Roles of the endoplasmic reticulum–resident, collagen-specific molecular chaperone Hsp47 in vertebrate cells and human disease

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Heat shock protein 47 (Hsp47) is an endoplasmic reticulum (ER)-resident molecular chaperone essential for correct folding of procollagen in mammalian cells. In this Review, we discuss the role and function of Hsp47 in vertebrate cells and its role in connective tissue disorders. Hsp47 binds to collagenous (Gly–Xaa–Arg) repeats within triple-helical procollagen in the ER and can prevent its local unfolding or aggregate formation, resulting in accelerating triple-helix formation of procollagen. Hsp47 pH-dependently dissociates from procollagen in the *cis*-Golgi or ER–Golgi intermediate compartment and is then transported back to the ER. Although Hsp47 belongs to the serine protease inhibitor (serpin) superfamily, it does not possess serine protease inhibitory activity. Whereas general molecular chaperones such as Hsp70 and Hsp90 exhibit broad substrate specificity, Hsp47 has narrower specificity mainly for procollagens. However, other Hsp47-interacting proteins have been recently reported, suggesting a much broader role for Hsp47 in the cell that warrants further investigation. Other ER-resident stress proteins, such as binding immunoglobulin protein (BiP), are induced by ER stress, whereas Hsp47 is induced only by heat shock. Constitutive expression of Hsp47 is always correlated with expression of various collagen types, and disruption of the *Hsp47* gene in mice causes embryonic lethality due to impaired basement membrane and collagen fibril formation. Increased Hsp47 expression is associated with collagen-related disorders such as fibrosis, characterized by abnormal collagen accumulation, highlighting Hsp47's potential as a clinically relevant therapeutic target.

Heat shock protein 47 (Hsp47) is a collagen-specific molecular chaperone

Collagen is the most abundant protein in mammals, making up a third of the total protein (1). In general, collagen functions as a major component of the extracellular matrix (ECM),[2] where it forms a specialized network around cells and is essential for cell–cell interactions and cell attachment to the basement membrane. To date, 28 different types of collagen have been identified in mammalian cells, all sharing a common structural feature: a triple-helical domain composed of the Gly–Xaa–Yaa three amino acid repeat, in which Xaa and Yaa are often proline and hydroxyproline, respectively (2). Type I collagen is a typical fibril-forming collagen consisting of two α1-chains and one α2-chain, each of which is co-translationally inserted into the endoplasmic reticulum (ER). The proline residue at the Tyr position is hydroxylated by prolyl 4-hydroxylase (3), and two α1-chains and one α2-chain assemble and form inter-chain disulfide bonds among the C-propeptide regions of each peptide (4). Triple-helix formation proceeds from the C to the N terminus in a zipper-like manner, and correctly folded procollagens are transported from the ER to the cell surface via the large coat protein complex II (COPII) vesicle and Golgi apparatus (5, 6). When procollagen reaches the outer surface of the cell, its N- and C-propeptides are cleaved off by N- and C-propeptidases, respectively, followed by formation of collagen bundles in the ECM (7, 8). Efficient post-translational modification and subsequent folding of procollagens in the ER require several chaperones. Although binding immunoglobulin protein (BiP) or protein-disulfide isomerase is shared with other secreted proteins (9, 10), heat shock protein 47 (Hsp47) is specifically required for collagen folding (11).

Hsp47 was initially identified as a collagen-binding heat shock protein residing in the ER (12) and was later reported to function as a collagen-specific molecular chaperone that is essential for the correct folding of procollagen in the ER. Hsp47 is encoded by the *SerpinH1* gene and belongs to the serine protease inhibitor (serpin) superfamily, but it does not inhibit serine proteases (13). Hsp47 transiently associates with triple-helical procollagens in the ER and dissociates at the *cis*-Golgi, returning to the ER via its ER retention signal (14). *In vitro*, Hsp47 directly binds collagens and prevents

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[2] The abbreviations used are: ECM, extracellular matrix; ER, endoplasmic reticulum; dpc, days post-coitus; UPR, unfolded protein response; OI, osteogenesis imperfecta; MSNP, mesoporous silica nanoparticle; SH3, Src homology 3; BiP, binding immunoglobulin protein; COPII, coat protein complex II; HSC, hepatic stellate cell; RA, rheumatoid arthritis; IRE1α, inositol-requiring enzyme 1α; LH2, lysyl hydroxylase; COPII, coat protein complex II; ERGIC, ER-Golgi intermediate compartment; MEF, mouse embryonic fibroblast.

collagen fibril formation (15). Hsp47 recognizes an Arg residue at the Yaa^0 position of Gly–Xaa–Yaa^0 repeats within the triple-helical form of collagen, as well as the amino acid in the Yaa^{-3} position in the sequence Yaa^{-3}–Gly–Xaa–Arg (16, 17). The residue in the Xaa position does not contribute to the interaction. Although many enzymes responsible for post-translational modification of procollagen bind the monomer form of procollagen, Hsp47 barely binds nontriple-helical procollagen (18). Information on the Hsp47–collagen interaction was gleaned from the co-crystal structures of canine Hsp47 and collagen model peptides (19). Hsp47 residues Asp-385 and Arg-222 (numbering based on canine Hsp47) interact with Arg at the Yaa^0 and Yaa^{-3} positions of the Yaa^{-3}–Gly–Xaa–Arg sequence, respectively. The crystal structure also revealed that Leu-381 and Tyr-383 of Hsp47 are responsible for hydrophobic interactions with triple-helical collagens, and Hsp47 undergoes no significant conformational changes upon collagen binding.

Importantly, the interaction between Hsp47 and collagen is pH-dependent: Hsp47 binds gelatin (denatured collagen)-Sepharose resin at neutral pH (~7.4), but it is eluted at low pH (~6.3) (20). This is reflected in the dissociation constant (K_D) between Hsp47 and a collagen model peptide, which ranges from 0.74 μM at pH 7.5 to 6.23 μM at pH 6.0 (21). In this pH-dependent release mechanism of Hsp47, histidine residues with pK_a values of ~6.1 are suggested to act as triggers. The *in vitro* and cellular experiments suggested a cycle in which Hsp47 transiently associates with procollagen in the ER (neutral pH) and then dissociates from procollagen once the complex is transported from the ER to the *cis*-Golgi or ER-Golgi intermediate compartment (ERGIC; low pH). Hsp47 itself is then recycled back to the ER via interaction with the KDEL receptor (14, 22).

Hsp47 knockout (KO) mice are embryonic lethal beyond 11.5 days post-coitus (dpc) (23). At 10.5 dpc, embryos of *Hsp47* KO mice are still viable but much smaller than wildtype (WT) embryos, and they also contained fewer somites, indicating developmental retardation. In *Hsp47* KO embryos, the mature, propeptide-processed form of collagen type I and fibril structures of type I collagen in mesenchymal tissues were barely detectable. Additionally, basement membranes were discontinuously disrupted because type IV collagen was also affected. By contrast, heterozygous *Hsp47* KO mice appeared phenotypically normal. *Hsp47* chondrocyte-specific KO mice (*col2a1*-Cre; *Hsp47*-flox/flox) died just before or soon after birth, and they exhibited severe generalized chondrodysplasia and bony deformities, with lower levels of type II and type XI collagen (24), demonstrating that Hsp47 is indispensable for well-organized cartilage and normal formation of endochondral bone.

In *Hsp47* KO cells, the secretion of procollagens is delayed relative to *Hsp47* WT cells, resulting in accumulation of procollagen in the ER (25, 26). Trypsin digestion experiments can be used to evaluate the triple-helical conformation of procollagens (27), and type I and type IV collagen secreted from *Hsp47* WT cells is resistant to trypsin digestion, whereas that from *Hsp47* KO cells is sensitive (23, 25). Fibrils of type I collagen produced by *Hsp47* KO cells are abnormally thin and frequently branched, and N-propeptides of secreted collagens are not processed or retained, even in the ECM (26). These findings suggest that procollagens are not correctly folded into the triple-helical form in the ER of *Hsp47* KO cells. Misfolded collagens in *Hsp47* KO cells form detergent-insoluble aggregates in the ER following ER stress, as confirmed by the splicing of X-box–binding protein 1 (XBP-1) mRNA, the up-regulation of C/EBP homologous protein, and apoptosis (26, 28, 29). These aggregated procollagens are eliminated through autophagy (30). Thus, Hsp47 prevents aggregation of procollagen in the ER, thereby ensuring efficient transport of procollagens from the ER to the Golgi apparatus. Based on these *in vivo* and *in vitro* experiments, Hsp47 appears to be indispensable for secretion of stable triple-helical collagen into the ECM, and it has two functions as a molecular chaperone: inhibition of local unfolding of procollagen and inhibition of procollagen aggregation (Fig. 1).

Transcriptional regulation of Hsp47 expression

Hsp47 was identified as a heat shock protein and is the only heat-inducible chaperone in the ER of mammalian cells (12). Upon heat shock, heat shock factor 1 binds a heat shock element located −180 bp from the transcription initiation site of *Hsp47* and activates the transcription of *Hsp47* mRNA (31). Although many ER-resident chaperones, including BiP and Grp94, are induced by accumulation of misfolded proteins in the ER, Hsp47 is not induced by ER stress-response pathways (32, 33). During embryonic development in medaka fish, ER stress occurs physiologically (34). Two unfolded protein response (UPR) transducer and transcriptional factors, ATF6 and BBF2H7, are required when notochord cells differentiate into sheath cells, which occurs concomitantly with the synthesis of type II collagen. ATF6 adjusts expression levels of ER chaperones, and BBF2H7 regulates a set of genes (Sec proteins, *Tango1, Sedlin*, and *KLHL12)* that are essential for the enlargement of COPII vesicles to export type II collagen. However, *Hsp47* mRNA expression is not affected by *BBF2H7* KO or *ATF6α/β* double KO (35).

From an evolutionary perspective, the *KAR2* gene of *Saccharomyces cerevisiae* encoding BiP has a functional heat shock element and a UPR element that are involved in the induction of *Bip* mRNA by unfolded proteins, and the two elements regulate transcription of *KAR2* independently (36). However, in mammals, the *Bip* gene does not include a heat shock element. Hsp47 is conserved at least in vertebrates, although collagen is conserved in all multicellular animals (37, 38). Analysis of the gene structure and genomic organization of *Hsp47/SerpinH1* in vertebrate genomes revealed an ancestral *Hsp47/SerpinH1* locus in Japanese lamprey (*Lethenteron japonicum*), which has remained on the same or similar locus for ~500 million years (37). A single copy of the *serpinH1* gene was detected in the genomes of human, chicken, and frog (*Xenopus*) using homology detection tools. However, the number of *Hsp47* genes is variable in fish; there are two copies in the genomes of takifugu and medaka, but three copies in the genomes of cave fish and zebrafish (37).

Notably, Hsp47 specifically binds to procollagens, whereas other molecular chaperones such as Hsp60, Hsp70, BiP, and Hsp90 exhibit broad substrate specificity (39). Although Hsp47

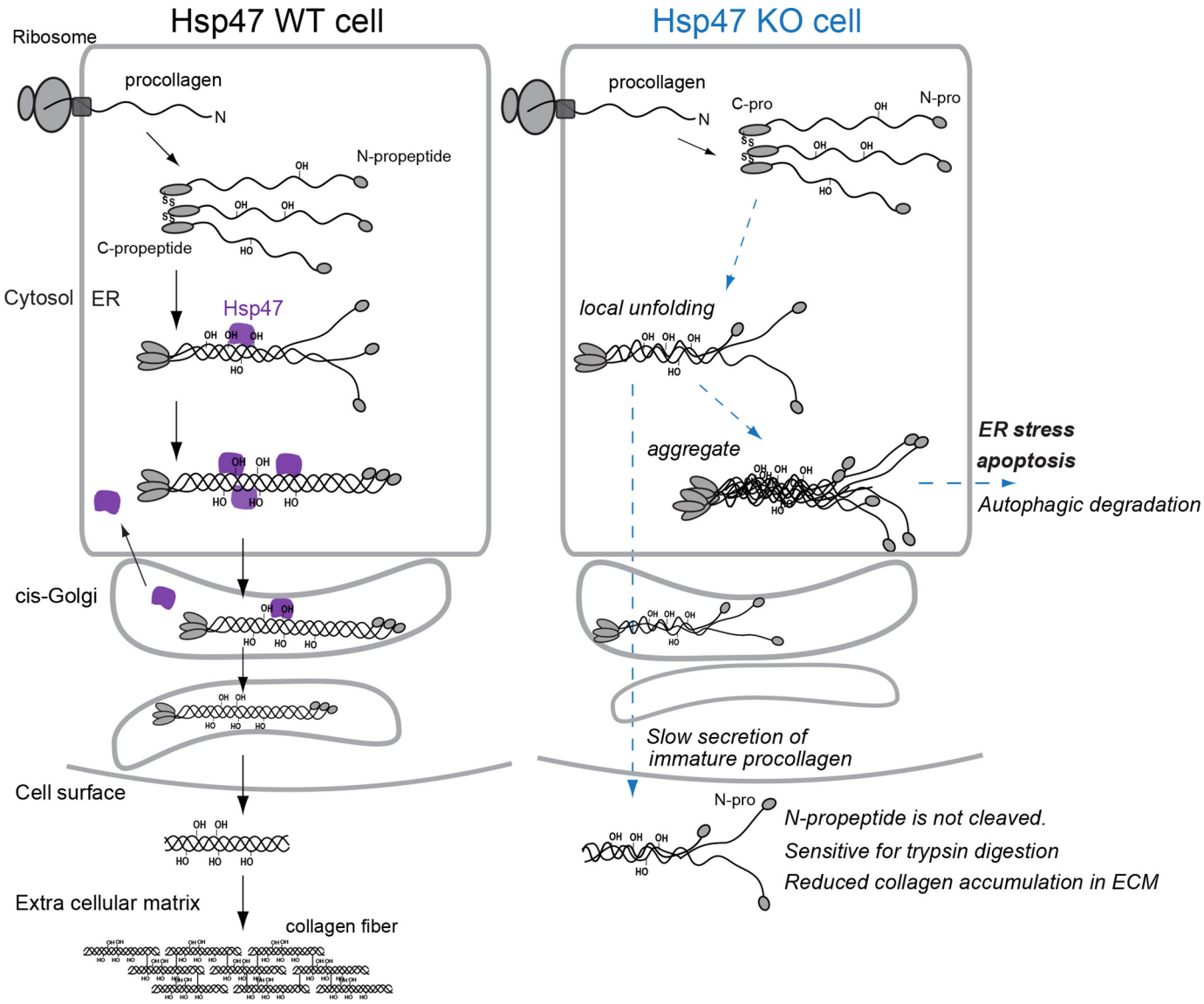

Figure 1. Procollagen folding in the ER comparing Hsp47 WT and *Hsp47* KO cells. Newly synthesized procollagen is inserted into the ER where it forms a trimer with a triple-helical structure. The collagen-specific molecular chaperone Hsp47 binds to triple-helical procollagen in the ER and dissociates in the *cis*-Golgi under low pH. Hsp47 prevents the local unfolding and aggregation of procollagens. In *Hsp47* KO cells, collagen folding is impaired, and a fraction is retained in the ER. Detergent-insoluble aggregates of procollagen and induction of ER stress are observed, ultimately triggering apoptosis. N-propeptides of procollagen secreted from *Hsp47* KO cells are not processed due to improper folding of the triple helix.

is induced by heat stress, the constitutive expression of Hsp47 is invariably correlated with expression of various types of collagens in multiple tissue, cell types, and collagen-related pathological conditions, including fibrotic diseases. Basal expression of Hsp47 requires a binding site for the Sp1 transcriptional factor, whereas tissue-specific expression is regulated by two domains in the first and second introns. *Hsp47* mRNA contains a binding site for microRNA-29 (miR-29) in the 3′-untranslated region (UTR) (40). The simultaneous down-regulation of miR-29 and up-regulation of *Hsp47* has been reported in pancreatic, gastric, and cervical cancers (41–43). The miR-29 family plays an important role in the regulation of ECM-related genes, suggesting that miR-29 might inhibit cancer cell migration and invasion. The introduction of miR-29 or silencing of *Hsp47* in breast cancer cells also suppresses malignant phenotypes by reducing collagen deposition (40).

Clinical relevance of Hsp47

Osteogenesis imperfecta (OI)

Hsp47 is a collagen-specific molecular chaperone that is essential for collagen synthesis at least in vertebrates. Thus, unsurprisingly, Hsp47 is tightly associated with collagen-related diseases, including OI, keloid, and fibrosis. OI, also known as brittle bone disease, is a genetic disease of connective tissue characterized by bone fragility, bone deformity, growth deficiency, and shortened life span. Most cases involving autosomal dominant inheritance are caused by mutations in type I collagen genes, which are associated with defective molecular assembly of bone collagen. There are four types of OI (Sillence's classification) based on clinical features and disease severity (44): OI type I (mild, common, with blue sclera); OI type II (perinatal lethal form); OI type III (severe and progressively

Table 1
Hsp47/SerpinH1 osteogenesis imperfecta mutations
OI type is based on Sillence's classification. Homo, homozygous; hetero, heterozygous.

Gene mutation	Amino acid change	Hetero-/homozygous	Species	OI type	Ref.
c.338–357del	Premature termination	Homo	Human	II	46
c.233T→C	p.L78P	Homo	Human	III	47
c.149T→G/c.1214G→A	p.L50R/p.R405H	Compound hetero	Human	IV	94
c.314–325del	p.delE105_H108	Homo	Human	IV	95
c.710T→C	p.M237T	Homo	Human	IV	48
c.977C→T	p.L326P	Homo	Dog	NA[a]	49

[a] NA means not available.

deforming, with normal sclera); and OI type IV (moderate severity with normal sclera). There is no cure for OI; treatment is directed toward preventing fractures, controlling symptoms, and developing bone mass.

In the last decade, recessive forms of OI resulting from mutations in collagen-modifying enzymes and chaperones, such as CRTAP, P3H1, CyPB, FKBP65, and Hsp47, have also been identified (45). Mutations in Hsp47 that lead to an OI phenotype have been reported in both humans and dachshunds (Table 1), comprising five missense mutations, including c.233T>C (p.L78P), c.710T>C (p.M237T), c.977C>T (p.L326P), and c.149T>G (p.L50R)/c.1214G>A (p.R405H), and two deletion mutations, c.338_357del22 and c.314_325del12 (p. deletion of Glu-105–His-108). The most severe symptoms were associated with deletion mutant c.338_357del22, resulting in a premature termination codon and nonsense-mediated decay of the abnormal mRNA. The patient was delivered by cesarean section at 36 weeks' gestation with a birth weight of 1600 g, and he died at 8 days of age with hemodynamic instability and pulmonary hemorrhage (46).

It is difficult to understand genotype–phenotype correlations in OI patients with SerpinH1 mutations because the sample size is small. However, it seems that there is a correlation between a decrease in the expression level of Hsp47 and the severity of the phenotype. The L78P mutant of Hsp47 was hardly detected in skin fibroblasts from the patient (47). In contrast, about half the amount of Hsp47 was detected in M237T and L326P mutants compared with WT levels (48, 49). L78P presented a more severe OI phenotype. The overmodification of procollagens was observed in Hsp47 L326P mutant cells but not in the human OI L78P patient. To compare the molecular features of Hsp47 between mutants, stability and proteasome sensitivity were examined by transfection of L78P and L326P mutants into *Hsp47* KO mouse embryonic fibroblast (MEF) cells (50). The amount of Hsp47 in both OI mutants was reduced due to ER-associated degradation of these structurally unstable proteins by the ubiquitin–proteasome system. The solubility of both Hsp47 mutants was considerably lower than that of WT Hsp47, and neither mutant bound to collagen, suggesting that these Hsp47 mutants lack the ability to bind procollagen in the ER. Thus, the molecular mechanism of OI in human and dog appears to involve not only a decrease in the amount of soluble Hsp47 in the ER, but also a reduced ability of Hsp47 to bind procollagens as a molecular chaperone.

Fibrosis

Fibrotic diseases, including liver, heart, kidney, and idiopathic pulmonary fibrosis, are characterized by the abnormal accumulation of ECM components, including collagen, followed by the onset of chronic inflammatory events. Long-term pathological accumulation of collagen in the ECM disrupts the normal structure and integrity and impairs the normal functions of the organ (51). Although a vast number of patients suffer from fibrotic diseases, no specific treatment is currently available (52). An imbalance between collagen synthesis and degradation caused by chronic inflammation results in abnormal accumulation of collagen. Thus, regulation of collagen biosynthesis and secretion offers a promising target for the treatment of these diseases.

Expression of both collagen and Hsp47 is increased dramatically with the onset of liver fibrosis, idiopathic pulmonary fibrosis, intestinal fibrosis, and glomerulonephritis (53–55). In an experimental glomerulosclerosis model induced by anti-Thy-1 antibodies, knockdown of *Hsp47* using antisense oligodeoxynucleotides decreases collagen accumulation in mouse kidneys (56). Knockdown of *Hsp47* also suppresses peritoneal fibrosis (57) and scar formation in rats (58). These studies clearly indicate a promising strategy for fibrosis treatment; inhibition of Hsp47 could suppress collagen accumulation and thus reduce the progression of fibrotic diseases.

Several *Hsp47* siRNA delivery systems have been developed. In one system, injection of biodegradable cationized gelatin microspheres containing *Hsp47* siRNA can continuously release siRNA over 21 days as a result of microsphere degradation, which suppresses collagen expression and prevents peritoneal fibrosis (59). In another system, mesoporous silica nanoparticles (MSNPs), which are biodegradable and have low toxicity *in vivo*, can decrease reactive oxygen species (60). In a bleomycin-induced scleroderma (skin fibrosis) mouse model, intradermal administration of si*Hsp47*–MSNPs effectively reduced Hsp47 protein expression in skin to normal levels, and reduced the pro-fibrotic markers, collagen type I, α-smooth muscle actin, and NADPH oxidase 4 (Nox4), as well as skin thickness (61). However, the most successful delivery system to date involves vitamin A-coupled liposomes encapsulating siRNA that targets *Hsp47*, and this system efficiently and preferentially targets stellate cells that store vitamin A (62). Chronic injury causes fibrosis in several organs by inducing collagen production. When stimulated by reactive oxygen intermediates or inflammatory cytokines, stellate cells are activated and transform into myofibroblasts, which actively produce and secrete collagen into the ECM (63). Thus, stellate cells are largely responsible for fibrosis, and vitamin A-coupled liposomes siRNA targeting *Hsp47* can improve liver, pancreatic, pulmonary, and skin fibrosis (62, 64–66).

During regression of liver fibrosis, half of activated hepatic stellate cells (HSCs) undergo apoptosis, and the other half escape apoptosis and revert to inactivated HSCs, which are more rapidly reactivated in response to fibrogenic stimuli than quiescent HSCs. The reactivation of these HSCs is regarded as a risk factor for fibrosis (67). Treatment of siRNA targeting *Hsp47* induces apoptosis in HSCs (62); thus, dysfunction of Hsp47 could alleviate fibrosis in two concomitant ways: inhibition of collagen secretion and induction of apoptosis in collagen-producing cells. Down-regulation of *Hsp47* or chemical inhibition of Hsp47 function therefore represents a novel therapeutic strategy for treating various fibroses (29).

Hsp47 mutants lacking the ability to bind to procollagen fail to recover collagen secretion in *Hsp47* KO fibroblasts (68), suggesting that inhibitors targeting Hsp47–procollagen binding in the ER offer another promising strategy for treating fibrotic diseases. Some potential inhibitors of this interaction have been identified by *in silico* screening based on the crystal structure (69) and by screening for the ability to prevent collagen fibrogenesis *in vitro* (70). However, no compound that inhibits Hsp47 function both at the cellular level and *in vivo* has yet been reported. Recently, we identified a small molecule compound (Col003) that inhibits the interaction between Hsp47 and collagen by screening chemical libraries. Col003 competitively inhibits the Hsp47–collagen interaction, inhibits collagen secretion by destabilizing the collagen triple helix, and decreases accumulation of collagen in the ECM (68). Structural information obtained by nuclear magnetic resonance (NMR) spectroscopy analysis revealed that Col003 competitively binds to the collagen-binding site of Hsp47, which could provide a basis for designing more effective therapeutic drugs for managing fibrosis.

Hsp47 on the cell surface in rheumatoid arthritis (RA) and thrombosis

Although Hsp47 localizes in the ER as the collagen-specific molecular chaperone, Hsp47 was also reported to localize on the cell surface in RA-related cell lines and platelets (71, 72). RA is an inflammatory autoimmune disease, in which pain and deformation of joints of arms and legs are caused by self-immunity. Hsp47 was reported as RA-related antigen protein from a human chondrosarcoma-derived chondrocytic cell line (71). When cells are treated with inflammatory cytokines such as TNFα, Hsp47 was detected on the cell surface by immunofluorescence staining. The altered localization of Hsp47 to the cell surface or the secretion into the blood may be used as the marker of RA.

Hsp47 on the surface of human platelets was also reported by proteomic analysis (72). Platelets, anucleate blood cells critical for hemostasis, adhere to collagens at sites of vessel wall injury, and form platelet aggregation that plugs the wound and prevents blood loss. Hsp47 is detected on the surface of platelet progenitor megakaryocytes and platelets by immunofluorescence and immunoblot (73). Inhibition of the interaction between Hsp47 and collagen using Hsp47 antibody diminished the formation of platelet aggregation. Platelet-specific *Hsp47* KO mice (*Pf4* (platelet factor 4)-Cre; *Hsp47*-flox/flox) reduced thrombosis induced by laser in cremaster muscle arterioles and needed more bleeding time. These data suggest that not only well-known platelet collagen receptor glycoprotein VI but also Hsp47 on the platelet surface interacts with collagen, stabilizes platelet adhesion, and thrombus formation (73).

The above two studies suggest intriguing new aspects of Hsp47 function. However, it is not well understood how Hsp47 localizes to the cell surface. One possible mechanism of the transport of Hsp47 to the cell surface is that overexpression of Hsp47 may saturate the Hsp47-anchoring protein in the ER, such as the KDEL receptor, and overflow beyond the Golgi apparatus (74). Because several ER oxidoreductases, ERp57 and ERp72, were observed on the platelet surface (75, 76), the specificity of ER protein localization and function on platelets should be investigated in the future.

New interactors of Hsp47

Recently, novel Hsp47-interacting proteins were identified (Fig. 2). These binding partners could help to elucidate as-yet-undefined biological roles of Hsp47.

Inositol-requiring enzyme 1α (IRE1α)

The UPR in the ER is a dynamic signaling network that helps to maintain ER proteostasis (77). The UPR adjusts and matches the protein folding capacity of the ER physiologically and pathologically (78). Inositol-requiring enzyme 1α (IRE1α), a type I ER transmembrane protein with serine/threonine protein kinase and endoribonuclease activities, is the most conserved UPR transducer that determines the cell fate under ER stress (79). Binding of the ER chaperone BiP to the luminal domain of IRE1α maintains it in a monomeric inactive state. Under ER stress, BiP preferentially associates with unfolded proteins, releasing the inhibitory effects that which allow the dimerization and autophosphorylation of IRE1α, triggering the activation of its RNase domain. IRE1α catalyzes the unconventional splicing of the mRNA encoding XBP-1, which modulates the expression of ER components that respond to ER stress. Although IRE1α is known to regulate ER stress via the UPR, the mechanistic details of the regulation of IRE1α itself remain poorly understood.

Interactome screening of IRE1α regulators identified Hsp47 as a candidate (80). Hsp47 directly binds to the ER luminal domain of IRE1α with high affinity *in vitro*, displacing the negative regulator BiP from the complex to facilitate IRE1α oligomerization. Co-immunoprecipitation assays showed that binding of endogenous Hsp47 to IRE1α is enhanced a short time after ER stress induction, correlating with the release of BiP from IRE1α. This indicates that Hsp47 is a novel IRE1α interactor that adjusts IRE1α signaling and may be important for a flexible and adaptive UPR pathway. Further investigation will hopefully clarify the novel role of Hsp47 in the UPR, especially the connection between collagen folding and ER stress regulation.

TANGO1

Procollagens folded in the ER form rigid rod-like structures ~300 nm in length (81) that are too large to enter conventional COPII-coated vesicles, which are less than 90 nm in diameter, suggesting that procollagen secretion from the ER requires specialized factors. TANGO1 has been identified as a cargo recep-

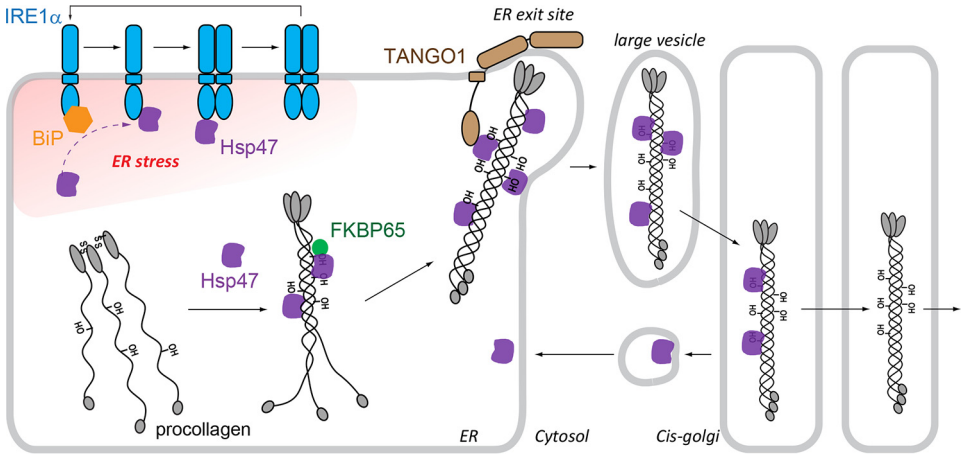

Figure 2. Newly discovered molecules that interact with Hsp47. Hsp47 directly binds to the ER luminal domain of IRE1α, displacing the negative regulator BiP from the complex to facilitate IRE1α oligomerization and modulate IRE1α signaling. Hsp47 also interacts with FKBP65, an ER-resident peptidylprolyl isomerase involved in collagen cross-linking via LH2. These proteins work together during procollagen maturation, contributing to the molecular stability and post-translational modification of type I procollagen. Hsp47 interacts with the SH3 domain of TANGO1, anchoring the molecule between TANGO1 and collagens at the ER exit site. TANGO1 is the cargo receptor required for the enlargement of COPII vesicles to accommodate large proteins for secretion from the ER.

tor for large proteins, including procollagens and pre-chylomicrons (6, 82). In mice, KO of TANGO1 results in delayed secretion of various types of collagens, including types I, II, III, IV, and VII, resulting in delayed chondrocyte and bone maturation (83). TANGO1 forms a complex with cTAGE5, interacts with Sec12 and Sec16 at the ER exit site, and tightly regulates the Sar1 GTPase cycle to accomplish large cargo secretion (84). The Src homology 3 (SH3) domain of TANGO1, located on the inside of the ER, reportedly recognizes type VII collagen because a mutant lacking the SH3 domain of TANGO1 does not bind collagen type VII secreted from RDEB/FB/C7 cells in pulldown assays (6). SH3 domains are small protein modules mediating protein–protein interactions related to cell proliferation, migration, and cytoskeletal modifications (85).

Recently, the collagen-specific molecular chaperone Hsp47 was identified as a candidate guide molecule for directing collagens to special vesicles by interacting with the SH3 domain of TANGO1 (86). Purified chicken Hsp47 directly binds the recombinant SH3 domain of human TANGO1, with a K_D of 0.26 μM. The binding orientation between collagens, the SH3 domain, and Hsp47 was evaluated by surface plasmon resonance, and binding of the SH3 domain to Hsp47 is not competitive with the binding of Hsp47 to type I collagen. Therefore, Hsp47 can function as an anchor molecule between the SH3 domain of TANGO1 and collagens. This finding may solve the important question of how TANGO1 is able to recognize different types of collagens (83). Additional studies are required to reveal the catch and release mechanisms of Hsp47, procollagen, and TANGO1 in the ER exit site, because TANGO1 does not enter the large COPII vesicles, whereas Hsp47 and procollagens are packed into these vesicles, and Hsp47 is dissociated from procollagens at the ERGIC or *cis*-Golgi. It would be intriguing to investigate whether the TANGO1 and Hsp47 system can evaluate and select the quality of procollagen at the ER exit site.

FKBP65

Collagens are structural ECM proteins that provide mechanical support to tissues (87). To gain stability, collagens can form intermolecular covalent cross-links between collagen telopeptide and helical domains, following telopeptide lysine hydroxylation by lysyl hydroxylase 2 (LH2) (88, 89). FKBP65, a 65-kDa FK506-binding protein encoded by the *Fkbp10* gene, is an ER-resident peptidylprolyl isomerase that forms complexes with LH2 (90). FKBP65 is involved in collagen cross-linking by specifically mediating the dimerization of LH2, which is required for LH2 activity. *Fkbp10* KO mice die before birth due to a growth delay and tissue fragility. Type I collagen isolated from these mice revealed less stable cross-links at telopeptide lysines (91). Furthermore, in *Fkbp10* KO MEFs, procollagen secretion was delayed, resulting in dilated ER, suggesting that FKBP65 also possesses chaperone activity for procollagens. Indeed, FKBP65 inhibits the thermal aggregation of citrate synthase and is involved in refolding denatured rhodanese (92). FKBP65 interacts with collagen and inhibits the *in vitro* fibril formation of type I collagen.

In Hsp47 OI mutant (M227T) cells, Hsp47 is destabilized and mislocalized, and FKBP65 is also destabilized at the protein level (48), suggesting that Hsp47 and FKBP65 act cooperatively during post-translational maturation of type I procollagen and that FKBP65 and Hsp47 fail to properly interact in M227T cells. *In situ* localization of the interaction between Hsp47 and FKBP65 was detected using proximity ligation assays with *Fkbp10* KO fibroblasts as controls, and a significant reduction in signal was observed in Hsp47 mutant cells compared with WT cells (48). These results suggest that Hsp47 and FKBP65 interact or work in very close proximity. Using purified endogenous proteins, interactions between Hsp47, FKBP65, and collagen were examined *in vitro*, and Hsp47 and FKBP65 were found to engage in a direct but weak interaction, whereas FKBP65 preferentially interacts with Hsp47 rather than type I collagen (93). Taken together, the findings indicate that FKBP65, LH2, and Hsp47 work together during procollagen maturation, contributing to the molecular stability and post-translational modification of type I procollagen.

Although Hsp47 has been identified as a collagen-specific molecular chaperone, newly discovered interacting proteins of Hsp47, including IRE1α, TANGO1, and FKBP65 as above, indicate a much broader role for Hsp47 that warrants further investigation.

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