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Invited critical review

The potential role of heat shock protein 27 in cardiovascular disease

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ABSTRACT

Heat shock proteins (Hsps) comprise several families of proteins expressed by a number of cell types following exposure to stressful environmental conditions that include heat, free radicals, toxins and ischemia, and are particularly involved in the recognition and renaturation of mis-folded proteins. Heat shock protein-27 (Hsp27) is a member of the small Hsp (sHsp) family with a molecular weight of approximately 27 KDa. In addition to its chaperoning functions, Hsp27 also appears to be involved in a diverse range of cellular functions, promoting cell survival through effects on the apoptotic pathway and plays important roles in cytoskeleton dynamics, cell differentiation and embryogenesis. Over the past two decades there has been an increasing interest in the relationship between Hsp27 and cardiovascular disease. Hsp27 is thought to exert an important role in the atherosclerotic process. Serum Hsp27 concentrations appear to be a biomarker of myocardial ischemia. In this review, we will focus on the possible protective and immuno-modulatory roles of Hsp27 in atherogenesis with special emphasis on their changes following acute coronary events and their potential as diagnostic and therapeutic targets.

Contents

1. Introduction

Heat shock proteins (Hsps) comprise several classes of functionally related families of heat-responsive cell stress proteins found in all organisms that are principally characterized by their chaperone functions. They were initially identified in 1962 when Ritossa and colleagues [1] observed that exposing larval salivary glands from Drosophila to heat, induced specific genes in the giant chromosomes of the gland cells. It is now known that these genes encode proteins called Hsps. The expression of these proteins is low under normal physiological conditions but several types of environmental stress

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factors have been shown to induce synthesis of similar proteins in tetrahymena and avian cells and hence Hsps are also called stress proteins. In addition to stresses such as hyperthermia, hypoxia, nutritional deficiency, oxidative stress and ultraviolet radiation, the expression of these proteins is also increased in conditions associated with whole body stress such as response to reperfusion following ischemic injury. Hsps are normally intracellular proteins and when they are expressed outside the cell may trigger an autoimmune response [2,3].

Hsps are commonly divided into seven families based on their size and molecular structure: HSP10, small HSPs (15–30 kDa), HSP40, HSP60, HSP70, HSP90, HSP100 (Table 1) [4].

Small HSPs (sHSP), including HSP27, are widely expressed across species, and range in size from 15–42 kDa for their monomeric forms. The small HSPs differ from some of the other HSPs with respect to the factors regulating their expression. Although a large body of evidence involved whole family of Hsps in mouse model or other in vitro systems, limited number of clinical studies is reported on Hsp70 and Hsp27.

2. Atherosclerosis

Atherosclerosis is a chronic multifactorial disease that underlies the pathophysiology of cardiovascular disease (CVD), stroke and peripheral vascular disease (PVD), and is the major cause of mortality globally [5,6]. It is characterized by the accumulation of lipids and extracellular matrix in the intima of large and medium sized arteries. It is associated with mononuclear cell infiltration, and smooth muscle proliferation [7]. Risk factors for CVD include: age, male gender, family history of CVD, hypertension, hypercholesterolemia, smoking, diabetes mellitus, socioeconomic status, and obesity [7].

There are several emerging risk factors for CVD including markers of oxidative stress, inflammation, and autoimmunity. Atherogenesis is a progressive, multi-step process requiring an ordered sequence of events, in which progress along the disease pathway is driven by risk factors that may differ with the stage of disease, vary between individuals and may not require the inclusion of any of the traditional risk factors [8]. A cell stress response appears to be a pivotal early event in atherogenesis. In addition to their chaperoning function, Hsps such as Hsp27 may be involved in an autoimmune response as they may themselves be altered and be recognized as foreign proteins by the innate and/or adaptive immune system. Hsp27 also undergoes several posttranslational modifications. Site-specific phosphorylation and oligomerization of Hsp27 have been observed following various stressors, and are associated with specific activity and survival roles of Hsp27. The cellular signaling pathways of Hsp27 regulating these modifications appear to involve archetypal phosphorylation cascades, such as MAPKAP and PRAK, and are observable within minutes following exposure to stress. The rapidity of post-translational modifications implicates Hsp27 in the early response to stress, well before transcriptional activation [9].

3. Hsp27 and the small Hsp family

Hsp27 belongs to a family of widespread stress proteins termed small Hsps (sHsps), which are found widely in nature. sHsps vary in size from 15 to 30 kDa and comprise Hsp27, p20, HspB3, MKBP/HspB2, HspB8, HspB9, cvHsp, α-A crystallin and α-B crystalline [10–17] (Table 1). The HSPB1-10 genes are distributed over nine chromosomes. The transcripts of several sHsp genes, notably HSPB7 which is a Hsp27 homologue, display low levels of alternative splicing, which may result in the elaboration of small amounts of protein isoforms [18].

During the stress response an increase in the level of Hsp27 expression is preceded by a phosphorylation-induced reorganization of the multimeric structure of the protein. Phosphorylation and differential oligomerization occurs following various stressors and are associated with specific activities of Hsp27. This rapid post-translational modification underscores the specific role of Hsp27 in the early stress response.

4. HSP27 gene structure and transcription

The HSP27 gene contains three exons encoding a 205- amino acid protein (Fig. 1) [19], and contains two functional HSE binding sites. The first occurs at about −200 bp upstream of the exon 1 [20], the second occurs within the first intron [21]. Recently, an atypical cAMP response element (CRE) was found −678 bp upstream of the exon 1 [22]. This atypical CRE sequence was found to be recognized by both ATF3 and ATF5, where binding of these transcription factors induced transactivation of the HSP27 gene and appears to play an important role in cell survival [22,23]. Several studies suggest that the specific trans-activation of HSP27 is stimulus dependent. During mitosis, HSF2 binds to the HSE of HSP27, whereas HSF1 was found to bind during hemin treatment [21,24]. Additionally, several HIF-1 binding sites were found to be necessary for endogenous upregulation of HSP27 in retinal neurons subjected to sub-lethal ischemic stress [25].

Phosphorylation occurs on several serine residues. Ser-15 and Ser-86 in rodent HspB1 or Ser-15, Ser-78 and Ser-82 in human HspB1.

Table 1
Properties of alpha-Crystallin-related Small Heat-shock Proteins (sHSPs), HSPB1–HSPB10, alternative name, and chromosomal locus and distribution; modified from Yang et al. reference [4], Publisher and year of copyright: Wiley-Liss, 2008.

HSPB	Molecular weight (kDa)	Alternative name	Gene location	Preferential expression	Tissue localization
HSPB1	27	HSP27	7p12.3	Lens, heart, stomach, colon, lung, and bladder	Breast tumours, uterus, skin, platelets, lymphoid tumours, Endometrium, cervix, myocardium, glomerular epithelial and mesangial cells, ovarian and endometrial carcinoma
HSPB2	22	MKBP	11q22–q23	Heart and muscle	Skeletal and myocardial muscle
HSPB3	17/27	–	5q11.2	Heart and muscle	Skeletal and cardiac muscle
HSPB4	20	αA-crystallin	1q22.3	Lens	Lens of eye, spleen and thymus (of rat)
HSPB5	20	αB-crystallin	11q22.3–q23.1	Lens, heart, and muscle	Glioma cells, astrocytes, myocardium, vascular wall cells, lens of eye, head and neck tumours, platelets, lung, kidney, brain (murine)
HSPB6	20	HSP20	19q13.13	Heart and muscle	Myocardium, stomach, liver, lung, kidney, brain, skeletal muscle, platelet
HSPB7	23/25	cvHSP	1p36.23–p34.3	Cardiovascular insulin-sensitive tissues	Heart and skeletal muscle
HSPB8	22–25	HSP22	12q24.23	Muscle, heart, and brain	Heart, Brain, liver, lung, kidney, stomach, skeletal muscle,myocardium (pig)
HSPB9	17.5	–	17q21.2	Testis	Testis
HSPB10	27/85	ODF1	8q22	Testis	Testis

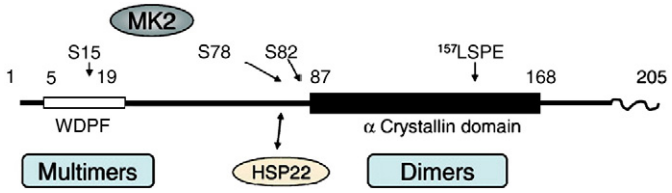

Fig. 1. Domain structure of human Hsp27. Human Hsp27 is a protein of 205 amino acids with the defining feature of a small HSP, an α-crystallin domain at residues Glu87–Pro168. The N-terminus of Hsp27 contains a WDPF motif necessary for multimer formation and chaperone activity which is adjacent to a mitogen-activated protein kinases associated protein(MAPKAP) kinases (MK2) phosphorylation site at Ser15. The WDPF motif is linked to the α-crystalline domain by a region that differs in length depending on species. The C-terminal 18–20 amino acids include an α-crystallin motif that is highly conserved among species and are thought to form a flexible structure important in chaperone function. Modified from Reference [27]. Publisher and year of copyright: Elsevier, 2008. Permission for reproduction/adaptation was granted by the copyright holder.

Phosphorylation promotes dissociation of the large HspB1 oligomers as previously eluded to the smaller ones [26,27]. This induced phosphorylation of Hsp27 is catalysed by MAPKAP kinases 2 and 3, [28–30] which in turn are activated through phosphorylation by p38 MAP kinase [31].

The sequence homology of the N-terminal domain of sHSPs is poorly conserved across species, although the hydrophobic motif within this domain is an exception to this [32,33]. This latter motif is responsible for stabilizing the formation of the oligomers [34] on the other hand, the secondary structure of the C-terminal of sHSPs is highly conserved [20,35], this C-terminal end requires the presence of the N-terminal sequence to form oligomers [36].

5. Post-translational modifications

In vitro data have revealed that phosphorylation of recombinant sHsp causes a decrease in the size of oligomers [37]. However, large oligomers of sHsps are necessary for chaperone activity and resistance against oxidative stress, whereas phosphorylation reduces these effects [37].

This phosphorylation of Hsp27 is likely to occur via MAP kinase activated protein (MAPKAP) kinase 2/3, and protein kinases C and D [38]. Mammalian sHsps are promptly phosphorylated at two or three serine residues in response to various extracellular stresses that is considered to be important in maintaining their function. The polymeric forms of Hsp27 are dissociated when cells are exposed to chemical stressors like sodium arsenite and cadmium chloride [20,39].

Hsp27 acts independently of ATP and the regulation of Hsp27 protein binding occurs at the level of phosphorylation and oligomerization.

Negative regulation of Hsp27 phosphorylation occur either by the inhibition of upstream signaling pathways, or through activation of phosphatases; these lead to dephosphorylation of Hsp27, and the production of high molecular weight oligomers. PP2A, PP1 and PP2B have all been reported in the process of Hsp27 dephosphorylating [40]. Phosphatase PP2A is considered the most powerful agent involved in Hsp27 dephosphorylation [41–43].

Large aggregates of Hsp27 are able to confer protection against reactive oxygen species (ROS) and TNFalpha-induced injury [44]. Phosphorylated Hsp27 was shown not to be protective against ischaemic damage in adult rat cardiomyocytes. Recent evidence suggests that Hsp27 may also be phosphorylated by the delta isoform of protein kinase C, [45] however, it is considered to occur only in response to a specific stimuli, [46] such as treatment with phorbol esters.

6. Functions of Hsp27

Hsp27, also called *HspB1* in humans *and Hsp25* in mice, is one of the most widely distributed of the small heat hock proteins [47].

The cellular roles of Hsp27 include protein chaperone activity, regulation of cellular glutathione levels, apoptotic signaling, inhibition of actin polymerization, and stabilization of actin filament arrays [48]. Intracellular Hsps27 provides cells with a mechanism of defense against external stressors such as heat shock, oxidative, or mechanical stress [49]. Hsp27 is known to be expressed during both skeletal and cardiac muscle development in several organisms, including human, mouse, pig, and zebra fish and has been shown to be involved in a diverse array of cellular processes [48].

Several other roles for Hsp27 during cellular stress have been proposed that account for its cytoprotective properties. The ability of Hsp27 to facilitate recovery of protein synthesis and RNA synthesis following exposure to heat shock may provide the cell with a survival advantage [50]. Hsp27 can bind to proteins and affect their function, and there is evidence that Hsp27 inhibits enzymes in the pro-death pathway. Several stimuli induce apoptosis by activating one or more signal transduction pathways, which can lead to the activation of a conserved family of aspartic-acid specific cysteine proteases (Caspases) resulting in the characteristic biochemical and morphological changes associated with apoptosis [51]. Hence, in Hsp27-mediated cellular survival, Hsp27 has many cellular and molecular targets [52] in addition to its conventional role as a chaperone (Table 2).

6.1. Hsp27 and apoptosis

Recent evidence confirms that phosphorylated Hsp27 is a potent anti-apoptotic molecule [53,54].Hsp27 can interact with Daxx and inhibit Fas-mediated apoptosis. Several other models have also demonstrated enhanced protection by Hsp27 via mechanisms other

Table 2
Multiple functions of Hsp27 and the corresponding interactions with protein or peptide targets; Taken from Arrigo et al. reference [60], publisher and year of copyright: Elsevier, 2007.

Function(s)	Targets
Lens transparency and protection	αA-crystallin and other crystallin proteins
Heart protection	Titin, Hsp20
Cytoskeletal architecture and protection	F-actin Intermediate filament proteins (desmin, vimentin, GFAP, neurofilaments, filensin, phakinin, lamin) Microtubules and microtubule-associated proteins
Apoptosis resistance	Pro-caspase 3, cytochrome c, Smac/Diablo, Akt, DAXX, STAT3, Bcl-xs, Bax, P53
Ubiquitin–proteasome system	Fbx4, C8/a7 subunit of 20S proteasome, eIF4F and eIF4G complex, ubiquitin
Cell cycle regulation	Cyclin D1, p27kip1, P53
Redox homeostasis	Glutathione, G6PDH
Protein intracellular transport	Microtubule, SMN, neurofilaments
Stress signalling pathway	P38 cascade, I Kappa B kinase
Hormone signalling pathway	ERb (Estrogen cascade), hGMEB1 (glucocorticoid hormones cascade)
Unknown nuclear function(s)	SMN, SC35
Unknown cytosolic function(s)	αB-crystallin, Hsp20, Hsp22, Hsp27
Pathological-related misfolded proteins	Desmin, GFAP, neurofilaments, ZASP, filamin C, myotiline, parkin, a-synuclein, prion protein, tau, b-amyloid, huntingtin, serpin, SOD, P150 dynactin, αA-crystallin, αB-crystallin, Hsp20, Hsp22, Hsp27
Virus	NS5A protein from Hepatitis C
Immune response	CD10, b2-microbulin
Unknown function in Sertoli cells	PASS 1
Golgi architecture	GM130

GFAP, glial fibrillary acidic protein; DAXX, death domain-associated protein 6; STAT3, signal transducer and activator of transcription 3; Fbx4, Fbox only protein 4; eIF4F, eukaryotic translation initiation factor 4F; eIF4G, eukaryotic translation initiation factor 4G; G6PDH, glucose-6-phosphate dehydrogenase; SMN, survival motor neuron protein; SOD, superoxide dismutase; ER, estrogen receptor; SC35, splicing factor; hGMEB1, human glucocorticoid modulatory element-binding protein 1; NS5A, non-structural protein 5A; ZASP, LIM domain-binding protein 3; PASS 1, protein associated with small stress protein 1. GM130, golgi matrix-protein 130.

than interaction with the apoptotic processes [55]. Hsp27 prevents the release of the proapoptotic molecule Smac/DIABLO by blocking the intrinsic pathway through the retention of cytochrome c9 [56]. The sHsps are also known to interfere with programmed cell death induced by TNFα and Fas ligand [57]. Hsp27 negatively regulates the activation of pro-caspase-9 by an ability to interact with cytochrome *c*, thus preventing the correct formation/ function of the apoptosome complex [58,59].

7. Mechanism of cytoprotection by Hsp27 chaperone activity

The sHsps are capable of binding misfolded proteins. However, unlike the larger family members, the ability of sHsps to refold proteins to their native conformation remains contested. Hsp27 forms a complex with non native proteins, preventing their non-specific aggregation and allowing them to be restored to their native structure in co-operation with ATP-dependent chaperones such as Hsp70 [60,61]. The binding of sHsps is thought to lead to enhanced proteasomal degradation [62], decreased aggregation of misfolded proteins [63–66], or inhibition of cell death signaling. Excessive amounts of damaged proteins within the cell, trigger the activation of apoptotic machinery [67]. Increased expression of Hsp27 facilitates the repair or elimination of these proteins and hence enhances cell recovery.

Moreover, the molecular chaperone function of Hsp27 is responsible for the regulation of apoptosis through the interaction of Hsp27 with protein kinase B. Activation of this protein has been demonstrated to inhibit apoptosis in a variety of systems [68–72]. Some studies have revealed that over-expression of Hsp27 reduces apoptotic cell death triggered by several stimuli, which include: hyperthermia, oxidative stress, staurosporine treatment, ligation of the Fas/CD95 death receptor, and cytotoxic drugs [73–76].

The chaperone function of Hsp27 is only evident when it is present as large, unphosphorylated oligomers [37]. However, several studies have reported enhanced cellular protection associated with over-expression of mutant forms of Hsp27 that are not phosphorylatable.

Some studies have reported the ability of Hsp27 to block endothelial migratory signals and to inhibit endothelial cell proliferation and migration stimulated via endostatin and thrombospondin-1 [77]. Lavoie et al. suggested that stress causes phosphorylation-induced conformational changes in the Hsp27 oligomers which modulate the activity of the protein at the level of microfilament dynamics, resulting in a higher state of stability and recovery of the filaments [26]. Hsp27 is required for mediating the chemotactic effects. Studies have suggested that Hsp27 regulates migration by affecting the generation of lamellipodia microfilaments [78]. Hsp27 regulates fibroblast adhesion, elongation and migration [79]. Hsp27 also confers resistance to TNFα-independent lysis by monocytes. Hsp27 has important roles in platelet function. There is evidence suggesting a potential role for Hsp27 in the regulation of transglutaminase activity in stabilizing fibrin–platelet clots. Hsp27 is phosphorylated by cGMP-dependent protein kinase (cGK), a signaling system important for the inhibition of platelet aggregation [80]. Phosphorylation of Hsp27 may also contribute to the inhibitory effects of cGK on platelet function [81]. Hsp27 may be involved in platelet aggregation by modulating actin polymerization [82].

7.1. Resistance to oxidative stress

It is suggested that the C-terminal domain of Hsp27 is essential for its protective activity against oxidative stress [83]. In a study of stable transformants of an immortalized human fibroblast cell line, the investigators observed that cells expressing high levels of Hsp27 were more sensitive to growth inhibition by a lower dose of hydrogen peroxide than those expressing low levels. Over expression of a non-phosphorylatable mutant Hsp27 did not affect sensitivity to oxidative stress [84]. These results suggested that a high constitutive expression of Hsp27 in this particular cell line make them more susceptible to oxidative stress resulting in growth arrest, and this may be due to an effect on Hsp27 phosphorylation [85].

7.2. Regulation of actin filament dynamics based on the phosphorylation state of Hsp27

Hsp27 may promote filament stabilization during stressful conditions. A reduction in the wound closure rate has been reported for Hsp27 knockdown cells [86] which demonstrates the importance of Hsp27 in regulating cell motility. It is implied that Hsp27 may be essential for the movement of unstressed cells. Hsp27 may act as an actin sequestrator, which is known to be important for maintaining a large pool of G-actin and actin filament turnover [87].

8. Atherosclerosis and inflammation

Inflammation is now thought to play a central role in atherosclerosis [88]. The potential role of inflammation in atherosclerosis was first proposed in the 1850 s [89]. Since then, many studies have confirmed that inflammatory processes are involved in atherogenesis [90,91]. The earliest lesions in atherogenesis, are fatty streaks, and can be seen in infants and young children [92]. They consist of a relative paucity of lipid accumulation and comparative abundance of intimal inflammatory cells that include activated T lymphocytes (helper, suppressor, and regulator), mast cells, macrophages, dendritic cells [93] and less commonly granulocytes and NK cells [94–96]. In several longitudinal studies, serum inflammatory biomarkers including C-reactive protein (CRP), fibrinogen and interleukin-6 (IL-6) have been associated with increased risk of coronary heart disease (CHD). Serum CRP concentrations have been reported to be a stronger independent predictor of coronary events than low density lipoprotein (LDL) cholesterol levels [97–100]. It has also been reported that elevated levels of soluble intercellular adhesion molecule (ICAM)-I, a marker of endothelial cell activation, are associated with increased risk of coronary events [101] and is expressed higher in human atherosclerotic lesions [102]. Some studies have proposed a role of complement activation [103–105] in atherogenesis that could contribute to the vascular damage associated with the autoimmune responses to modified LDL [106] or denatured Hsps [107].

9. Hsp27 and atherosclerosis

The role of several larger Hsps in atherosclerosis has been studied previously because they represent the response of cells of the vessel wall to different stress inducing factors agents, including several classical atherosclerosis risk factors [108]. Furthermore the Hsps are potential targets for immune responses, as they may be themselves altered during exposure to the stress response, and are usually not found in the extracellular milieu; they may therefore directly contribute to the inflammatory process [109]. These immune responses may initially be directed against antigens of pathogenic organisms and then cross-react with homologous host Hsps being elaborated by cells of the vascular wall, including endothelial cells [110]. The anti-Hsp antibodies may lead to endothelial injury by antibody-dependent, complement mediated cellular cytotoxicity, Whilst anti-Hsp antibodies are probably produced for the primary purpose of eliminating infectious organisms they may lead to endothelial injury. Mayr et al. [111] have reported that serum anti-Hsp-antibodies to E. coli and C. pneumonia can mediate endothelial cell lysis of stressed, but not unstressed endothelial cells. Alternatively, these immune responses may be induced following exposure of Hsps on the surface of infected endothelial cells.

SMCs have an important role in atherogenesis and can be induced to express Hsps as part of a survival mechanism following exposure to a variety of stressors, for example, exposure to high blood pressure. Hsps have an important role in the function of cells involved in immune

system and as a result of disperse distribution and homologous structure in different species they can serve as an antigen to the immune system. A positive relationship has been observed between the immune responses to Hsps and subsequent atherosclerosis in a rabbit model [112].

Most research on Hsps in atherosclerosis has focused on Hsp60/ 65 and Hsp70. Several studies have recently addressed the role of Hsp27 in atherogenesis. Park et al. studied the expression of Hsp27 in human atherosclerotic plaques and plasma levels of Hsp27 in patients with acute coronary syndrome. Using 2-dimensional gel electrophoresis and Western blotting, the investigators analyzed arterial tissues for the expression of Hsp27, ie, atherosclerotic plaques versus normal arterial tissue. They found significantly increased Hsp27 expression in the adjacent normal-appearing vessel areas compared with unaffected reference vessels. In addition, in the lesion core region a lower degree of HSP27 expression was found compared with areas adjacent to plaques [113].

Based upon several observations showing that atherosclerotic plaques contain proteases [114,115], Ventura et al. hypothesized that the lower levels of Hsp27 expressed by atherosclerotic plaques could be a result of its degradation by proteolysis. Diminished Hsp27 release was also related to the complexity of the plaque [116]. The fact that the highest degree of Hsp27 expression was observed in the still normal-appearing area adjacent to the atherosclerotic plaque is probably because this is where the inflammatory processes is most active. The decrease of Hsp27 expression toward the atherosclerotic core may be due to an increase of proteolytic activity in this region of the plaque [113].

It has been reported that Hsp27 stimulates monocyte production of anti-inflammatory cytokines such as IL-10 [117] and also inhibits their expression of toll like receptor-4 (TLR-4) and their differentiation into dendritic cells. Wick et al. [108] have hypothesized that the autoimmune responses to Hsps could be crucial in the initiation of atherosclerosis [118,119] and this is supported by human studies [97,111,120–122]. In a rat model in which aortic atherosclerosis is induced by a high cholesterol diet, 46 proteins were differentially expressed in the aortic tissue; among these differences was a decreased level of phosphorylated Hsp-27 [123]. Cardiac biopsies from heart transplant recipients has shown a 20-fold greater expression of phosphorylated Hsp27 in biopsies from patients without disease compared with those who had developed atherosclerosis [124].

It has also been reported that phosphorylated Hsp27 is present in large amounts in the conditioned media of undiseased endarterectomy samples while it was rarely found in atherosclerotic conditioned media [116]. Apoptosis of vascular smooth muscle cells (VSMCs) occurs in the process of atherothrombosis and is involved in the weakening of the fibrous cap and therefore plaque rupture [125]. Hsp27 may be protective in this process because it plays an important role in the regulation of the mobility of SMC and coordination of actin filament dynamics intracellular.

IκB kinases (IKK)-1 and -2 are related kinases that are induced by stimuli such as TNF or IL-1 to phosphorylate serines 32 and 36 of IκBα, the regulatory subunit of the transcription factor NF-κB [126]. Hsp27 interacts with IKK protein and may prevent the activation of nuclear factor κB, which is thought to be critical in the inflammatory response and therefore in determining plaque stability [127,128]. VSMCs respond to survival signals derived from their interaction with the extracellular matrix. Several mechanisms involved in the degradation of the extra cellular matrix, operating via plasminogen activation may interfere with these signals, thereby promoting apoptosis [129–131]. It is reported that Hsp27 may increase the stability of atherosclerotic plaques via the modulation of plasmin and other extracellular potential mediators of VSMC apoptosis [132]. It has been known that Hsp27 is induced by estrogens and in some way associated with estrogen receptors in different cells like platelets (Fig. 2).

Mechanical forces play an important role in the remodeling associated with the pathogenesis of atherosclerosis. The changes in expression of proteins associated with hemodynamical stress have shown that Hsp27 levels are altered [133].

Further studies are needed to fully identify and reveal the role of Hsp27 in atherogenesis and its potential role as a biomarker and therapeutic target for cardiovascular disease.

10. Hsp27 in human heart disease

Hsp27 expression has been found to be altered in cardiovascular diseases, e.g. congestive heart failure [134]. It is also proposed that Hsp27 is upregulated in stressed or vascular endothelial cell growth factor–activated human endothelial cells [135]. It has also been suggested that pro-inflammatory mediators and cytokines (interleukin-6, tumor necrosis factor) may upregulate the expression of HSP27 [113]. It is well established that the expression of Hsp27 in the myocardium is upregulated by acute ischemia [136]. Hsps have an important role in protecting myocardial cells from a number of environmental stressors and Martin et al. [137,138] have reported that both Hsp27 and Hsp70 have protective effects on cardiac myocytes against ischemia; furthermore decreasing the level of endogenous Hsp27 resulted in an enhancement of the damaging effects of exposure to ischemia. However, it is not clear whether the phosphorylated form of Hsp27 is required for this protective effect [139]. The induction of Hsp27 phosphorylation and myofilament translocation were observed following cardioplegia and cardiopulmonary bypass [140], supporting the observations from studies in animal models.

Although the consequences of myofilament translocation following ischemia are not well understood, it is thought that non-phosphorylated Hsp27 may stabilize cytoskeletal components, such as actin, via its chaperone function [139]. It is proposed that phosphorylated-Hsp27 in conjunction with tropomyosin may play a role in stabilizing the cytoskeleton and be protective against ischaemic injury. Tropomyosin interacts with phosphorylated-Hsp27 in SMCs [141]. An increased resistance to ischemia/reperfusion in type 1 diabetic rats, improved post-ischemic mechanical function has been described, which attenuated reperfusion arrhythmias and reduced CPK release. Furthermore, a significantly greater amount of phosphorylated-Hsp27 was observed in the hearts from diabetic rats compared with non-diabetic animals [142].

Increased levels of Hsp27 are reported to play an important role in cardioprotection by maintaining the integrity of microtubules and actin cytoskeleton, and may protect endothelium from ischemia. The phosphorylation and translocation of Hsp27 from cytosol to myofibril or nucleus has been shown to be particularly important for protecting against actin fragmentation and microtubule degradation [142].

Vimentin is a member of the intermediate filaments of the cytoskeleton that have a role in cell integrity, mobility, and differentiation. Hsp27 interacts with vimentin and can prevent its proteolytic degradation under conditions of stress, thus maintaining cell integrity [143]. Therefore, it is possible that with reduced phosphorylated-Hsp27, vimentin is no longer protected and its intact form decreases due to fragmentation. Increased expression of transgelin is reported in IHD [144]. It is hypothesized that in ischaemia, phosphorylated transgelin can no longer bind actin and promotes actin stabilization. This is supported by the report that deletion of transgelin in ApoE$^{-/-}$ mice (atherosclerosis prone mice) resulted in an increased atherosclerotic lesion area and increased proliferation of SMCs [145]. Feil et al. suggested that transgelin may regulate SMC differentiation during vascular remodeling. Robinson et al. have reported the presence of Hsp27 in all of its phosphorylated forms in both SMC and ECs in human coronary arteries. There appeared to be a selective reduction of the Ser15, Ser78, and Ser82 phosphorylated forms of Hsp27, and a reduction in phospho-p38 MAPK, in the arteries of patients with IHD [144].

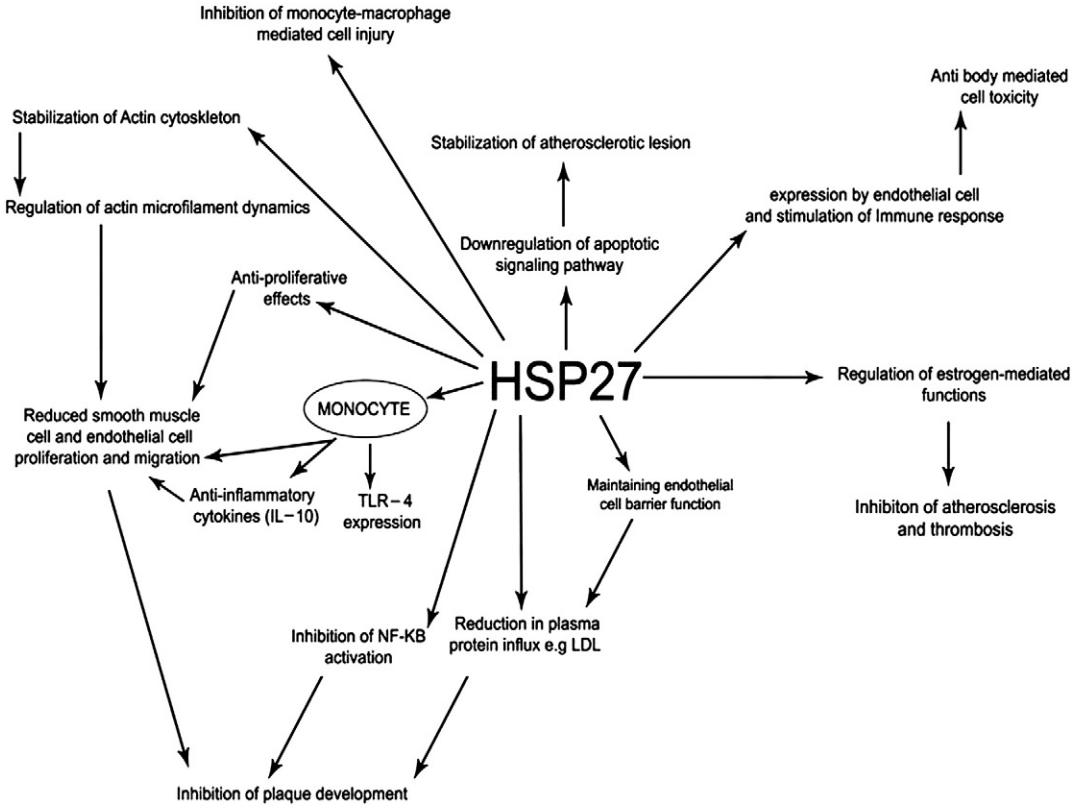

Fig. 2. Possible mechanisms of involvement of Hsp27 in atherosclerosis. Hsp27 is expressed by endothelial cells and may stimulate autoimmune response. Hsp27 have roles in stabilizing atherosclerotic plaque, inhibition of plaque development and inhibition of endothelial cell injury through different mechanisms.

11. Serum Hsp27 antigen and antibody concentrations and their relationship to cardiovascular risk

Park et al. have previously shown that there is no difference in serum Hsp27 concentrations between a normal healthy individuals and a group with risk factors for coronary artery disease. Circulating levels of Hsp27 antigen did not appear to be related to risk factors for coronary artery disease, but significantly higher levels of Hsp27 antibodies are seen in patients with acute coronary syndrome. In a prospective nested control study Kardys et al. [146] evaluated plasma concentrations of Hsp27 at baseline among 255 initially healthy women for any kind of cardiovascular disease or stroke during a follow up period of 5.9 years and matched same number of women for age and smoking who did not developed these diseases. They observed no association between baseline plasma Hsp27 concentration and any future cardiovascular events. Hsp27 concentration is inversely associated with age and not linked with other established cardiovascular risk factors. The odds ratios for cardiovascular disease for the highest vs. lowest tertile of Hsp27 plasma concentration was 0.99. In addition, this study did not support an association between plasma Hsp27 concentrations and established cardiovascular risk factors. The difficulty in interpreting these studies is that the appearance of significant quantities of Hsp27 antigen in the serum may be a relatively late phenomenon, and therefore not predictive many years in advance.

There are conflicting reports with respect to the interplay of anti Hsp antibody titers and human cardiovascular disease [121,147–150]. Whilst previous studies have shown a positive association between plasma antibody titres to Hsps-60/65 and −70/72 and the severity of cardiovascular disease [110,121,122,147,151–154], there are limited data on the role of the smaller Hsp antibodies in atherosclerosis. Although Hsp 27 is present within human atherosclerotic lesions, it is not fully established whether its serum levels are altered in patients with atherosclerotic disease [116,155]. Antibodies to Hsp 27 have been detected in serum of patients with coronary heart disease [26]. But the correlation between antibody titres and the extent of CAD remains unresolved. Several studies have reported an increased expression of Hsps in infarcted myocardial tissue, and also their release into the peripheral circulation several hours following a stroke [156–158].

Shams et al. [159] have reported that serum concentrations of Hsp27 IgG antibodies were significantly higher in patients with chest pain compared to the healthy control group. They concluded that an increase in antibody concentrations is not directly associated with the acute coronary event, instead contributing to the underlying atherogenic process. They found that age was a significant determinant of anti-Hsp27 IgG concentrations, particularly in subjects with chest pain but there was an inverse relationship between blood pressure and antibody concentrations.

Anti-Hsp27 antibody concentrations were strongly associated with age, gender, hypertension, and weakly with diabetes in patients with acute coronary syndrome [159]. However, other cardiovascular risk factors have not been found to be associated with serum anti-Hsp27 IgG antibody concentrations. We were unable to demonstrate an association between anti-Hsp27 antibody levels and several coronary risk factors in an Iranian cohort [149]. Plasma Hsp27 concentrations have also been reported to be correlated with total serum cholesterol concentrations in patients with acute coronary syndrome [155]. Moreover, patients with hyperlipidemia and hypertension had lower levels of anti-Hsp27 antibody than those with neither [150].

Several studies have reported the over expressed Hsp27 in cardiac myocytes following ischaemia–reperfusion, and have suggested the cardioprotective role of Hsp27 [156,160]. In a small study of 56 patients it has been reported that there is a significant increase in serum Hsp27 antigen levels among patients with acute coronary syndrome (ACS) [155]. The authors concluded that Hsp27 is apparently up regulated in ischemia and found at higher concentration in plasma of ACS diseased

patients than non-diseased individuals. We [149] have previously reported that in patients with ACS, Hsp27 antibody titers are high during the first 12 h following an event, then fall to near normal levels after about 12 h. In a recent study, we compared plasma concentrations of Hsp27 antigen of 75 patients with diagnosis of ACS with 75 healthy individuals on admission and 12 h after the onset of chest pain. We found that serum Hsp27 concentrations are significantly elevated in the early hours post cardiac event, but falls to levels near to healthy subjects after about 12 h after the onset of chest pain. In this study, we observed no association between Hsp27 and any CAD risk factors. Serum Hsp27 concentrations have been compared in patients with ACS and with chronic stable CAD. Concentrations of Hsp27 were shown to be higher in patients with ACS in comparison to controls, while patients with stable CAD had non significantly higher concentration of Hsp27 [9]. Levels of Hsp27 were also higher in the patients with 2- and 3-vessel CAD compared with patients with 1-vessel disease. Whereas the number of affected coronary arteries with significant stenosis affects the extent of ischaemia, authors concluded that there is a significant relationship between Hsp27 concentrations in plasma and the ischaemic myocardium and hence serum Hsp27 may serve as a marker of severe myocardial ischaemia typical for 2- or 3-vessel CAD [9]. In a recent study of 400 patients with suspected CAD based on coronary angiography, we observed that patients with three-vessel disease had higher anti-Hsp27 titers compared with both two-vessel disease (2VD) and one-vessel disease (1VD) subgroups [161]. We concluded that serum anti-Hsp27 titers may be associated with the presence and severity of CAD. In another recent study, we have measured anti-Hsp27 titers in 168 patients in the first 24 hours after the onset of stroke and 80 control participants. We found that serum anti-Hsp27 concentrations are significantly elevated in patients with stroke [162]. However, the utility and specificity of Hsp27 as a biomarker of CVD remains a matter of debate. Several other conditions and co-morbidities may affect the serum concentrations of Hsp27. Furthermore, the serum concentrations of Hsp27 in healthy individuals may be affected by large intra-individual variation, and assays to date have lacked appropriate standardization. The latter is particularly difficult for the antibody assays.

Further clinical studies are required to define the role of Hsp27 in CAD and its kinetic release in plasma in relation to atherosclerosis and these are currently in progress.

12. Therapeutic implications

Hsps may act as auto-antigens that exacerbate the immune response that may result in a vascular injury cycle. It is proposed that tolerization to these antigens may inhibit atherogenesis and thus may be a practical therapeutic approach. Hsp27 may be a potential therapeutic target for other reasons. Because Hsp27 has anti-proliferative effects, reduced expression in the presence of growth factors and inflammatory mediators might favor smooth muscle growth and perhaps contribute to plaque formation. One approach to counter these effects would be to enhance or maintain normal levels of expression of the small Hsps including Hsp27. Wolfgang et al. propose that Hsp27 exerts a protective effect against simulated ischemia. They proposed a model whereby proteins that are not in their final folding state bind to the outside of the large oligomeric small heat shock protein complexes which act as a sheltered place. After the ischemia is resolved these proteins can be released and result in proteins in their final folding state, which can assume their normal activity in cells recovered from ischemic injury [163]. It has been proposed that knowing an individual's Hsp27 "status" using a blood test for example, may be predictive of atherogenesis and may determine who should receive estrogen therapy, because the development of undesirable side effects (e.g., venous thrombosis, malignancy) could be caused by loss of Hsp27 regulation of estrogen-mediated transcription [164].

Targeted over expression of Hsp27 in tissue susceptible to ischemic injury may be beneficial. The use of pharmacological inducers or enhancers of the endogenous Hsp expression in the target cells, in particular, inducers such as Herbimycin A and Geranylgeranylacetone have been shown to possess a cytoprotective potential on testing in models with simulated ischemia. Geranylgeranylacetone (GGA) is a drug which is often used for boosting HSP expression. The mechanism for induction of HSP expression is through activation of the heat shock transcription factor HSF1. Oral administration of GGA rapidly upregulates general HSP expression in response to a variety of stressors, while its effect is attenuated under non-stress conditions [165].

13. Conclusion

The Hsp27 is a ubiquitous small heat shock protein with chaperoning activity that has several other potential functions in cells involved in CVD, including: VSMC migration, apoptosis, resistance to oxidative stress and toxins and anti-inflammation that are all involved in atherosclerosis. Cells involved in atherosclerosis express large quantities of Hsp27 in response to exposure to stressors that may also promote atherosclerosis. Antibodies to Hsp27 have been detected in patients with coronary artery disease. Measures of Hsp27 expression, including serum antigen, or antibody concentrations may be useful as markers of disease susceptibility and progression although these levels do not associate with known coronary risk factors and data are inconsistent. This can be as a result of the complex interactions between Hsp27 production, release and clearance. Hsp27 may also be involved in initiation of cardiovascular disease by inducing a pro-inflammatory autoimmune response against endothelial cells. It may also play roles in inhibition of platelet aggregation, cardio-protection in ischemic events and enhancing post ischemic outcome.

Further long-term prospective studies in diverse populations will be required to assess the time course of appearance of the Hsp27 antigens and antibodies relative to the development of clinical events and evaluate their potential values as diagnostic and therapeutic targets in atherosclerosis.

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