We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Diagnostic and Prognostic Application of Heat Shock Proteins and their Post-Translational Modifications from Liquid Biopsies

Byron Baron

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79737>

Abstract

Liquid biopsies contain numerous proteins coming from extracellular vesicles (EVs), be it microvesicles or exosomes, released by both normal and tumour cells, as well as the presence of any circulating tumour cells (CTCs). Such proteins can be used as biomarkers for early diagnosis, prognostic assessment, disease progression monitoring, therapy selection and treatment response, particularly in oncology. EVs have been identified as mediators of cell-to-cell communication in both normal and pathological conditions and suggested to play a role in promoting and maintaining cancer dissemination and progression by altering the tumour microenvironment through immune suppression, angiogenesis and metastasis. One class of proteins garnering particular interest are extracellular heat shock proteins (HSPs) (secreted despite no consensus secretory sequence), and their post-translational modifications (PTMs), which are thought to act as key players in intercellular crosstalk and activation of signalling pathways during stress conditions. This review will focus on how characterising and quantifying these proteins can indicate the condition of the physiological system in a variety of pathological contexts.

Keywords: extracellular vesicles, biomarkers, heat shock proteins (HSPs), post-translational modifications (PTMs), intercellular crosstalk

1. Introduction

Liquid biopsies may contain a wide variety of biomolecules including DNA, RNA, proteins and metabolites. When considering the presentation of the numerous proteins within a liquid

biopsy, these can be free in the plasma, encapsulated within or on the surface of extracellular vesicles (EVs) or still inside cells within the biopsy (such as in the case of circulating tumour cells (CTCs)).

One class of proteins garnering particular interest as part of liquid biopsies are extracellular heat shock proteins (HSPs), and their post-translational modifications (PTMs), mainly because they should not be present in body fluids at the concentrations observed due to their lack of an export sequence and also as a result of the growing evidence supporting the notion that these proteins can mediate intercellular crosstalk and act as messengers that activate signalling pathways during stress conditions.

1.1. Heat shock proteins (HSPs)

HSPs are a class of chaperone proteins ubiquitously expressed in the cells of both prokaryotic and eukaryotic organisms. HSPs have been traditionally named and subdivided into six groups or families based on their molecular weight, namely, the small HSPs (which include HSP27), HSP40, HSP60, HSP70, HSP90 and HSP100 family. However, more recently, a new nomenclature and classification system based on the naming issued by the Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC) has been proposed for classifying human HSPs into the following groups: HSPA (HSP70), HSPB (small HSPs including HSP27), HSPC (HSP90), HSPD/HSPE (HSP60/HSP10), HSPH (HSP110) and DnaJ (HSP40) [1]. Each of these families has members that are constitutively expressed and others that are inducible upon stress.

Under normal physiological conditions, constitutive HSPs fulfil important regulatory roles in a wide range of cellular processes including the synthesis, folding, translocation, assembly and in some cases activation of the proteins they interact with. On the other hand, after an episode of cellular stress, inducible HSPs help to refold and prevent aggregation of misfolded proteins, as well as assist in the proteasomal degradation of misfolded proteins which cannot be recovered. Moreover, HSPs can block apoptotic signalling and increase tolerance to subsequent insults [2].

However, it is now starting to emerge that during stress, the role of HSPs goes beyond what is expected to be their intracellular chaperoning functions for recovery from multiple stress conditions. Despite HSPs acting predominantly intracellularly, they have also been found expressed in the cell plasma membrane and in the extracellular space. Numerous HSPs have been reported to be present in the extracellular space and general circulation, activating a range of signalling pathways depending on the effector cell type or target organ. The role of such extracellular HSPs appears to be that of a systemic warning system of stressful events or chronic conditions, acting by priming the body, of which the immune system is a major effector, in order to prepare for and counteract the spread of the stress insult. Extracellular HSPs thus seem to act as a form of intercellular communication system during stress conditions, particularly those responses linked to oxidative stress, immunity or inflammation [3].

1.2. The presence of HSPs outside cells

When HSPs are present outside cells, they can be found as free proteins in solution or forming part of EVs. EVs can be of various types, with distinct structural and biochemical properties

as well as intracellular site of origin. These include large microvesicles (up to 1500 nm) that are heterogeneous in shape and produced from the plasma membrane, small (50–100 nm) and more uniformly shaped exosomes released from endosomes via the endocytic pathway and apoptotic vesicles produced upon cell death [4, 5].

EVs are released by almost all cell types, both healthy and diseased (including tumour cells). Such vesicles carry a wide range of biologically active molecules including growth factors, cytokines, mRNAs and microRNAs, extracellular matrix constituents and also proteins [6]. The protein fraction consists of cytosolic or plasma membrane components, either inside or on the surface. Their molecular contents have been shown to mediate intercellular communication in a variety of cellular processes, in both normal and pathological conditions, with the transfer of such biomolecules altering the function of the target cells. In the context of cancer, for example, EVs can modulate both the tumour microenvironment and cells and tissues which are located at a distance, affecting the immunity in the area, promoting angiogenesis and bringing about metastasis [7, 8].

EVs are also released by cells in response to being exposed to a stressor or as a result of chronic cellular stress. Such EVs contain particular molecules, including HSPs, whose expression level is directly linked to or induced by the stress insult. Upon reaching their effector cells, and especially when interacting with cells of the immune system, some EV components act as signalling molecules, activating a response in the effector cells which pre-empts the stress insult prior to its spread [3].

Proteomic studies have shown that EVs from serum, saliva, milk or plural effusions contain HSP27, HSP60, HSP70 and HSP90 [9–16] at high concentrations, with the ability to synergise with other encapsulated factors [3]. The delivery of HSPs in EVs provides a much stronger signal to effector cells as exemplified by EVs containing HSP70 producing a 250-fold higher activation of macrophages than an equal concentration of HSP70 in solution [17].

The HSPs encapsulated within or presented on the surface of such EVs, together with changing levels in free HSPs, can thus be valuable disease biomarkers for early detection, diagnosis and therapy selection. However, in order to access them, these proteins need to be purified from the body fluids of patients, characterised, quantified and compared to what is known in the healthy condition.

2. Secretion and uptake of extracellular heat shock proteins

When cells are exposed to a stressor, which includes but is not limited to heat shock, osmotic stress, exposure to heavy metals, hypoxia, ischemia or pathogens, these release signalling molecules in order to alert the rest of the system that a stressful condition is being experienced in some part of the organism and which might potentially lead to a situation of systemic damage. Among the stress signals which can be released by cells in response to an incidence of cellular stress are HSPs and other components of the chaperone (**Figure 1**). It is worth noting that most HSPs lack the consensus signal required for secretion via the classical endoplasmic reticulum (ER)-Golgi pathway [3, 18]. So far, it appears that the secretion of HSPs is achieved via a number of alternative pathways; however, these are still not well defined. Presently, the HSP release mechanisms identified are (but might not be limited to) processes via:

- i. Cell lysis—where the process can be the result of a physiologically regulated release of cytokines or necrosis resulting from a pathological condition. Extracellular HSP70 has been suggested to be released into circulation under a variety of pathological conditions which cause widespread cell death as well as the following necrosis of tumour cells [19].
- ii. Endolysosomal pathway—where the HSP is translocated into lysosomes and instead of being degraded is translocated out of the cell via endocytosis. HSP27 (dephosphorylated at S15 and S82) [20] and HSP70 [21] have been shown to enter endolysosomes, which are then secreted extracellularly in an ATP-dependent manner, from both tumour cells and macrophages possibly via some pathway analogous to the ATP-binding cassette (ABC) transport system [21].
- iii. Exosomal pathway—where the HSP is contained in secretory vesicles (exosome lumen) which rupture or are lysed once present in the extracellular space. A number of HSPs have been detected within extracellular vesicles including HSP27, HSP70, HSC70, GRP75, GRP78 and HSP90 [22–25].
- iv. Inclusion in the exosomal membrane—where the HSP is inserted into the membrane of the secretory vesicles rather than being in the lumen. The isolation of HSP70-containing vesicles, derived from the plasma membrane, indicates that the surface of the vesicle can be used as an export system [17, 26, 27].
- v. Secretory-like granules—where the vesicles used to transport the HSP are neither lipid bodies, nor endosomes, or lysosomes. Tumour cells were found to release HSP70 in structures that were only positive for chromogranin A, which is a marker of secretory granules [28].

Once in the extracellular space or general circulation, these HSPs can stimulate a wide range of cell types. However, similar to the secretion mechanisms, the recognition and uptake of HSPs by cells, as well as the role that extracellular HSPs play in cell activation, are poorly understood. HSPs have been reported to bind to a wide variety of receptors on target cells, among which are:

- i. Low-density lipoprotein (LDL) receptor-related protein 1 (LRP1; CD91)—a receptor involved in receptor-mediated endocytosis, which is found on numerous cell types including antigen-presenting cells (APCs), known to bind to HSP70, HSP90 and calreticulin [29, 30].
- ii. CD40—a member of the tumour necrosis factor (TNF) receptor family that is essential in mediating a broad variety of immune and inflammatory responses and can bind to HSP70 [31, 32].
- iii. C-C chemokine receptor type 5 (CCR5; CD195)—a receptor on white blood cells involved in the process by which T cells are attracted to target areas via cytokines, which has also been shown to bind to mycobacterial HSP70 [33].
- iv. Toll-like receptors (TLRs)—of the ten TLR receptors found in humans, only TLR2 and TLR4 are so far known to act as HSP receptors. They are known to bind to HSP60, HSP70 and HSP90 [34–37]. It has been suggested that TLR activation by HSP is most likely not the result of a direct binding of HSP70 to these receptors but rather either a low affinity interaction or a secondary activation involving the prior binding of HSP to another receptor [38].

- v. CD14—a co-receptor for TLR4 activation, which was found to be also required for HSP70 induction of cytokines [35].
- vi. Scavenger receptors (SR)—a family of receptors currently classified into ten subclasses (A–J) based on structure and biological function [39]. At least three SRs bind to and internalise HSPs, namely, lectin-like oxidised LDL receptor 1 (LOX-1), scavenger receptor expressed by endothelial cell 1 (SREC-1) and fasciclin and EGF-like, laminin-type EGF-like and link domain-containing scavenger receptor 1 (FEEL-1)/common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER-1), with HSP70 binding to all three, HSP60 binding to LOX-1, HSP90 binding to LOX-1 and SREC-1 and calreticulin binding SREC-1 but not LOX-1 [38, 40–43]. Furthermore, scavenger receptor-A (SR-A) can bind to and internalise HSP90 and calreticulin as well as HSP110 and GRP170 [42, 44]. The sialic acid-binding immunoglobulin-type lectin (Siglec) receptors Siglec-5 and Siglec-14 have also been found to bind to HSP70 [45, 46].

Figure 1. Heat shock proteins (HSPs) are exported into the extracellular space and general circulation via a number of different processes including cell lysis, secretory vesicles, lysosomal endosomes or export vesicles. Once these extracellular HSPs reach the target tissues, they bind to a variety of receptors, which initiate an alarm response. When these extracellular HSPs are collected from patients with chronic diseases and quantified, they can have diagnostic or prognostic value.

3. Biomarker potential of extracellular heat shock proteins

Changes in extracellular HSPs have been detected and implied to be actively involved in many chronic pathological conditions including arthritis, cardiovascular disease, cancer, type 2 diabetes mellitus (T2DM), chronic obstructive pulmonary disease (COPD) and neurodegenerative diseases. However, in order for these extracellular proteins to be used as biomarkers for early diagnosis, prognostic assessment, disease progression monitoring, therapy selection or treatment response, it is essential to characterise their functions and quantify their levels in the selected body fluids for liquid biopsies under both normal physiological conditions and the various pathological contexts.

For example, with respect to cancer, a wide range of studies have linked changes in extracellular HSPs to key mechanisms involved in either the process of malignant transformation or the progression of a tumour via evasion of apoptosis, increased cell proliferation and immortality, invasiveness and metastasis. On the other hand, when it comes to T2DM, because the biochemical mechanisms are not well understood, it is more difficult to link extracellular HSPs to the aetiology of the condition. However, T2DM patients present a two- to fourfold higher risk of developing macrovascular diseases, including coronary artery disease, stroke and peripheral vascular disease, making episodes of cardiovascular complications the major fatality in such patients [47]. Moreover, the sustained hyperglycaemia brings about cellular dysfunction via systematic biochemical changes due to oxidative stress, accumulation of advanced glycated end products (AGEs) and chronic inflammation [48], which are processes highly associated with HSPs.

3.1. HSP27

Extracellular HSP27 has been so far linked to three major functions, immune response modulation, angiogenesis and atheroprotection through a number of mechanisms, which in the contexts of cancer and T2DM can have a significant contribution to the aetiology or progression of the disease.

Immune signalling is activated by extracellular HSP27 via interaction with receptors on the surface of immune or endothelial cells, leading to the differential production and release of cytokines and growth factors, in order to modulate the immune response, cellular migration and proliferation. Extracellular HSP27 interacts with TLR2, TLR3 and TLR4, bringing about NF- κ B transcriptional activation and the upregulation of intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1), leading to the secretion of TNF- α , IL-6, IL-8, IL-10, IL-1 β , IL-12p35 and IL-12p40, colony-stimulating factor 2 (CSF2) and vascular endothelial growth factor (VEGF) [49–52]. The release of IL-10 induced by extracellular HSP27 was found to involve the phosphorylation of p38 and MAPKAPK-2, whilst the upregulation of TNF- α was attributed to the activation of both p38 and ERK1/ERK2 signalling pathways [53]. HSP27 was also found to interact with oestrogen receptor- β (ER- β) [54, 55].

In cancer, extracellular Hsp27 has been reported to exert pro-angiogenic effects via the stimulation of the transcription of the vascular endothelial growth factor (VEGF) gene [50]. Increased

VEGF expression promoted HSP27 phosphorylation through the stress-activated protein kinase 2 (SAPK-2)/p38 pathway, resulting in cytoskeletal rearrangements and endothelial cell migration [56]. Furthermore, HSP27 phosphorylation not only reduced the release of HSP27 in the extracellular space, where the released HSP27 binds to and blocks VEGF [20], but also enhanced intracellular VEGF expression by interacting with the TLR3 on endothelial cells [50].

In the context of diabetes, T2DM patients with cardiovascular disease presented no significant change in serum HSP27 than non-diabetic controls [57]. However, extracellular HSP27 levels were found to be inversely correlated to progression, complexity and instability of plaques found in atherosclerotic human coronary arteries [54, 58], with HSP27 secretion being greatly reduced in atherosclerotic lesions and almost absent in complicated plaques [59]. Lower levels of serum HSP27 were described as being predictive of subsequent heart attacks, strokes or cardiovascular death within the following 5 years [60]. Atheroprotection is thought to be mediated through oestrogen (for the extracellular release of HSP27) as well as via modulation of various processes involved in atherosclerosis, such as cholesterol homeostasis and trafficking, regional inflammation (including mobility of immune cells in plaques and macrophage activation into foam cells) and plaque remodelling by extracellular HSP27 [61]. Extracellular HSP27 seems to be involved in reduced lipid engulfment by macrophages and foam cell formation through the blocking and downregulation of macrophage scavenger receptor A [62, 63], as well as the promotion of cholesterol efflux by enhancing ATP-binding cassette (ABC) transporter activity via the TLR4-induced and NF- κ B-mediated release of CSF2 [64]. A similar activation of NF- κ B in endothelial cells via TLR2, TLR3 and TLR4 may further worsen the condition [50, 51]. Moreover, patients with T2DM presented accelerated platelet aggregation correlated with the release of phosphorylated HSP27 from platelets induced by thrombin receptor-activating protein (TRAP) activation of Akt and p38 MAP kinase [65, 66].

3.2. HSP60

Till now, extracellular HSP60 has not been linked to any specific function. What has been explored so far is mostly related to its release mechanism. It has been shown that HSP60 is released into the extracellular space via the exosomal pathway, with most of the HSP60 tightly bound to (as opposed to embedded in) the exosomal membrane, rather than housed in the lumen of the exosomes. Moreover, evidence indicates that exosomal HSP60 is at least in part ubiquitinated (but not poly-ubiquitinated, i.e. not marked for degradation), which might act as a signal for the sorting of HSP60 to exosomes [12]. This ties in with its presence in cancer and T2DM, although the significance of its role in the aetiology or disease progression have not been well investigated.

When looking at the cancer context, tumours often tend to present HSP60 in the cell membrane [67] as well as secreted via exosomes [68]. It is hypothesised that cellular stress results in ubiquitination and possibly other post-translational modifications on cytosolic HSP60, which lead to its localisation in the cell membrane and consequently internalisation via lipid rafts, accumulation in multivesicular bodies and release into the extracellular space via the exosomal pathway [12]. Once secreted (either alone or in conjunction with other biomolecules), it then fulfils an as-yet unspecified but probably immunomodulatory extracellular function [69, 70].

Bioinformatic analysis of colorectal cancer (CRC) pointed at the HSP60 gene as one of the best indicators for diagnosis [71] and proteomic studies have corroborated this finding [72] giving it diagnostic and prognostic value. Similarly, HSP60 has also been found to be linked to Crohn's disease and ulcerative colitis [73], two conditions with a high risk for CRC development, probably having a pro-inflammatory role in the remodelling of the colonic mucosa via a TLR4-ERK-dependent mechanism [74].

Extracellular HSP60 is also thought to play a role in diabetes, as stresses associated with diabetes result in the expression of HSP60 on the cell surface as well as its extracellular release, such that it has been detected in both the serum and the saliva of T2DM patients [75, 76]. Moreover, T2DM patients with cardiovascular disease were associated with higher levels of circulating HSP60 compared to control subjects without cardiovascular disease [77]. Extracellular HSP60 has been associated with the severity of atherosclerosis and has been proposed as a biomarker for coronary heart disease [78, 79].

3.3. HSP70

Extracellular HSP70 has been shown to have important immunostimulatory properties, activating macrophages, monocytes, dendritic cells (DCs) and natural killer (NK) cells, by acting either as a cross presenter of immunogenic peptides via major histocompatibility complex (MHC) antigens, as a chaperone stimulating both innate and adaptive immunities, or as a stimulator and target for innate immune responses mediated by NK cells [35, 80, 81]. In contrast, some studies have shown that it can also have anti-inflammatory effects by activating both immunosuppressive regulatory T cells (Tregs) and Siglec receptors that block the inflammatory process by interacting with TLRs [82]. Moreover, extracellular HSP70 bound to vesicle membranes has been shown to induce an immunosuppressive effect [27], supporting the notion that HSP70 fulfils different roles depending on the composition, source and effector of the vesicles it is associated with.

Apart from immunity, extracellular HSP70 has been implicated in a wide array of conditions including cancer, diabetes, chronic inflammation, cardiovascular disease, hypertension, pre-eclampsia, Alzheimer's disease (inhibiting amyloid β aggregation) and ischemia [3, 83, 84].

When it comes to the cancer setting, serum HSP70 levels have been correlated with treatment response and tumour volume [85], making extracellular HSP70 a potential biomarker for cancer [86] both as a candidate biomarker for tumour detection and monitoring clinical outcome of radiotherapy [87], as well as a prognostic marker, such as in CRC, associated with rapid disease progression and poor survival [88]. In some contexts, extracellular HSP70 has even shown potential in discriminating between infection or inflammation and cancer (e.g. chronic hepatitis, liver cirrhosis and hepatocellular carcinoma) [89]. Extracellular HSP70 has been found to increase MMP9 expression by activating NF- κ B and AP-1 and that the subsequent increase in pro-MMP9 secretion results in enhanced cell motility and invasiveness [90]. HSP70 was also isolated from the surface of tumour-derived exosomes [26], in which setting it can interact with myeloid-derived suppressor cells, so as to suppress T-cell activation and promote cancer development [27]. Extracellular HSP70 has also been used as a cancer vaccine, such that immunisation of mice with a vaccine made of HSP70-peptide complexes extracted

from fusions between DCs and radiation-enriched tumour cells resulted in a T-cell-mediated immune response against radioresistant tumour cells [91].

In vitro experiments of diabetes have shown that extracellular HSP70 plays a role in diabetic nephropathy in T2DM by promoting inflammation in the proximal tubule cells via a TLR4-NF- κ B pathway. HSP70 release induced by the albumin in the proximal tubule cells triggered the overexpression of the inflammatory cytokines monocyte chemoattractant protein 1 (MCP-1), tumour necrosis factor alpha (TNF- α) and macrophage inflammatory protein 2 (MIP2) [92]. Similar results were obtained in diabetic mice where TLR4 deletion or HSP70 inhibition reduced albuminuria and markers of inflammation and tubular injury [92]. Further supporting these findings, patients with T2DM with albuminuria showed higher serum HSP70 levels [93] as well as an association between urinary HSP70 levels and albuminuria [94]. Serum HSP70 was also found to be higher in patients with diabetic retinopathy, together with HIF-1 α compared with subjects without [95] and correlated well with asymmetric dimethylarginine (ADMA) and C-reactive protein (CRP) levels in T2DM patients compared with healthy controls [96].

An inverse association has been reported between levels of HSP70 with the presence and severity of cardiovascular disease [97–100]. Moreover, an inverse correlation was found between HSP70 levels and the risk of future development of atherosclerosis in subjects with established hypertension [101]. Extracellular Hsp70 levels have also been inversely correlated with the risk of cardiovascular disease [97, 101, 102] and the severity and survival after chronic heart failure [103].

3.4. GRP78

Extracellular GRP78 has been documented [104, 105], but it has been studied much more extensively at the cell surface than in the extracellular space or in circulation. GRP78 could be detected in plasma as both full-length and C-terminus fragments [106]. GRP78 is secreted from cells via exosomes, and the release appears to be at least partly controlled by acetylation since the use of histone deacetylase (HDAC) inhibitors could block GRP78 release, causing aggregation in the ER. Suppression of HDAC6 activity leads to GRP78 acetylation, which is then bound to vacuolar protein sorting 34 (VPS34), a class III phosphoinositide-3 kinase, preventing GRP78 from being sorted into multivesicular bodies [107]. Since it has been shown that ER stress can actively promote the expression of GRP78 on the cell surface, and that over-expression of GRP78 can result in similar cell surface localisation, independent of ER stress [102], this might also hold true for extracellular release of GRP78. Once in the plasma membrane GRP78 binds to a wide selection of proteins, which in turn causes signalling cascades through multiple pathways that can result both in cell survival and cell death [108, 109], however the potential interaction or competition of extracellular GRP78 has not been explored. Interestingly, HSP40 (DnaJ) seems to be involved in GRP78 cell surface localisation and silencing of the murine homolog, MTJ-1 abolished cell surface localisation of GRP78 [110], but so far its possible involvement in extracellular release instead has not been investigated.

When looking at cancers, extracellular GRP78 is not commonly investigated; however, some tumours secrete significant levels of GRP78 into the tumour microenvironment [105], and in one study, extracellular GRP78 was identified exclusively in the sera of 28% of gastric cancer

patients but not in healthy controls [111]. It is speculated that ER stress and activation of the unfolded protein response (UPR), an evolutionarily conserved mechanism in which survival or apoptotic pathways are activated in response to ER stress, induce GRP78 in tumour cells leading to increased secretion of GRP78, and by binding to cell surface receptors of endothelial cells, extracellular GRP78 activates ERK and AKT pathways [105].

Useful inferences could be made by looking at cell surface GRP78 which is expressed significantly in human tumours and generally associated with cell proliferation, cell survival, angiogenesis and metastasis [112]. Cell surface GRP78 interacts with α 2-macroglobulin, a plasma protease inhibitor, through its amino-terminal domain-activating the PI3K/Akt, ERK1/ERK2 and p38 MAPK pathways, promoting cell proliferation and cell survival via Akt and NF- κ B signalling cascades, by inducing the UPR [105, 113, 114]. Moreover, interaction of cell surface GRP78 with teratocarcinoma-derived growth factor 1 (TDGF1; Cripto-1), a small, glycosylphosphatidylinositol (GPI)-anchored protein, modulates activin-A, activin-B, nodal and transforming growth factor- β (TGF- β)-dependent signalling of several ligands via the MAPK/PI3K and Smad2/3 pathway and promotes cell proliferation, downregulates E-cadherin (which decreased cell adhesion) and promotes pro-proliferative responses to activin-A and nodal [115, 116]. Of particular interest is that specifically on the surface of cancer cells but not healthy cells, GRP78 interacts via its amino-terminal domain with extracellular prostate apoptosis response 4 (Par-4), which together with tumour necrosis factor-related apoptosis-inducing ligand or Apo 2 ligand (TRAIL/Apo2L) mediates apoptosis via an extrinsic apoptotic pathway (dependent on ER stress and the Fas-associated death domain (FADD)/caspase-8/caspase-3 pathway) [117]. Similarly, plasminogen kringle 5 (K5), an angiogenesis inhibitor, interacts with cell surface GRP78 via the carboxy-terminal domain, on hypoxic and cytotoxic stressed tumour cells, mediating anti-angiogenic and pro-apoptotic activity following the internalisation of GRP78 by the scavenger receptor low-density lipoprotein receptor-related protein 1 (LRP1) and activation of p38 mitogen-activated protein kinase [118, 119].

The isolation of a tumour-specific variant of GRP78 containing an O-linked carbohydrate moiety with a molecular weight of 82 kDa opens up numerous therapeutic possibilities not only of targeting tumours by specific variants of GRP78 [120] but also of searching for the presence of tumour-specific variants in circulation, as a diagnostic marker.

Once again in the context of diabetes, extracellular GRP78 is poorly investigated. However, data from cell surface expression of GRP78 indicates that the extracellular counterpart might play some role in the cardiovascular complications linked to T2DM. GRP78 has been detected on microparticles shed from activated endothelial cells indicating that GRP78 expression may be involved in regulating thrombosis [121]. Expression of cell surface GRP78 in arterial atherosclerotic lesions negatively regulates the initiation of the tissue factor(TF)-mediated coagulation cascade [122, 123], attenuating procoagulant activity similar to the effect observed from the binding of K5 to cell surface GRP78 on stimulated endothelial cells [119]. Atherosclerotic lesions also present an increase in truncated cadherin (T-cadherin) expression, which interacts with cell surface GRP78, similar to the interaction on vascular endothelial cells [124] and on endothelial cells during tumour angiogenesis [125], promoting cell survival and indicating that this interaction plays a role in vascular tissue remodelling related to stress.

3.5. HSP90

As with most other HSPs, extracellular HSP90 has been mainly studied in relation to inflammation and immunity [126]. However, no specific roles, processes or mechanisms have been elucidated yet.

In the context of cancer, extracellular HSP90 (mainly not only HSP90a but also HSP90b) is known to be involved in tumour cell migration, invasion and metastasis [127–131]. Serum levels of extracellular Hsp90a were significantly higher in the patient groups with tumour burden, with a positive correlation with tumour malignancy and metastasis [132]. The interaction of extracellular Hsp90 with the LRP1 receptor as well as HER-2 activates AKT1/AKT2 (in the phosphatidylinositol-3-kinase (PI3K) signalling pathway) and ERK1/ERK2 signalling cascades giving rise to increased cell migration, supporting growth and survival [128, 133, 134]. AKT activation is sustained by the phosphorylation of the receptor tyrosine kinase ephrin type-A receptor 2 (EPHA2), which is a downstream product of the interaction between LRP1 and extracellular Hsp90 [135]. Also, critical for cell migration is the presence of extracellular HSP90 for the interaction between Src and integrin β 1 at focal adhesion points between the cell and ECM [130]. The interaction of extracellular HSP90 with TLR4 also signals through Src, and this transactivates the epithelial growth factor receptor (EGFR), which increases cell migration [136]. It has also been shown that extracellular HSP90 can have a role in ECM remodelling or stabilisation via its direct interaction with fibronectin [137]. Work in colorectal cancer cells showed that extracellular Hsp90 promotes epithelial-to-mesenchymal transition (EMT) via an LRP1-NF- κ B pathway [138], whilst exposure of prostate cancer cells to extracellular Hsp90 promoted EMT via a process requiring both matrix metalloprotein 9 (MMP9) and ERK activity [139]. Extracellular Hsp90 was also shown to interact with MMP2 [140]. The activation of ERK by extracellular Hsp90 has also been shown to increase expression of the polycomb repressor complex methyltransferase enhancer of zeste homologue 2 (EZH2), bringing about the epigenetic repression of E-cadherin [141], further supporting the EMT process.

Extracellular HSP90 has not been studied much in the context of diabetes, with the majority of studies investigating HSP90 inhibition in general and thus focusing on intracellular mechanisms whilst not excluding effects by extracellular HSP90. In response to oxidative stress, vascular smooth muscle cells secrete HSP90a, and the stimulation of these cells by HSP90a induces MAPK activity [142]. Similarly, endothelial cells also secrete HSP90 upon activation, and this stimulates angiogenesis [143]. Experiments in diabetic rats have shown that annexin II on endothelial cells interacts with extracellular HSP90a, modulating plasminogen activation to plasmin [144]. Furthermore, HSP90 levels were found to be higher in the serum of patients with atherosclerosis [145]. Exosomes collected from cultured fibrocytes contained HSP90a (among other biomolecules) and enhanced cellular migration and proliferation as well as secretion of type I collagen (COL1) and type III collagen (COL3) and expression of α -smooth muscle actin (α -SMA) [146]. Inhibition of total HSP90 disrupts the IKK complex [147] and JAK2 protein stability [148], blocking the activity of the transcription factors NF- κ B [149] and STAT [150], respectively, together with a downregulation in the expression of pro-atherogenic cytokines and chemokines. Dysregulated NF- κ B and STAT pathways contribute to diabetic nephropathy [150, 151] and atherosclerosis [152, 153]. The inhibition of HSP90 thus

modulates inflammation and oxidative stress, improving diabetes-associated renal damage and atheroprogession [154], insulin sensitivity [155], high-fat-diet-induced renal failure [156] and diabetic peripheral neuropathy [157].

4. Conclusion

The need to identify biomarkers for complex systemic and chronic diseases is pressing, with an increasing push towards the successful development of therapies aimed at modulating serum levels, blocking receptor binding or inhibiting signalling cascades. HSPs hold great potential as therapeutic targets for those conditions with underlying mechanisms involving accumulation of misfolded or damaged proteins, oxidative stress, altered mitochondrial bioenergetics or dysregulated apoptosis, particularly as a result of their non-chaperoning functions. Studies presented herein suggest that circulating HSP levels may be exploited as biomarkers of such conditions, with cancer and cardiovascular complications linked to T2DM being the contexts used to exemplify.

A major limitation of most studies performed on extracellular HSPs is that their functions and roles in disease have not been elucidated yet. As a result the biochemistry and signalling are investigated very poorly, such as testing for a single downstream product of a complex cascade which can be affected by multiple inputs. Similarly, the PTMs on extracellular HSPs are still in their majority obscure both in abundance and functional significance. Studies conducted retrospectively, on single HSPs in isolation, using small patient groups and without adjustment for confounding effects offer a very poor analysis of the predictive power of HSPs for early diagnosis or prognostic assessment. Thus, in future research, it is important to take into consideration that HSPs do not work in isolation, but act within a network, rather than just detect changes in the total extracellular expression levels of individual HSPs and analyse changes in both total HSP and specific PTMs within groups of chaperone proteins that are functionally relevant to either the development of or resultant from the progression of the condition under investigation. Furthermore, this needs to be performed in large cohorts of well-characterised patients, with prospective validation of promising biomarker panels, if the intent is really their application in a clinical setting.

Conflict of interest

The author declares no conflict of interest.

Author details

Byron Baron

Address all correspondence to: byron.baron@um.edu.mt

Centre for Molecular Medicine and Biobanking, Faculty of Medicine and Surgery,
University of Malta, Msida, Malta

References

- [1] Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress and Chaperones*. 2009;**14**(1):105-111
- [2] Bellini S, Barutta F, Mastrocola R, Imperatore L, Bruno G, Gruden G. Heat shock proteins in vascular diabetic complications: Review and future perspective. *International Journal of Molecular Sciences*. 2017;**18**(12):2709. DOI: 10.3390/ijms18122709
- [3] De Maio A. Extracellular heat shock proteins, cellular export vesicles, and the stress observation system: A form of communication during injury, infection, and cell damage. *Cell Stress and Chaperones*. 2011;**16**:235-249
- [4] Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nature Reviews Immunology*. 2009;**9**:581-593
- [5] Horstman LL, Jy W, Minagar A, Bidot CJ, Jimenez JJ, Alexander JS, Ahn YS. Cell-derived microparticles and exosomes in neuroinflammatory disorders. *International Review of Neurobiology*. 2007;**79**:227-268
- [6] Record M, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. *Biochemical Pharmacology*. 2011;**81**(10):1171-1182
- [7] Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *Journal of Proteomics*. 2010;**73**:1907-1920
- [8] Henderson MC, Azorsa DO. The genomic and proteomic content of cancer cell-derived exosomes. *Frontiers in Oncology*. 2012;**2**:38
- [9] Thery C, Zitvogel L, Amigorena S. Exosomes: Composition, biogenesis and function. *Nature Reviews Immunology*. 2002;**2**(8):569-579
- [10] Bard MP, Hegmans JP, Hemmes A, Luider TM, Willemsen R, Severijnen LAA, van Meerbeeck JP, Burgers SA, Hoogsteden HC, Lambrecht BN. Proteomic analysis of exosomes isolated from human malignant pleural effusions. *American Journal of Respiratory Cell and Molecular Biology*. 2004;**31**(1):114-121
- [11] Admyre C, Johansson SM, Qazi KR, Filén JJ, Laheesmaa R, Norman M, Neve EP, Scheynius A, Gabrielsson S. Exosomes with immune modulatory features are present in human breast milk. *Journal of Immunology*. 2007;**179**(3):1969-1978
- [12] Gupta S, Knowlton AA. HSP60 trafficking in adult cardiac myocytes: Role of the exosomal pathway. *American Journal of Physiology. Heart and Circulatory Physiology*. 2007;**292**(6):H3052-H3056
- [13] Graner MW, Alzate O, Dechkovskaia AM, Keene JD, Sampson JH, Mitchell DA, Bigner DD. Proteomic and immunologic analyses of brain tumor exosomes. *The FASEB Journal*. 2009;**23**(5):1541-1557
- [14] Ogawa Y, Miura Y, Harazono A, Kanai-Azuma M, Akimoto Y, Kawakami H, Yamaguchi T, Toda T, Endo T, Tsubuki M, Yanoshita R. Proteomic analysis of two types of exosomes in human whole saliva. *Biological and Pharmaceutical Bulletin*. 2011;**34**(1):13-23

- [15] Kharaziha P, Ceder S, Li Q, Panaretakis T. Tumor cell-derived exosomes: A message in a bottle. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2012;**1826**(1):103-111
- [16] Kalra H, Adda CG, Liem M, Ang CS, Mechler A, Simpson RJ, Hulett MD, Mathivanan S. Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma. *Proteomics*. 2013;**13**(22):3354-3364
- [17] Vega VL, Rodríguez-Silva M, Frey T, Gehrmann M, Diaz JC, Steinem C, Multhoff G, Arispe N, De Maio A. Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages. *Journal of Immunology*. 2008;**180**:4299-4307
- [18] Calderwood SK, Gong J, Murshid A. Extracellular HSPs: The complicated roles of extracellular HSPs in immunity. *Frontiers in Immunology*. 2016;**7**:159
- [19] Pockley AG. Heat shock proteins, inflammation, and cardiovascular disease. *Circulation*. 2002;**105**:1012-1017
- [20] Lee YJ, Lee HJ, Choi S, Jin YB, An HJ, Kang JH, Yoon SS, Lee YS. Soluble HSPB1 regulates VEGF-mediated angiogenesis through their direct interaction. *Angiogenesis*. 2012;**15**:229-242
- [21] Mambula SS, Calderwood SK. Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes. *Journal of Immunology*. 2006;**177**:7849-7857
- [22] Pilzer D, Fishelson Z. Mortalin/GRP75 promotes release of membrane vesicles from immune attacked cells and protection from complement-mediated lysis. *International Immunology*. 2005;**17**:1239-1248
- [23] Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z. Induction of heat shock proteins in B-cell exosomes. *Journal of Cell Science*. 2005;**118**:3631-3638
- [24] Chaput N, Flament C, Viaud S, Taieb J, Roux S, Spatz A, Andre F, LePecq JB, Boussac M, Garin J, Amigorena S. Dendritic cell derived-exosomes: Biology and clinical implementations. *Journal of Leukocyte Biology*. 2006;**80**:471-478
- [25] Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gill D, Matthiesen R, Valle M, Elortza F, Lu SC, Mato JM, Falcon-Perez JM. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. *Journal of Proteome Research*. 2008;**7**:5157-5166
- [26] Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Research*. 2005;**65**:5238-5247
- [27] Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, Boireau W, Rouleau A, Simon B, Lanneau D, De Thonel A. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *The Journal of Clinical Investigation*. 2010;**120**:457-471

- [28] Evdonin AL, Martynova MG, Bystrova OA, Guzhova I, Margulis B, Medvedeva N. The release of Hsp70 from A431 carcinoma cells is mediated by secretory-like granules. *European Journal of Cell Biology*. 2006;**85**:443-455
- [29] Binder RJ, Han DK, Srivastava PK. CD91: A receptor for heat shock protein gp96. *Nature Immunology*. 2000;**1**:151-155
- [30] Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity*. 2001;**14**:303-313
- [31] Wang Y, Kelly CG, Karttunen JT, Whittall T, Lehner PJ, Duncan L, MacAry P, Younson JS, Singh M, Oehlmann W, Cheng G. CD40 is a cellular receptor mediating mycobacterial heat shock protein 70 stimulation of CC-chemokines. *Immunity*. 2001;**15**:971-983
- [32] Becker T, Hartl FU, Wieland F. CD40, an extracellular receptor for binding and uptake of Hsp70-peptide complexes. *The Journal of Cell Biology*. 2002;**158**:1277-1285
- [33] Floto RA, MacAry PA, Boname JM, Mien TS, Kampmann B, Hair JR, Huey OS, Houben EN, Pieters J, Day C, Oehlmann W. Dendritic cell stimulation by mycobacterial Hsp70 is mediated through CCR5. *Science*. 2006;**314**:454-458
- [34] Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annual Review of Immunology*. 2003;**21**:335-376
- [35] Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK. Novel signal transduction pathway utilized by extracellular HSP70: Role of toll-like receptor (TLR) 2 and TLR4. *The Journal of Biological Chemistry*. 2002;**277**:15028-15034
- [36] Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Hacker H, Wagner H. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *The Journal of Biological Chemistry*. 2001;**276**:31332-31339
- [37] Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H. HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *The Journal of Biological Chemistry*. 2002;**277**:15107-15112
- [38] Theriault JR, Mambula SS, Sawamura T, Stevenson MA, Calderwood SK. Extracellular HSP70 binding to surface receptors present on antigen presenting cells and endothelial/epithelial cells. *FEBS Letters*. 2005;**579**:1951-1960
- [39] PrabhuDas M, Bowdish D, Drickamer K, Febbraio M, Herz J, Kobzik L, Krieger M, Loike J, Means TK, Moestrup SK, Post S. Standardizing scavenger receptor nomenclature. *Journal of Immunology*. 2014;**192**(5):1997-2006
- [40] Delneste Y, Magistrelli G, Gauchat JF, Haeuw JF, Aubry JP, Nakamura K, Kawakami-Honda N, Goetsch L, Sawamura T, Bonnefoy JY, Jeannin P. Involvement of LOX-1 in dendritic cell-mediated antigen cross-presentation. *Immunity*. 2002;**17**:353-362
- [41] Theriault JR, Adachi H, Calderwood SK. Role of scavenger receptors in the binding and internalization of heat shock protein 70. *Journal of Immunology*. 2006;**177**:8604-8611

- [42] Berwin B, Hart JP, Rice S, Gass C, Pizzo SV, Post SR, Nicchitta CV. Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. *The EMBO Journal*. 2003;**22**:6127-6136
- [43] Berwin B, Delneste Y, Lovingood RV, Post SR, Pizzo SV. SREC-I, a type F scavenger receptor, is an endocytic receptor for calreticulin. *The Journal of Biological Chemistry*. 2004;**279**:51250-51257
- [44] Facciponte JG, Wang XY, Subjeck JR. Hsp110 and Grp170, members of the Hsp70 superfamily, bind to scavenger receptor-A and scavenger receptor expressed by endothelial cells-I. *European Journal of Immunology*. 2007;**37**:2268-2279
- [45] Multhoff G. Activation of natural killer cells by heat shock protein 70. *International Journal of Hyperthermia*. 2002;**18**:576-585
- [46] Fong JJ, Sreedhara K, Deng L, Varki NM, Angata T, Liu Q, Nizet V, Varki A. Immunomodulatory activity of extracellular Hsp70 mediated via paired receptors Siglec-5 and Siglec-14. *The EMBO Journal*. 2015;**34**:2775-2788
- [47] Low Wang CC, Hess CN, Hiatt WR, Goldfine AB. Clinical update: Cardiovascular disease in diabetes mellitus. *Circulation*. 2016;**133**:2459-2502
- [48] Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiological Reviews*. 2013;**93**:137-188
- [49] Salari S, Seibert T, Chen YX, Hu T, Shi C, Zhao X, Cuerrier CM, Raizman JE, O'Brien ER. Extracellular HSP27 acts as a signaling molecule to activate NF-kappaB in macrophages. *Cell Stress and Chaperones*. 2013;**18**(1):53-63. DOI: 10.1007/s12192-012-0356-0
- [50] Thuringer D, Jegu G, Wettstein G, Terrier O, Cronier L, Yousfi N, Hébrard S, Bouchot A, Hazoumé A, Joly AL, Gleave M. Extracellular HSP27 mediates angiogenesis through toll-like receptor 3. *The FASEB Journal*. 2013;**27**(10):4169-4183. DOI: 10.1096/fj.12-226977
- [51] Jin C, Cleveland JC, Ao L, Li J, Zeng Q, Fullerton DA, Meng X. Human myocardium releases heat shock protein 27 (HSP27) after global ischemia: The proinflammatory effect of extracellular HSP27 through toll-like receptor (TLR)-2 and TLR4. *Molecular Medicine*. 2014;**20**(1):280-289. DOI: 10.2119/molmed.2014.00058
- [52] Yusuf N, Nasti TH, Huang CM, Huber BS, Jaleel T, Lin HY, Xu H, Elmetts CA. Heat shock proteins HSP27 and HSP70 are present in the skin and are important mediators of allergic contact hypersensitivity. *Journal of Immunology*. 2009;**182**(1):675-683. DOI: 10.4049/jimmunol.182.1.675
- [53] De AK, Kodys KM, Yeh BS, Miller-Graziano C. Exaggerated human monocyte IL-10 concomitant to minimal TNF-alpha induction by heat-shock protein 27 (Hsp27) suggests Hsp27 is primarily an antiinflammatory stimulus. *Journal of Immunology*. 2000;**165**(7):3951-3958. DOI: 10.4049/jimmunol.165.7.3951
- [54] Miller H, Poon S, Hibbert B, Rayner K, Chen YX, O'Brien ER. Modulation of estrogen signaling by the novel interaction of heat shock protein 27, a biomarker for atherosclerosis,

and estrogen receptor beta: Mechanistic insight into the vascular effects of estrogens. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005;**25**(3):e10-e14. DOI: 10.1161/01.ATV.0000156536.89752.8e

- [55] Al-Madhoun AS, Chen YX, Haidari L, Rayner K, Gerthoffer W, McBride H, O'Brien ER. The interaction and cellular localization of HSP27 and ER β are modulated by 17 β -estradiol and HSP27 phosphorylation. *Molecular and Cellular Endocrinology*. 2007; **270**(1-2):33-42
- [56] Sawada J, Li F, Komatsu M. R-Ras inhibits VEGF-induced p38MAPK activation and HSP27 phosphorylation in endothelial cells. *Journal of Vascular Research*. 2016;**52**:347-359
- [57] Park HK, Park EC, Bae SW, Park MY, Kim SW, Yoo HS, Tudev M, Ko YH, Choi YH, Kim S, Kim DI. Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. *Circulation*. 2006;**114**:886-893
- [58] Lapedda AJ, Cigliano A, Cherchi GM, Spirito R, Maggioni M, Carta F, Turrini F, Edelstein C, Scanu AM, Formato M. A proteomic approach to differentiate histologically classified stable and unstable plaques from human carotid arteries. *Atherosclerosis*. 2009;**203**(1):112-118. DOI: 10.1016/j.atherosclerosis.2008.07.001
- [59] Martin-Ventura JL, Duran MC, Blanco-Colio LM, Meilhac O, Leclercq A, Michel J-B, Jensen ON, Hernandez-Merida S, Tuñón J, Vivanco F, Egido J. Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. *Circulation*. 2004;**110**:2216-2219
- [60] Seibert TA, Hibbert B, Chen YX, Rayner K, Simard T, Hu T, Cuerrier CM, Zhao X, de Belleruche J, Chow BJ, Hawken S. Serum heat shock protein 27 levels represent a potential therapeutic target for atherosclerosis: Observations from a human cohort and treatment of female mice. *Journal of the American College of Cardiology*. 2013;**62**(16):1446-1454. DOI: 10.1016/j.jacc.2013.05.041
- [61] Batulan Z, Pulakazhi Venu VK, Li Y, Koumbadinga G, Alvarez-Olmedo DG, Shi C, O'Brien ER. Extracellular release and signaling by heat shock protein 27: Role in modifying vascular inflammation. *Frontiers in Immunology*. 2016;**7**:285
- [62] Rayner K, Chen YX, McNulty M, Simard T, Zhao X, Wells DJ, De Belleruche J, O'Brien ER. Extracellular release of the atheroprotective heat shock protein 27 is mediated by estrogen and competitively inhibits acLDL binding to scavenger receptor-A. *Circulation Research*. 2008;**103**:133-141
- [63] Raizman JE, Chen YX, Seibert T, Hibbert B, Cuerrier CM, Salari S, Zhao X, Hu T, Shi C, Ma X, Simard T. Heat shock protein-27 attenuates foam cell formation and atherogenesis by down-regulating scavenger receptor-A expression via NF- κ B signaling. *Biochimica et Biophysica Acta*. 1831;**2013**:1721-1728
- [64] Pulakazhi Venu VK, Adijiang A, Seibert T, Chen YX, Shi C, Batulan Z, O'Brien ER. Heat shock protein 27-derived atheroprotection involves reverse cholesterol transport that

is dependent on GM-CSF to maintain ABCA1 and ABCG1 expression in ApoE^{-/-} mice. *The FASEB Journal*. 2017;**31**:2364-2379

- [65] Tokuda H, Kuroyanagi G, Tsujimoto M, Enomoto Y, Matsushima-Nishiwaki R, Onuma T, Kojima A, Doi T, Tanabe K, Akamatsu S, Iida H. Release of phosphorylated HSP27 (HSPB1) from platelets is accompanied with the acceleration of aggregation in diabetic patients. *PLoS One*. 2015;**10**:e0128977
- [66] Tokuda H, Kuroyanagi G, Tsujimoto M, Matsushima-Nishiwaki R, Akamatsu S, Enomoto Y, Iida H, Otsuka T, Ogura S, Iwama T, Kojima K. Thrombin receptor-activating protein (TRAP)-activated Akt is involved in the release of phosphorylated-HSP27 (HSPB1) from platelets in DM patients. *International Journal of Molecular Sciences*. 2016;**17**(5):737
- [67] Shin BK, Wang H, Yim AM, Le Naour F, Brichory F, Jang JH, Zhao R, Puravs E, Tra J, Michael CW, Misek DE. Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. *The Journal of Biological Chemistry*. 2003;**278**:7607-7616
- [68] Merendino AM, Bucchieri F, Campanella C, Marcianò V, Ribbene A, David S, Zummo G, Burgio G, Corona DF, de Macario EC, Macario AJ. Hsp60 is actively secreted by human tumor cells. *PLoS One*. 2010;**5**:e9247
- [69] Cohen-Sfady M, Nussbaum G, Pevsner-Fischer M, Mor F, Carmi P, Zanin-Zhorov A, Lider O, Cohen IR. Heat shock protein 60 activates B cells via the TLR4-MyD88 pathway. *Journal of Immunology*. 2005;**175**:3594-3602
- [70] Osterloh A, Kalinke U, Weiss S, Fleischer B, Breloer M. Synergistic and differential modulation of immune responses by Hsp60 and lipopolysaccharide. *The Journal of Biological Chemistry*. 2007;**282**:4669-4680
- [71] Mahata P, Mahata K. Selecting differentially expressed genes using minimum probability of classification error. *Journal of Biomedical Informatics*. 2007;**40**:775-786
- [72] Derijks-Engwegen JY, Cats A, Smits ME, Schellens JH, Beijnen JH. Improving colorectal cancer management: The potential of proteomics. *Biomarkers in Medicine*. 2008;**2**:253-289
- [73] Rodolico V, Tomasello G, Zerilli M, Martorana A, Pitruzzella A, Marino Gammazza A, David S, Zummo G, Damiani P, Accomando S, Conway de Macario E, Macario AJL, Cappello F. Hsp60 and Hsp10 increase in colon mucosa of Crohn's disease and ulcerative colitis. *Cell Stress and Chaperones*. 2010;**15**(6):877-884
- [74] Zhao Y, Zhang C, Wei X, Li P, Cui Y, Qin Y, Wei X, Jin M, Kohama K, Gao Y. Heat shock protein 60 stimulates the migration of vascular smooth muscle cells via Toll-like receptor 4 and ERK MAPK activation. *Scientific Reports*. 2015;**5**:15352
- [75] Yuan J, Dunn P, Martinus RD. Detection of Hsp60 in saliva and serum from type 2 diabetic and non-diabetic control subjects. *Cell Stress and Chaperones*. 2011;**16**(6):689-693
- [76] Wick C. Tolerization against atherosclerosis using heat shock protein 60. *Cell Stress and Chaperones*. 2016;**21**(2):201-211

- [77] Shamaei-Tousi A, Stephens JW, Bin R, Cooper JA, Steptoe A, Coates ARM, Henderson B, Humphries SE. Association between plasma levels of heat shock protein 60 and cardiovascular disease in patients with diabetes mellitus. *European Heart Journal*. 2006; **27**:1565-1570
- [78] Zhang X, He M, Cheng L, Chen Y, Zhou L, Zeng H, Pockley AG, Hu FB, Wu T. Elevated heat shock protein 60 levels are associated with higher risk of coronary heart disease in Chinese. *Circulation*. 2008; **118**:2687-2693
- [79] Pockley AG, Wu R, Lemne C, Kiessling R, de Faire U, Frostegard J. Circulating heat shock protein 60 is associated with early cardiovascular disease. *Hypertension*. 2000; **36**:303-307
- [80] Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nature Medicine*. 2000; **6**:435-442
- [81] Pockley AG, Muthana M, Calderwood SK. The dual immunoregulatory roles of stress proteins. *Trends in Biochemical Sciences*. 2008; **33**:71-79
- [82] Chen G-Y, Brown NK, Wu W, Khedri Z, Yu H, Chen X, Van De Vlekkert D, D'Azzo A, Zheng P, Liu Y. Broad and direct interaction between TLR and Siglec families of pattern recognition receptors and its regulation by Neu1. *eLife*. 2014; **3**:e04066
- [83] Molvarec A, Prohaszka Z, Nagy B, Szaley J, Fust G, Karadi I, Rigo Jr J. Association of elevated serum heat-shock protein 70 concentration with transient hypertension of pregnancy, preeclampsia and superimposed preeclampsia: A case-control study. *Journal of Human Hypertension*. 2006; **20**:780-786
- [84] Evans CG, Wisen S, Gestwicki JE. Heat shock proteins 70 and 90 inhibit early stages of amyloid beta-(1-42) aggregation in vitro. *The Journal of Biological Chemistry*. 2006; **281**:33182-33191
- [85] Gunther S, Ostheimer C, Stangl S, Specht HM, Mozes P, Jesinghaus M, Vordermark D, Combs SE, Peltz F, Jung MP, Multhoff G. Correlation of Hsp70 serum levels with gross tumor volume and composition of lymphocyte subpopulations in patients with squamous cell and adeno non-small cell lung cancer. *Frontiers in Immunology*. 2015; **6**:556
- [86] Pockley AG, Henderson B, Multhoff G. Extracellular cell stress proteins as biomarkers of human disease. *Biochemical Society Transactions*. 2014; **42**:1744-1751
- [87] Bayer C, Liebhardt ME, Schmid TE, Trajkovic-Arsic M, Hube K, Specht HM, Schilling D, Gehrmann M, Stangl S, Siveke JT, Wilkens JJ. Validation of heat shock protein 70 as a tumor-specific biomarker for monitoring the outcome of radiation therapy in tumor mouse models. *International Journal of Radiation Oncology • Biology • Physics*. 2014; **88**(3):694-700
- [88] Kocsis J, Madaras B, Tóth ÉK, Füst G, Prohászka Z. Serum level of soluble 70-kD heat shock protein is associated with high mortality in patients with colorectal cancer without distant metastasis. *Cell Stress and Chaperones*. 2010; **15**(2):143-151

- [89] Gehrmann M, Cervello M, Montalto G, Cappello F, Gulino A, Knape C, Specht HM, Multhoff G. Heat shock protein 70 serum levels differ significantly in patients with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. *Frontiers in Immunology*. 2014;**5**:307
- [90] Lee KJ, Kim YM, Kim DY, Jeoung D, Han K, Lee ST, Lee YS, Park KH, Park JH, Kim DJ, Hahn JH. Release of heat shock protein 70 (Hsp70) and the effects of extracellular Hsp70 on matrix metalloproteinase-9 expression in human monocytic U937 cells. *Experimental & Molecular Medicine*. 2006;**38**:364-374
- [91] Weng D, Song B, Koido S, Calderwood SK, Gong J. Immunotherapy of radioresistant mammary tumors with early metastasis using molecular chaperone vaccines combined with ionizing radiation. *Journal of Immunology*. 2013;**191**:755-763
- [92] Jheng HF, Tsai PJ, Chuang YL, Shen YT, Tai TA, Chen WC, Chou CK, Ho LC, Tang MJ, Lai KTA, Sung JM. Albumin stimulates renal tubular inflammation through an HSP70-TLR4 axis in mice with early diabetic nephropathy. *Disease Models & Mechanisms*. 2015;**8**:1311-1321
- [93] Morteza A, Nakhjavani M, Larry M, Nargesi AA, Esteghamati A. Heat shock protein 70 and albuminuria in patients with type 2 diabetes: A matched case control study. *Cell Stress and Chaperones*. 2013;**18**:815-819
- [94] El-Horany HE-S, Abd-Ellatif RN, Watany M, Hafez YM, Okda HI. NLRP3 expression and urinary HSP72 in relation to biomarkers of inflammation and oxidative stress in diabetic nephropathy patients. *IUBMB Life*. 2017;**69**:623-630
- [95] Sayed KM, Mahmoud AA. Heat shock protein-70 and hypoxia inducible factor-1 α in type 2 diabetes mellitus patients complicated with retinopathy. *Acta Ophthalmologica*. 2016;**94**:e361-e366
- [96] Nakhjavani M, Morteza A, Asgarani F, Khalilzadeh O, Ghazizadeh Z, Bathaie SZ, Esteghamati A. The dual behavior of heat shock protein 70 and asymmetric dimethylarginine in relation to serum CRP levels in type 2 diabetes. *Gene*. 2012;**498**:107-111
- [97] Zhu J, Quyyumi AA, Wu H, Csako G, Rott D, Zalles-Ganley A, Ogunmakinwa J, Halcox J, Epstein SE. Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003;**23**:1055-1059
- [98] Herz I, Rosso R, Roth A, Keren G, George J. Serum levels of anti heat shock protein 70 antibodies in patients with stable and unstable angina pectoris. *Acute Cardiac Care*. 2006;**8**:46-50
- [99] Martin-Ventura JL, Leclercq A, Blanco-Colio LM, Egidio J, Rossignol P, Meilhac O, Michel J-B. Low plasma levels of HSP70 in patients with carotid atherosclerosis are associated with increased levels of proteolytic markers of neutrophil activation. *Atherosclerosis*. 2007;**194**:334-341

- [100] Dulin E, García-Barreno P, Guisasola MC. Extracellular heat shock protein 70 (HSPA1A) and classical vascular risk factors in a general population. *Cell Stress and Chaperones*. 2010;**15**:929-937
- [101] Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegård J. Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. *Hypertension*. 2003;**42**:235-238
- [102] Zhang X, Xu Z, Zhou L, Chen Y, He M, Cheng L, Hu FB, Tanguay RM, Wu T. Plasma levels of Hsp70 and anti-Hsp70 antibody predict risk of acute coronary syndrome. *Cell Stress and Chaperones*. 2010;**15**:675-686
- [103] Genth-Zotz S, Bolger AP, Kalra PR, von Haehling S, Doehner W, Coats A, Volk HD, Anker SD. Heat shock protein 70 in patients with chronic heart failure: Relation to disease severity and survival. *International Journal of Cardiology*. 2004;**96**:397-401
- [104] Delpino A, Castelli M. The 78 kDa glucose-regulated protein (GRP78/BIP) is expressed on the cell membrane, is released into cell culture medium and is also present in human peripheral circulation. *Bioscience Reports*. 2002;**22**(3-4):407-420
- [105] Kern J, Untergasser G, Zenzmaier C, Sarg B, Gastl G, Gunsilius E, Steurer M. GRP-78 secreted by tumor cells blocks the antiangiogenic activity of bortezomib. *Blood*. 2009;**114**:3960-3967
- [106] Laverriere A, Landau R, Charvet I, Irion O, Bischof P, Morales M, Cohen M. GRP78 as a marker of pre-eclampsia: An exploratory study. *Molecular Human Reproduction*. 2009;**15**(9):569-574
- [107] Li Z, Zhuang M, Zhang L, Zheng X, Yang P, Li Z. Acetylation modification regulates GRP78 secretion in colon cancer cells. *Scientific Reports*. 2016;**6**:30406
- [108] Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: A multifunctional receptor on the cell surface. *Antioxidants & Redox Signaling*. 2009;**11**(9):2299-2306
- [109] Wang M, Wey S, Zhang Y, Ye R, Lee AS. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. *Antioxidants & Redox Signaling*. 2009;**11**(9):2307-2316
- [110] Misra UK, Gonzalez-Gronow M, Gawdi G, Pizzo SV. The role of MTJ-1 in cell surface translocation of GRP78, a receptor for alpha 2-macroglobulin-dependent signaling. *Journal of Immunology*. 2005;**174**:2092-2097
- [111] Tsunemi S, Nakanishi T, Fujita Y, Bouras G, Miyamoto Y, Miyamoto A, Nomura E, Takubo T, Tanigawa N. Proteomics-based identification of a tumor-associated antigen and its corresponding autoantibody in gastric cancer. *Oncology Reports*. 2010;**23**:949-956
- [112] Lee AS. GRP78 induction in cancer: Therapeutic and prognostic implications. *Cancer Research*. 2007;**67**:3496-3499

- [113] Shu CW, Sun FC, Cho JH, Lin CC, Liu PF, Chen PY, Chang MD, Fu HW, Lai YK. GRP78 and Raf-1 cooperatively confer resistance to endoplasmic reticulum stress-induced apoptosis. *Journal of Cellular Physiology*. 2008;**215**:627-635
- [114] Misra UK, Deedwania R, Pizzo SV. Activation and cross-talk between Akt, NF- κ B, and unfolded protein response signaling in 1-LN prostate cancer cells consequent to ligation of cell surface-associated GRP78. *The Journal of Biological Chemistry*. 2006;**281**:13694-13707
- [115] Kelber JA, Panopoulos AD, Shani G, Booker EC, Belmonte JC, Vale WW, Gray PC. Blockade of Cripto binding to cell surface GRP78 inhibits oncogenic Cripto signaling via MAPK/PI3K and Smad2/3 pathways. *Oncogene*. 2009;**28**:2324-2336
- [116] Shani G, Fischer WH, Justice NJ, Kelber JA, Vale W, Gray PC. GRP78 and Cripto form a complex at the cell surface and collaborate to inhibit transforming growth factor beta signaling and enhance cell growth. *Molecular and Cellular Biology*. 2008;**28**:666-677
- [117] Burikhanov R, Zhao Y, Goswami A, Qiu S, Schwarze SR, Rangnekar VM. The tumor suppressor Par-4 activates an extrinsic pathway for apoptosis. *Cell*. 2009;**138**:377-388
- [118] Davidson DJ, Haskell C, Majest S, Kherzai A, Egan DA, Walter KA, Schneider A, Gubbins EF, Solomon L, Chen Z, Lesniewski R. Kringle 5 of human plasminogen induces apoptosis of endothelial and tumor cells through surface-expressed glucose-regulated protein 78. *Cancer Research*. 2005;**65**(11):4663-4672
- [119] McFarland BC, Stewart J Jr, Hamza A, Nordal R, Davidson DJ, Henkin J, Gladson CL. Plasminogen kringle 5 induces apoptosis of brain microvessel endothelial cells: Sensitization by radiation and requirement for GRP78 and LRP1. *Cancer Research*. 2009;**69**:5537-5545
- [120] Rauschert N, Brändlein S, Holzinger E, Hensel F, Müller-Hermelink HK, Vollmers HP. A new tumor-specific variant of GRP78 as target for antibody-based therapy. *Laboratory Investigation*. 2008;**88**(4):375
- [121] Banfi C, Brioschi M, Wait R, Begum S, Gianazza E, Pirillo A, Mussoni L, Tremoli E. Proteome of endothelial cell-derived procoagulant microparticles. *Proteomics*. 2005;**5**:4443-4455
- [122] Bhattacharjee G, Ahamed J, Pedersen B, El-Sheikh A, Mackman N, Ruf W, Liu C, Edgington TS. Regulation of tissue factor-mediated initiation of the coagulation cascade by cell surface grp78. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005;**25**:1737-1743
- [123] Liu C, Bhattacharjee G, Boisvert W, Dilley R, Edgington T. In vivo interrogation of the molecular display of atherosclerotic lesion surfaces. *The American Journal of Pathology*. 2003;**163**:1859-1871
- [124] Philippova M, Ivanov D, Joshi MB, Kyriakakis E, Rupp K, Afonyushkin T, Bochkov V, Erne P, Resink TJ. Identification of proteins associating with glycosylphosphatidylinositol-

- anchored T-cadherin on the surface of vascular endothelial cells: Role for Grp78/BiP in T-cadherin-dependent cell survival. *Molecular and Cellular Biology*. 2008;**28**:4004-4017
- [125] Ivanov D, Philippova M, Allenspach R, Erne P, Resink T. T-cadherin upregulation correlates with cell-cycle progression and promotes proliferation of vascular cells. *Cardiovascular Research*. 2004;**64**:132-143
- [126] Basu S, Srivastava PK. Heat shock proteins: The fountainhead of innate and adaptive immune responses. *Cell Stress and Chaperones*. 2000;**5**:443-451
- [127] Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G, Scroggins BT. Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. *Nature Cell Biology*. 2004;**6**:507-514
- [128] Sidera K, Gaitanou M, Stellas D, Matsas R, Patsavoudi E. A critical role for HSP90 in cancer cell invasion involves interaction with the extracellular domain of HER-2. *The Journal of Biological Chemistry*. 2008;**283**:2031-2041
- [129] Li W, Li Y, Guan S, Fan J, Cheng CF, Bright AM, Chinn C, Chen M, Woodley DT. Extracellular heat shock protein-90alpha: Linking hypoxia to skin cell motility and wound healing. *The EMBO Journal*. 2007;**26**:1221-1233
- [130] Tsutsumi S, Scroggins B, Koga F, Lee M-J, Trepel J, Felts S, Carreras C, Neckers L. A small molecule cell-impermeant Hsp90 antagonist inhibits tumor cell motility and invasion. *Oncogene*. 2007;**27**(17):2478-2487
- [131] Suzuki S, Kulkarni AB. Extracellular heat shock protein HSP90 β secreted by MG63 osteosarcoma cells inhibits activation of latent TGF- β 1. *Biochemical and Biophysical Research Communications*. 2010;**398**(3):525-531
- [132] Wang X, Song X, Zhuo W, Fu Y, Shi H, Liang Y, Tong M, Chang G, Luo Y. The regulatory mechanism of Hsp90a secretion and its function in tumor malignancy. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;**106**(50):21288-21293
- [133] Cheng C-F, Fan J, Fedesco M, Guan S, Li Y, Bandyopadhyay B, Bright AM, Yerushalmi D, Liang M, Chen M, Han YP. Transforming growth factor alpha (TGFalpha)-stimulated secretion of HSP90alpha: Using the receptor LRP-1/CD91 to promote human skin cell migration against a TGFbeta-rich environment during wound healing. *Molecular and Cellular Biology*. 2008;**28**(10):3344-3358
- [134] Tsen F, Bhatia A, O'Brien K, Cheng C-F, Chen M, Hay N, Stiles B, Woodley DT, Li W. Extracellular heat shock protein 90 signals through subdomain II and the NPVY motif of LRP-1 receptor to Akt1 and Akt2: A circuit essential for promoting skin cell migration in vitro and wound healing in vivo. *Molecular and Cellular Biology*. 2013;**33**(24):4947-4959

- [135] Gopal U, Bohonowych JE, Lema-Tome C, Liu A, Garrett-Mayer E, Wang B, Isaacs JS. A novel extracellular Hsp90 mediated co-receptor function for LRP1 regulates EphA2 dependent glioblastoma cell invasion. *PLoS One*. 2011;**6**(3):e17649
- [136] Thuringer D, Hammann A, Benikhlef N, Fourmaux E, Bouchot A, Wettstein G, Solary E, Garrido C. Transactivation of the epidermal growth factor receptor by heat shock protein 90 via toll-like receptor 4 contributes to the migration of glioblastoma cells. *Journal of Biological Chemistry*. 2011;**286**(5):3418-3428
- [137] Hunter MC, O'Hagan KL, Kenyon A, Dhanani KCH, Prinsloo E, Edkins AL. Hsp90 binds directly to fibronectin (FN) and inhibition reduces the extracellular fibronectin matrix in breast cancer cells. *PLoS One*. 2014;**9**(1):e86842
- [138] Nagaraju GP, Long T-E, Park W, Landry JC, Taliaferro-Smith L, Farris AB, Diaz R, El-Rayes BF. Heat shock protein 90 promotes epithelial to mesenchymal transition, invasion, and migration in colorectal cancer. *Molecular Carcinogenesis*. 2015;**54**(10): 1147-1158
- [139] Hance MW, Dole K, Gopal U, Bohonowych JE, Jezierska-Drutel A, Neumann CA, Liu H, Garraway IP, Isaacs JS. Secreted Hsp90 is a novel regulator of the epithelial to mesenchymal transition (EMT) in prostate cancer. *Journal of Biological Chemistry*. 2012;**287**(45):37732-37744
- [140] Stellas D, El Hamidieh A, Patsavoudi E. Monoclonal antibody 4C5 prevents activation of MMP2 and MMP9 by disrupting their interaction with extracellular HSP90 and inhibits formation of metastatic breast cancer cell deposits. *BMC Cell Biology*. 2010;**11**:51
- [141] Nolan KD, Franco OE, Hance MW, Hayward SW, Isaacs JS. Tumorsecreted Hsp90 subverts polycomb function to drive prostate tumor growth and invasion. *Journal of Biological Chemistry*. 2015;**290**(13):8271-8282
- [142] Liao D-F, Jin Z-G, Baas AS, Daum G, Gygi SP, Aebersold R, Berk BC. Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells. *Journal of Biological Chemistry*. 2000;**275**(1):189-196
- [143] Song X, Luo Y. The regulatory mechanism of Hsp90 α secretion from endothelial cells and its role in angiogenesis during wound healing. *Biochemical and Biophysical Research Communications*. 2010;**398**:111-117
- [144] Lei H, Romeo G, Kazlauskas A. Heat shock protein 90 α -dependent translocation of annexin II to the surface of endothelial cells modulates plasmin activity in the diabetic rat aorta. *Circulation Research*. 2004;**94**:902-909
- [145] Businaro R, Profumo E, Tagliani A, Buttari B, Leone S, D'Amati G, Ippoliti F, Leopizzi M, D'Arcangelo D, Capoano R, Fumagalli L. Heat-shock protein 90: A novel autoantigen in human carotid atherosclerosis. *Atherosclerosis*. 2009;**207**:74-83
- [146] Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. *Biochemical and Biophysical Research Communications*. 2015;**467**:303-309

- [147] Salminen A, Paimela T, Suuronen T, Kaarniranta K. Innate immunity meets with cellular stress at the IKK complex: Regulation of the IKK complex by HSP70 and HSP90. *Immunology Letters*. 2008;**117**:9-15
- [148] Marubayashi S, Koppikar P, Taldone T, Abdel-Wahab O, West N, Bhagwat N, Caldas-Lopes E, Ross KN, Gönen M, Gozman A, Ahn JH. HSP90 is a therapeutic target in JAK2-dependent myeloproliferative neoplasms in mice and humans. *The Journal of Clinical Investigation*. 2010;**120**:3578-3593
- [149] Hertlein E, Wagner AJ, Jones J, Lin TS, Maddocks KJ, Towns WH, Goettl VM, Zhang X, Jarjoura D, Raymond CA, West DA. 17-DMAG targets the nuclear factor- κ B family of proteins to induce apoptosis in chronic lymphocytic leukemia: Clinical implications of HSP90 inhibition. *Blood*. 2010;**116**:45-53
- [150] Marrero MB, Banes-Berceli AK, Stern DM, Eaton DC. Role of the JAK/STAT signaling pathway in diabetic nephropathy. *American Journal of Physiology. Renal Physiology*. 2006;**290**:F762-F768
- [151] Mezzano S, Aros C, Droguett A, Burgos ME, Ardiles L, Flores C, Schneider H, Ruiz-Ortega M, Egido J. NF- κ B activation and overexpression of regulated genes in human diabetic nephropathy. *Nephrology, Dialysis, Transplantation*. 2004;**19**:2505-2512
- [152] Recio C, Oguiza A, Lazaro I, Mallavia B, Egido J, Gomez-Guerrero C. Suppressor of cytokine signaling 1-derived peptide inhibits Janus kinase/signal transducers and activators of transcription pathway and improves inflammation and atherosclerosis in diabetic mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014;**34**:1953-1960
- [153] Baker RG, Hayden MS, Ghosh S. NF- κ B, inflammation, and metabolic disease. *Cell Metabolism*. 2011;**13**:11-22
- [154] Madrigal-Matute J, López-Franco O, Blanco-Colio LM, Muñoz-García B, Ramos-Mozo P, Ortega L, Egido J, Martín-Ventura JL. Heat shock protein 90 inhibitors attenuate inflammatory responses in atherosclerosis. *Cardiovascular Research*. 2010;**86**:330-337
- [155] Lee JH, Gao J, Kosinski PA, Elliman SJ, Hughes TE, Gromada J, Kemp DM. Heat shock protein 90 (HSP90) inhibitors activate the heat shock factor 1 (HSF1) stress response pathway and improve glucose regulation in diabetic mice. *Biochemical and Biophysical Research Communications*. 2013;**430**:1109-1113
- [156] Zhang HM, Dang H, Kamat A, Yeh CK, Zhang BX. Geldanamycin derivative ameliorates high fat diet-induced renal failure in diabetes. *PLoS One*. 2012;**7**:e32746
- [157] Urban MJ, Pan P, Farmer KL, Zhao H, Blagg BS, Dobrowsky RT. Modulating molecular chaperones improves sensory fiber recovery and mitochondrial function in diabetic peripheral neuropathy. *Experimental Neurology*. 2012;**235**:388-396