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MOLECULAR CHAPERONES IN THE KIDNEY

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■ **Abstract** The normal milieu of the kidney includes hypoxia, large osmotic fluxes, and an enormous amount of fluid/solute reabsorption. Renal adaptation to these conditions requires a host of molecular chaperones that stabilize protein conformation, target nascent proteins to their final intracellular destination, and prevent protein aggregation. Under physiologic or pharmacologic stress, inducible molecular chaperones provide additional mechanisms for repairing or degrading non-native proteins and for inhibiting stress-induced apoptosis. In contrast to intracellular chaperones, chaperones present on the cell surface regulate the immune system and have cytokine-like effects. A diverse range of chaperones and chaperone functions provide the renal cell with an armamentarium of responses to improve the chances of survival.

INTRODUCTION

The normal kidney offers a challenging milieu. Despite a filtration volume of 160–180 liters each day, only 1–2 liters of urine are excreted. Over 98% of the glomerular filtrate must be reabsorbed. The work of solute and fluid reabsorption requires substantial aerobic energy production and extracts a large amount of oxygen from the blood stream. Due in part to the high oxygen extraction ratio and the countercurrent mechanism required for urinary concentration, the medulla operates on the brink of hypoxia (1). In humans the renal inner medullary cells are chronically exposed to a relatively hyperosmotic interstitium, and during changes in hydration state, these cells can experience wide swings in the osmolality ranging from 50 to 1200 mOsm. Thus even under normal circumstances, the kidney experiences a substantial level of stress. The kidney is also exposed to a variety of disease states and adverse challenges such as hypoxia, energy deprivation, and toxins that can compromise cell survival. To adapt to stressful conditions, kidney cells, like cells of all living organisms, utilize

inducible cytoprotective mechanisms involving molecular chaperones or stress proteins.

The stress response has been extensively reviewed elsewhere (2–6). Using information obtained from recent investigations, the present review focuses on the role of molecular chaperones in mediating common physiologic and chemical insults that alter renal epithelial cell function. These insults include ischemia, nephrotoxin exposure, glomerulonephritis, kidney transplantation, and osmotic stress. Individual molecular chaperones that modulate cell injury during stress and/or recovery are discussed with an emphasis on the their mechanism(s) of action.

STRESS PROTEINS: A HISTORICAL PERSPECTIVE

In 1962, Ferruccio Ritossoa observed a characteristic puffing of the specific chromosomes after subjecting isolated *Drosophila* salivary glands to temperature shock (7). Twelve years later, Tissieres and colleagues noted that the de novo synthesis of only six prominent proteins (i.e., heat shock proteins) accounted for 30% of the total new protein synthesis in the salivary glands of heated larvae (8). Subsequent studies characterized many heat shock proteins that are divided into families based primarily on their molecular mass. These include HSP 20–30, HSP 50–60, HSP 70 (including HSP 68–78 kDa), HSP 90, and HSP 100–110 kDa. Stress proteins are arbitrarily designated as constitutively expressed (cognate stress proteins) or stress-inducible, although constitutively expressed HSPs can be modestly up-regulated and inducible HSPs may be constitutively expressed (9). Constitutively expressed stress proteins participate in normal cell maintenance. In contrast, accumulation of inducible stress proteins requires an acute stress.

ROLE OF MOLECULAR CHAPERONES IN NORMAL RENAL FUNCTION

Stress proteins serve diverse functions (Table 1). In the kidney, their best-characterized role is that as a molecular chaperone (Figure 1). As the term implies, a molecular chaperone binds various protein substrates, particularly nascent polypeptides and proteins, in a non-native (denatured) conformation. It is now recognized that the primary amino acid sequence is not sufficient to determine tertiary protein structure. A host of molecular chaperones, present in multiple cellular compartments, work in a coordinated and substrate-specific fashion to produce a protein in its final conformation (10). Chaperones present in the endoplasmic reticulum stabilize and fold peptide intermediates (11). Cytosolic chaperones exert quality control in assuring that nascent proteins achieve their mature conformation. Molecular chaperones also collaborate in the maturation of signal transducing proteins including the steroid receptor (12) and protein kinases (13). To deliver newly

TABLE 1 Stress proteins and their diverse functions

Housekeeping functions
Fold proteins into native conformation
Assemble multimeric complexes
Deliver proteins across organelle membranes (“unfoldase”)
Facilitate degradation of malformed proteins
Uncoat clathrin-coated vesicles (after endocytosis)
Stress functions
Repair denatured proteins
Prevent protein aggregate formation
Facilitate degradation of severely damaged proteins
Deliver replacement proteins to target organelles
Inhibit cell death (apoptosis)
Stabilize the cytoskeleton
Repair nuclear DNA damage
Immune modulating functions
Stimulate immune system (cytokine-like function)
Act as target for generating auto-antibodies
Induce apoptosis

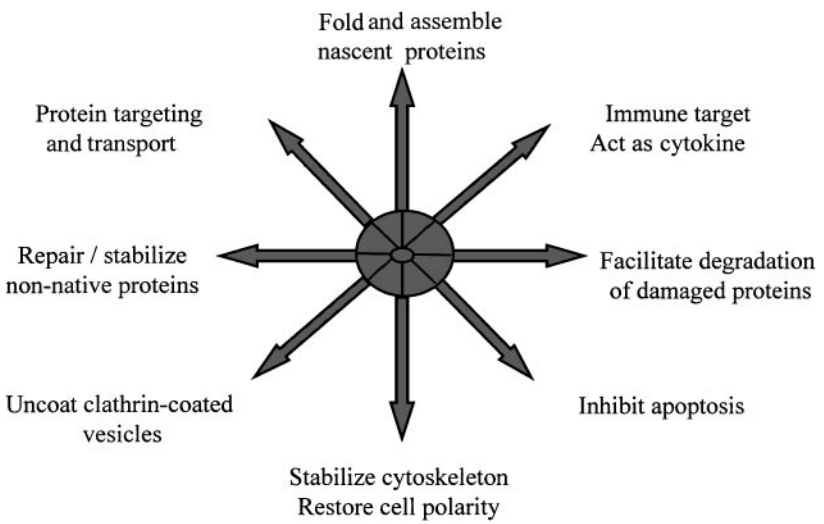

Figure 1 Functional roles of renal molecular chaperones. The roles of stress proteins in the kidney include both chaperone and non-chaperone functions. Constitutively expressed stress proteins fulfill most of these functions under normal circumstances. To enhance resistance to injury and improve cell survival during physiologic or pharmacologic stressors, inducible stress proteins accumulate in the kidney.

synthesized proteins destined for organelles, chaperones maintain peptides in an unfolded state, traffic the peptides across biologic membranes, and then orchestrate re-folding within the lumen (14). Protein complexes made up of multimers are assembled in the cytosol with the assistance of chaperones (10). Most organelles possess unique sets of molecular chaperones. Many of these chaperones coordinate the final re-folding of peptides that cross their membranes (15). This assembly line process assures final delivery of the mature protein to its destination. Importantly, these chaperones also prevent newly synthesized proteins from forming large and potentially toxic aggregates (14, 16–18). The burden of unfolded or denatured proteins appears to be an important sensor for regulating the synthesis of molecular chaperones. In the kidney, chaperones mediate effective binding of mineralocorticoid to its receptor. HSP 90 has been directly implicated in this process (19). Members of the HSP 70 family assist in re-cycling of clathrin-coated vesicles involved in the endocytosis and transport of hormone receptors, glucose, and other molecules (20).

When proteins exist in a non-native state due to an error in synthesis or an adverse stress, molecular chaperones attempt either to refold and repair the non-native protein or to facilitate its degradation (10, 21, 22). The function of stress proteins extends beyond those of a protein chaperone. Intracellular accumulation of stress proteins inhibits apoptosis (23–28). In contrast, expression of the same stress proteins on the cell surface can precipitate cell death (29, 30), activate the immune system (3, 31), or serve as a target for the generation of autoantibodies in systemic autoimmune disease (32–34). These observations demonstrate that the effects of stress proteins are highly variable and may be stress and cell-type specific.

Induction of the Cellular Stress Response

Hundreds of diverse stimuli have been shown to stimulate the induction of molecular chaperones (35). Changes in intracellular pH, temperature, reduced ATP content, hypoxia, infection, cancer, exposure to radiation or toxins, or even non-toxic drugs have all been shown to illicit the stress response. Surprisingly, cold-shock also induces molecular chaperones (4). Although each stimulus could operate through a unique biochemical pathway, several investigators conceptualize protein denaturation as a unifying model for the cellular stress response (16, 36). In this model, misfolded proteins tend to self-aggregate into larger, nonfunctional protein complex proteins or aggresomes (17). This model system appears to be highly consistent with the mechanism for inducing HSP 70 (72 kDa), the best characterized of the inducible stress proteins. Denaturation of intracellular proteins, especially those proteins that tend to form large complexes, is a potent stimulus for up-regulating HSP 72 (37). Constitutively expressed molecular chaperones (e.g., HSP 73 kDa or HSC 70) appear to act as a quality control mechanism for recognizing nascent proteins with normal conformation (10, 14, 38). Several chaperones cooperate with HSP 70 members to stabilize intrinsically unstable folding

intermediates. These co-chaperones include HSP 40, HSP 60, and HSP 90 (10, 39). The presence of both constitutive and inducible chaperones permits a flexible response that exhibits substrate, cell, and stress specificity (40).

When confronted with an increased burden of non-native proteins, the cellular stress response is activated (41). Once induced, molecular chaperones have the onerous task of distinguishing irreparably damaged proteins from those that can be refolded (21). Substantial evidence suggests that selected enzymes can be successfully refolded by molecular chaperones after partial denaturation (21, 42). Proteins that cannot be refolded are destined to be degraded either by lysosomes (38) or by a ubiquitin-mediated proteolysis pathway (22, 43). HSP 70 members have been shown to facilitate both processes of protein degradation (43). Although the consequences of accumulating denatured intracellular proteins is poorly characterized, denatured proteins have the potential to cause injury, referred to as proteotoxicity (16).

The cytoprotection afforded by molecular chaperones represents a potentially elegant and complex system. Renal cells, like the cells of other organs, possess an array of compartment-specific chaperones capable of responding to alterations in protein conformation (44). Both the inducible and constitutively expressed chaperones have numerous isoforms, demonstrate distinct peptide-binding preferences (45), and exhibit stimulus specificity (46). Furthermore, different regions of a single chaperone (e.g., HSP 72) may be critical for mediating cytoprotection against a specific insult (25, 47). In some circumstances, the ATPase region of the chaperone is required to permit high-affinity substrate binding and to mediate release of the repaired peptide, a process referred to as the chaperone function (25). In other cases, non-chaperone domains are equally effective cytoprotectants (25).

In sum, the essential purpose of constitutively expressed molecular chaperones is to assure that nascent polypeptides achieve their native conformation, to target delivery of newly synthesized proteins across biologic membranes and to facilitate the timely degradation of misfolded proteins. In contrast, the inducible molecular chaperones improve the likelihood of cell survival following a noxious insult. A host of diverse renal diseases have been associated with the induction of molecular chaperones (Table 2). The protective effects of molecular chaperones in renal disease are mediated by one or more potential mechanisms (Table 3).

Renal Ischemia/ATP Depletion

Ischemia, often resulting from a transient decrease in blood flow, is a common cause of acquired renal failure. Ischemic renal injury frequently occurs in the presence of hypovolemia, hypotension, sepsis, cardiopulmonary bypass, and during renal transplantation. Both ischemia of the intact kidney and ATP depletion in vitro (a surrogate model for ischemia) result in characteristic changes in renal epithelial cell morphology (48–50). In vivo, the most dramatic changes are observed in epithelial cells within the S_3 segment of the proximal tubule (51). Alone or in combination, collapse of the actin cytoskeleton, loss of cell-cell contact,

TABLE 2 Role of molecular chaperones in renal disease

Disease state	Reference
Glomerulonephritis	(3, 122, 123, 125)
Obstruction	(113, 116, 117)
Interstitial nephritis	(123)
Ischemia	
Nephrotoxins	(111)
Heavy metals	
Cadmium	(73, 153, 154)
Mercury	(106, 109)
Cisplatin	
Iron	(112)
Antibiotics	
Gentamicin	(104, 105, 155)
Hypoxia	(156)
Renal Transplantation	
Ischemia	(87)
Rejection	(136, 137)

and disruption of cell adhesion to the substratum cause epithelial cell dysfunction (52, 53). Cell polarity is disrupted and as a result, Na^+,K^+-ATPase, normally restricted to the basolateral membrane, is re-distributed (54, 55), thereby compromising vectoral solute transport (54). Loss of cell-cell contact permits paracellular backleak, one cause of decreased renal function (53). Intact and/or necrotic cells form casts that obstruct tubular flow and exacerbate renal dysfunction (48, 50). In addition to cytoskeletal and cell contact sites, mitochondria are a primary target for ischemic or ATP depletion-mediated injury (49, 56). Both mitochondrial injury (57) and loss of cell contact sites (58) precipitate apoptosis, a primary form of cell death after transient renal ischemia (28, 59).

TABLE 3 Potential cytoprotective pathways mediated by chaperones

Site of action	Reference
Endoplasmic Reticulum	
Glucose-regulated proteins (GRP78 or BiP)	
Bind misfolded ER proteins	(11, 24)
Promote degradation of misfolded proteins	(38, 69)
Prevent calcium redistribution	(24)
Inhibit oxidant stress	(24)

TABLE 3 (*Continued*)

Site of action	Reference
Calnexin/calreticulin	
Protein folding/assembly	(10)
Maturation of glycoproteins	(10)
Cytosol	
HSP 90	
Translocate cell surface receptors	(10)
Regulate apoptosis	(157)
Assemble enzyme complexes	(10)
HSP 70 (72 and 73 kDa)	
Re-fold damaged proteins	(10)
Prevent protein aggregation	(21)
Facilitate protein degradation	(22)
Replace damaged organelle proteins	(15)
Stabilize actin cytoskeleton	(77, 78)
Stabilize centrosome, microtubules	(158)
Re-establish cell polarity	(55, 159)
Regulate protein kinase(s) (e.g., c-Src)	(234)
Act as a cytokine-like molecule	(31)
Regulate the cell cycle	(160, 161)
Mediate inflammatory response	(111)
Suppress apoptosis	
Inhibit caspase activation	(162)
Prevent apoptosome assembly	(163)
Inhibit JNK activation	(23)
Augment Bcl-2 effect	(28)
Inhibit TNF action	(164)
Inhibit FAS-Fas-ligand effect	(164)
Augment apoptosis in damaged cells	(137)
HSP 47	
Regulate collagen processing and deposition	(104) (125)
HSP 27	
Stabilize cytoskeleton (microfilaments, intermediate filaments)	(158)
Prevent nuclear protein aggregation	(165)
Inhibit apoptosis	(166)
Osmotic stress protein 94	
Molecular protein chaperone?	(149)
Mitochondria	
Mitochondrial HSP 70 (mt70)	(44)
Protein translocation into mitochondria	(167)
Chaperonin 60 (cpn60)	
Protein translocation into mitochondria	(15, 158)
Fold proteins into native conformation	(15)
Re-fold damaged proteins	(42)

Substantial evidence suggests that molecular chaperones participate in ischemic injury and repair. Within hours after even brief renal ischemia, enhanced expression of several molecular chaperones, including the cytoprotectant protein HSP 72, is observed (60). HSP 72 is the major inducible molecular chaperone found in virtually all mammalian cells. HSP 72 localizes to the cytosol in quiescent cells but rapidly accumulates in nucleoli after stress (61), a pattern observed in most eukaryotic cells (62). During recovery from ATP depletion, HSP 72 translocates to the cytosol where its content exceeds the pre-stress level (63). Expression of HSP 72 is most robust in the inner medulla (60), the region of the kidney least susceptible to ischemic injury (51). Increased expression of HSP 72 is likely to be precipitated by stress-induced aggregation of cytoskeletal proteins including actin (36). ATP depletion (perhaps by perturbing protein conformation) has been shown to stimulate HSF-1, a major transcriptional factor that regulates HSP 72 expression (64). Akcetin and colleagues recently demonstrated that two HSP-70 genes coding for HSP 72 respond to renal ischemia. HSP 70-1 increased after brief renal ischemia, whereas HSP 70-2 increased after more prolonged ischemic stress (65), suggesting that the kidney has a sophisticated stress response system. In order to respond to cell stress, it is likely that HSP 72 requires co-chaperones and regulatory factors including HSP 40 (66), BAG-1 (67), Hip-Hop (12), and others. Although less robust than the HSP 72 response, renal ischemia alters the expression and/or intracellular distribution of other chaperones such as HSP 25, HSP 73, HSP 90, and GRP 78 (BiP).

A host of resident molecular chaperones in the endoplasmic reticulum (ER) assist with routine protein folding (68). After stress, some of these chaperones are up-regulated. GRP 78, an ER member of the HSP 70 family, is induced by stressors that precipitate protein denaturation (69). Interestingly, selective induction of ER chaperones using tunicamycin or A23187 protects against subsequent cell membrane injury caused by ATP depletion (70). These important observations demonstrate that perturbations in protein conformation accompany ischemic stress and that molecular chaperones in the ER are key mediators of protein repair.

HSP 25, an actin-stabilizing protein, normally localizes to the brush border of cortical renal tubules. After transient ischemia, HSP 25 accumulates in a cell fraction of kidney homogenates that also contains cytoskeletal proteins (71). This finding was substantiated by the observation that HSP 25 is constitutively expressed in the kidney in a detergent-soluble protein fraction but reversibly accumulates in the detergent-insoluble fraction during 45 min of ischemia (72). Under basal conditions, HSP 25 co-localized with microfilamentous actin in the brush border but exhibited a diffuse cytosolic pattern similar to the distribution of actin aggregates during ischemia and recovery, supporting the hypothesis that HSP 25 participates in stabilizing or repairing the actin cytoskeleton after an ischemic insult (71, 72). After ischemia in vivo, HSP 25 and α-B crystallin, another small molecular weight HSP, accumulate in the cytosol of the proximal tubule (73).

In contrast to the rapid and dramatic changes in HSP 25 and HSP 72 that accompany renal ischemia, HSP 73 and HSP 90 do not exhibit marked alterations in

distribution or expression. No acute changes in HSP 73 or 90 were observed in ATP-depleted renal epithelial cells (63). In the intact kidney, HSP 73 modestly increased 3–6 days after ischemia, whereas HSP 90 accumulation peaked during days 5–7, suggesting that these HSPs may participate in the renal repair process (74). HSP 90 operates much like HSP 72 with regard to its dependence upon ATP hydrolysis to function as a chaperone (75). HSP 90 is often found in complexes that contain HSP 70, suggesting that the two chaperones cooperate in repairing non-native proteins (39, 40). HSP 90 also exerts an important role in the maturation process of protein tyrosine kinases including c-Src (13). c-Src regulates the assembly of key structural proteins that maintain cell contact sites (13). The role of HSP 90 in modulating protein kinase activity after ischemic stress has not been reported.

Elegant studies showed that HSP 70 members have three distinct functional domains: a C-terminal peptide recognition and binding site, a short linking sequence, and an N-terminal ATPase domain (76). Tsang generated a novel hypothesis that the ATPase domain of HSP 70 binds to and stabilizes actin (77). Proximity between HSP 70 and actin in oxidant-stressed cells has been shown using electron microscopy (78). In renal and non-renal cells, a variety of stressors precipitate the formation of large cytosolic aggregates that contain actin, structural proteins, and HSP 72 (36, 79). In renal epithelial cells, prior heat stress, sufficient to induce HSP 72, ameliorates collapse of the actin cytoskeleton during ATP depletion (53). Either as a result of improved actin stability or via an independent mechanism, accumulation of HSP 72 is also associated with improved integrity of the tight junction (53). A recent observation suggests that HSP 72 may interact with c-Src in ATP-depleted renal cells (80). By mediating the activity or distribution of protein kinases such as c-Src, HSP 72 could alter protein tyrosine phosphorylation of key regulatory proteins that mediate cell contact sites (81). Changes in cell-cell and cell-substrate adhesion have important implications in the pathogenesis of acute renal failure. HSP 72 may assist in the restoration of epithelial cell polarity after an ischemic insult. Na^+,K^+-ATPase, a membrane protein responsible for vectoral solute transport, is lost from the basolateral cell surface when integrity of the junctional complex is compromised (55). HSP 72 co-localizes with the Na^+,K^+-ATPase and appears to facilitate its re-insertion into the proper target membrane in an ATP-dependent manner (79). Together, these studies suggest that HSP 72 is important for stabilizing and repairing cell structures that are critical for maintaining epithelial cell function. A recent study has also implicated HSP 25 in preserving microfilamentous actin in the post-ischemic rat renal cortex (72).

Renal cells subjected to ischemia or ATP depletion undergo apoptosis (57–59). Maneuvers that increase HSP 72 content inhibit apoptotic cell death after diverse stresses (23, 25, 28, 41, 82, 83). HSP 72 inhibits Jun N-terminal kinase (JNK), a stress-activated kinase, from initiating the apoptotic pathway in non-renal cells subjected to heat stress (23). A similar effect of HSP 72 was shown after proteasome inhibition, another cause of apoptosis (26). The ATPase domain appears to be unnecessary for prevention of JNK-mediated apoptosis after UV irradiation or exposure to interleukin-1, suggesting that cytoprotection is independent of protein

re-folding (47). Recent work by Meriin et al. showed that stress-induced activation of JNK is caused by the inhibition of the phosphatases that dephosphorylate and deactivate JNK (41). HSP 72 antagonizes the effect of stress on phosphatase activity, thereby promoting the deactivation of JNK (41). The anti-apoptotic effects of HSP 72 are not limited to JNK inhibition; however, the chaperone function of HSP 72 is required for inhibiting the activation of pro-apoptotic proteases (procaspases 9 and 3) after lethal heat stress (25). In vitro, HSP 70 also interferes with the assembly of the apoptosome, a complex comprised of cytochrome *c*, apoptosis-activating factor (APAF-1), and procaspase 9 (83). In ATP-depleted renal epithelial cells, HSP 72 binds Bcl-2, an anti-apoptotic protein and increases the Bcl-2:BAX ratio (28), a determinant of the apoptotic setpoint or rheostat in stressed cells. Importantly, the cellular distribution of HSPs (such as HSP 70) could determine a cell's fate. Up-regulation of HSP 70 in the cytosol or nucleus appears to be cytoprotective, whereas expression on the cell's surface may initiate cell destruction by the immune system (3, 29). In addition to HSP 70, the potential contribution of the small HSPs (e.g., HSP 25–27) as regulators of the apoptotic pathway has recently been recognized (84).

To date, attempts to enhance resistance to ischemic injury in the intact kidney by up-regulating HSP 72 in situ have been inconsistent. Joannidis and colleagues failed to show protection from tubular necrosis in the isolated perfused kidney after subjecting the intact rat to whole-body hyperthermia (85). Although renal ischemia in the intact rat protected against subsequent hypoxic damage in a suspension of proximal tubule, the resistance to injury did not correlate with HSP 72 content (86). In contrast, others showed that subjecting the isolated kidney to hyperthermia prior to transplantation increases HSP 72 and decreases ischemic injury (87). In myocardial cells, HSP 72 accumulation affords consistent cytoprotection against ATP depletion and ischemia. In addition, selective overexpression of HSP 72 protected against cardiac ischemia in a transgenic mouse (88). Pharmacologic approaches have been attempted to induce stress proteins and improve cytoresistance to ischemic injury. Bimoclomol, an hydroxylamine derivative, increases expression of HSPs without significant toxicity and protects mice against ischemic tissue injury (89). Nontoxic methods for inducing molecular chaperones in the kidney hold promise for improving renal epithelial resistance to anticipated episodes of ischemia.

Nephrotoxin Exposure

Acute renal failure is a well-recognized complication of exposure to intravenous contrast agents, nephrotoxic antibiotics, heavy metals, or a variety of chemotherapeutic agents (e.g., cisplatin). The mechanisms of cytotoxicity appear to differ for these insults. Intravenous contrast agents may be directly toxic to renal epithelial cells (90) and can precipitate vasoconstriction, an important cause of cellular ischemia (91). Heavy metals target the mitochondria (92) and cause protein denaturation (93). Cisplatin induces both renal epithelial cell apoptosis and necrosis in a dose-dependent manner (94, 95).

Although cisplatin-induced apoptosis has been assumed to be the direct result of DNA damage (96, 97), other pathways may contribute to cell death. Activation of the Fas/Fas-L system by cisplatin can promote apoptotic renal cell death (98). Cisplatin activates interleukin-1 β-converting enzyme (ICE) proteases in the apoptotic cascade (99). Recently, cisplatin-mediated phosphorylation of α-adducin, an actin-capping protein, was shown to precede caspase activation (100). Once activated, however, caspase 3 led to irreversible cleavage of α-adducin. This observation suggests that the actin stress fibers and focal adhesions might be additional target sites for cisplatin-mediated apoptosis in renal cells (100). Although HSP 72 content did not increase after exposure to cisplatin, the distribution to the nucleus was dramatically altered in HeLa cells (97). Several studies demonstrated that molecular chaperones increase cytoresistance to nephrotoxic injury. In noncancerous renal cells, resistance to cisplatin-induced cell death was observed in transfected epithelial LLC-PK1 cells that overexpressed HSP 72 (103). The ability of HSP 72 to regulate cisplatin-induced apoptosis is not surprising given that HSP 72 inhibits multiple steps in the apoptotic pathway (27, 28, 41). An increase in HSP 70 content may alter the prognosis for renal cell carcinomas (101), perhaps by altering the sensitivity of cancer cells to chemotherapeutic drugs (102).

Exposure to gentamicin, a nephrotoxic antibiotic known to cause acute tubular necrosis, induces the expression of renal molecular chaperones. Increased expression of HSP 47 and HSP 73 was observed in rat kidneys after subcutaneous injection of gentamicin (104). Accumulation of HSP 47 was maximal at day 3, several days after the appearance of ATN. In this same study, HSP 47 immunostaining was most abundant in the tubular epithelial cells and interstitial cells in the regions of collagen III deposition, suggesting that HSP 47 may participate in interstitial repair or fibrosis (104). In contrast, HSP 73 rapidly accumulated within lysosomes of damaged proximal tubular epithelial cells after gentamicin exposure (105), suggesting that this chaperone may facilitate lysosomal protein degradation.

Heavy metals such as mercuric ($HgCl_2$) or cadmium chloride ($CdCl_2$) cause marked toxicity to renal cells. Heavy metals also induce the synthesis of molecular chaperones. Although heavy metals cause protein denaturation (93), a potent stimulus for chaperone induction (37), other events may contribute. Exposure of LLC-PK1 cells to $HgCl_2$ generates a substantial oxidant stress from endogenous hydrogen peroxide that originates, at least in part, from damaged mitochondria (106). In addition to mitochondrial injury, the lysosomal proton gradient required for normal protein degradation is disrupted (106). The protective effect afforded by overexpressing anti-apoptotic genes of the Bcl-2 family against $CdCl_2$ injury suggests a central role of mitochondrial injury caused by $CdCl_2$ (106, 107). As a response to either mitochondrial injury or the protein-damaging effects of hydrogen peroxide, molecular chaperones are induced. An increase in the de novo synthesis of both HSP 72 and HSP 90 was observed in slices of rat kidney after heavy metal exposure (108). Even a single dose of $HgCl_2$, sufficient to induce only single epithelial cell necrosis, induced HSP 72 in the rat renal cortex (109).

However, accumulation of HSP 72 was most abundant in undamaged distal convoluted cells, making its role in proximal tubular cell injury less clear in this study. Other investigators detected HSP 65 and HSP 72 in cortical tubules with the most overt histologic injury (110). In the later study, immunoelectron microscopy of severely damaged cells showed abundant HSP 65 in mitochondria and nucleoli, whereas HSP 72 was overexpressed in the cytoplasm, mitochondria, lysosomes, the cytoskeleton, and in the nucleus (110). Activation of T lymphocytes may also contribute to the inflammatory process that causes chronic interstitial nephritis. After prolonged exposure to cadmium, kidney-derived T cells were capable of inducing interstitial nephritis after passive transfer to cadmium-exposed mice before the onset of overt nephritis (111). These investigators suggested that HSP 70 is an important target for T cell–mediated inflammation and chronic renal injury. Increases in HSP 90 within the renal proximal renal tubule have also been reported after exposure to toxic doses of iron (112).

Obstructive Nephropathy

Urinary tract obstruction is a common cause of both acute and chronic renal dysfunction. Obstruction produces interstitial renal injury with inflammation and promotes chronic apoptosis with progressive renal failure and interstitial fibrosis (113–115). Unilateral obstruction in the rat is associated with an increase in HSP 72 only in the obstructed kidney (113, 116, 117), possibly resulting from localized renal oxidant stress kidney (116). In addition, HSP 47, a collagen-binding stress protein, accumulates in the mouse kidney after unilateral obstruction (118). HSP 47 mRNA expression is increased within 12 h of acute obstruction (118). In this study, administration of either an AII receptor antagonist or an ACE inhibitor decreased HSP 47 and type I collagen mRNA levels by ~60% and prevented interstitial fibrosis. These observations support the hypothesis that interstitial fibrosis after urinary tract obstruction involves molecular chaperones and may be amenable to therapeutic treatment.

Glomerulonephritis (GN) and Immune-Mediated Injury

Glomerular injury represents a balance between insults delivered by infiltrating leukocytes and platelets that elaborate damaging cytokines, eicosanoids, complement, and oxygen radical species and the ability of the glomerulus to resist injury (119). Noxious stimuli result in vascular injury, alterations in basement membrane composition, damage the glomerular epithelial cell and podocytes, stimulate cell proliferation and, ultimately, precipitate glomerular fibrosis.

Stress proteins may be an important component of resistance to glomerular injury. The presence of constitutive and inducible stress proteins has been demonstrated in animal kidneys with experimental GN (120–122). An increase in stress proteins was detected in human kidneys with various forms of acute GN (120, 123–125). Proteinuria, a potential cause of renal tubular injury that often accompanies GN, increases the expression of HSP 72 (123). The presence of protein in the

tubular lumen was increased with pro-apoptotic stimuli, including tumor necrosis factor (TNF), a potent initiator of apoptosis (126). In non-renal cells, the selective overexpression of HSP 72 blocks TNF-induced cell death (127).

Stress proteins have also been implicated in the pathogenesis of autoimmune diseases including systemic lupus erythematosis. In the kidney, molecular chaperones may serve as antigenic targets for immune-mediated injury (111, 128, 129). An allele of HSP70 has been linked to the human leukocyte antigen (HLA) haplotypes that are associated with increased susceptibility to SLE in a Spanish population (34). The pathogenic significance of this finding awaits further clarification.

Increasing evidence implicates HSP 70, HSP 90, and HSP 110 members as potential antigen targets for cell-mediated inflammation (3, 111, 129). Recent investigations also support a possible role for HSP 47, 65, 70, and 90 in the pathogenesis of some forms of glomerulopathy (122, 123, 125). Work by Warr and colleagues suggests that cross reactivity between microbial and human HSPs may stimulate a T cell subset that has been implicated in the pathogenesis of progressive IgA nephritis in humans (124). In this study, a peptide derived from mycobacterial HSP 65 stimulated T cell proliferation. In experimental nephritis caused by injection of Thy1.1 antigen (a form of mesangioproliferative GN), HSP 90 appears to regulate the response to pro-mitogenic signals required for mesangial cells to enter G1 or to progress through the S-phase (120). Mesangial cell proliferation is an important precursor of glomerular dysfunction.

In sum, molecular chaperones may mediate glomerular injury via immune and non-immune mediated mechanisms. Some renal chaperones appear likely to alter the acute infiltration of pro-inflammatory cells, perhaps by inadvertently mimicking an invading micro-organism (124). Other chaperones modulate cell proliferation, determine the susceptibility to pro-apoptotic stimuli, or the propensity to undergo irreversible fibrosis.

Chronic, Progressive Renal Failure

Although the mechanism is debated, many forms of renal injury can lead to progressive renal failure even in the absence of the initial insult (130). Many factors have been proposed to cause progressive renal injury including hemodynamic stress, phosphate deposition, hyperlipidemia, reactive oxygen species, immune-mediated injury resulting from increased ammoniagenesis, and dysregulation of apoptosis leading to unrelenting cellular dropout (49). One or more of these insults could be responsible for the progressive interstitial and glomerular fibrosis that are important hallmarks of chronic, progressive renal failure. In aging rats, increased accumulation of both HSP 47 (131) and HSP 72 (132) have been observed, suggesting that progressive loss of renal function is associated with an increased burden of non-native proteins.

The expression of HSP 47, a collagen-binding protein, in glomeruli with segmental sclerosis paralleled the expression of type I, III, and IV collagen in rats subjected to subtotal nephrectomy (131). These rats showed glomerulosclerosis

with marked tubulointerstitial damage, as well as interstitial fibrosis with increased collagen and HSP 47 deposition in glomeruli, tubular epithelial cells, and interstitial cells of remnant rat kidneys (131). Administration of HSP 47 antisense oligodeoxynucleotides suppressed the collagen deposition and attenuated the histologic manifestations of the glomerular fibrosis (131). HSP 47 is unlikely to act alone in processing collagen. GRP 78 and GRP 94 cooperate with the HSP 47-procollagen complex in metabolically stressed cells, preventing the proper folding and release of procollagen (133).

Renal Transplantation

Removal, storage, and re-implantation of a kidney are accompanied by a variable degree of ischemic damage (87, 134). Ischemic renal injury is an important cause of early graft failure (135) and may increase the likelihood of subsequent episodes of acute or chronic rejection (134). Although therapy for acute allograft rejection with active inflammation is often effective, no treatment presently exists for chronic progressive rejection associated with progressive interstitial fibrosis. Accumulation of HSP 47 in the interstitium positively correlates with interstitial fibrosis in allografts with chronic progressive dysfunction (136). A novel HSP (45 kDa or HJD-2) was recently identified in human kidney biopsies that exhibited either acute or chronic rejection. In contrast, normal, pretransplant kidneys and transplanted kidneys without histologic evidence of rejection (acute or chronic) showed no HJD-2 (137). These investigators suggest that this novel HSP might be an antigen against which cytotoxic T cells that mediate acute rejection are directed. However, HSP 60 was also increased in allografts with rejection (137), making it difficult to ascribe causality to a specific HSP. Although expression of molecular chaperones after a nonlethal ischemic insult is assumed to confer cytoprotection, these same chaperones expressed on the cell surface may mark cells for apoptosis (29) or precipitate cell infiltration and inflammation (3, 129).

Osmotic Stress

Over a decade ago, Cohen and colleagues observed a brisk induction of HSP 72 in renal MDCK cells subjected to physiologic, hyperosmolar stress (138). The transcriptional response to hyperosmotic stress has been recently reviewed (139, 140). Renal medullary stress is a consequence of physiological changes in extracellular osmolality associated with normal fluctuations in water, urea, and ion excretion. Cells acutely exposed to hypo- or hyperosmolar stress exhibit changes in cytoskeletal organization (141), membrane transporter activities (142), and stimulation of the cell death pathway (139, 143). To compensate for this adverse physiologic situation, kidney medullary cells constitutively overexpress molecular chaperones, with an increasing gradient of molecular chaperones evident from cortex to medulla (139, 144).

Intracellular cytoprotective osmolytes (i.e., sorbitol, betaine, inositol, taurine, and glycerophosphorylcholine) are accumulated during hypertonic stress (142), although the rate of their accumulation is relatively slow (145). Similarly, cells

exposed to a hypo-osmolar environment respond by dumping organic osmolytes, inhibiting intracellular osmolyte production, and increasing their degradation (141). These adaptive mechanisms are likely inadequate to protect renal cells from rapid changes in extracellular osmolality. Thus constitutive expression of inducible HSPs or their rapid induction with severe osmotic stress could fill an acute cytoprotective gap (146, 147).

Several osmotic-sensitive molecular chaperones have been implicated as potential cytoprotectants, including HSP 25, 60, 72, 78, 110, 200, and the osmotic stress protein OSP 94. Expression of HSP 25 and HSP 72 is enhanced in vivo in rat inner medulla in response to dehydration-mediated hyperosmolar stress (139, 143, 148). In addition, molecular chaperones of 46, 60, 78, and 200 kDa have been identified in renal epithelial cells subjected to hypertonic NaCl (147). More recently, OSP 94, a member of the HSP 110 family, was found to be highly expressed in murine inner medulla in vivo (149). In the dehydrated mouse, increased medullary expression of OSP 94 and HSP 110 were observed, emphasizing the importance of these proteins in the adaptive response to hyperosmotic stress (144).

Studies of renal cells in culture yielded comparable results. After exposure to hypertonic NaCl, murine inner medullary collecting duct (IMCD3) cells increased HSP 72 mRNA levels prior to increases in either OSP 94 or HSP 110 (144). The relatively rapid induction of HSP 70 in response to increased osmolality suggests that this chaperone is a critical, but not sufficient, adaptive response to perturbations in protein conformation. An assay for ATP-dependent binding to an unfolded protein identified additional hyperosmotic stress-inducible proteins including mitochondrial HSP 70, as well as 60 and 200 kDa proteins (147). Furthermore, preconditioning with heat stress, a well-established cause of protein denaturation and inducer of heat shock proteins, protects IMCD3 cells against subsequent osmotic stress (144). The existence of this cross tolerance suggests that hyperosmolality and thermal stress share many key features such as protein denaturation and refolding.

Recent work has sought to identify specific molecular chaperones responsible for cytoprotection during hyperosmotic stress. In MDCK cells, a priming osmotic stress correlated with protection against a more severe osmotic shock. MDCK cells exposed to 600 mOsm NaCl were more likely to survive a subsequent urea stress (143). Improved cell survival correlated with the accumulation of HSP 72 (as well as increased levels of betaine and glycerophosphorylcholine) but not HSP 25, suggesting that HSP 72 may mediate cytoprotection (143). Other investigators detected constitutive expression of HSP 72 in vivo, especially in the regions of the medulla exposed to the highest osmolality. However, in vivo water restriction failed to elicit an induction of HSP 72 above constitutively high levels in mice or rats in some studies (144, 145). In fact, water-restricted mice showed increased expression of medullary OSP 94 and HSP 110, suggesting that HSP 72 may not be the only cytoprotectant during osmolar stress (144, 145).

What is the primary stimulus for molecular chaperone induction in medullary cells exposed to increased osmolality? In vivo, urea may be a primary stimulus for inducing molecular chaperones (139, 148). This is an attractive hypothesis

because high concentrations of urea cause protein denaturation in vitro (42). Stress kinases appear to signal the osmotic stress response, and induction of HSP 72 appears dependent upon p38 kinase-mediated regulation of Jun N-terminal kinase (JNK) (46). In contrast, thermal induction of HSP 72 is independent of p38 kinase (46). Another in vitro study suggests that vasopressin activates heat shock transcription factor (HSF), a precursor for the induction of HSP 70 (150). In this study, blockade of the V_2 receptor prevented HSP 70 induction, suggesting that the response to osmotic stress is renal specific and may be mediated by through the cyclic-AMP pathway. Ultimately, osmotic stress has the potential to cause cell death. Given that acute hyperosmolality activates stress kinases, it is not surprising that exposure to hypertonic NaCl or urea precipitates apoptosis in inner medullary collecting duct cells (151, 152), a process that could be modulated by the presence of HSPs.

FUTURE DIRECTIONS

In kidney, accumulation of molecular chaperones is a critical step in inducing cytoprotection under adverse circumstances. Induction of chaperones can be achieved experimentally using drugs, heat exposure, or molecular strategies that stimulate overexpression of heat shock transcription factor (HSF)-specific stress proteins. Although this preemptive approach might appear limited in most clinical settings, many episodes of acute renal injury can be anticipated. Renal injury caused by exposure to intravenous contrast agents or nephrotoxins (e.g., aminoglycosides, cisplatin), surgical procedures involving cardiopulmonary bypass, and cold storage of kidneys prior to organ transplantation could potentially be prevented by pre-induction of molecular chaperones. Ultimately, induction of molecular chaperones or the administration of cytoprotective domains of specific chaperones could prevent or ameliorate acute renal failure in high-risk situations. In the meantime, appreciation of the impact of molecular chaperones on cell function has provided a better understanding of the pathways that mediate cellular injury and survival.

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