Pediatr Nephrol (2016) 31:1561–1570
DOI 10.1007/s00467-015-3297-x

REVIEW

# Heat shock proteins in the kidney

**Rajasree Sreedharan**[1] · **Scott K. Van Why**[1]

Received: 19 August 2015 / Revised: 1 December 2015 / Accepted: 7 December 2015 / Published online: 25 February 2016


**Abstract** Heat shock proteins (Hsps) are essential to cell survival through their function as protein chaperones. The role they play in kidney health and disease is varied. Hsp induction may be either beneficial or detrimental to the kidney, depending on the specific Hsp, type of cell, and context. This review addresses the role of Hsps in the kidney, including during development, as osmoprotectants, and in various kidney disease models. Heat shock transcription factor, activated by a stress on renal cells, induces Hsp elaboration and separately regulates immune responses that can contribute to renal injury. Induced Hsps in the intracellular compartment are mostly beneficial in the kidney by stabilizing and restoring cell architecture and function through acting as protein chaperones. Intracellular Hsps also inhibit apoptosis and facilitate cell proliferation, preserving renal tubule viability after acute injury, but enhancing progression of cystic kidney disease and malignancy. Induced Hsps in the extracellular compartment, either circulating or located on outer cell membranes, are mainly detrimental through enhancing inflammation pathways to injury. Correctly harnessing these stress proteins promises the opportunity to alter the course of acute and chronic kidney disease.

**Keywords** Heat shock proteins · Stress proteins · Heat shock transcription factor · Kidney · Osmoprotectant · Inflammation

✉ Scott K. Van Why
svanwhy@mcw.edu

[1] Pediatrics, Nephrology, Medical College of Wisconsin, 999 N. 92nd St., Suite C510, Milwaukee, WI 53226, USA

## Introduction

Three decades ago, heat shock proteins (Hsps) were identified as fundamental to cell survival through their identified functions as protein chaperones [1]. Thereafter, the role of Hsps has been explored in a variety of in vitro and in vivo models of kidney disease, including in human disease. Initially, it was thought by some investigators that Hsps provided nearly ubiquitous benefit, providing protection against a wide range of insults to the kidney. Subsequent investigations have confirmed benefit provided to renal cells by Hsps in a variety of contexts, but have also found detrimental effects contributed by Hsps in other settings.

Hsps, also called stress proteins, are highly conserved across species and are found in nearly every organism. They are classified according to size and apparent function. Each Hsp family has constitutively expressed members that appear necessary for routine maintenance functions in cells. Inducible Hsps are regulated by heat shock transcription factor (HSF) activation, the most proximal component of the stress response. HSF activation is rapid after a cellular insult, and significant Hsp induction soon follows, within minutes to hours. Stress protein induction was first found in cells after injury caused by heat, anoxia, and a number of toxic agents. Hsps appear to be essential to basic cell function and survival by providing routine assistance in intracellular protein handling and trafficking (see Fig. 1) [1]. The stress protein families most studied in the kidney are the 90-kDa (Hsp90), 70-kDa (Hsp70), and the small Hsp 25/27 class (Hsp25 in rodents, Hsp27 in humans). Other Hsps, including a member of the larger 110-kDa class have been examined in only a couple of isolated studies in the kidney. Each of these Hsps acts as a chaperone to newly formed proteins, allowing proper folding and preventing inappropriate peptide interactions or aggregation. In a like manner, they can assist in the translocation of proteins across intracellular membranes and assist denatured proteins in refolding or reassembling into

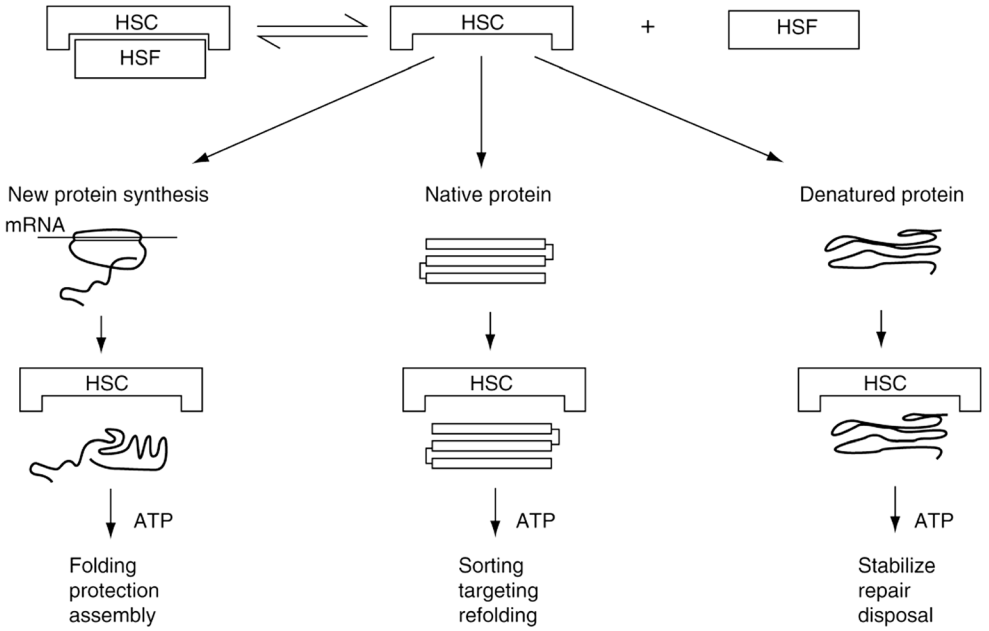


**Fig. 1** Function of heat shock proteins as protein chaperones. Essentially all Hsps act as protein chaperones. Many have ATP binding domains, and hydrolyze ATP to complete the specific protein chaperone function. Constitutively expressed members of each heat shock protein (Hsp) family (shown here as HSC) routinely process nascent cellular proteins during production and movement to final correct configuration and cellular localization. The HSCs can also act to stabilize and repair denatured proteins, or to chaperone them into disposal pathways as needed. Cellular injury increases the demand on HSCs by increasing the quantity of denatured proteins. HSCs, primarily from the 70- and 90-kD families, also reversibly bind the heat shock transcription factor (HSF). HSF is released when HSC demand is increased in injured cells, rapidly activating the stress response for prompt production of all inducible heat shock proteins. The induced Hsps then assist stabilization and recovery of the cell through several mechanisms, as discussed in the text

normal configuration as part of the process of repair (Fig. 1). Specific functions of the stress proteins in the kidney appear to have multiple aspects.

While initial reports seemed to suggest that Hsps were promiscuous in the target of their protective effects in renal cells, subsequent studies have shown that each Hsp interacts with specific intracellular proteins depending on the renal cell type and the insult applied. Furthermore, the initial presumption by some that the stress proteins provide universal benefit, which was fueled by the glut of such reports, was subsequently shown to be a limited view. Depending on the kidney disease model, especially with in vivo models, Hsp induction may, in particular settings, have adverse effects on cell injury and disease progression.

## Hsps in kidney development

For several reasons, it seemed likely that Hsps would prove essential to normal renal development. First, their central function of chaperoning other proteins through a variety of intracellular processes would appear crucial for normal cell differentiation, and by extension to normal organ development. Furthermore, it has long been recognized that stress proteins are critical to developing organisms and that Hsp regulation and expression varies during development [1, 2]. Knockout models of individual constitutively expressed chaperones (Hsp cognates) in primitive and unicellular organisms made them highly susceptible to injury from even mild stress, in some cases proved lethal without an additional stress, and typically caused impaired growth of the organism. So it was reasonable to anticipate that knocking out individual stress proteins or ablating their induction might well cause significant developmental abnormalities of the kidney. However, such has not been reported to date. While not explicitly described, no renal developmental abnormalities have been reported in the few mouse models where individual inducible Hsps have been knocked out.

We specifically examined kidneys in the HSF-1 knockout (KO) mouse for histological signs of abnormal renal development, prior to studying acute kidney injury in this model [3]. HSF-1 is the transcription factor responsible for the rapidly induced expression of all Hsps after a cellular stress is applied. While litters produced lower-than-expected numbers of HSF-1 KO animals compared with the wild type, kidneys from HSF-1 KO animals had no identifiable renal developmental abnormalities on histological examination. Compared to the wild type, the HSF-1 KO mice had equivalent baseline renal expression of Hsp25/27 and Hsp70, but could not induce Hsp further after the stress of ischemia. So, it may be that constitutive expression of the inducible Hsps or their cognates is sufficient for normal renal development, so long as no additional stress is applied in the developing animal.

Nevertheless, Hsp regulation appears to differ during renal development compared with later in life. HSF is constitutively more active in neonatal rat kidneys, which have not completed development, compared with mature animals. This translates into increased constitutive and inducible expression of Hsp70 in the immature rat kidney [4, 5]. Furthermore, the cognate of Hsp70, Hsc70, as well as Hsp90 are constitutively higher, while Hsp60 is lower in immature rat kidneys compared with the adult [6]. Therefore, the various Hsps are differentially regulated in the developing rodent kidney. This finding may speak to the diverse and specific roles that individual Hsps play, including chaperoning other proteins and affecting pathways in cell differentiation, cell cycle, and apoptosis, all pertinent to renal development [7–9]. The overlap and occasional redundancy of function of individual Hsps may also explain why one Hsp knockout can be embryonic lethal while a different Hsp knockout allows for globally normal development, including in the complex development of the kidney. Regardless, as discussed below, the differential regulation of Hsps in the developing kidney appears to contribute significantly to the long-recognized resistance of the immature kidney to injury.

## Osmoprotectant role of stress proteins

The environment of the renal medulla is arguably the most chronically stressed in the human body. Cells in this region not only face chronic relative hypoxia compared with other body compartments, but also are routinely subjected to tenfold variations in extracellular osmolarity. Rapid changes of such magnitude would be uniformly lethal to most mammalian cells, but cells in the renal medulla survive. Since changes in intracellular organic osmolytes in medullary cells may not keep pace with the rapid extracellular changes in osmolarity, it was suspected that other mechanisms protect these cells. It was not surprising, then, when it was found that the constitutive expression of the cytoprotectant proteins Hsp25 and 70 in the rat kidney, and Hsp27 in human kidney, were highest in medullary collecting ducts [10–12].

While constitutively higher levels of Hsps in kidney medullary cells may protect them from their routinely hostile environment, these proteins are also acutely induced by changes in that environment. Vasopressin administration activates HSF and increases Hsp70 specifically in the kidney, in the medulla, an effect prevented by $V_2$ receptor blockade [13]. Hsp70 is induced in response to changes in medullary sodium and urea concentration, and water restriction induces Hsp60 and Hsp25 in the papilla of rat kidneys [14, 15]. Furthermore, impairment of the normal adaptive efflux of organic osmolytes from medullary cells subjected to a hypotonic environment increased the expression of Hsp25 and Hsp70.

Understanding the uniquely stressed environment of the renal medulla afforded the opportunity to discover new stress proteins. These included several putative protein chaperones isolated from inner medullary collecting duct cells in hyperosmolar conditions. The common features of the newly identified proteins included the ability to bind to denatured proteins with release by the addition of ATP, typical characteristics of several Hsps (see Fig. 1). One such protein isolated was identified to be the mitochondrial chaperone mtHsp-70 [16].

Osmotic stress protein 94 (Osp94) was separately discovered and characterized in mouse kidney, primarily in the renal medulla [17]. Its homology to the Hsp70 family, including amino terminal ATP binding domain and C-terminal probable peptide binding domain, made it a suspected additional protein chaperone that could provide protection to osmotically challenged medullary cells. Indeed, Osp94 expression increases in the inner medulla in response to water restriction, and in cultured inner medullary collecting duct cells subjected to hyperosmotic stress, concomitant with increased expression of Hsp70 isoforms and increased phosphorylation of Hsp27 [17–20]. Furthermore, the regulatory region of the Osp-94 gene, along with a tonicity response element, contains the consensus heat shock element (HSE) to which HSF binds [21]. Finally, Osp-94 has been found to be induced in the brain following ischemia, and in the cochlea in an acute endolymphatic hydrops model [22]. These findings, combined with the several other characteristics that this protein shares with previously defined stress proteins, suggests that Osp-94 may play a broader role as a stress protein in response to insults other than merely osmotic, and in other organs than the kidney.

In sum, several stress proteins, both classical Hsps and novel stress proteins first identified in the context of hyperosmotic conditions, appear to protect cells in the highly stressed environment of the renal medulla. Constitutively higher levels, along with further rapid induction of these several stress proteins in response to acute changes in tonicity, appear to work together to provide supplemental protection to cells in the renal medulla beyond that provided by regulation of intracellular organic osmolytes.

## Acute kidney injury

By far the most intensely studied role of inducible Hsps in the kidney has been in models of acute kidney injury (AKI), and mostly in models of ischemic injury. Heat shock transcription factor-1 (HSF-1) activation is rapid after onset of renal ischemia, and significant induction of inducible Hsp72 and Hsp25/27 in the kidney soon follows [10, 23–25]. Furthermore, progressive activation of HSF-1 is tightly linked to progressive alterations in intracellular calcium and disruption of cell structure in renal cells subjected to graded reductions in cellular ATP [26]. Renal tubule cells that suffer loss of structural integrity in AKI regain their normal architecture and polarity through remodeling [27]. Recycling of damaged proteins is

a primary mechanism of cellular repair following ischemia in vivo or ATP depletion in vitro [28, 29]. The chaperone capacity of Hsps may be central to this repair process. Hsp72 binds specifically to disrupted Na/K-ATPase, and Hsp25/27 binds specifically to disorganized actin filaments in renal epithelial cells injured by ischemia or ATP depletion [30–32]. Furthermore, both Hsp72 and Hsp25/27 have been shown to be protective against injury from in vitro ischemia in proximal tubule cells [31–33]. Hsp72 and 25/27 appear to work synergistically to preserve renal cell structure [34].

In addition to the in vitro kidney cell studies, proximal tubule cells isolated from transgenic mice that overexpress human Hsp27 are resistant to hydrogen peroxide injury. However, these same mice are more susceptible to ischemic AKI in vivo [35]. The more severe renal outcome in vivo appeared to be due to increased pro-inflammatory gene expression, along with increased neutrophil and lymphocyte infiltration into the kidney early after ischemia. Selective renal overexpression of Hsp27 protected against ischemic AKI in vivo, in direct contrast to the finding in animals with systemic Hsp27 overexpression [36]. With renal-specific Hsp27 overexpression, protection against AKI was attributed to decreased neutrophil infiltration of kidneys and reduced pro-inflammatory gene expression.

Long recognized by clinicians is that the immature kidney appears to be resistant to insults that produce profound AKI in older patients. A more actively primed stress response may contribute to this phenomenon. As observed in patients, immature rat kidneys are more resistant to an ischemic insult than are their mature counterparts. The neonatal rat kidney has increased constitutive activity of HSF-1 compared with mature animals, with accompanying increased expression of Hsp72 [4, 5]. Blocking HSF-1 function with decoy heat shock element, the target of HSF-1, reduces Hsp72 expression and the tolerance of isolated immature renal tubules to anoxia [4, 5, 37]. Stress proteins, then, appear to be central players in the resistance of immature kidneys to ischemic or anoxic injury.

That these basic science studies might be translatable to the clinical realm was suggested by a study of human neonates. Specific genetic polymorphisms of Hsp72 were found to be associated with neonatal susceptibility to develop AKI. The Hsp72 (1267) GG allele has low inducibility of the Hsp72 protein. Neonates carrying the Hsp72 (1267) GG variation were found to have increased risk of AKI [38, 39].

So, it was widely thought that the cellular stress response regulated by HSF-1 would also be essential to providing resistance against in vivo AKI in mature animals. It was a surprise, then, when it was found that HSF-1 knockout mice, with absent Hsp induction following renal ischemia, are resistant to developing AKI after an ischemic insult [3]. HSF-1 knockout mice were found to have an increased baseline level of immunomodulatory Foxp3+ regulatory T cells resident in the kidney, which appears to prevent development of usual features of AKI. First, the pro-inflammatory environment that develops in wild-type mice kidneys shortly following an ischemic insult does not develop in the HSF-1 knockout. Second, the typical medullary vascular congestion seen in wild-type kidneys after ischemia was nearly absent in the HSF-1 knockout.

Thus, stress proteins regulated by HSF-1 clearly provide protection at the cellular level in renal tubules, but the systemic effect of inducible Hsps may instead be harmful in the setting of an in vivo ischemic insult. Detrimental effects of inducible Hsps, outside of the renal tubule and in the microvasculature, may conflict with their beneficial effects within renal tubule cells subjected to ischemia. The inducible stress response appears to augment an ischemic insult in vivo by facilitating pro-inflammatory mechanisms of renal injury. The most recent studies, then, uniquely connect the rapidly inducible cellular stress response, regulated by HSF-1, to pathways of inflammation in ischemia-induced AKI.

## Hsps in malignancy

Resistance to apoptotic cell death and unregulated cell proliferation are characteristic of malignancy. The finding of increased expression of Hsps in a variety of cancers is suggestive that their role as cytoprotectants may bolster the endurance of malignant cells. Treatment of a renal carcinoma cell line with 17-allylamino-17-demethoxygeldanamycin (17-AAG), an inhibitor of the chaperone function of Hsp90, in combination with the HIV protease inhibitor Ritonavir suppressed expression of Hsp27, 70, and 90 in addition to HSF-1, similar to treatment with siRNA against HSF-1 [40]. Furthermore, suppression of the stress response with this combination of agents resulted in increased apoptosis and decreased proliferation of the renal carcinoma cells. This suggests that overall inhibition of Hsps, through impeding function of HSF-1, is a potential therapeutic approach in renal cell cancer. However, it is not yet evident on which specific pathway, apoptosis or cell proliferation, that altering HSF-1 function has its primary impact in malignant renal cells.

On the other hand, increased immunogenicity of cells in the presence of overexpression of Hsps can be used as a basis for immunotherapy. This could be a strategy for treatment of malignancy. For example, Hsp72 overexpression induced by hyperthermia causes a renal carcinoma cell line to be more susceptible to injury from IL-2 stimulated NK cells [41].

## Hsps in polycystic kidney disease

Similar to the concept that Hsp90 may enhance cell survival and proliferation of malignant cells, supporting the use of anti-Hsp90 agents in cancer treatment, such agents may also be effective in polycystic kidney disease (PKD). Several of the proteins that are effectors of the pathological changes in autosomal dominant

polycystic kidney disease (ADPKD) are also clients of Hsp90. Therefore, it is not surprising that treatment of animals with STA-2842, a specific Hsp90 inhibitor, results in early and late inhibition of renal cyst development in ADPKD models [42].

Primary cilia have a significant role in normal embryonic development and differentiation of kidney epithelia. Ciliary dysfunction has been linked to multiple developmental anomalies, including PKD, and is associated with abnormal chemical and mechanical signals. The actin cytoskeleton mediates many of these signals, and Hsp27 is integral in maintaining normal actin cytoskeletal structure and function. Disruption of primary cilia structure or function, as seen in PKD, is associated with suppression of Hsp27-dependent actin organization, and is restored by overexpression of Hsp27 [43]. Therefore, the role of stress proteins in PKD appears to depend on the specific stress protein studied. Hsp90 function appears to facilitate disease progression in PKD, whereas enhancing Hsp27 expression and function potentially could inhibit development of PKD.

## Tubulointerstitial diseases

Mutations in the gene for Tamm-Horsfall protein (THP) have been described in a group of hereditary tubulointerstitial diseases encompassing familial juvenile hyperuricemic nephropathy, medullary cystic kidney disease type II, and glomerulocystic kidney disease that progress to end-stage renal disease. The mutation results in decreased cytosolic transport of this protein in cells of the thick ascending limb of the loop of Henle to the luminal surface. This effect appears to be associated with decreased cytosolic Hsp70. In vitro studies have restored the transport of THP to the cell surface by chemically increasing the cytosolic abundance of Hsp70 [44]. So, enhancing the function of these protein chaperones could be an important therapeutic approach in THP mutation-associated renal diseases, which have otherwise relatively limited options.

## Glomerular disease

While initial studies focused on Hsp effects in the tubulointerstitial compartment of the kidney, it has become apparent that a variety of Hsps have a role in glomerular disease. In contrast to findings in osmotic stress and AKI, where the actions of Hsps appear to be largely beneficial, their role in glomerular disease is more varied, and have often been implicated to contribute to the disease process. Hsp25/27 has been most studied in models of glomerular disease. Already highly expressed in normal glomerular epithelial cells compared with tubule cells, Hsp25 expression was significantly enhanced further in rat glomeruli in the puromycin aminonucleoside model of nephrotic syndrome [45]. Furthermore, phosphorylation of Hsp25 in glomeruli was also increased after puromycin treatment. In an in vitro model of podocyte injury, Hsp27 protected against puromycin-induced microfilament disruption [46]. Toxic metals also induce and alter phosphorylation of Hsp25/27 in podocytes, along with induction of Hsp70 [47]. Induction of the stress proteins was correlated in this model with resistance to injury from the toxicants. Because of its known function in regulating actin polymerization, these studies suggest a role for Hsp25/27 in the maintenance of normal foot process structure through modulating cytoskeletal changes that occur in glomerular epithelial cells subjected to toxicant injury, or during development of the nephrotic syndrome. Finally, proteomic analysis found that dexamethasone increased the expression of several proteins in cultured podocytes, including Hsp25/27, indicating a potential therapeutic pathway mediated by Hsps in glomerular disease [48].

Heat shock proteins have a diverse effect in glomerulonephritis, which depends on the cause of the lesion and also the class of stress protein involved. In an animal model of proliferative glomerulonephritis, MK2 and MK3 genes mediate an MK2/MK3 genotype-specific stress response within the renal cortex, without a systemic stress response [49]. The study suggested that protein kinases MK2/MK3 regulate the renal stress response, and play a crucial role in development of glomerulonephritis. Hsp60 is known to stimulate T cell-mediated immune response in vitro. In a rat model of immune-mediated glomerulonephritis, Hsp60 administration aggravated the renal pathology [50]. The pathway through which Hsp60 contributes to this detrimental effect is yet to be clarified, but appears to be T-cell-mediated though not associated with a systemic Th1/Th2 shift. Hsp90 is upregulated in mesangial cells in a rat model of mesangial proliferative glomerulonephritis. Hsp90 appears to control the proliferative capacity of mesangial cells by facilitating cell cycle progression, such that upregulated Hsp90 exacerbates the glomerulonephritis [51]. Hsp47 is a collagen synthesis/assembly regulating stress protein. Hsp47 co-expression with collagen is increased in kidneys of patients with diabetic nephropathy and IgA nephropathy compared with control specimens that have minor renal abnormalities [52]. Taken together, these studies indicate that Hsps may be responsible for aggravating glomerulonephritis either via inflammatory or non-inflammatory pathways.

There is a close linkage between the Hsp70-2B allele and HLA haplotype carrying systemic lupus erythematosus (SLE) susceptibility [53]. Hsp90 and its endoplasmic reticulum homologue HSPgp96 are also closely associated with autoimmune diseases including SLE [54, 55]. Hsp90 has been found to be deposited in glomeruli of lupus patients [56]. MRL/lpr mice with lupus nephritis have elevated Hsp90 expression in kidneys [54]. Inhibition of Hsp90 in this model decreased proteinuria, anti-dsDNA titer, and was associated with altered T cell differentiation. This indicates that Hsp90 may well have a direct detrimental effect in facilitating the nephritis in lupus, primarily through immune mechanisms. In the same model, another study

demonstrated that treatment with Hsp10, an innate and adaptive immunity suppressor, improved renal function, albuminuria, and scores of disease activity and chronicity [57]. CD24, the receptor for exogenous Hsps, though an inhibitor of inflammatory cytokines appears to mediate autoimmune diseases [58, 59]. In a mouse model of SLE by forced expression of HSPgp96, deletion of CD24 led to augmented T regulatory cells and decreased nephritis [59]. Thus, CD24 appears to be a key player in SLE-related inflammation mediated by Hsps and should be evaluated as a therapeutic target for autoimmune diseases, including lupus nephritis. Collectively, most Hsps appear to initiate or augment autoimmune and inflammatory responses that aggravate glomerulonephritis, but certain classes, such as Hsp10, can be anti-inflammatory and thereby beneficial.

So in sum, the role that stress proteins play in glomerular disease is not straightforward. In isolated glomerular cell injury as in nephrotic syndrome, the small Hsp25/27 appears to have primarily a beneficial function in preserving podocyte architecture. In glomerulonephritis, however, the story appears more complex. While some Hsps may afford benefit in glomerulonephritis, it appears that most of the stress proteins enhance injurious inflammatory pathways to glomerular injury.

## Hypertension

A role for autoimmunity in contributing to the pathophysiology of essential hypertension was proposed following findings of increased autoantibodies in patients with essential hypertension [60, 61]. Animal studies of salt-sensitive and spontaneous hypertension have found increased renal expression of Hsps and antibodies against Hsp70 in the serum of affected animals, giving rise to the speculation that Hsps play an important but yet undefined pathological role in hypertension [62–64]. Consistent with this hypothesis, patients with hypertension have also been found to have elevated serum anti-Hsp70 and anti-Hsp65 [65]. Identifying the exact pathophysiological role Hsps may play in the development of hypertension might then provide additional targets for treating established or intractable hypertension.

## Chronic kidney disease

The role of heat shock proteins in chronic kidney disease (CKD) need be considered from two aspects. The first aspect is whether a specific Hsp has a role in either the pathophysiology of progression of the disease to CKD or improvement of the disease such that no progression to CKD occurs. The second aspect is whether therapeutic modulation of either the beneficial or detrimental effects of a specific Hsp in a particular disease is possible and might alter the expected course of the disease toward or away from CKD. As such, the etiology of the chronic kidney disease will likely determine the role of Hsps in prevention or augmentation of chronicity. For example, ischemia reperfusion injury induces Hsp25/27, Hsp72, and Hsp90, and each play a role in protecting against injury at a cellular level (see AKI section above). All studies to date that examine the role of these stress proteins in ischemia reperfusion injury are during the acute phase of the disease process, thereby giving no definitive indication of their role during recovery and progression to the chronic phase after the acute insult. The presumption is, if an Hsp provides benefit during the acute phase to limit the injury, that there will accompany later benefit with less progression to chronic kidney disease. The answer, however, may not be so simple.

Selective Hsp72 activation has been shown to slow the development of fibrosis in an animal model of obstructive nephropathy [66]. On the contrary, systemic suppression of Hsp induction following renal ischemia reperfusion injury resulted in decreased inflammatory cells in the kidney after the insult, along with protection against the acute injury (see AKI section above). Since inflammation pathways contribute significantly to the pathophysiology of progression to chronic injury in many disease processes, the immediate cellular benefit provided by induction of the stress proteins might then have detrimental longer-term effects if they then contribute to the resulting inflammation. Pertinent is the different effect Hsps appear to have depending on location. Intracellular Hsps act as protein chaperones and typically are protective, but extracellular or cell membrane-bound Hsps are immunogenic, resulting in an inflammatory response [67].

Several reports indicate that the stress proteins can influence systemic manifestations of chronic kidney disease. Animal studies suggest that Hsp25/27 limits the development of atherosclerosis following subtotal nephrectomy [68]. There is limited research on Hsps in humans with CKD. The profile of serum Hsps and anti-Hsp antibodies along with factors known to predispose to atherosclerosis such as hyperlipidemia, endothelial cell activation, and inflammation have been examined in children with CKD compared with healthy control children. Anti-Hsp60, known to be associated with increased risk for dyslipidemia and coronary disease, is elevated in CKD. Hsp90α levels were also elevated in the serum of CKD patients along with soluble E-selectin, a marker of endothelial dysfunction. The findings of elevated Hsp90α, anti-Hsp60, sE-selectin, and dyslipidemic profile in CKD patients suggest a potential role for these proteins as markers of atherosclerosis in CKD patients [69]. Finally, decreased Hsp72 expression has been described in peripheral monocytes of adult CKD patients. It was suggested that this finding is a manifestation of exhausted adaptive mechanisms, leading to increased apoptosis and poor immunity in CKD patients [70].

Supportive care for severe CKD includes hemodialysis, peritoneal dialysis, and kidney transplantation. Stress proteins have been examined in each of these contexts. Studies of Hsps in hemodialysis focus mainly on Hsp72. These studies demonstrate decreased constitutive expression and also decreased induction of Hsp72 in peripheral monocytes in adult

hemodialysis patients, but not in pediatric patients. In addition, hemodialysis patients had higher anti-Hsp60 levels compared with patients on peritoneal dialysis, suggesting worse long-term effects in patients treated with chronic hemodialysis, especially on the cardiovascular system [69]. Exposure of peritoneal mesothelial cells in vivo to peritoneal dialysis solution causes both disruption of the cytoskeleton and induction of Hsps [71]. Pharmacological manipulation of dialysis solution or heat preconditioning to induce Hsps results in less mesothelial detachment in that same animal model and in an in vitro model, but needs validation in the chronic setting and in humans [71, 72].

Kidney transplantation from deceased donors commonly results in variable levels of ischemic injury to the transplanted kidney. It was found that Hsp72 could be detected in the urine of children shortly after kidney transplantation, but not in the urine of children with normal renal function or with various forms of CKD. Therefore, it appears that Hsp72 could be used as a urinary marker for acute tubule cell injury in patients [73]. While studies remain consistent in showing that at the cellular level Hsp72 and 25/27 have a protective role against various insults, as discussed above, in vivo studies of acute kidney injury show that systemic activation of HSF-1 or overexpression of Hsps is not beneficial against ischemia-induced AKI, likely due to an increased inflammatory response. This latter fact is especially pertinent in the setting of kidney transplantation. Acute induction of stress proteins may be important to the restoration of renal tubule cell architecture disrupted by the ischemia a transplanted kidney suffers, but may then have detrimental effects later. Specifically, local induction of Hsps from the transient renal ischemia, or systemic activation of the broad stress response mediated by HSF-1, may then facilitate immune mechanisms injurious to the kidney, including precipitation of acute allograft rejection. This putative mechanism may then be an explanation for the long-known relationship that a kidney transplant patient who suffers delayed graft function, an ischemia-induced injury to the kidney transplant, has a significantly increased risk of subsequently developing early acute rejection.

## Conclusions

The role that heat shock proteins play in kidney health and disease is varied. Defining the precise localization and specific function of individual stress proteins in the kidney is beginning to clarify why this variation exists, why in certain contexts Hsps are beneficial but in others are harmful to the kidney. On review of the studies outlined above, themes of stress protein function in the kidney are developing (see Fig. 2). HSF-1 activation,

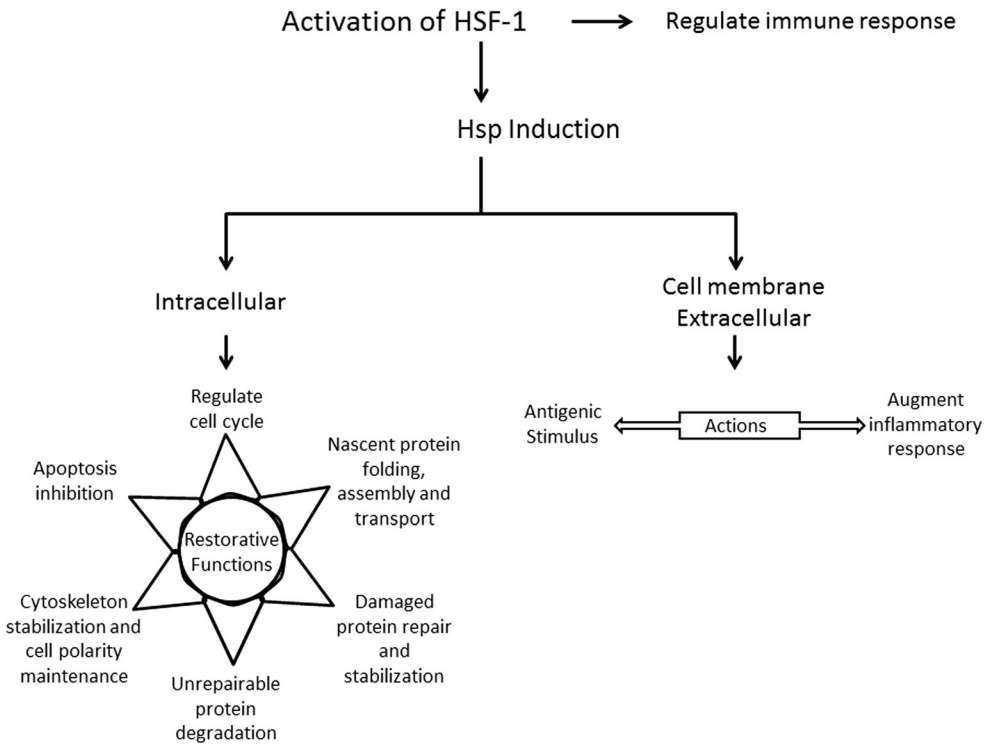


**Fig. 2** The role of stress response in the kidney. Heat shock transcription factor 1 (HSF-1), activated by a variety of cellular insults, induces heat shock protein (Hsp) elaboration and separately regulates immune responses that can contribute to renal injury. Induced Hsps in the intracellular compartment are mostly beneficial in the kidney by stabilizing and restoring cell architecture and function through acting as protein chaperones. Intracellular Hsps also inhibit apoptosis and facilitate cell proliferation, preserving renal tubule viability after acute injury, but enhancing progression of cystic kidney disease and malignancy. Induced Hsps in the extracellular compartment, either circulating or located on outer cell membranes, are mainly detrimental to the kidney through enhancing inflammation pathways to injury

essential to stress response induction, has recently been found separately to regulate immune responses that can lead to detrimental effects in the kidney. Once induced by HSF-1, the role of Hsps appears to be largely driven by location. Hsps located in the intracellular compartment appear mostly to be beneficial. Through their action as protein chaperones, they can stabilize and repair the structure and function of acutely, sublethally injured renal cells. In more severely injured cells, stress proteins can contribute to tubule survival by inhibiting progress to apoptotic cell death, and regulating the cell cycle to facilitate proliferation and repopulation of the nephron. However, while the latter two functions are beneficial in the acute setting, they may be detrimental to patients in the contexts of kidney malignancy and PKD.

Inducible Hsps that are extracellular, either circulating or found on the outer cell membrane, for the most part have been found to be harmful to the kidney. This appears to be from Hsp contribution to injurious inflammatory pathways. Stress proteins may directly augment the broad inflammatory response, or an individual Hsp may be part of the specific antigenic stimulus. The latter mechanism, while harmful in inflammatory and autoimmune kidney disease, might be turned into another tool to treat kidney malignancy.

Harnessing and directing these fundamental cellular proteins, then, promises the opportunity to alter significantly the course of a variety of kidney diseases. Doing so will require continuing to define precisely the individual Hsp function in each specific form of renal disease. As shown in the studies outlined in this review, this clear vision is needed. Blindly inducing or directing these potent proteins may cause harm instead of help, and we must first do no harm.

**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflicts of interest.

## References


1. Nover L (ed) (1991) Heat shock response. CRC Press, Boca Raton, FL
2. Le Masson F, Christians E (2011) HSFs and regulation of Hsp70.1 (Hspa1b) in oocytes and preimplantation embryos: new insights brought by transgenic and knockout mouse models. Cell Stress Chaperones 16:275–285
3. Sreedharan R, Chen S, Miller M, Haribhai D, Williams CB, Van Why SK (2014) Mice with an absent stress response are protected against ischemic renal injury. Kidney Int 86:515–524
4. Gaudio KM, Thulin G, Mann A, Kashgarian M, Siegel NJ (1998) Role of heat stress response in the tolerance of immature renal tubules to anoxia. Am J Physiol 274:F1029–1036
5. Vicencio A, Bidmon B, Ryu J, Reidy K, Thulin G, Mann A, Gaudio KM, Kashgarian M, Siegel NJ (2003) Developmental expression of HSP-72 and ischemic tolerance of the immature kidney. Pediatr Nephrol 18:85–91
6. D'Souza SM, Brown IR (1998) Constitutive expression of heat shock proteins Hsp90, Hsc70, Hsp70 and Hsp60 in neural and non-neural tissues of the rat during postnatal development. Cell Stress Chaperones 3:188–199
7. Kennedy D, Jager R, Mosser DD, Samali (2014) A Regulation of apoptosis by heat shock proteins. IUBMB Life 66:327–338
8. Velichko AK, Markova EN, Petrova NV, Razin SV, Kantidze OL (2013) Mechanisms of heat shock response in mammals. Cell Mol Life Sci 70:4229–4241
9. Verghese J, Abrams J, Wang Y, Morano KA (2012) Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. Microbiol Mol Biol Rev 76:115–158
10. Emami A, Schwartz JH, Borkan SC (1991) Transient ischemia or heat stress induces a cytoprotectant protein in rat kidney. Am J Physiol 260:F479–485
11. Muller E, Neuhofer W, Ohno A, Rucker S, Thurau K, Beck FX (1996) Heat shock proteins HSP25, HSP60, HSP72, HSP73 in isoosmotic cortex and hyperosmotic medulla of rat kidney. Pflugers Arch 431:608–617
12. Khan W, McGuirt JP, Sens MA, Sens DA, Todd JH (1996) Expression of heat shock protein 27 in developing and adult human kidney. Toxicol Lett 84:69–79
13. Xu Q, Ganju L, Fawcett TW, Holbrook NJ (1996) Vasopressin-induced heat shock protein expression in renal tubular cells. Lab Investig 74:178–187
14. Cowley BD Jr, Muessel MJ, Douglass D, Wilkins W (1995) In vivo and in vitro osmotic regulation of HSP-70 and prostaglandin synthase gene expression in kidney cells. Am J Physiol 269:F854–862
15. Medina R, Cantley L, Spokes K, Epstein FH (1996) Effect of water diuresis and water restriction on expression of HSPs-27, -60 and -70 in rat kidney. Kidney Int 50:1191–1194
16. Rauchman MI, Pullman J, Gullans SR (1997) Induction of molecular chaperones by hyperosmotic stress in mouse inner medullary collecting duct cells. Am J Physiol 273:F9–17
17. Kojima R, Randall J, Brenner BM, Gullans SR (1996) Osmotic stress protein 94 (Osp94). A new member of the Hsp110/SSE gene subfamily. J Biol Chem 271:12327–12332
18. Santos BC, Chevaile A, Kojima R, Gullans SR (1998) Characterization of the Hsp110/SSE gene family response to hyperosmolality and other stresses. Am J Physiol 274:F1054–1061
19. Cai Q, Ferraris JD, Burg MB (2004) Greater tolerance of renal medullary cells for a slow increase in osmolality is associated with enhanced expression of HSP70 and other osmoprotective genes. Am J Physiol Renal Physiol 286:F58–67
20. Valkova N, Kultz D (2006) Constitutive and inducible stress proteins dominate the proteome of the murine inner medullary collecting duct-3 (mIMCD3) cell line. Biochim Biophys Acta 1764:1007–1020
21. Kojima R, Randall JD, Ito E, Manshio H, Suzuki Y, Gullans SR (2004) Regulation of expression of the stress response gene, Osp94: identification of the tonicity response element and intracellular signalling pathways. Biochem J 380:783–794
22. Kim CH, Kim YC, Choi BY, Lee HS, Oh SH, Kim YH (2012) Expression of osmotic stress protein 94 in murine endolymphatic hydrops model. Acta Otolaryngol 132(Suppl 1):S118–123
23. Van Why SK, Mann AS, Thulin G, Zhu XH, Kashgarian M, Siegel NJ (1994) Activation of heat-shock transcription factor by graded reductions in renal ATP, in vivo, in the rat. J Clin Invest 94:1518–1523
24. Van Why SK, Hildebrandt F, Ardito T, Mann AS, Siegel NJ, Kashgarian M (1992) Induction and intracellular localization of HSP-72 after renal ischemia. Am J Physiol 263:F769–775

25. Aufricht C, Ardito T, Thulin G, Kashgarian M, Siegel NJ, Van Why SK (1998) Heat-shock protein 25 induction and redistribution during actin reorganization after renal ischemia. Am J Physiol 274: F215–222
26. van Why SK, Kim S, Geibel J, Seebach FA, Kashgarian M, Siegel NJ (1999) Thresholds for cellular disruption and activation of the stress response in renal epithelia. Am J Physiol 277:F227–234
27. Molitoris BA (1991) New insights into the cell biology of ischemic acute renal failure. J Am Soc Nephrol 1:1263–1270
28. Molitoris BA, Dahl R, Hosford M (1996) Cellular ATP depletion induces disruption of the spectrin cytoskeletal network. Am J Physiol 271:F790–798
29. Van Why SK, Mann AS, Ardito T, Siegel NJ, Kashgarian M (1994) Expression and molecular regulation of Na(+)-K(+)-ATPase after renal ischemia. Am J Physiol 267:F75–85
30. Aufricht C, Lu E, Thulin G, Kashgarian M, Siegel NJ, Van Why SK (1998) ATP releases HSP-72 from protein aggregates after renal ischemia. Am J Physiol 274:F268–274
31. Riordan M, Sreedharan R, Wang S, Thulin G, Mann A, Stankewich M, Van Why S, Kashgarian M, Siegel NJ (2005) HSP70 binding modulates detachment of Na-K-ATPase following energy deprivation in renal epithelial cells. Am J Physiol Renal Physiol 288: F1236–1242
32. Van Why SK, Mann AS, Ardito T, Thulin G, Ferris S, Macleod MA, Kashgarian M, Siegel NJ (2003) Hsp27 associates with actin and limits injury in energy depleted renal epithelia. J Am Soc Nephrol 14:98–106
33. Riordan M, Garg V, Thulin G, Kashgarian M, Siegel NJ (2004) Differential inhibition of HSP72 and HSP25 produces profound impairment of cellular integrity. J Am Soc Nephrol 15:1557–1566
34. Sreedharan R, Riordan M, Thullin G, Van Why S, Siegel NJ, Kashgarian M (2011) The maximal cytoprotective function of the heat shock protein 27 is dependent on heat shock protein 70. Biochim Biophys Acta 1813:129–135
35. Chen SW, Kim M, Song JH, Park SW, Wells D, Brown K, Belleroche J, D'Agati VD, Lee HT (2009) Mice that overexpress human heat shock protein 27 have increased renal injury following ischemia reperfusion. Kidney Int 75:499–510
36. Kim M, Park SW, Chen SW, Gerthoffer WT, D'Agati VD, Lee HT (2010) Selective renal overexpression of human heat shock protein 27 reduces renal ischemia-reperfusion injury in mice. Am J Physiol Renal Physiol 299:F347–358
37. Sreedharan R, Riordan M, Wang S, Thulin G, Kashgarian M, Siegel NJ (2005) Reduced tolerance of immature renal tubules to anoxia by HSF-1 decoy. Am J Physiol Renal Physiol 288:F322–326
38. Fekete A, Treszl A, Toth-Heyn P, Vannay A, Tordai A, Tulassay T, Vasarhelyi B (2003) Association between heat shock protein 72 gene polymorphism and acute renal failure in premature neonates. Pediatr Res 54:452–455
39. Vasarhelyi B, Toth-Heyn P, Treszl A, Tulassay T (2005) Genetic polymorphisms and risk for acute renal failure in preterm neonates. Pediatr Nephrol 20:132–135
40. Sato A, Asano T, Ito K (2012) 17-Allylamino-17-demethoxygeldanamycin and ritonavir inhibit renal cancer growth by inhibiting the expression of heat shock factor-1. Int J Oncol 41: 46–52
41. Roigas J, Wallen ES, Loening SA, Moseley PL (1998) Heat shock protein (HSP72) surface expression enhances the lysis of a human renal cell carcinoma by IL-2 stimulated NK cells. Adv Exp Med Biol 451:225–229
42. Seeger-Nukpezah T, Proia DA, Egleston BL, Nikonova AS, Kent T, Cai KQ, Hensley HH, Ying W, Chimmanamada D, Serebriiskii IG, Golemis EA (2013) Inhibiting the HSP90 chaperone slows cyst growth in a mouse model of autosomal dominant polycystic kidney disease. Proc Natl Acad Sci U S A 110:12786–12791
43. Jones TJ, Adapala RK, Geldenhuys WJ, Bursley C, AbouAlaiwi WA, Nauli SM, Thodeti CK (2012) Primary cilia regulates the directional migration and barrier integrity of endothelial cells through the modulation of hsp27-dependent actin cytoskeletal organization. J Cell Physiol 227:70–76
44. Ma L, Liu Y, El-Achkar TM, Wu XR (2012) Molecular and cellular effects of Tamm-Horsfall protein mutations and their rescue by chemical chaperones. J Biol Chem 287:1290–1305
45. Smoyer WE, Gupta A, Mundel P, Ballew JD, Welsh MJ (1996) Altered expression of glomerular heat shock protein 27 in experimental nephrotic syndrome. J Clin Invest 97:2697–2704
46. Smoyer WE, Ransom RF (2002) Hsp27 regulates podocyte cytoskeletal changes in an in vitro model of podocyte process retraction. FASEB J 16:315–326
47. Eichler TE, Ransom RF, Smoyer WE (2005) Differential induction of podocyte heat shock proteins by prolonged single and combination toxic metal exposure. Toxicol Sci 84:120–128
48. Ransom RF, Vega-Warner V, Smoyer WE, Klein J (2005) Differential proteomic analysis of proteins induced by glucocorticoids in cultured murine podocytes. Kidney Int 67:1275–1285
49. Guess AJ, Ayoob R, Chanley M, Manley J, Cajaiba MM, Agrawal S, Pengal R, Pyle AL, Becknell B, Kopp JB, Ronkina N, Gaestel M, Benndorf R, Smoyer WE (2013) Crucial roles of the protein kinases MK2 and MK3 in a mouse model of glomerulonephritis. PLoS One 8:e54239
50. Lang A, Benke D, Eitner F, Engel D, Ehrlich S, Breloer M, Hamilton-Williams E, Specht S, Hoerauf A, Floege J, von Bonin A, Kurts C (2005) Heat shock protein 60 is released in immune-mediated glomerulonephritis and aggravates disease: in vivo evidence for an immunologic danger signal. J Am Soc Nephrol 16: 383–391
51. Pieper M, Rupprecht HD, Bruch KM, De Heer E, Schocklmann HO (2000) Requirement of heat shock protein 90 in mesangial cell mitogenesis. Kidney Int 58:2377–2389
52. Razzaque MS, Kumatori A, Harada T, Taguchi T (1998) Coexpression of collagens and collagen-binding heat shock protein 47 in human diabetic nephropathy and IgA nephropathy. Nephron 80:434–443
53. Pablos JL, Carreira PE, Martin-Villa JM, Montalvo G, Arnaiz-Villena A, Gomez-Reino JJ (1995) Polymorphism of the heat-shock protein gene HSP70-2 in systemic lupus erythematosus. Br J Rheumatol 34:721–723
54. Shimp SK 3rd, Chafin CB, Regna NL, Hammond SE, Read MA, Caudell DL, Rylander M, Reilly CM (2012) Heat shock protein 90 inhibition by 17-DMAG lessens disease in the MRL/lpr mouse model of systemic lupus erythematosus. Cell Mol Immunol 9: 255–266
55. Liu B, Dai J, Zheng H, Stoilova D, Sun S, Li Z (2003) Cell surface expression of an endoplasmic reticulum resident heat shock protein gp96 triggers MyD88-dependent systemic autoimmune diseases. Proc Natl Acad Sci U S A 100:15824–15829
56. Kenderov A, Minkova V, Mihailova D, Giltiay N, Kyurkchiev S, Kehayov I, Kazatchkine M, Kaveri S, Pashov A (2002) Lupus-specific kidney deposits of HSP90 are associated with altered IgG idiotypic interactions of anti-HSP90 autoantibodies. Clin Exp Immunol 129:169–176
57. Kulkarni OP, Ryu M, Kantner C, Sardy M, Naylor D, Lambert D, Brown R, Anders HJ (2012) Recombinant chaperonin 10 suppresses cutaneous lupus and lupus nephritis in MRL-(Fas)lpr mice. Nephrol Dial Transplant 27:1358–1367
58. Sanchez E, Abelson AK, Sabio JM, Gonzalez-Gay MA, Ortego-Centeno N, Jimenez-Alonso J, de Ramon E, Sanchez-Roman J, Lopez-Nevot MA, Gunnarsson I, Svenungsson E, Sturfelt G, Truedsson L, Jonsen A, Gonzalez-Escribano MF, Witte T, Alarcon-Riquelme ME, Martin J (2007) Association of a CD24

gene polymorphism with susceptibility to systemic lupus erythematosus. Arthritis Rheum 56:3080–3086
59. Wang L, Lin S, Rammohan KW, Liu Z, Liu JQ, Liu RH, Guinther N, Lima J, Zhou Q, Wang T, Zheng X, Birmingham DJ, Rovin BH, Hebert LA, Wu Y, Lynn DJ, Cooke G, Yu CY, Zheng P, Liu Y (2007) A dinucleotide deletion in CD24 confers protection against autoimmune diseases. PLoS Genet 3:e49
60. Kristensen BO, Andersen PL (1978) Autoantibodies in untreated and treated essential hypertension. I. Acta Med Scand 203:55–59
61. Cui H, Wang YM, Kuang YZ (1992) Association between essential hypertension and immunology. Zhonghua Nei Ke Za Zhi 31:21–23, **60**
62. Ishizaka N, Aizawa T, Ohno M, Usui Si S, Mori I, Tang SS, Ingelfinger JR, Kimura S, Nagai R (2002) Regulation and localization of HSP70 and HSP25 in the kidney of rats undergoing long-term administration of angiotensin II. Hypertension 39:122–128
63. Bravo J, Quiroz Y, Pons H, Parra G, Herrera-Acosta J, Johnson RJ, Rodriguez-Iturbe B (2003) Vimentin and heat shock protein expression are induced in the kidney by angiotensin and by nitric oxide inhibition. Kidney Int Suppl:S46-51
64. Parra G, Quiroz Y, Salazar J, Bravo Y, Pons H, Chavez M, Johnson RJ, Rodriguez-Iturbe B (2008) Experimental induction of salt-sensitive hypertension is associated with lymphocyte proliferative response to HSP70. Kidney Int Suppl:S55-59
65. Pockley AG, De Faire U, Kiessling R, Lemne C, Thulin T, Frostegard J (2002) Circulating heat shock protein and heat shock protein antibody levels in established hypertension. J Hypertens 20: 1815–1820
66. Mao H, Li Z, Zhou Y, Zhuang S, An X, Zhang B, Chen W, Nie J, Wang Z, Borkan SC, Wang Y, Yu X (2008) HSP72 attenuates renal tubular cell apoptosis and interstitial fibrosis in obstructive nephropathy. Am J Physiol Renal Physiol 295:F202–214
67. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C (2007) Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol 81:15–27
68. Lin YP, Hsu ME, Chiou YY, Hsu HY, Tsai HC, Peng YJ, Lu CY, Pan CY, Yu WC, Chen CH, Chi CW, Lin CH (2010) Comparative proteomic analysis of rat aorta in a subtotal nephrectomy model. Proteomics 10:2429–2443
69. Musial K, Szprynger K, Szczepanska M, Zwolinska D (2010) The heat shock protein profile in children with chronic kidney disease. Perit Dial Int 30:227–232
70. Marzec L, Zdrojewski Z, Liberek T, Bryl E, Chmielewski M, Witkowski JM, Rutkowski B (2009) Expression of Hsp72 protein in chronic kidney disease patients. Scand J Urol Nephrol 43:400–408
71. Endemann M, Bergmeister H, Bidmon B, Boehm M, Csaicsich D, Malaga-Dieguez L, Arbeiter K, Regele H, Herkner K, Aufricht C (2007) Evidence for HSP-mediated cytoskeletal stabilization in mesothelial cells during acute experimental peritoneal dialysis. Am J Physiol Renal Physiol 292:F47–56
72. Bender TO, Bohm M, Kratochwill K, Lederhuber H, Endemann M, Bidmon B, Aufricht C (2010) HSP-mediated cytoprotection of mesothelial cells in experimental acute peritoneal dialysis. Perit Dial Int 30:294–299
73. Mueller T, Bidmon B, Pichler P, Arbeiter K, Ruffingshofer D, VanWhy SK, Aufricht C (2003) Urinary heat shock protein-72 excretion in clinical and experimental renal ischemia. Pediatr Nephrol 18:97–99