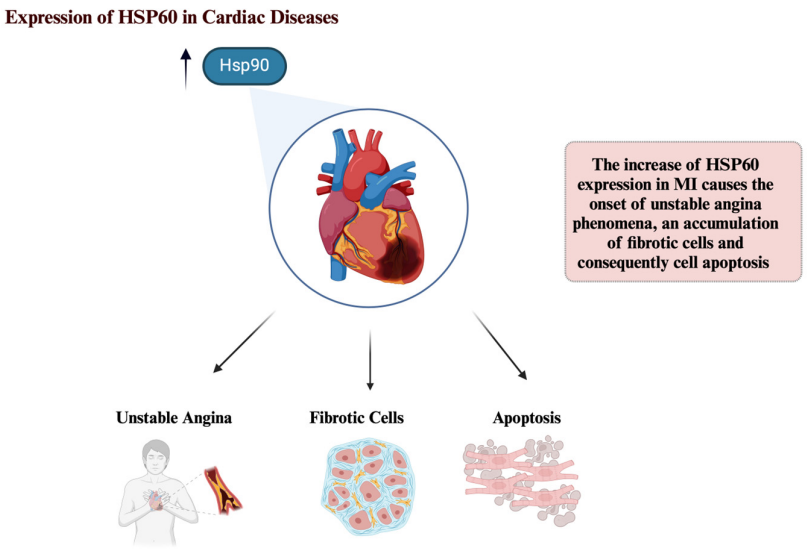

Figure 2. Expression of HSP60 in Cardiac Disease.

Hsp60 has been identified as one of the markers of MI, as demonstrated in both human and animal models. There are many papers demonstrating the over-expression and release of Hsp60 in the blood stream of animals and patients with signs of heart failure or coronary heart disease. For example, in a study on a population of Chinese individuals, it was shown that Hsp60 levels increase both on the first day and seven days after MI [26]. While in animal studies, intracellular overexpression and release of Hsp60 into the interstitial space of cardiac tissue were observed in C57BL/6 mice with induced MI [27]. The increase in Hsp60 was supposed to be related to apoptosis.

However, recent studies have identified Hsp60 as a ligand for TLR-2 and TLR-4, capable of modulating the immune response when released into the extracellular space. The expression of Hsp60 is not merely a reaction to a hypoxic environment but is finely regulated by cardiac-related micro-RNAs. In an animal model of MI in rats, researchers demonstrated the overexpression of miR-1 after the induction of MI and the simultaneous downregulation of Hsp60. Carvedilol, a nonselective β-adrenergic receptor (β-AR) antagonist, was shown to reduce miR-1 expression and restore Hsp60 levels in response to tissue damage [28]. The negative regulation of miR-1 on Hsp60 expression was previously demonstrated in a 2012 study on a mouse model of cardiac ischemia-reperfusion injury.

In atrial fibrillation (AF), an age-related disease associated with congestive heart failure and cardiovascular disease, there is a documented depletion of HSPs. It has been established that AF-induced proteostasis derailment and consequent electropathological remodeling are caused by abnormalities in the heat shock response. In two other studies, the mitochondrial heat shock proteins HSP60 and HSP10 were found to be overexpressed in atrial tissue from AF patients [29].

Increased blood levels of HSP60 can cause reactions in several organs and distant cells, such as endothelial cells and other components of blood vessel walls and heart muscle [20]. HSP60 is a known risk factor due to its strong correlation with the development and progression of atherosclerosis. However, ample data indicates that HSP60 is also important in the later stages of cardiac illness development [30].

The cardiovascular system's endothelium may operate less effectively, and heart muscle injury may result from the potential of HSP90 and HSP60 to increase angiotensin-2 function [31]. HSP60 plays a role in the development of atherosclerosis and heart failure. In patients with heart failure, it has been observed to migrate to the surface of the heart muscle before being released into the bloodstream [32]. Elevated levels of HSP60 were found to be correlated with increased apoptosis and worsening heart failure [33].

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A study found that under stressful circumstances, adult cardiac myocytes release ubiquitinated HSP60 inside exosomes [18], however there is no evidence that HSP60 is then released by exosomes. Another study suggests that HSP60 seems to remain unchanged in exosomes released under various conditions [34]

The heart's chambers have different protein compositions, particularly regarding HSP60 profile. Research indicates that the upper and lower sections of the left and right ventricles differ in protein composition. In one study, the upper left ventricle (LV) showed a significant increase in five proteins, including HSP60. This finding has significant implications for diseases such as acute myocardial infarction (AMI), as this region is most affected by ischemic injury. However, the base-apical protein profile of the right ventricle did not show such an increase in the levels of HSP60 [35].

A study investigated the effects of recurrent exposure to high temperatures through quick hot water baths as a mean of reducing total blood pressure, minimizing heart remodeling, and enhancing mechanical performance in patients with hypertension-induced cardiac hypertrophy. HSPs, including HSP90, HSP70, and HSP60, were measured in LV tissue samples due to their sensitivity to elevated temperatures, which lead to increased expression [36]. Some studies suggest a connection between variations in atrial myolysis during distinct stages of atrial fibrillation and changes in HSP60 expression [37]. Trandopril was shown to have benefits in left ventricular dysfunction following acute myocardial infarction in an animal experiment by preventing the reduction in mitochondrial activity, lowering reactive oxygen species formation, and altering HSP60 synthesis [38].

Cardiovascular diseases (CVDs) result from various stressors that damage the heart's structure and function by affecting cardiac tissues, especially the myocardium. A major factor in the progressive reduction of oxygen and nutrition supply to the myocardium is plaque accumulation in the coronary arteries. Furthermore, localized inflammation plays a significant role in ongoing detrimental processes, including resident cell release of cytokines that trigger inflammatory reactions and attract immune cells to affected areas. In these settings, recurrent tissue damage and the initiation of programmed cell death are common outcomes that lead to organ dysfunction if the initial stressor is not removed [39].

Researchers found that the absence of HSP60 caused the heart chambers to enlarge, and the left ventricle performed less effectively in another study. Early mortality and the lung-to-body weight ratio both significantly increased concomitantly. According to their research, the loss of HSP60 in mature cardiomyocytes leads to dilated cardiomyopathy, which in turn causes heart failure and deadly consequences [40]. In mice, HSP60 is essential for maintaining normal cardiac function and structure. The absence of HSP60 in mature cardiac muscle cells causes dilated cardiomyopathy (DCM) and heart failure by disrupting the equilibrium of mitochondrial proteins and impairing mitochondrial function [40].

In a study by Knowlton et al., the levels of several HSPs in patients with ischemic cardiomyopathy, DCM, and a control group were examined. According to the study, DCM patients had twice the levels of HSP27 and HSP60 compared to the control group [41]. In another study, Niizeki T et al. discovered a relationship between HSP60 levels and the prognosis and severity of congestive heart failure in 112 patients. Additionally, they observed that elevated HSP60 levels were associated with an increased risk of progressive heart failure [42].

According to a study by T. Niizeki and colleagues, individuals with Chronic Heart Failure (CHF) had significantly higher levels of HSP60 than those in a control group. The study also found that, compared to the control group, patients with CHF had higher serum levels of HSP60, with this increase being even more pronounced in patients with a higher New York Heart Association (NYHA) functional class. This increase was particularly notable for patients with severe CHF assigned to NYHA functional class IV [42].

In a study, scientists measured the amounts of HSP90, HSP60, and HSF-1 mRNA in the tissues of the heart, brain, and muscle on days 12, 14, 16, and 18. Compared to the control group, thermal modification resulted in a significant increase in HSF-1, HSP60, and HSP90 expression. While peak expressions in the heart occurred on embryonic days (ED) 12 and 16, the highest levels of HSP60 were

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found in muscle tissue on ED 12 and 18. The strongest expressions in the brain were observed on EDs 14 and 16 [43].

HSP60 affects both innate and adaptive immune responses and is essential for immune system regulation. It induces apoptosis to promote cell death when its cellular location is disturbed in patients with cardiovascular diseases. On the other hand, exogenous HSP60 stimulates the immune system, leading to a systemic inflammatory state marked by increased production of TNF-α and other inflammatory mediators, which further accelerates the progression of heart failure [30]. These hypothesis are shown in Figure 3.

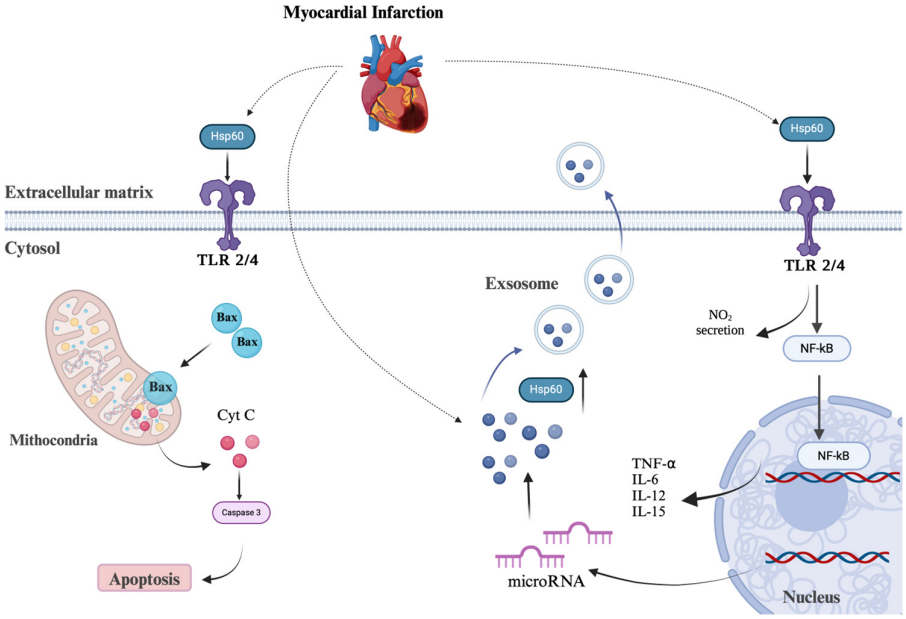

Figure 3. This diagram is showing how Hsp60 may play an important role in cardiovascular disease such as myocardial infarction.

5. Conclusions

Heart muscle cells have a complex structure and their function is strictly dependent on the crucial role of mitochondria. The role of mitochondria in generating ATP is inevitably for cardiomyocytes to perform their energy-requirement functions along with redox balance, calcium homeostasis, and lipid synthesis. They are so abundant in the cardiac cells that they appear as lines of mitochondria all around the myofibrils. The abundancy of mitochondria is important to preserve energy requirements and structural integrity of the heart tissue. As an outcome, mitochondria health is necessary to avoid cardiac disorders, while malfunction and dysfunction play an important role in commencing of cardiomyopathy and heart failure.

In the context of cardiac physiology and disease, the role of Hsp60 becomes more crucial. It has a wide variety of functions: from the role in the process of protein folding to setting and establishing immunological responses. The localization of Hsp60 varies inside the cell: known is its distribution inside mitochondria, in the cytoplasm, and inside extracellular vesicles [21].

The expression of this chaperonin seems to be closely related to heart failure, especially in response to cardiac stresses. Some of heart diseases like heart failure, myocardial infarction, and other cardiac disorders show an increase in the levels of Hsp60, suggesting a role of Hsp60 as a biomarker for stress and injury also in the heart.

Hsp60's simultaneous involvement in immune regulation and apoptosis adds another level of complexity to its function in heart disorders. Hsp60's participation in the inflammatory response after cardiac injury is highlighted by its interaction with TLRs and its function as a DAMP. The importance

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of Hsp60 in heart homeostasis is pointed up in the fact that heart tissue specific micro-RNAs may influence its expression, highly linked to heart failure and cardiac remodeling.

Future studies need to focus on the therapeutic possibilities of influencing mitochondrial function and Hsp60 to alleviate heart disease. Understanding the precise processes underlying the release of Hsp60 and its interaction with the cardiac milieu could facilitate the development of modern interventions to maintain heart health and prevent heart attacks. Furthermore, exploring the regulatory movements that interact with Hsp60 and mitochondrial dynamics may provide us with a new approach for the development of cardioprotective interventions.

In short, the link between mitochondrial activity, cardiomyocyte morphology and Hsp60 is described as a complex network necessary for the maintenance of cardiac health. By expanding our knowledge and understanding of the various functions and processes implied in this article, as well as the fundamental role that disruption in this network plays in heart disease, we may be able to effectively treat heart disorders.

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Abbreviations

Oxidative phosphorylation	OXPHOS
Electron transport chain	ETC
Mitochondrial antioxidant manganese superoxide dismutase	MnSOD, SOD2
Endoplasmic reticulum	ER
Inner mitochondrial membrane	IMM
Toll-like receptors	TLRs
Damage Associated Molecular Pattern	DAMP
Nitric oxide	NO
Interleukin	IL
Major histocompatibility complex	MHC
Antigen-presenting cells	APCs
Myocardial infarction	MI
Atrial fibrillation	AF
Left ventricle	LV
Acute myocardial infarction	AMI
Cardiovascular diseases	CVDs
Dilated cardiomyopathy	DCM
Chronic Heart Failure	CHF
Embryonic days	ED

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