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Heat Shock Protein 47 and Renal Fibrogenesis

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Abstract

Recent research has greatly increased our knowledge regarding the molecular mechanisms of collagen synthesis and processing. Heat specific shock protein (HSP47) is a collagen-specific molecular chaperone, and helps in post-translational modifications of procollagens, during biosynthesis of collagen. Both in vivo and in vitro studies have convincingly demonstrated that HSP47 is localized in the endoplasmic reticulum of collagen-producing cells, and that its synthesis is closely associated with the rate of procollagen assembly. Recent studies are directed towards the pathological relevance of HSP47 in tissue scarring, a process that is characterized by excessive accumulation of collagens. It appears likely that increased levels of HSP47 in fibrotic diseases assist in increased assembly of procollagen, and thereby help in excessive accumulation of collagens in the fibrotic mass. Such profibrotic effects of HSP47 suggest that modulation of HSP47 expression in scarring diseases might alter the course of fibrotic diseases. In this brief article, we review the role of HSP47 in renal fibrotic diseases and its relevance to other scarring diseases.

Introduction

Most living tissues are capable of regenerative repair in response to an injury. In normal wound healing, this regenerative process is self-limiting. However, in tissue scarring, excessive accumulation of matrix proteins, partly due to their uncontrolled metabolism, alters the structural integrity of the involved tissues or organs, and thereby affects their functional activities. The pathogenesis of late-stage tissue scarring appears to be independent of early initiating events or diseases, and a final common pathway for scarring is

believed to exist for tissue scarring for all affected organs, including kidneys, heart, lungs, and liver. Our understanding of the underlying molecular mechanisms of renal fibrosis may therefore reap benefits in fibrotic diseases in general. The multistep, multifactorial chronic scarring diseases usually progress to irreversible end-stage organ failure, resulting in increased disability and eventual death. Extensive research has been performed to elucidate the underlying mechanisms of tissue scarring, and one of the molecules that have been comprehensively studied in the scarring process is collagen. Collagen is an abundant extracellular matrix protein, and is involved in the maintenance of the structural integrity of cells and tissues. In certain pathological states, proliferation of matrix-producing cells with subsequent overproduction, and excessive accumulation of essentially insoluble collagen fibers, lead to the development of tissue scarring. On the other hand, defects in the genes encoding for different types of collagens are associated with various debilitating diseases, including Ehlers-Danlos syndrome, osteogenesis imperfecta, Alport syndrome, epidermolysis bullosa, Schmid's metaplasia chondrodysplasia (SMCD), and Bethlem myopathy. Furthermore, autoantibodies against different types of collagens have been detected in a number of autoimmune diseases.

Collagen

Collagen is a widely distributed structural protein that is rich in three nonessential amino acids: glycine, proline and hydroxyproline. About one third of the all-mammalian protein is collagen, and it is essential for providing the necessary mechanical support for a variety of hard and soft tissues. So far, more than 40 distinct polypeptide chains, forming more than 25 different types of collagens, have been identified. The typical collagen molecule consists of three collagen polypeptide chains, called α-chains, which are wrapped around one another to form a triple-stranded helical structure; every third amino acid within the helix is glycine, while the remaining positions in the chain are filled with proline and hydroxyproline. Thus, the sequence of the α-chain can be expressed as $(Gly\text{-}X\text{-}Y)_n$, where X and Y represent amino acids, proline and hydroxyproline respectively [1]. Hydroxyproline is vital for the stability of the collagen molecules; and vitamin C is required to convert proline to hydroxyproline.

A number of complex cotranslational and post-translational modifications are required for collagen biosynthesis. The synthesis of the α-polypeptide chains, their hydroxylation, and formation of stable triple-helical procollagen molecules are important intracellular events in collagen synthesis; the intracellular processing requires a number of specific enzymes, including prolyl 4-hydroxylase, prolyl

3-hydroxylase, lysyl hydroxylase, hydroxylysyl galactosyltransferase and galactosyl-hydroxylysyl glucosyltransferase, while the extracellular modifications require procollagen N-proteinase, procollagen C-proteinase and lysyl oxidase [2–7]. In addition to the above-mentioned enzymes, recent studies have documented the essential roles of a number of chaperones and folding proteins during procollagen synthesis, including protein disulphide isomerase, and heat shock protein 47 (HSP47) [8–10]. Protein disulphide isomerase is believed to bind with C-propeptides of procollagen, and forms intra- and inter-chain disulphide bonds, resulting in the formation of a trimer of procollagen at the C-terminus. Upon assembly of C-propetides into a correctly aligned trimer, triple-helix formation proceeds from the C-terminus to the N-terminus [11]. Recently, HSP47, a collagen-binding heat shock protein, has also been found to be involved in the post-translational modifications, and triple-helix formation of procollagen molecules (fig. 1). We will be briefly presenting the pathogenic relevance of HSP47 in human and experimental fibrotic diseases.

HSP47

HSP47 is a 47-kDa glycoprotein protein (two potential *N*-glycosylation sites), resides in the endoplasmic reticulum of collagen-producing cells, and is involved in the post-translational modifications of procollagen molecule [10]. At the N-terminus, HSP47 has a signal sequence that targets the molecule to the endoplasmic reticulum, while at the C-terminus it has RDEL, the endoplasmic reticulum retention signal [12]. Once the RDEL signal is removed from the C-terminus, the mutant protein is rapidly secreted out of cells [13].

HSP47 has a unique collagen binding ability and specifically binds to various types of collagens [10]. The binding abilities of HSP47 to procollagens have been demonstrated by co-immunoprecipitation studies [14]. Both native HSP47 (from chick embryos) and synthetic HSP47 (produced in *E. coli*) has been shown to bind various collagens (types I to V), as demonstrated by in vitro pull-down studies by using surface plasmon resonance [15, 16]. Recent in vitro binding analysis of a synthetic peptide model of collagen resulted in the identification of a specific HSP47 binding sequence; binding of HSP47 to typical collagen model peptides $(Pro\text{-}Pro\text{-}Gly)_n$ occurred when n was more than 8 [17]. The probability of HSP47 binding to the triplet repeats was higher with greater numbers of repeats.

HSP47 was initially identified by Kurkinen et al. [18], from murine parietal endoderm cells. Thereafter, species-specific collagen-binding proteins were characterized in human and rat as gp46 [19], in the mouse as J6 [20] and in the chick and rabbit as HSP47 [21, 22]. All these proteins were later found

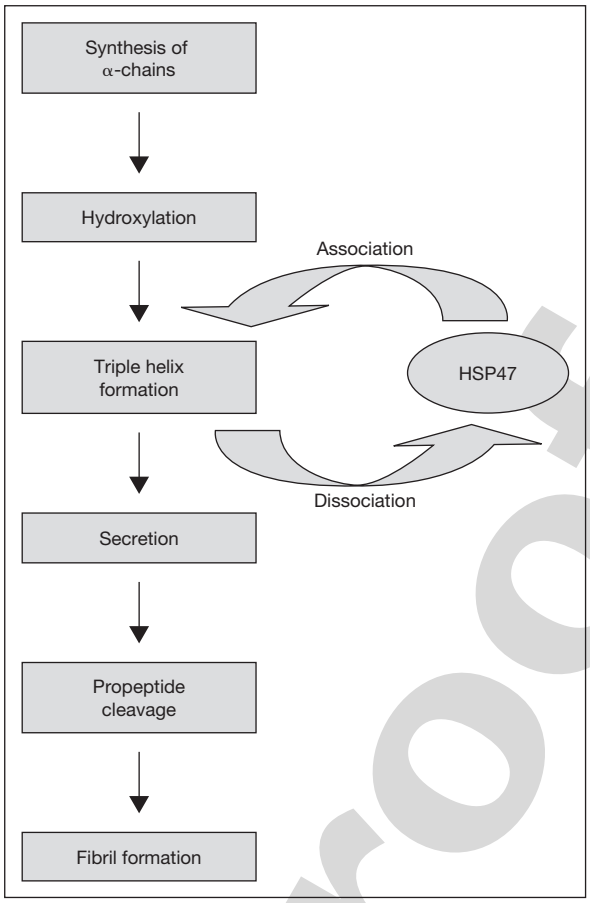

Fig. 1. Simplified schematic diagram showing involvement of HSP47 during biosynthesis of collagen.

to be the same group of molecules with common collagen binding abilities. There is ample evidence for the involvement of HSP47 in the folding, assembly and/or post-translational modification of procollagen [10]. The essential role of HSP47 in collagen biosynthesis has been documented in various in vivo and in vitro studies; *HSP47* disruption resulted in embryonic lethality in mice. The *HSP47* null mice died at 11.5 days postcoitus, and these mice showed abnormal collagen formation and impaired organogenesis [23]. Having identified the essential role of HSP47 in the synthesis of collagen, recent studies have focused on its role during tissue scarring. A number of experimental studies have found a close association between overexpression of HSP47 and

increased deposition of collagens in various human and experimental fibrotic diseases [24–27].

Renal Fibroproliferative Diseases

Most renal diseases, irrespective of glomerular, tubulointerstitial or vascular involvements, develop renal fibrosis in chronic and advanced stages. These diverse groups include, but are not limited to, diabetic nephropathy, lupus nephritis, hypertensive nephropathy, renal scleroderma, IgA nephropathy, sickle cell nephropathy, glomerulonephritis, interstitial nephritis, toxic- and drug-induced nephropathy, and chronic allograft nephropathy. The extent of renal fibrosis is a prognostic indicator of most of the above-mentioned renal diseases. One common feature of renal fibrosis is excessive accumulation of matrix proteins due to uncontrolled synthesis and/or degradation. Most of the renal fibrotic diseases are progressive, and gradual expansion of scarring tissues eventually leads to the destruction of normal renal tissues. The degree of renal fibrosis correlates with a progressive loss of renal function, and eventual end-stage renal disease. Although the exact molecular mechanisms of renal fibroproliferative diseases are not yet clear, increased synthesis and deposition of types I, III IV, and VI collagens have been detected in chronic progressive fibrotic renal diseases including IgA nephropathy, diabetic nephropathy (fig. 2) and hypertensive nephrosclerosis [28–30]. Morphological changes in various stages of renal diseases range from activation and proliferation of intrarenal cells to severe glomerulosclerosis and tubulointerstitial fibrosis. Although numerous studies have convincingly demonstrated that increased synthesis with excessive deposition of collagens are mainly responsible for the initiation and progression of renal fibrotic diseases, our knowledge of intracellular processing of the collagen molecules during fibrotic diseases is still very limited. Recent studies have shown a correlation between the expression of HSP47, a collagen-specific molecular chaperone, and increased deposition of collagens in human and experimental fibrotic renal diseases [31–33].

HSP47 in Experimental Models of Nephritis

Dysregulated activation and proliferation of resident glomerular cells produce in due course increased levels of collagen, and facilitate the development of progressive glomerular scarring, an important cause of irreversible end-stage renal failure [34]. Anti-thymocyte serum-induced nephritis is a widely used experimental model characterized by mesangial cell proliferation

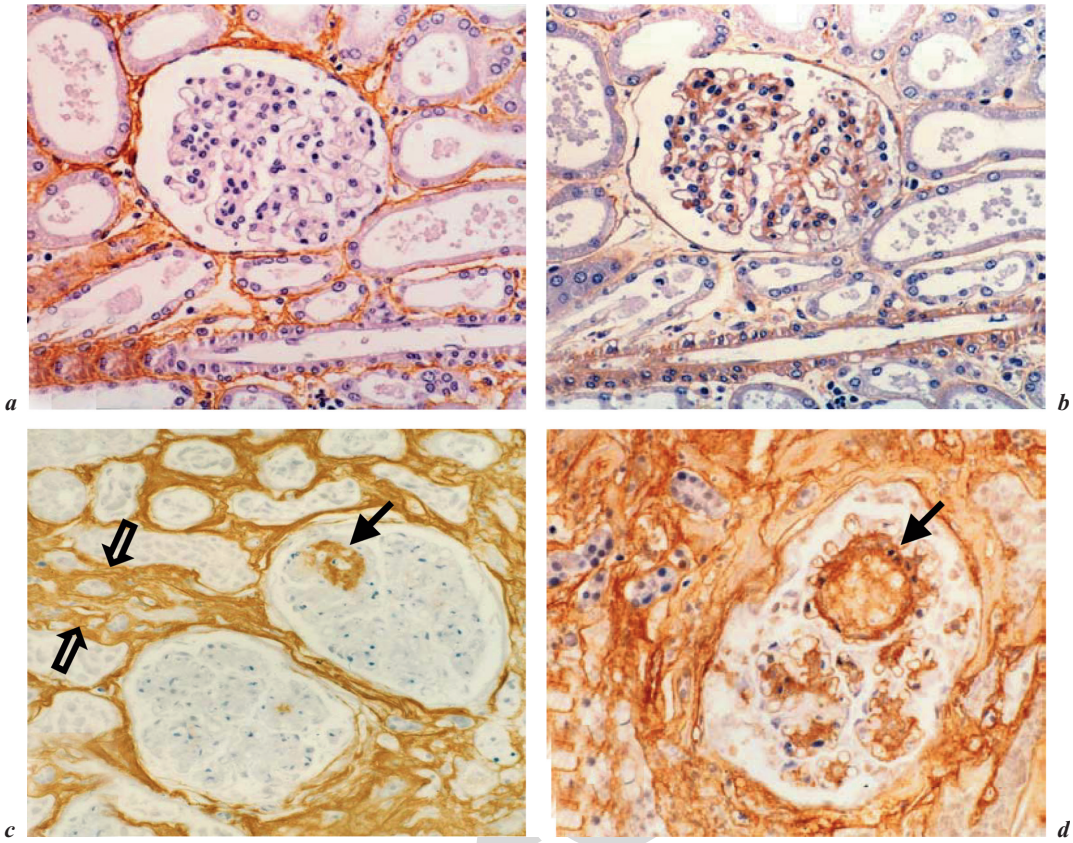

Fig. 2. Expression of type III and type IV collagens in renal tissues collected from a control subject (***a***, ***b***) and from a patient with diabetic nephropathy (***c***, ***d***). Type III collagen (***a***) is mostly present in the interstitium and absent from the glomeruli, while type IV collagen (***b***) is present in the mesangium, and along the glomerular and tubular basement membranes in control kidney. In contrast to the control, increased accumulation of type III collagen (***c***) and type IV collagen (***d***) is detected in the sclerotic glomeruli (open arrow) and fibrotic interstitium (arrow) in diabetic nephropathy.

and sclerotic changes in the glomeruli [35]; increased glomerular expression of HSP47 in this experimental model was associated with excessive accumulation of collagens in the scleroproliferative glomeruli [36]. Furthermore, phenotypically altered collagen producing glomerular myofibroblasts (α-smooth muscle actin positive), and glomerular epithelial cells (desmin-positive) are the main HSP47-producing cells in the scleroproliferative glomeruli [35–37]. All these HSP47-expressing glomerular cells are collagen-producing cells, as well. Since

HSP47 is a molecular chaperone intimately involved in the synthesis of procollagens, it is likely that high levels of glomerular HSP47 might help in enhancing the production rate of collagens, and thus contribute to the glomerular sclerotic process. A similar increase in the expression of HSP47, and excessive accumulation of collagens in the glomeruli was also noted in other experimental models of glomerulosclerosis, including diabetic nephropathy.

HSP47 in Experimental Models of Tubulointerstitial Fibrosis

Tubulointerstitial fibrosis is characterized by interstitial accumulation of collagens, produced by phenotypically altered interstitial cells and tubular epithelial cells. Expression of α-smooth muscle actin in renal interstitial cells is indicative of acquiring myofibroblastic phenotype [38], while expression of intermediate filament vimentin in tubular epithelial cells is suggestive of phenotypical alteration of renal tubular epithelial cells [39]. Earlier studies demonstrated that increased synthesis of collagens by phenotypically altered interstitial myofibroblasts and tubular epithelial cells plays an important role in the initiation and progression of tubulointerstitial fibrotic process. As expected, in various experimental models of tubulointerstitial fibrosis, including in cisplatin nephropathy, aged F-344 rats and hypertensive nephrosclerosis, increased expression of HSP47 was always detected in collagen-producing interstitial myofibroblasts and tubular epithelial cells (fig. 3) [24, 31–33, 40, 41]. Elevated expression of HSP47 was associated with excessive accumulation of collagens, and mostly detected in and around interstitial fibrotic areas. No such expression of HSP47 was detected in infiltrating monocytes/macrophages in the interstitium.

HSP47 in Human Scarring Renal Diseases

Until now, only a few studies have examined the role and expression of HSP47 in human fibrotic diseases [42]. The first such human study was conducted using renal biopsy tissues of IgA nephropathy, and diabetic nephropathy. In adult human kidneys, a weak expression level of HSP47 was detected in glomerular cells, tubular epithelial cells and interstitial cells. In contrast, enhanced expression of HSP47 was detected in the early sclerotic glomeruli of IgA nephropathy, diabetic nephropathy and crescentic nephritis. HSP47 was also expressed in tubulointerstitial cells in areas around interstitial fibrosis [42]. The glomerular and tubulointerstitial expression of HSP47 in renal biopsy tissues was closely associated with glomerular accumulation of

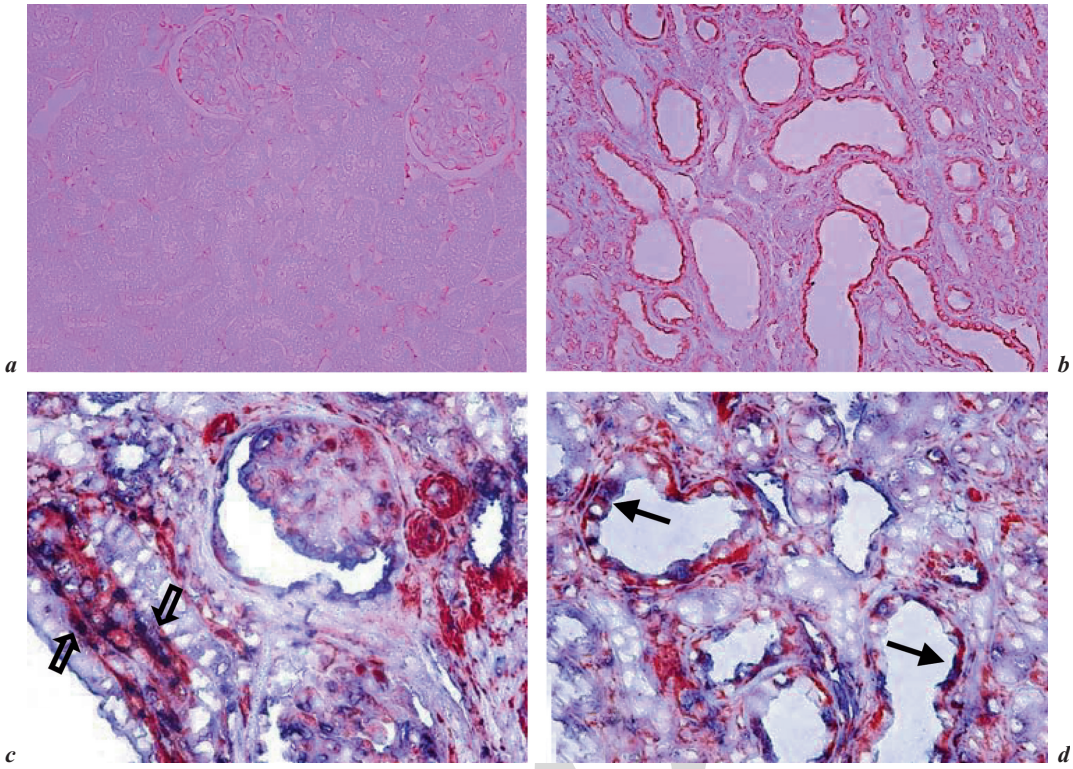

Fig. 3. Expression of HSP47 in renal tissues collected from an age-matched control rat (***a***) and from rats with unilateral ureteral obstruction [(UUO); after 21 days] (***b***). HSP47 is weakly expressed in the glomeruli and in the interstitial cells in control rat kidney and absent from the tubules (***a***). In contrast to the control, an increased expression of HSP47 is detected in UUO-induced fibrotic rat kidney (***b***). Double staining shows HSP47 expressing cells (black) are α-smooth muscle actin-positive (red) myofibroblasts (***c***, open arrow), and vimentin-positive (red) tubular epithelial cells (***d***, arrow) in UUO-induced fibrotic rat kidney.

type IV collagen, and interstitial accumulation of types I and III collagens, respectively. Although further studies are needed, it appears likely at this stage that irrespective of primary diseases, upregulation of HSP47 is a common phenomenon during collagenization of glomeruli and tubulointerstitium.

HSP47 in Non-Renal Scarring Diseases

Similar to the renal fibrotic diseases, HSP47 is also upregulated in various fibrotic diseases involving the lung, liver, heart, eye and skin. Upregulated

expression of HSP47 with accumulation of collagen was detected in bleomycin-induced pulmonary fibrosis [26]. Consistent with the experimental data, a similar correlation in the expression of HSP47 and excessive accumulation of collagen has been detected in human pulmonary fibrotic diseases [43]. In human dermal fibrosis of patients with keloid [44], and cicatricial pemphigoid [25], increased dermal expression of HSP47 is believed to be partly responsible for enhanced accumulation of interstitial collagens in the scarring tissues. In a separate study, the correlation of HSP47 expression and collagen accumulation was also documented in human conjunctival scarring diseases in patients with ocular cicatricial pemphigoid [45], and Stevens-Johnson syndrome (unpubl data). Similar profibrogenic role of HSP47 has been proposed in the development of fibrotic lesions involving the liver and heart [46, 47].

Regulatory Mechanisms of HSP47 Expression

Several studies have recently documented the possible regulatory roles of known fibrogenic molecules, and the expression of HSP47. For instance, stimulation of mouse osteoblast MC3T3-E1 cells by transforming growth factor-β_1 (TGF-β_1) resulted in a dose-dependent induction of both HSP47 and type I collagen [48]. Similar in vitro induction of HSP47 by TGF-β_1 was also noted in various human cells, including conjunctival fibroblasts, dermal fibroblasts, and aortic smooth muscle cells [25, 45, 49]. Using human embryonic lung fibroblasts, it has been shown that both TGF-β_1 and IL-β_1 could induce trimer formation of heat shock transcription factor 1, which then bound to heat shock element of HSP47, resulting in increased expression of HSP47 [50]. In addition, certain other profibrogenic cytokines, including IL-4, IL-13, and connective tissue growth factor have shown to induce the expression of HSP47 [51–53]. Human conjunctival fibroblasts, treated with various concentrations of IL-4 [51] and IL-13 [52], could induce the expression of both HSP47 and collagens. Recent in vitro studies and in vivo experimental model of diabetic nephropathy, reported that advanced glycation end-products could induce the expression of HSP47 in association with collagens, and thereby could play a role in diabetic nephrosclerosis. [54].

Conclusion

Fibrosis accounts for considerable chronic morbidity in various renal diseases and could be a potential target of therapy. Recent studies have identified a number of important molecules that are involved in the regulation of chronic fibrotic processes, and some of these molecules are potential therapeutic candidates

for modulating renal fibroproliferative diseases. However, because of complex molecular interactions, despite identifying crucial molecules involved in fibrogenesis, there are difficulties in developing therapeutic strategies for the treatment of these chronic progressive diseases. Moreover, regardless of the in vitro efficacy of targeting a number of these identified fibrogenic molecules, it is not always easy to predict the in vivo effects of similar targeting, because of the complex in vivo microenvironment. Taking into consideration the altered microenvironment of the affected tissues, the design of therapeutic strategies that include targeting relevant molecules involved in the regulation of excessive accumulation of collagens in the fibrotic mass, may be a more practical and effective approach. Since excessive accumulation of collagens is one of the main pathological events of scarring diseases [28–30, 42, 55–58], therapies should be designed to prevent excessive synthesis and accumulation of such matrix molecules. In view of the fact that HSP47 is involved in the biosynthesis of collagen molecules, selective blockage of this molecule in fibrotic diseases, not only offers an attractive therapeutic strategy, but also provides a functional explanation of why this therapeutic approach might be successful. In fact recent in vivo studies have documented the beneficial effects of interfering with the expression of HSP47 in fibrotic renal diseases [59, 60]. In an experimental model of nephritis, interference with the enhanced expression of HSP47 by the administration of antisense oligodeoxynucleotides resulted in relatively less glomerular accumulation of collagens [59]. Furthermore, improvement of age-related renal scarring was achieved by prolonged caloric restriction, possibly by modulating renal expression of HSP47 and accumulation of collagens [60]. These preliminary studies provide the basis for the development of antifibrotic therapies that control chronic fibroproliferative diseases by targeting HSP47. Needless to say, pharmacological amelioration of renal fibrosis may require stage-specific modulation of multiple factors involved in a certain stage of the fibrotic process; since HSP47 appears to be involved in nearly all stages of the fibrotic process, by facilitating accumulation of collagens, a targeted modulation of its activities might be a useful approach to control the progression of fibrotic diseases. Furthermore, as HSP47 plays a role in renal fibrogenesis, monitoring the expression of HSP47 may help in defining those patients at risk for developing fibrotic complications, and in assessing the response to conventional and selective therapies.

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