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Review

Heat Shock Protein 47: A Novel Biomarker of Phenotypically Altered Collagen-Producing Cells

Takashi Taguchi[1], Arifa Nazneen[1], Abdulmonem A. Al-Shihri[2], Khadijah A. Turkistani[3] and Mohammed S. Razzaque[1,3]

[1]Department of Pathology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [2]Department of Restorative Dentistry and Biomaterials Sciences, Harvard School of Dental Medicine, Boston, MA, USA and [3]Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA, USA

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Heat shock protein 47 (HSP47) is a collagen-specific molecular chaperone that helps the molecular maturation of various types of collagens. A close association between increased expression of HSP47 and the excessive accumulation of collagens is found in various human and experimental fibrotic diseases. Increased levels of HSP47 in fibrotic diseases are thought to assist in the increased assembly of procollagen, and thereby contribute to the excessive deposition of collagens in fibrotic areas. Currently, there is not a good universal histological marker to identify collagen-producing cells. Identifying phenotypically altered collagen-producing cells is essential for the development of cell-based therapies to reduce the progression of fibrotic diseases. Since HSP47 has a single substrate, which is collagen, the HSP47 cellular expression provides a novel universal biomarker to identify phenotypically altered collagen-producing cells during wound healing and fibrosis. In this brief article, we explained why HSP47 could be used as a universal marker for identifying phenotypically altered collagen-producing cells.

Key words: HSP47, collagen, fibrosis, biomarker

I. Heat Shock Proteins

Heat shock proteins (HSPs) are a distinctive class of proteins that play an important role in the assembly and folding of intracellular polypeptides and help to restore the biological activities of abnormal proteins. HSPs also assist in the recovery of the cell from stress, either by refolding damaged proteins or by degrading them, and provide a cellular defense against a wide range of stresses and injuries by restoring protein homeostasis. Furthermore, these proteins can play important roles in signal transduction by maintaining and stabilizing intracellular microenvironments.

The heat shock response was first observed by Ritossa in *Drosophila* in 1962 [55] and is now widely accepted to be a universally conserved cellular defense system. The heat shock response is mediated by a group of HSPs; this response has been observed in both eukaryotic and prokaryotic cells. Some HSPs are strictly stress-inducible, whereas others may be constitutively expressed or developmentally regulated. A number of HSPs are constitutively expressed and actively involved in maintaining cellular homeostasis by acting as molecular chaperones [3, 6, 9]. HSPs regulate the folding and assembly of nascent and unfolded peptides, help in transporting proteins to a particular subcellular compartments and assist in the degradation of misfolded proteins [8].

Both *in vivo* and *in vitro* studies have documented important roles of HSPs in the pathogenesis of various diseases, ranging from autoimmune diseases (arthritis and diabetes) to tumors and fibrotic diseases. In this article, we will briefly explain how HSP47 can be used as a novel biomarker to identify phenotypically altered collagen-producing cells during wound healing and fibrosis.

Correspondence to: M. Shawkat Razzaque, MD, PhD, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Room: 304, 188 Longwood Avenue, Boston, MA 02115, USA.
E-mail: mrazzaque@hms.harvard.edu; razzaque@med.nagasaki-u.ac.jp

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II. HSP47

HSP47 was first characterized by Kurkinen *et al.* in murine parietal endoderm cells [13]. HSP47 is a stress-inducible 47 kD collagen-binding glycoprotein that is present in the endoplasmic reticulum of collagen-secreting cells [21, 61]. HSP47 can transiently interact with procollagen during its folding, assembly and transport from the endoplasmic reticulum of mammalian cells. The collagen binding ability of HSP47 has been demonstrated by co-immunoprecipitation studies [26]. HSP47 can bind various types of collagens (types I to V), as determined by *in vitro* pull-down studies using surface plasmon resonance [22, 57]. Studies have suggested that HSP47 can potentially stabilize the correctly folded collagen helix and prevent heat denaturation before its transport from the endoplasmic reticulum [64, 65]. The essential role of HSP47 in collagen synthesis was further established in *Hsp47* knockout mice, in which the genetic inactivation of *Hsp47* resulted in abnormal collagen formation and impaired organogenesis. Knockout of *Hsp47* is embryonically lethal, and the mice usually die at embryonic day 11.5 [57]. Interestingly, no collagen fibrils are present in the mesenchyme-epithelial cell junctions of *Hsp47* disrupted mice [57]. Taken together, the data from these *in vitro* and *in vivo* studies clearly suggest that HSP47 plays an important role during collagen production and may also play a role in subsequent fibrogenesis [45, 58].

III. HSP47 in Fibrotic Diseases

The development of irreversible tissue fibrosis is a relatively late change that occurs in most organs following chronic inflammation. The excessive production of matrix proteins by the activated and phenotypically altered resident cells gradually leads to the development of organ fibrosis. The increased expression and deposition of collagens (type I, type III and type IV) were detected in various organ fibroses in human diseases [40–42], a pattern that is similar to the organ fibrosis observed in experimental models [5, 15, 72, 73]. Advanced stages of fibrosis compromise the functionality of the involved tissues and lead to the development of complications related to end-stage organ failure [1, 12, 29, 38, 53, 61, 68, 69]. For instance, renal fibrosis can alter the water, electrolyte and mineral ion balance, inducing vascular calcification and skeletal mineralization defects [27, 28, 30–33, 49]. Similarly, advanced lung and liver fibrosis can lead to organ failure and increase the overall mortality levels of affected patients [18, 68]. Irrespective of organ involvement, a fibrogenic role of HSP47 is consistently observed in fibrotic diseases.

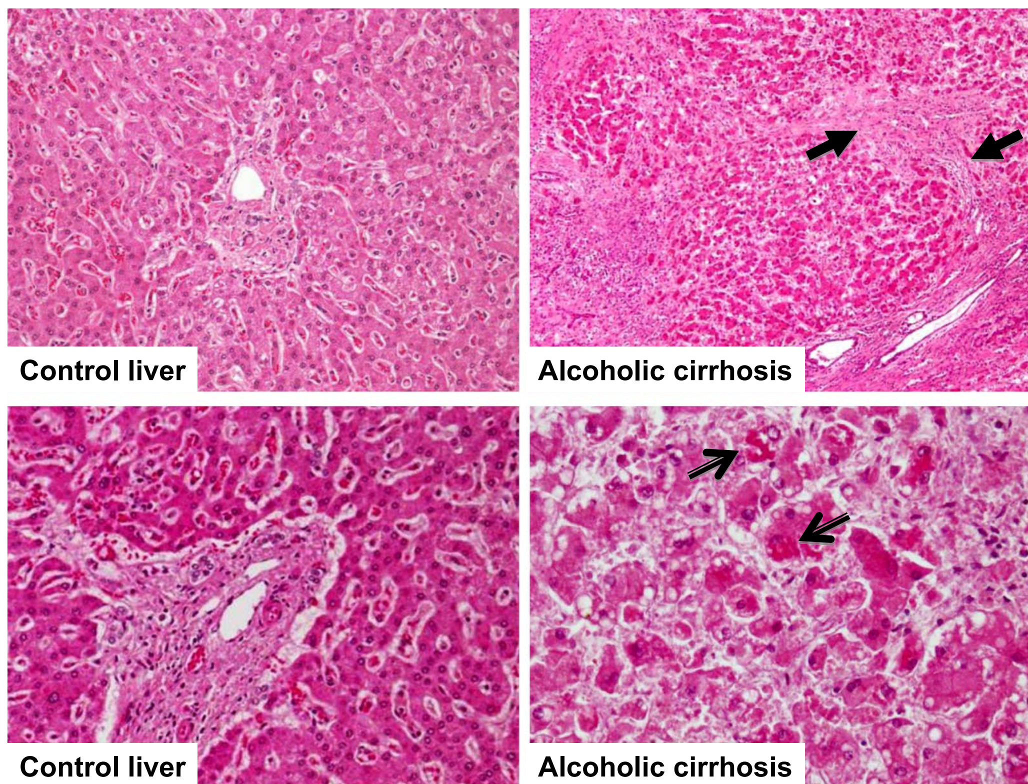

Fig. 1. Alcoholic liver cirrhosis in a 56-year-old female patient showing severe fibrotic changes. Typical hepatic nodular lesions (upper panels) are observed, as shown by both hematoxylin and eosin (HE) staining. Fatty changes in the liver with characteristic Mallory-bodies (*arrows*) are shown in the lower panels. No such changes are noted in the control liver obtained during autopsy from a 59-year-old female patient of progressive systemic sclerosis.

Increased glomerular expression of HSP47 has been shown to be correlated with an increased accumulation of collagens in scleroproliferative glomeruli in an anti-thymocyte serum-induced experimental model of nephritis [50]. Phenotypically altered collagen-producing glomerular myofibroblasts (alpha-smooth muscle actin-positive) and glomerular epithelial cells (desmin-positive) are the main HSP47-producing cells in the scleroproliferative glomeruli [50]. Such glomerulosclerosis in an experimental model of nephritis could be delayed by knocking down HSP47 *in vivo* using antisense therapy [59]. A similar induction of HSP47 expression, along with the excessive accumulation of collagens, is also noted in experimental models of diabetic nephropathy and hypertensive nephrosclerosis [16, 36]. Increased expression of HSP47 is always detected in collagen-producing interstitial myofibroblasts and tubular epithelial cells in various experimental models of renal tubulointerstitial fibrosis, such as age-associated nephropathy in F-344 rats and in radiation-induced tubulointerstitial nephritis [17, 47, 48].

Consistent with renal fibrotic diseases, increased expression of HSP47 and collagen accumulation are also detected in bleomycin-induced experimental pulmonary fibrosis [39]. As observed in the experimental fibrotic diseases, the expression of HSP47 correlates with the degree of collagen accumulation in various human fibrotic diseases [4, 14, 37, 38, 44, 46, 71]. For instance, increased expression of HSP47 in glomeruli and the tubulointerstitium correlates with glomerular and tubulointerstitial accumulation of type IV collagen and type III collagen, respectively, in human IgA nephropathy and diabetic nephropathy [44]. A similar correlation is also noted in human pulmonary fibrotic diseases [46]. Furthermore, a profibrogenic role of HSP47 has been proposed in the development of fibrotic lesions in the liver and heart [4, 62]. Fatty changes and extensive fibrosis are both severe complications observed in patients with chronic alcoholism (also referred as alcoholic liver cirrhosis) (Fig. 1). Increased expression of HSP47 is associated with the increased accumulation of collagen in alcoholic liver cirrhosis (Fig. 2). These HSP47-expressing cells in alcoholic liver cirrhosis are primarily fibroblasts and myofibroblasts. Similarly, in patients with keloids [25] and cicatricial pemphigoid [34], increased dermal expression of HSP47 is correlated with the accumulation of interstitial collagens around areas of dermal fibrosis; a similar correlation is also found in human conjunctival scarring diseases in patients with ocular cicatricial pemphigoid [35, 37, 38]. Hereditary gingival fibromatosis is usually characterized by increased accumulation of collagen in the gingival tissue of the affected patients; fibroblasts isolated from normal and fibrotic gingival tissues showed relatively increased expression of HSP47 with significantly higher

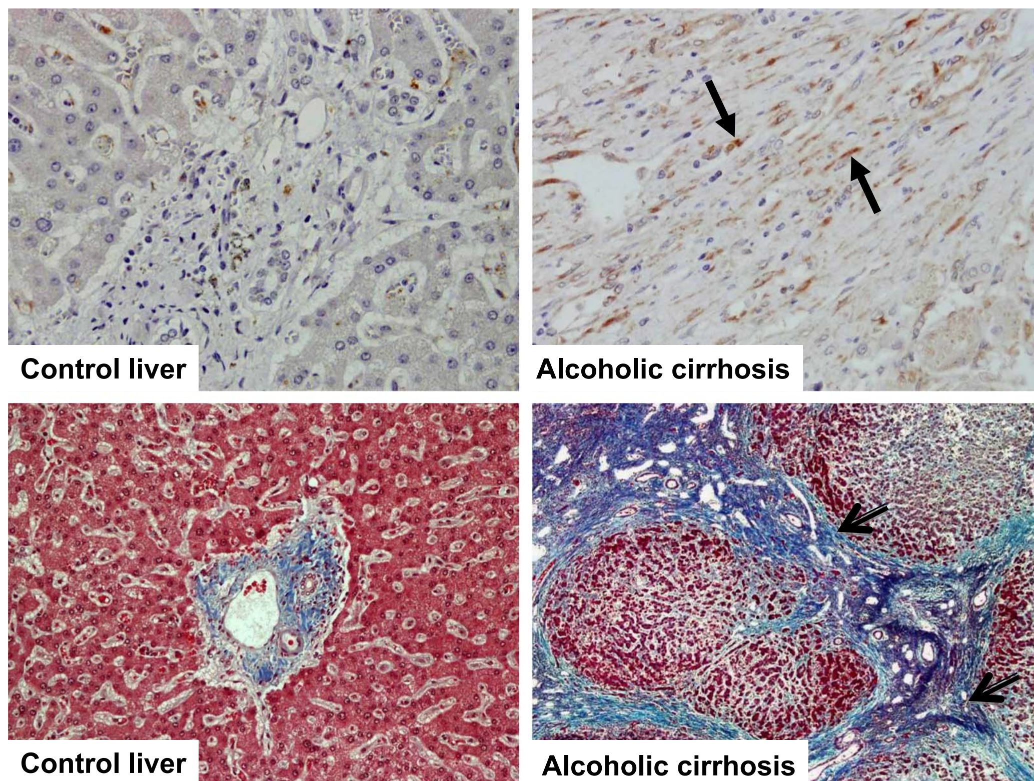

Fig. 2. Compared to the control liver, an increased expression of HSP47 is noted in alcoholic liver cirrhosis, as detected by immunohistochemistry (upper panel, *arrows*). Increased expression of HSP47 is associated with the increased accumulation of collagens (detected by Azan-Mallory stain, lower panel, *arrows*) in the fibrotic areas of alcoholic liver cirrhosis.

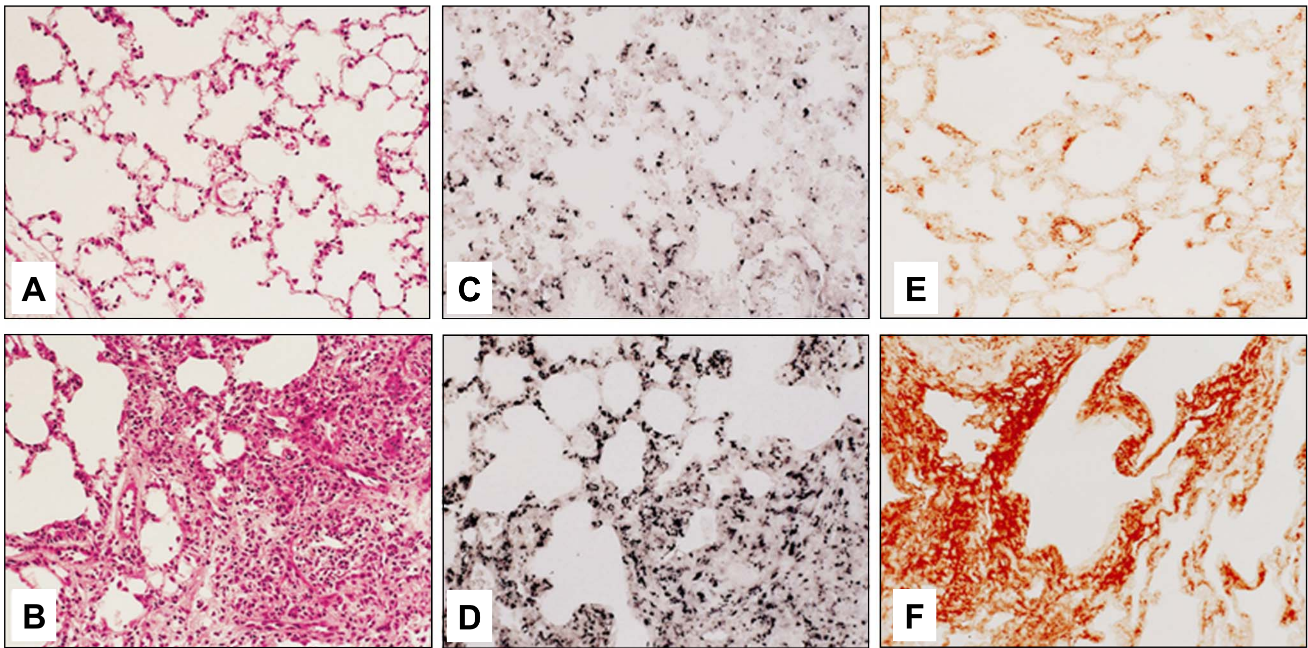

Fig. 3. Histological features of lung of a normal rat (**A**), showing no significant histological changes, while in a bleomycin-treated rat, there is marked fibrosis in the lung (**B**). Immunohistochemistry for HSP47 in a control lung, showing weak staining for HSP47, mainly located in the interstitial cells (**C**). In contrast, markedly increased HSP47 expression is noted, mainly in the stromal interstitial cells, in bleomycin-treated rat lungs (**D**). Weak immunostaining of type III collagens is noted in the control lungs (**E**). Increased accumulation of type III collagen is noted in the fibrotic areas of a bleomycin-treated lung (**F**). Please note that bleomycin-induced pulmonary fibrosis (**B**) is associated with increased expression of HSP47 (**D**) and excessive accumulation of collagens (**F**) in the fibrotic mass. (Reproduced from reference 39 with the permission from Springer publisher).

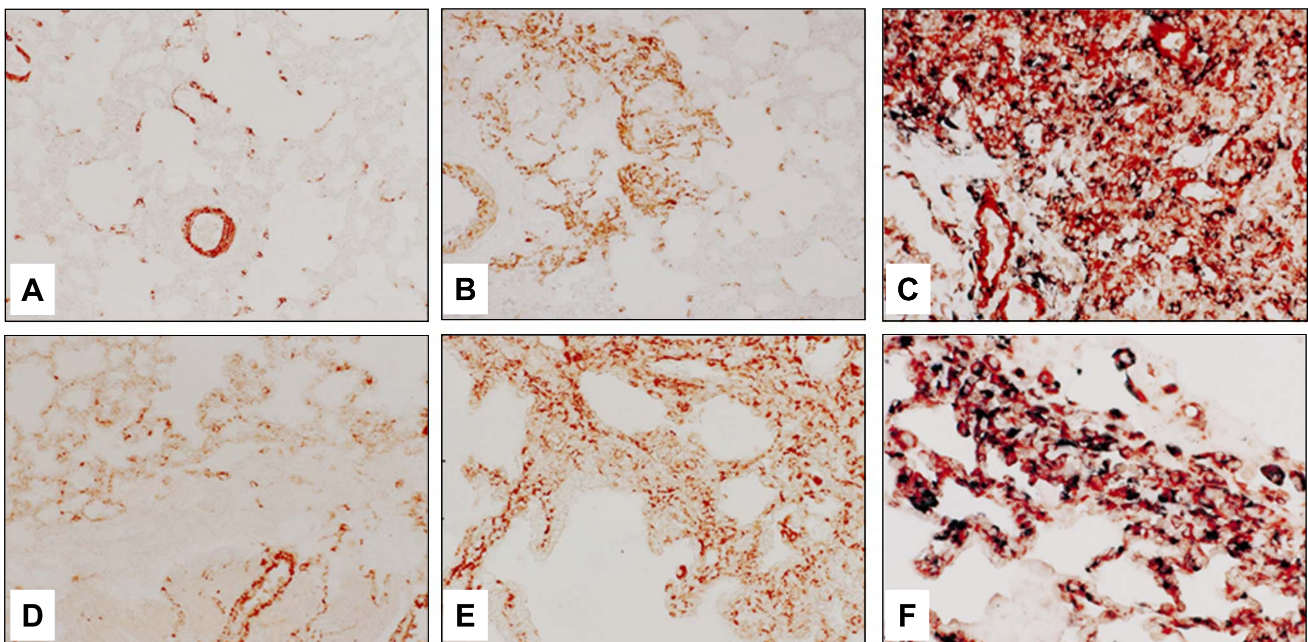

Fig. 4. Immunohistochemistry for α-smooth muscle actin in the control rat lung and is showing mostly in the vessel wall expression (**A**). Compared with the control lung, markedly increased expression for α-smooth muscle actin is noted in the bleomycin-treated rat lung (**B**). Immunohistochemistry for vimentin, showing weak immunostaining in the control rat lung (**D**), while, an increased number of vimentin-positive cells are noted in the bleomycin-treated rat lung (**E**). Please note that double staining shows that both α-smooth muscle actin-positive cells (**C**, *red staining*), and vimentin-positive cells (**F**, *red staining*) are also HSP47 expressing cells (*black*). (Reproduced from reference 39 with the permission from Springer publisher).

collagen production by the fibroblasts isolated from the patient with hereditary gingival fibromatosis [19]. An association between HSP47 expression and excessive deposition of collagen is also noted in patients with oral submucosal fibrosis [11]. In summary, findings from human and experimental fibrotic diseases clearly suggest that upregulation of HSP47 is a common phenomenon during collagenization of the involved organ, regardless of the primary disease.

Fibrogenic factors such as transforming growth factor (TGF)-β1 are produced by the activated and phenotypically altered resident cells and infiltrating inflammatory cells, and these have the potential to mediate both human and experimental fibrotic diseases by contributing to the increased production of collagens and thus matrix remodeling [34, 37, 61, 63]. TGF-β1 affects matrix remodeling by stimulating the transcription of genes encoding collagen proteins, while HSP47 plays an important role in fibrosis by post-transcriptional upregulation of collagens [16, 23, 48, 61, 63, 67]. Importantly, TGF-β1 can induce the expression of HSP47 [37, 70].

IV. HSP47 Is a Marker of Collagen-Producing Cells in Fibrotic Diseases

Organogenesis and fibrogenesis are complex process [10, 54, 56, 66]. During fibrosis, in addition to proliferation of fibroblasts, resident cells usually undergo phenotypic changes to produce excessive amounts of collagen, thus promoting fibrogenesis. To date, there is no universal histologic marker for collagen-producing cells to identify these cells in histological sections. For instance, during renal fibrosis, glomerular mesangial cells, tubular epithelial cells and interstitial cells change their phenotypes to produce increased amounts of collagen. The expression of alpha-smooth muscle actin by mesangial and interstitial cells is commonly used as a marker for phenotypic alteration in these cells, which produce increased amounts of collagen that induce glomerulosclerosis and interstitial fibrosis [24]. Similarly, the phenotypic transformation of renal tubular epithelial cells, which generate increased amounts of collagen leading to renal tubulointerstitial fibrosis [7], can be identified using mesenchymal markers like vimentin. Interestingly, all of the phenotypically altered glomerular mesangial cells, tubular epithelial cells and interstitial cells express HSP47, a common marker of collagen synthesis [5, 16, 17, 44, 47, 48, 50–52]. Likewise, increased numbers of alpha-smooth muscle actin-positive myofibroblasts and vimentin-positive fibroblasts are the main collagen-producing cells in both human and experimental fibrotic lung tissues [69, 74, 75]. Again, in both human and experimental pulmonary fibrotic diseases with increased accumulation of collagens (Fig. 3), these phenotypically altered cells, which are identified by different histological markers, are also HSP47-expressing cells [39, 46] (Fig. 4), suggesting the potential of HSP47 as a universal marker to identify phenotypically altered collagen-producing cells. It is worth mentioning that the expression of HSP47 in collagen-producing cells during fibrosis is a collective phenomenon, irrespective of organ involvement [34, 37, 43, 44, 46, 60]. As mentioned, co-localization studies suggest that phenotypically altered collagen-producing cells are always HSP47-expressing cells. Such observations lead us to propose that HSP47 is a novel biomarker of phenotypically altered collagen-producing cells during wound healing and fibrosis.

V. Conclusion

The synthesis and post-translational modification of collagen require the help of numerous enzymes and chaperones to generate a stable collagen protein in the correct conformation [2]. HSP47 is present in the endoplasmic reticulum of collagen-producing cells and helps in the correct formation of the collagen quaternary structure [22]. The expression of HSP47 positively correlates with the degree of collagen accumulation in human and experimental fibroproliferative diseases [5, 20, 37, 39, 44, 46, 47, 52]. Since HSP47 has a single substrate protein, collagen, the *de novo* expression of HSP47 acts as a novel histological biomarker to identify phenotypically altered collagen-producing cells during fibrosis and other relevant disorders. The commercial availability of HSP47 antibody and its sensitivity and reactivity on routine paraffin sections are additional advantage for the user. The specific identification of collagen-producing cells based on HSP47 expression will help in designing cell-based therapies to slow the progression of fibrotic diseases. Finally, given that HSP47 is involved in nearly all stages of the fibrotic process by facilitating the increased production of collagen, HSP47 presents a unique therapeutic target for selective inactivation with the goal of either preventing or delaying the progression of fibrotic diseases.

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