TRANSLATIONAL REVIEW

Fighting the Fiber: Targeting Collagen in Lung Fibrosis

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Abstract

Organ fibrosis is characterized by epithelial injury and aberrant tissue repair, where activated effector cells, mostly fibroblasts and myofibroblasts, excessively deposit collagen into the extracellular matrix. Fibrosis frequently results in organ failure and has been estimated to contribute to at least one-third of all global deaths. Also, lung fibrosis, in particular idiopathic pulmonary fibrosis (IPF), is a fatal disease with rising incidence worldwide. As current treatment options targeting fibrogenesis are insufficient, there is an urgent need for novel therapeutic strategies. During the last decade, several studies have proposed to target intra- and extracellular components of the collagen biosynthesis, maturation, and degradation machinery. This includes intra- and extracellular targets directly acting on collagen gene products, but also such that anabolize essential building blocks of collagen, in particular glycine (Gly) and proline (Pro) biosynthetic enzymes. Collagen, however, is a ubiquitous molecule in the body and fulfills essential functions as a macromolecular scaffold, growth factor reservoir, and receptor binding site in virtually every tissue. This review summarizes recent advances and future directions in this field. Evidence for the proposed therapeutic targets and where they currently stand in terms of clinical drug development for treatment of fibrotic disease is provided. The drug targets are furthermore discussed in light of *1*) specificity for collagen biosynthesis, maturation, and degradation, and *2*) specificity for disease-associated collagen. As therapeutic success and safety of these drugs may largely depend on targeted delivery, different strategies for specific delivery to the main effector cells and the extracellular matrix are discussed.

Keywords: LARP6; HSP47; FKBP10; prolyl hydroxylase; lysyl oxidase

Fibrosis is an aberrant tissue regeneration response following injury and can affect most tissue types and organs. Estimated to contribute to at least one-third of all deaths worldwide and even 45% of deaths in the developed world, fibrosis undoubtedly is a major global cause of mortality and morbidity (1, 2). Irrespective of affected tissue, fibrosis is characterized by increased extracellular matrix (ECM) deposition. The culprit effector cells are most often fibroblasts, which, upon specific activating cues, exit quiescence, proliferate, migrate, and secrete excessive amounts of ECM components. Deposition and subsequent crosslinking of the ECM lead to a drastic reduction of tissue functionality, ultimately resulting in organ failure when disease progresses (3, 4).

Constituting between 30% and 70% of ECM protein in all tissue types, collagen is the main component of the ECM, and its biosynthesis, deposition, and crosslinking are highly upregulated in fibrotic disease (4–6). Attenuation of collagen synthesis and deposition, therefore, is the most common readout when the efficacy of new potential drugs is evaluated *in vitro* and *in vivo*. Many drugs targeting central fibrogenic pathways, like transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), or mammalian target of rapamycin (mTOR) signaling have a pronounced effect on collagen synthesis and maturation, but, owing to the functional pleiotropy of their targets, do not specifically act on collagen (7–9). Clearly, targeting central upstream mechanisms of fibrogenesis can be advantageous as typically various profibrotic processes are affected, but this approach may also lead to unanticipated and undesired side effects (7, 10).

During the last decade, the collagen biosynthesis and maturation pathway itself has emerged as an attractive therapeutic

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target for fibrotic disease. This includes targets directly acting on collagen but also such that anabolize essential building blocks of collagen, in particular glycine (Gly) and proline (Pro) biosynthetic enzymes. However, it is important to acknowledge that collagen is a ubiquitous molecule in the body that fulfills essential functions as macromolecular scaffold, growth factor reservoir, and receptor binding site in virtually every tissue. The challenge, therefore, consists in targeting the disease-specific collagen. This review aims to provide a comprehensive overview of currently discussed drug targets in this context and offer conceptual insight into how fibrosis-specific targeting could be achieved. First, collagen types (Table 1) and biosynthesis, maturation, and degradation of fibrillar collagens are reviewed. Then, considering all types of organ fibrosis, the current state of potential antifibrotic drug targets within the collagen biosynthesis and maturation pathway is summarized (Table 2). This is followed by an assessment of the targets' disease specificity and a discussion on potential strategies for targeted delivery. Ultimately, such considerations may be crucial for the successful implementation of anti-collagen biosynthesis drugs to treat fibrogenesis.

The Collagen Superfamily

In vertebrates, the collagen superfamily to date comprises 28 members, numbered with Roman numerals I to XXVIII. Collagens contain other domains, too, but the presence of at least one triple-helical domain, termed collagen domain, is the most important unifying and defining feature for this superfamily. Every collagen molecule is assembled from three parallel polypeptides, the so-called α-chains, which are in a polyproline II-type helical conformation and wind around each other, forming a right-handed triple helix (11). The α-chains that make up collagen can be identical or different, depending on the collagen type. For instance, type III collagen is made up of three identical α_1-chains encoded by *COL3A1*. In contrast, type I collagen is a heterotrimer made of two identical α_1-chains and an α_2-chain encoded by *COL1A1* and *COL1A2*, respectively, and type VI collagen is even composed of three different chains.

Collagens are classified according to their extracellular supramolecular assembly (Table 1) (12–16). Fibril-forming collagens, in particular types I, III, and V, are well established as overexpressed in fibrotic disease, and most of what we know about collagen biosynthesis and maturation derives from fibril-forming collagens, above all from the archetypal type I collagen. Even though alterations of other collagen types in fibrosis have been described, including type IV, VI, VII, XV, XVIII, and XXVII (17–20), these have received much less attention to date, and their roles in disease pathogenesis have yet to be elucidated.

Unique Characteristics and Requirements of the Collagen Triple Helix

The triple-helical three-dimensional structure is a unique property of the collagen domain (Figures 1A–1C). It provides exceptional strength and stability and confers resistance to mechanical and tensile stress. Two amino acids, namely Pro and Gly, are of exceptional importance for triple helix formation, and their frequencies are much higher in collagen domains than in the average vertebrate protein (21) (Figure 1D).

Table 1. Classification of Collagens according to Supramolecular Assembly

Class	Collagen Types
Fibril-forming collagens	I, II, III, V, XI, XXIV, XXVII
FACITs	IX, XII, XIV, XVI, XIX, XX, XXI, XXII
Network-forming collagens	IV, VIII, X
Transmembrane collagens	XIII, XVII, XXIII, XXV
Endostatin-producing collagens (multiplexins)	XV, XVIII
Anchoring fibrils	VII
Beaded-filament-forming collagens	VI
Others	XXVI, XXVIII

Definition of abbreviation: FACITs = fibril-associated collagens with interrupted triple helices.

Overrepresentation of Pro increases the propensity of the individual α-chains to adopt the above-mentioned polyproline II-type helical conformation, which in turn decreases the entropic cost for triple helix folding (11). The use of Pro, however, is associated with a drawback: All collagen Pros must be in *trans* conformation to allow for linear chains because *cis*-Pros stereochemically induce bends and turns in proteins. While the *trans* conformation typically is energetically far more favorable for all other amino acids, this is not so pronounced for Pros. Up to 6% of all Pros in protein structures adopt the *cis* conformation (22); collagen folding, therefore, requires peptidyl-prolyl isomerases (PPIs), enzymes that interconvert *cis*- and *trans*-Pros.

In addition, amino acid repeats of Gly-Xaa-Yaa are essential for the assembly of the three α-chains because triple helix assembly positions the residue of every third amino acid toward the interior of the helix where the available space only accommodates the smallest side group possible, the single hydrogen atom of Gly (Figure 1C). This also means that all other amino acids in Xaa and Yaa positions point outwards (shown for Pro in Figure 1B). Fibrillar collagens, like type I and type III collagens, are typically composed of a single uninterrupted collagen domain once propeptide cleavage has occurred in the extracellular space. Consequently, Gly makes up more than 30% of their amino acid sequence (Figure 1D).

All amino acids can be obtained by diet, and endogenous Pro and Gly anabolism is generally not considered sufficient to maintain collagen biosynthesis on its own (23). However, within the last years, it has become increasingly clear that myofibroblast differentiation comes along with profound metabolic changes, including upregulation of glycolysis and glutaminolysis, which both feed into the equally upregulated *de novo* biosynthetic pathways for Pro and Gly (24). This metabolic reprogramming makes myofibroblasts much less dependent on dietary supplementation for collagen biosynthesis and provides interesting novel therapeutic strategies. Given the exceptional importance of Pro and Gly for collagen biosynthesis, their *de novo* biosynthetic pathways, including the most important enzymes, will be described in the following.

De Novo Pro Biosynthesis

Pro can, in principle, be synthesized from several precursors. The classical pathway of

Table 2. Suggested Drug Targets in the Context of Collagen Biosynthesis and Maturation and Status of Clinical Development

Potential Drug Target	Collagen Types Affected	Organ Fibrosis Types Assessed	Status of Validation	Clinical Trials (139)
GLS1	All (affects *de novo* Gly and Pro biosynthesis)	Lung fibrosis (26)	Therapeutic approach *in vitro* (human lung fibroblasts) (26, 35) Therapeutic approach *in vivo* (two independent mouse models of lung fibrosis) (37)	None
PHGDH	All (affects *de novo* Gly biosynthesis)	Lung fibrosis (34)	Therapeutic approach *in vitro* (human lung fibroblasts) (33) Therapeutic approach *in vivo* (mouse model of lung fibrosis) (34)	None
SHMT2	All (affects *de novo* Gly biosynthesis)	Lung fibrosis (33, 34)	Therapeutic approach *in vitro* (human lung fibroblasts) (33)	None
PSAT1	All (affects *de novo* Gly biosynthesis)	Lung fibrosis (26)	Therapeutic approach *in vitro* (human lung fibroblasts) (26)	None
ALDH18A1	All (affects *de novo* Pro biosynthesis)	Lung fibrosis (26)	Therapeutic approach *in vitro* (human lung fibroblasts) (26)	None
LARP6	I, III (129)	Liver fibrosis (130) Lung fibrosis (131)	Therapeutic approach *in vitro* (human lung fibroblasts, hepatic stellate cells) (130, 131) Therapeutic approach *in vivo* (rat models of liver fibrosis) (130)	None
miR-29a miR-29b miR-29c	I, II, III, IV, V, VII, VIII, XI, XV, XVI (225, 226)	Cardiac fibrosis (182, 183) Kidney fibrosis (227) Liver fibrosis (132, 134) Lung fibrosis (226) Muscle fibrosis (137) Skin fibrosis (136)	Therapeutic approach *in vitro* (rat cardiac fibroblasts, mouse cardiac myocytes, mouse lung fibroblasts, hepatic stellate cells, human skin fibroblasts) (132, 133, 138, 228) Therapeutic approach *in vivo* (mouse model of liver, lung, muscle, skin fibrosis) (134, 136–138)	First generation miR-29b mimic (MRG-201, remlarsen) for treatment of skin fibrosis: Phase 1: NCT02603224 Phase 2: NCT03601052 (136)
P4HA1 P4HA2 P4HA3	All collagens are considered substrates (229)	Bladder fibrosis (148) Cardiac fibrosis (146) Liver fibrosis (147) Lung fibrosis (145)	Therapeutic approach *in vitro* (human lung fibroblasts, hepatic stellate cells) Therapeutic approach *in vivo* (rat model of bladder and liver fibrosis, mouse model of lung fibrosis) (145, 147, 148)	None
FKBP10	I, III, V, VI (71, 72)	Lung fibrosis (71, 72, 151)	Therapeutic approach *in vitro* (human lung fibroblasts) (71, 72)	None

(*Continued*)

Table 2. (*Continued*).

Potential Drug Target	Collagen Types Affected	Organ Fibrosis Types Assessed	Status of Validation	Clinical Trials (139)
HSP47	I, II, III, IV, V, XI (156, 230)	Intestinal fibrosis Kidney fibrosis Liver fibrosis Lung fibrosis Pancreatic fibrosis Peritoneal fibrosis Skin fibrosis (156) (and references therein)	Proof-of concept *in vitro* and *vivo* linking HSP47 to collagen levels Therapeutic approach *in vitro* (keloid fibroblasts, hepatic stellate cells) Therapeutic approach *in vivo* (animal models of skin fibrosis, rat models of pulmonary fibrosis, mouse model of kidney fibrosis, mouse model of peritoneal fibrosis, rat model of liver fibrosis, rat model of pancreatic fibrosis) (156) (and references therein)	Vitamin A-coupled lipid nanoparticle containing anti-HSP47 siRNA (ND-L02-s0201, BMS-986263) for treatment of liver fibrosis/ idiopathic pulmonary fibrosis: Phase 1: NCT01858935 NCT03241264 NCT03142165 Phase 1b/2: NCT02227459 Phase 2: NCT03538301 NCT04267393 NCT03420768 (139, 156, 158)
TANGO1	I, II, III, IV, VII, IX (86, 159, 160)	Liver fibrosis (159)	Therapeutic approach *in vitro* (hepatic stellate cells) Therapeutic approach *in vivo* (mouse models of liver fibrosis) (159)	None
LOX LOXL2	I, II, III, IV, V, IX, XI (98)	Cardiac fibrosis (166, 231) Liver fibrosis (100) Lung fibrosis (100) Myelofibrosis (168, 169) Peritoneal fibrosis (167) Skin fibrosis (232)	Therapeutic approach *in vitro* and *ex vivo* (collagen hydrogels, decellularized human lung scaffolds) (174) Therapeutic approach *in vivo* (mouse models of cardiac, liver, lung, bone marrow, and peritoneal fibrosis) (100, 166–169)	LOXL2-specific antibody (simtuzumab) for treatment of liver fibrosis, idiopathic pulmonary fibrosis, and myelofibrosis: Phase 2: NCT01452308 NCT01759511 NCT01672853 NCT01769196 NCT01672879 NCT01672866 NCT01707472 NCT01369498 (170–172) pan-LOX Inhibitor (PXS-5505) for treatment of myelofibrosis: NCT04676529

Definition of abbreviations: ALDH18A1 = aldehyde dehydrogenase 18A1; FKBP10 = FK506-binding protein 10; GLS1 = glutaminase 1; HSP47 = heat shock protein 47; LARP6 = La-related protein 6; LOX = lysyl oxidase; LOXL2 = LOX-like 2; P4HA = prolyl-4-hydroxylase; PHGDH = phosphoglycerate dehydrogenase; PSAT1 = phosphoserine aminotransferase; SHMT2 = hydroxymethyltransferase 2; TANGO1 = transport and Golgi organization protein 1.

de novo Pro biosynthesis commences with α-ketoglutarate (αKG), an intermediate of the citric acid cycle (Figure 2). Glutamic-oxaloacetic transaminases (GOT) convert αKG into glutamate (Glu) under concomitant conversion of aspartate (Asp) to oxaloacetate (OA). Aldehyde dehydrogenase 18A1 (ALDH18A1) then catalyzes the two-step conversion from Glu to glutamate-semialdehyde, which spontaneously rearranges to 1-pyrroline-5-carboxylate (P5C). Finally, pyrroline-5-carboxylate reductases (PYCR) convert P5C to Pro (25).

Interestingly, TGF-β-induced collagen synthesis has been demonstrated to be independent of GOTs, suggesting that, in a profibrotic milieu, synthesis of Glu from αKG is dispensable for Pro biosynthesis. Instead, glutamine (Gln) is a far more important precursor for Glu in this context (26, 27) (Figure 2). Gln is the most abundant circulating amino acid in the blood and can replenish the citric acid cycle and act as a precursor for multiple biosynthetic pathways, including Pro biosynthesis (24, 28, 29). Glutaminase

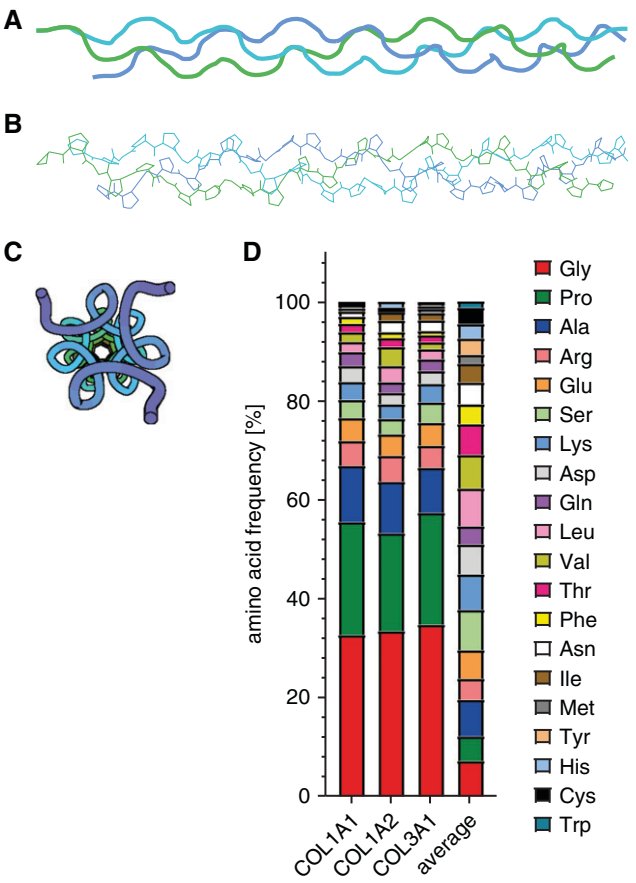

Figure 1. Glycine (Gly) and proline (Pro) are overrepresented in collagen sequences and crucial for formation of the triple helical structure. (*A–C*) Schematic representations of the collagen triple helical structure, given as polypeptide chain backbones (*A*); in ball-and-stick representation, showing the orientation of the pyrrolidine rings of Pro residues (*B*); and in cross-section, demonstrating the unique spatial restrictions inside the triple helix, which strictly require Gly as every third amino acid (*C*). Structures are retrieved from pdb file 1K6F (233) and created with BioRender. (*D*) Amino acid frequencies in three human mature collagen chains (COL1A1, COL1A2, COL3A1), representative for fibrillar collagens overexpressed in fibrosis, compared with the average observed amino acid distribution in vertebrate proteins (21). Collagen sequences without propeptides were extracted from the UniProt database (COL1A1: P02452, 162–1218; COL1A2: P08123, 80–1119; COL3A1: P02461, 154–1221).

(GLS) catalyzes the hydrolytic deamidation of Gln to generate Glu. GLS, all enzymes downstream of Glu in the biosynthesis of Pro (i.e., ALDH18A1, PYCR1, PYCR2, and PYCRL) (Figure 2), are induced by TGF-β, and at least GLS and ALDH18A1 are required for collagen biosynthesis in lung fibroblasts. In contrast, Gln is not required for mitochondrial oxygen consumption in response to TGF-β, indicating that Gln does not feed into the citric acid cycle under these conditions (26).

In addition, Pro can also be generated from arginine via action of arginase 1 or 2 (ARG1, ARG2), followed by deamination of ornithine by ornithine aminotransferase, a reaction which yields P5C and Glu (Figure 2). While this pathway appears to contribute to collagen biosynthesis and lung fibrosis in the mouse (30, 31), its significance for human fibrosis is less clear and requires further investigation.

De Novo Gly Biosynthesis

Endogenous Gly is mainly produced from serine, including dietary serine and *de novo* synthesized serine, by the action of serine hydroxymethyltransferases 1 and 2 (SHMT1 and SHMT2) (Figure 2) (32). Starting from the glycolysis intermediate 3-phosphoglycerate (3PG), the first reaction of *de novo* serine biosynthesis is the oxidation of 3PG to 3-phosphooxypyruvate catalyzed by the enzyme phosphoglycerate dehydrogenase (PHGDH). Then, phosphoserine aminotransferase (PSAT1) catalyzes amino group transfer from Glu, a reaction yielding phosphoserine and αKG. Finally, dephosphorylation catalyzed by phosphoserine phosphatase yields serine (Ser) (Figure 2). Similar to *de novo* Pro biosynthetic enzymes, enzymes of this pathway are upregulated by TGF-β and necessary for TGF-β-induced collagen synthesis in lung fibroblasts (33). Gln seems equally important as a precursor for Glu, which, here, serves as a cofactor in the PSAT1-mediated amino group transfer (Figure 2). Hence, GLS1-mediated hydrolytic deamidation of Gln generates Glu as an important precursor for both *de novo* Pro and Gly biosynthesis.

Finally, dietary intake of Pro, Gly, their precursor amino acids, and amino acids released by degradation of exogenous and endogenous proteins serve as additional pools for these amino acids. Interestingly, neither serine nor Gly deprivation protected mice from bleomycin-induced lung fibrosis (34), indicating that dietary supplementation may play a subordinate role in the context of collagen biosynthesis in fibrosis.

Proposed Targets in the Context of Pro and Gly Biosynthesis

A number of recent reports have highlighted the great potential that targeting myofibroblast-specific metabolic reprogramming holds for the treatment of fibrosis. Even though this is a relatively new concept, several *in vitro* and *in vivo* studies have already put forward a number of possible drug targets in Pro and Gly biosynthesis. With multiple collective evidence at hand, glutaminase 1 (GLS1), which generates Glu as an important cofactor for both biosynthetic pathways, may be the most promising emerging drug target in this context. Knocking down GLS1 in normal human lung fibroblasts or treatment with the GLS inhibitors CB-839 or Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide attenuated TGF-β-induced collagen synthesis (26, 35, 36). Conditional deletion of GLS1 in mouse fibroblasts protected against bleomycin-induced lung fibrosis, and CB-839 was therapeutically effective in two independent mouse models of lung fibrosis

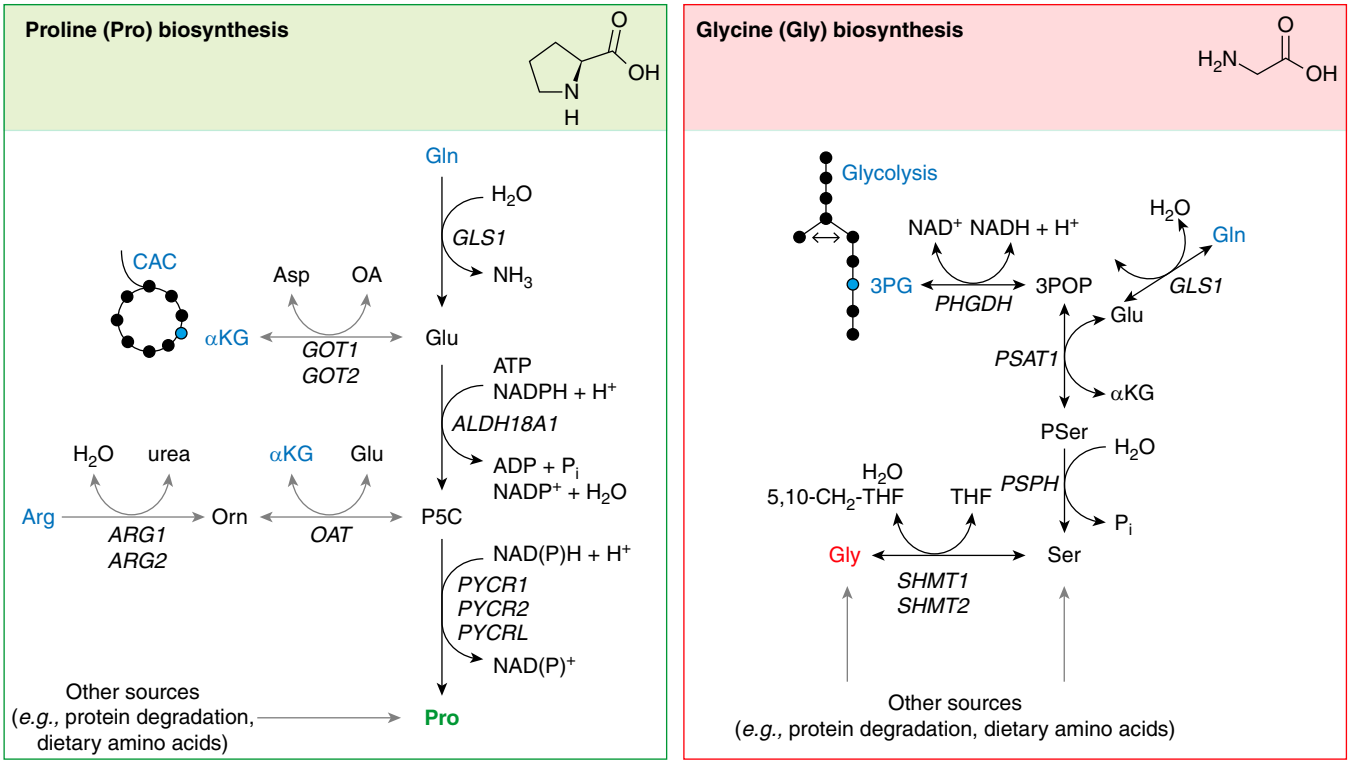

Figure 2. *De novo* Pro and Gly biosynthesis. Left box. *De novo* Pro biosynthesis starting from the citric acid cycle (CAC) intermediate α-ketoglutarate (αKG) (blue solid circle in the schematic representation of the CAC). Glutamic-oxaloacetic transaminases 1 and 2 (GOT1 and GOT2) convert αKG into glutamate (Glu) under concomitant conversion of aspartate (Asp) to oxaloacetate (OA). Aldehyde dehydrogenase 18A1 (ALDH18A1) catalyzes the two-step conversion from Glu to glutamate-semialdehyde, which spontaneously rearranges to 1-pyrroline-5-carboxylate (P5C). Finally, pyrroline-5-carboxylate reductases 1 and 2 convert P5C to Pro. Right box. *De novo* Gly biosynthesis starting from the glycolysis intermediate 3-phosphoglycerate (3PG) (blue solid circle in the schematic representation of glycolysis pathway). 3PG is oxidized to 3-phosphooxypyruvate (3POP) by phosphoglycerate dehydrogenase (PHGDH), followed by amino group transfer from Glu yielding phosphoserine (PSer) and αKG, catalyzed by phosphoserine aminotransferase (PSAT1). Dephosphorylation catalyzed by phosphoserine phosphatase (PSPH) yields serine (Ser). Finally, Ser hydroxymethyltransferases 1 and 2 (SHMT1 and SHMT2) catalyze the conversion of Ser to Gly. For both *de novo* Pro and Gly biosynthesis, Glu can be derived from glutamine (Gln) via the action of glutaminase 1 (GLS1). Arg = arginine; NAD = nicotinamide adenine dinucleotide; NAD^+, NADH = oxidized and reduced form of the coenzyme NAD; NADP = nicotinamide adenine dinucleotide phosphate; P_i = inorganic phosphate; THF = tetrahydrofolate; 5,10-CH_2-THF = 5,10-methylenetetrahydrofolate; $NAD(P)^+$, NAD(P)H = oxidized and reduced form of the coenzymes NAD and NADP.

(37). Notably, the GLS inhibitor CB-839 (telaglenastat) is currently under investigation in phase 1 and 2 anticancer clinical trials, and new classes of GLS inhibitors are being developed, emphasizing that this approach may be readily translated into antifibrotic treatment (28, 38).

Another drug target for which therapeutic proof-of-concept exists *in vivo* is the enzyme PHGDH (34). Hamanaka and colleagues, using a small molecule inhibitor of PHGDH (NCT-503), were able to show that blocking *de novo* Ser/Gly biosynthesis protected from the development of lung fibrosis in the mouse model of bleomycin-induced lung fibrosis (34). PHGDH is increasingly recognized as a drug target in the cancer field, and even though small molecule inhibitors for PHGDH have not entered clinical trials yet, lead compounds are being optimized (39).

Finally, *in vitro* evidence in primary human lung fibroblasts suggest that additional enzymes like SHMT2, PSAT1, and ALDH18A1 may qualify as drug targets. At least for SHMT2 specific preclinical small molecule inhibitors exist (40) while for PSAT1 and ALDH18A1, antisense strategies may hold the greatest potential to date.

Posttranscriptional Collagen Biosynthesis and Maturation

After transcription of collagen genes to mRNA, protein biosynthesis and maturation of collagen is a complex multistep process that involves regulatory events at the translational level, an elaborate ER-resident modification and folding machinery, but also extracellular processing and crosslinking (16) (Figure 3). As fibril-forming collagens, particularly types I, III, and V, are the collagens predominantly overexpressed in fibrotic disease, the following text will focus on the biosynthesis of those, with type I collagen as the best-described representative for this collagen class.

Collagen Chain Translation

Type I collagen is a heterotrimer composed of two α1(I) chains and one α2(I) chain, which are encoded by *COL1A1* and *COL1A2*, respectively. Translation efficacy depends on unique conserved sequences in the 5′- and 3′-UTRs of *COL1A1* and

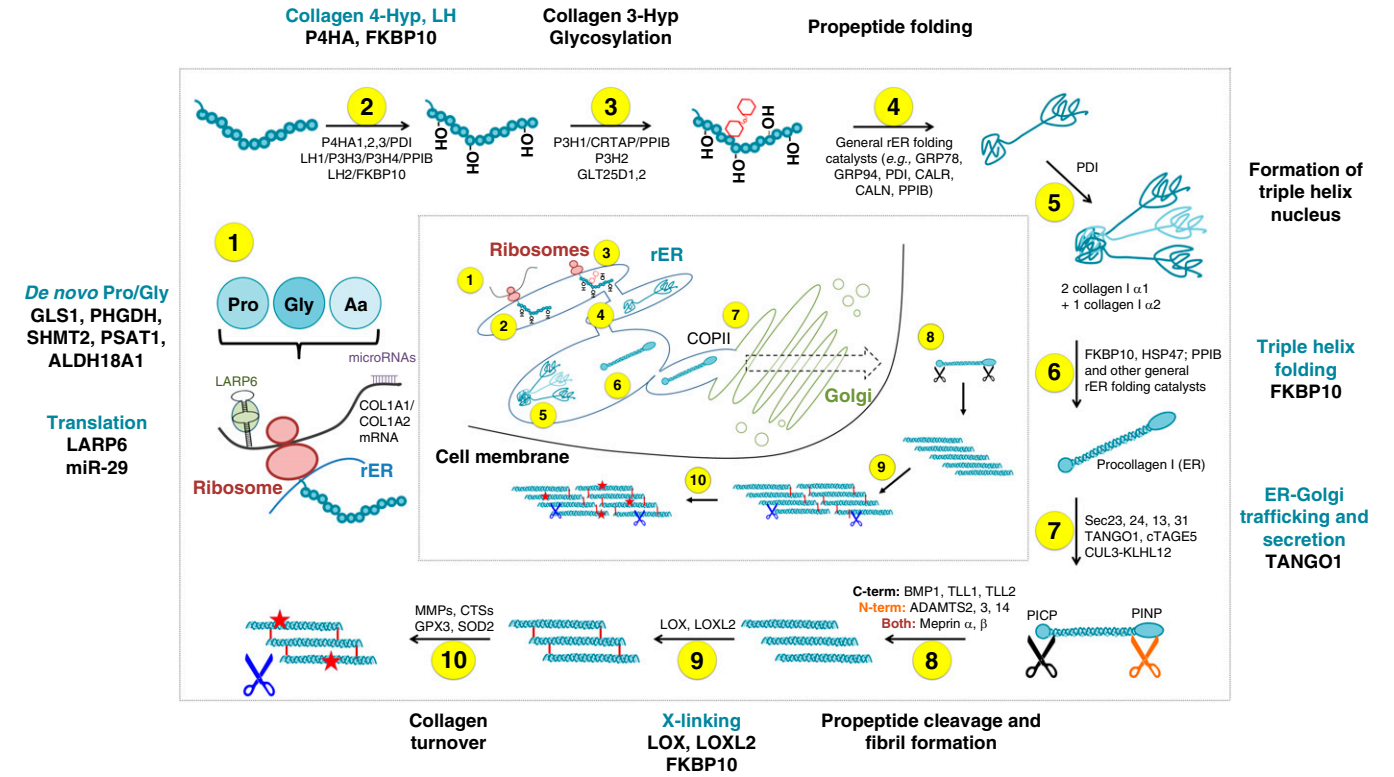

Figure 3. Collagen biosynthesis, processing, and maturation. Inner box: Overview on intra- and extracellular steps of collagen biosynthesis and maturation. Outer box. Individual steps given in more detail, including key regulators, enzymes, and chaperones. Potential drug targets (see also Table 2) are given outside of the boxes at the level of the relevant step: *1*) at the level of translation, amino acids (Aa) Pro and Gly are particularly important as overrepresented in triple-helical collagen sequences. LARP6 binds to a specific 5′ stem-loop structure in COL1A1, COL1A2, and COL3A1 mRNA and activates translation while microRNAs like has-miR-29 repress translation. *2*) During cotranslational collagen modification, prolyl-4- and lysyl hydroxylation is exerted by specific ER-resident multiprotein complexes. *3*) Further enzymes and enzyme complexes are involved in collagen prolyl-3-hydroxylation and glycosylation. *4*) A set of general rER folding catalysts mediate collagen propeptide folding. *5*) Chain selection and formation of the triple helix nucleus involves protein disulfide isomerase (PDI). *6*) Triple helix formation proceeds in a zipper-like fashion from the C- to the N-terminus and is supported by a set of collagen-specific and general PPIs and chaperones (e.g., FKBP10, HSP47, PPIB). *7*) Trafficking from the ER via the Golgi to the extracellular space is dependent on TANGO1 and cTAGE5 at the ER exit site, COPII coat proteins such as SEC13, SEC23, SEC24, and SEC31, and the ubiquitin ligase CUL3-KLHL12. *8*) Propeptide cleavage involves BMP1 or TLL1 or TLL2 at the C-terminus side and ADAMTS family members at the N-terminus, or the more recently discovered meprins, which are capable of cleavage at both sides. Propeptide cleavage triggers fibril formation. *9*) Fibrils are further stabilized by LOX-mediated crosslinking. *10*) Antioxidant proteins like GPX3 and SOD2 may protect from oxidative damage. Collagen turnover is exerted by MMPs and CTSs. Adapted from Reference 16. ADAMTS = A disintegrin and metalloproteinase with thrombospondin motifs; BMP1 = bone morphogenetic protein 1; CALN = calnexin; CALR = calreticulin; CRTAP = cartilage-associated protein; cTAGE5 = cutaneous T-cell lymphoma-associated antigen 5 or melanoma inhibitory activity protein 2; CTSs = cathepsins; FKBP10 = FK506-binding protein 10; GLT25 = glycosyl transferase 25; GPX3 = glutathione peroxidase 3; GRP = glucose regulated protein; HSP47 = heat shock protein 47; LARP6 = La-related protein 6; LH = lysyl hydroxylase; LOX(L) = lysyl oxidase(-like); MMP = Matrix metalloproteinase; P3H = prolyl-3-hydroxylase; P4HA = prolyl-4-hydroxylase; PDI = protein disulfide isomerase; PPIB = peptidyl prolyl isomerase B or cyclophilin B; Sec23, 24, 13, 31 = protein transport protein Sec23, 24, 13, 31; SOD2 = superoxide dismutase 2; TANGO1 = Transport and Golgi organization protein 1; TLL = Tolloid-like protein.

COL1A2 mRNAs. First, both transcripts (as well as *COL3A1* mRNA, which encodes the type III collagen homotrimer) are characterized by a unique 5′ stem-loop structure which is specifically recognized by the RNA binding protein La-related protein 6 (LARP6). LARP6 plays a crucial role in the acceleration of type I and III collagen biosynthesis upon profibrotic stimuli because it facilitates the recruitment of additional translational factors and the coordinated translation of these collagen chains. Second, just like the majority of mammalian genes (41), collagens are regulated by numerous microRNAs, which bind to the 3′-UTRs and induce translational repression. Here, the human micro-RNA family hsa-miR-29 stands out because it targets many collagens in concert, acting as a master regulatory hub for collagen expression (42, 43). Even if this is in addition to the regulation of other ECM components and ECM regulators, hsa-miR-29-mediated regulation of collagen types and proteins involved in collagen synthesis and crosslinking is clearly overrepresented (42, 43).

Co- and Posttranslational Modification

During translation at the ribosome, the growing polypeptide is directly extended into the lumen of the rER, where numerous modifications are inserted into the nascent unfolded chain in a cotranslational fashion.

These include hydroxylation of Pro and lysine residues as well as glycosylation of hydroxylysines, all of which are mediated by an rER-resident procollagen-specific molecular ensemble, enzymes existing in defined multiprotein complexes with other chaperones, and protein folding catalysts (Figure 3) (44). Many of these modifications are crucial for stability, triple-helix folding, and secretion of procollagen, as well as for the final extracellular supramolecular structure (12, 14, 44, 45). For instance, as mentioned previously, 4-hydroxylation of Pro residues in position Y (4-hydroxyproline) (Figure 4C) of the Gly-X-Y repeats is known to increase thermodynamic stability of the triple helix (44, 46–50). Also, the degree of lysine hydroxylation in the collagenous and telopeptide regions of fibrillar collagens defines the extent, type, and stability of extracellular intermolecular collagen crosslinks (51). At the same time, the functions of hydroxylysyl glycosylations (Figures 4D and 4E) and the comparatively rare prolyl-3-hydroxylation (3-hydroxyproline) (Figure 4B) in position X are far less understood.

Numerous enzymes and chaperones are involved in that process. Collagen prolyl-4-hydroxylation is mediated by prolyl-4-hydroxylases, which exist as $\alpha_2\beta_2$ heterotetramers composed of one of three catalytical α-subunits (encoded by *P4HA1*, *P4HA2*, and *P4HA3*) and the structural β-subunit protein disulfide isomerase (PDI) (encoded by *P4HB*). The latter helps to maintain the α-subunit in soluble active form and to retain the full complex in the ER (44, 49). Collagen prolyl-3-hydroxylation is a much less frequent and only poorly understood collagen PTM introduced by collagen prolyl-3-hydroxylases (P3H) (49, 52–59). Prolyl-3-hydroxylation is typically found on Pros in the X position of the Gly-X-Y repeat when Y is a 4-hydroxylated Pro; prolyl-3-hydroxylation is therefore thought to occur after prolyl-4-hydroxylation (44, 49). The collagen prolyl-3-hydroxylase, or leprecan family, comprises five members (P3H1–P3H4 and cartilage-associated protein [CRTAP]), of which only P3H1, P3H2, and P3H3 exert prolyl-3-hydroxylase activity. Also, these enzymes form multimeric complexes with other collagen-modifying proteins and chaperones. For instance, P3H1 forms a tight trimeric complex with CRTAP and the peptidyl-prolyl isomerase cyclophilin B (PPIB). CRTAP, despite no enzymatic activity of its own, promotes P3H1 activity (53, 60). P3H3 and P3H4 (also termed SC65) form a trimeric complex with lysyl hydroxylase 1 (LH1). Here, P3H4, also without a prolyl-3-hydroxylase activity of its own, seems to adopt a role similar to CRTAP, with which it is closely related (61, 62). In contrast, the enzymatic activity of P3H2 does not require CRTAP (63), and other interaction partners have not been identified so far.

Lysyl hydroxylases, which hydroxylate lysines in the C5 position (Figure 4A), comprise three enzymes termed LH1–LH3 and are encoded by *PLOD1–PLOD3*, respectively (44). LH1 and LH2 display domain-specific activities: LH1 preferentially acts on lysines in triple-helical regions of fibrillar collagens (64), and the resulting 5-hydroxylysine residues are often subject to O-glycosylation by glycosyltransferases (Figures 3, 4D, and 4E) (44, 64, 65). LH2, on the contrary, acts on lysines in the non-collagenous telopeptide regions of fibrillar collagens (64, 66), where the resulting 5-hydroxylysine residues are often subject to extracellular lysyl oxidase (LOX)-mediated crosslinking. While both unhydroxylated and hydroxylated telopeptide lysines can be crosslinked by LOX, the hydroxylation status of the involved lysines affects the crosslink in terms of chemical nature and stability (44, 51, 65, 66). LH1 and LH2 have been demonstrated to exist in multimeric complexes, LH1, as already mentioned above, in a complex with P3H3 and P3H4 (61, 62) and LH2 in a complex with FK506-binding protein 10 (FKBP10) (66). LH3 represents a special case in that context; it is considered the evolutionary ancestor of the LH family and capable not only of lysine hydroxylation but also of two consecutive O-glycosylation reactions, activities which the other LHs appear to have lost (44, 64, 65, 67). Finally, two collagen glycosyltransferases, GLT25D1 and GLT25D2, mediate *O*-glycosylation of collagen 5-hydroxylysines. In general, hydroxylysines can be *O*-glycosylated with a monosaccharide (β-d-galactopyranose) or a disaccharide [α-d-glucopyranosyl-(1->2)-β-d-galactopyranose], resulting in galactosyl hydroxylysine or glucosyl galactosyl hydroxylysine, respectively (Figures 4D and 4E) (44, 64, 65).

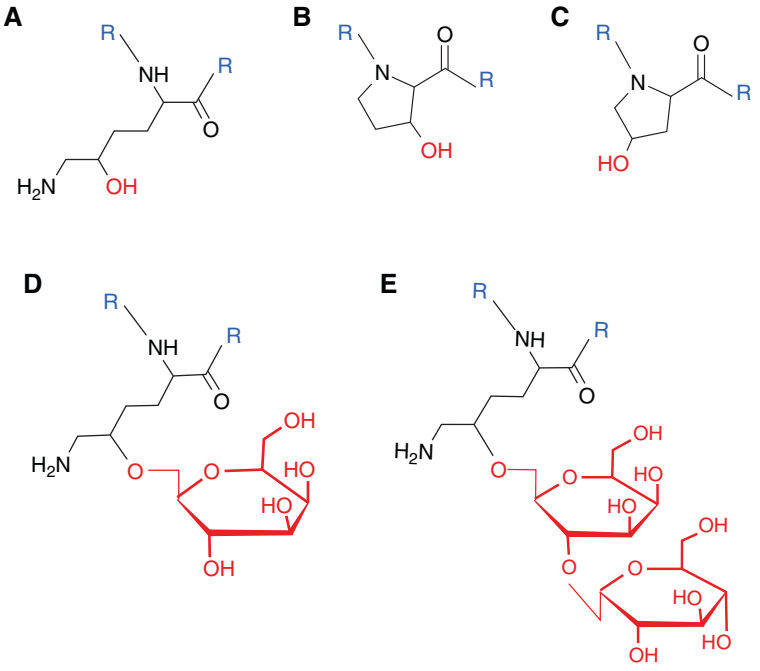

Figure 4. Frequent collagen posttranslational modifications mediated by ER-resident enzymes. Structures of (*A*) 5-hydroxylysine, (*B*) 3-hydroxyproline, (*C*) 4-hydroxyproline, (*D*) galactosyl hydroxylysine, and (*E*) glucosylgalactosyl hydroxylysine. Structures were generated using ACD/ChemSketch freeware.

Triple Helix Formation

After introducing collagen PTMs, the N- and C-terminal propeptides fold into globular domains, and the C-propeptides of three

α-chains assemble close to the ER membrane, forming the nucleus for triple helix formation (44, 68–70). Many general ER-folding folding catalysts, including Grp78 (BiP), Grp94, PDI, calreticulin, calnexin, and CypB, support this process (44). Once triple helix formation is initiated, it proceeds in a zipper-like fashion from the C- to the N-terminus. One of the rate-limiting steps is the *cis-trans* isomerization of Pro residues, which, as detailed above, are overrepresented in the collagenous domains and must be in the *trans* state to allow for the linear continuation of the triple helix. Many ER-resident peptidyl-prolyl isomerases (PPIases), including FKBP10, FKBP11, FKBP14, and PPIB, are involved in collagen folding, but to what extent they act as *cis-trans* isomerases or primarily as chaperones preventing premature fibril formation has not been fully elucidated (44, 70–75).

Heat Shock Protein 47 (HSP47) is Required for Multiple Steps

The collagen-specific chaperone HSP47 is considered essential for all ER-resident collagen biosynthetic processes, including collagen modifications and triple helix formation. HSP47 has been demonstrated to directly interact with several of the above-mentioned proteins, including PPIB, FKBP10, GRP78, and LH2 (44, 70, 75–82). In addition, HSP47 is also required for the transfer from the ER to the Golgi, where it binds to transport and Golgi organization protein 1 (TANGO1) and thus helps recruit procollagen molecules to the ER-Golgi intermediate structures. Finally, in the ER-Golgi intermediate compartment or the *cis*-Golgi, HSP47 dissociates from procollagen in a pH-dependent manner and returns to the ER (82, 83).

Trafficking to the Extracellular Space

Secretion of procollagen was previously thought to involve the transport of procollagen molecules via specialized, enlarged, coat protein complex II (COPII) vesicles from the rER to the Golgi. However, a recent study where the authors used live-cell imaging to track GFP-tagged procollagen molecules in the cell did not support the existence of such large COPII vesicles between the ER and the Golgi but instead rather argued for direct interconnections between organelles or less well-characterized intermediate carriers (84, 85). Either way, even if the exact mechanisms have not been fully clarified, it is well established that procollagen secretion is dependent on TANGO1, cutaneous T-cell lymphoma-associated antigen 5 (cTAGE5) at the ER exit site, and COPII coat proteins such as SEC13, SEC23, SEC24, and SEC31. TANGO1 recruits procollagen molecules to COPII proteins by binding to HSP47 on the one hand and COPII coat proteins on the other (86–89). Second, recruitment of Sedlin by TANGO1 stabilizes the inner layer of the ER-Golgi intermediate (90), and ubiquitination of the outer COPII coat protein SEC31 by the ubiquitin ligase CUL3-KLHL12 is also required for ER-Golgi trafficking of procollagen (91).

Propeptide Cleavage, Fibril Formation, and Crosslinking

After secretion of fibrillar-type procollagens, the N- and C-terminal propeptides are cleaved off by specific procollagen proteinases. While bone morphogenetic protein 1 (BMP1) or the tolloid-like proteins 1 or 2 cleave off the C-terminal propeptide, a member of the A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family (e.g., ADAMTS2, ADAMTS3, or ADAMTS14) cleaves off the N-terminal propeptides; the more recently discovered meprins are capable of cleaving off both propeptides (13, 14, 45, 92). Propeptide cleavage triggers the spontaneous quarter-staggered assembly of the triple-helical collagen molecules into fibrils (93, 94), which are then stabilized by intra- and intermolecular crosslinks.

Collagen crosslinking is mediated by enzymes of the LOX and transglutaminase (TGM) family (95–98). While TGM-mediated crosslinking fulfills pleiotropic functions in numerous physiological processes like blood clotting, skin cornification, and extracellular matrix remodeling (99), LOX enzymes preferentially crosslink collagens and elastin. There are five LOX family members, LOX and LOXL1–LOXL4. Among these, LOX and LOX-like 2 (LOXL2) are the best-established crosslinking enzymes for fibrillar collagens, introducing both intra- and intermolecular crosslinks (98, 100), which increase tissue stiffness and are critical for tissue formation during development and maintenance (65). In addition, antioxidant proteins like extracellular superoxide dismutase or glutathione peroxidase 3 associate with collagens and may protect them from oxidative damage (101–104).

Collagen Turnover

Mature collagen is subject to a typically slow but constant turnover, the rate of which is highly tissue-dependent (105–107). Collagen can be degraded by extracellular proteases or taken up into the cell and degraded by lysosomal proteases. In general, only members of two protease families, namely matrix metalloproteinases (MMPs) and cathepsins, are effective in collagen degradation (16, 108–111). Interestingly, these processes often release biologically active peptides, so-called matricryptins or matrikines (112, 113).

Extracellular collagen degradation is primarily exerted by MMPs and cathepsin K (catK). MMPs are extracellular zinc-dependent endopeptidases, many of which display collagenolytic activity (108, 109). MMPs recognize specific cleavage sites consisting of distinct amino acid sequences in the collagen molecule and need to unwind the collagen structure to access the cleavage site (114–116). In contrast, the cysteine protease cathepsin K can also target triple-helical collagen directly, without the need for unwinding of the helix (117) and therefore probably is the most effective protease for the extracellular degradation of fibrillar collagen (117, 118). The intracellular collagen degradation pathway is initiated by the uptake of collagen into the cell via three alternative pathways: *1*) Intact collagen fibrils are recognized by specific integrins and internalized by phagocytosis (119, 120); fragmented collagen (e.g., following MMP- or CTSK-mediated cleavage) can be internalized by *2*) macropinocytosis or *3*) receptor-mediated endosomal uptake (16). While macropinocytosis is actin cytoskeleton-driven and independent of membrane receptors, receptor-mediated endosomal uptake requires cell type-specific receptors (e.g., the urokinase plasminogen activator receptor-associated protein [uPARAP/Endo180 or C-type mannose receptor 2]) on fibroblasts (121–123) and macrophage mannose receptor 1 (MRC1) and lactadherin (MFGE8) on macrophages (124, 125). Collagen-containing phagosomes, macropinosomes, and endosomes all merge with lysosomes, which contain a range of cathepsins. Of these, cathepsins B, D, K, and L are known to cleave collagen into low-molecular-weight peptides (126–128).

Proposed Targets for Antifibrotic Therapy within the Collagen Biosynthesis and Maturation Pathway

Several drug targets within the collagen biosynthesis and maturation process, including representatives from many of the above described regulatory levels, have been suggested to treat fibrosis (summarized in Table 2 and Figure 3), and the relevant evidence will be briefly presented below.

LARP6

Upon profibrotic stimuli, the RNA binding protein LARP6 specifically binds to a unique 5′ stem-loop structure of Col1a1, Col1a2, and Col3a1 transcripts and facilitates the recruitment of additional translational factors and the coordinated translation of these collagen chains. Mutation of the 5′ stem-loop in Col1a1 attenuates liver fibrosis in mice (129), and lead compounds have been identified that block binding of LARP6 to the stem-loop and inhibit collagen biosynthesis *in vitro* and fibrogenesis *in vivo* (130, 131).

hsa-miR-29

MicroRNAs are short endogenous RNA molecules that act by repressing the translation of their target genes. In the context of collagen biosynthesis, the human microRNA family hsa-miR-29 acts as a master regulatory hub for collagen expression (42, 43). The hsa-miR-29 family consists of four members (hsa-miR-29a, hsa-miR-29b-1, hsa-miR-29b-2, and hsa-miR-29c). They are cotranscribed as polycistronic transcripts from two separate gene clusters on chromosome 7 (hsa-miR-29a, hsa-miR-29b-1) and chromosome 1 (hsa-miR-29b-2, hsa-miR-29c). The mature sequences of hsa-miR-29b-1 and hsa-miR-29b-2 are identical and collectively referred to as hsa-miR-29b. All mature miR-29 family members share identical seed regions, which determine the protein-coding targets by complementarity to the 3′ UTR in their transcript sequence. Hence, predicted targets and functions of all miR-29s largely overlap, even though evidence exists for isoform- and tissue-specific effects (42). Here, as the aim of this review is to provide a short overview, no further distinction is made between the different miR-29 isoforms.

Using hsa-miR-29 (mimics) to target fibrogenesis has been put forward for essentially every organ or tissue type that can develop fibrosis (see Table 2). Several lines of evidence, both *in vitro* and *in vivo*, in different cell types and organs, argue for its suitability to control fibrosis-associated overexpression of collagen (132–138). The first-generation miR-29b mimic MRG-201 (remlarsen, miRagen Therapeutics, now Viridian Therapeutics) for treatment of skin fibrotic disorders has already successfully passed a phase 1 clinical trial (NCT02603224) and just completed phase 2 (NCT03601052) (139). The same company developed a second-generation miR-29b mimic MRG-229 which is anticipated to enter clinical trials for idiopathic pulmonary fibrosis (IPF) soon (140).

Collagen Prolyl-4-Hydroxylase

Collagen prolyl-4-hydroxylases (P4HAs) catalyze the hydroxylation of Pro at C4 (4-hydroxyproline, 4-Hyp) (Figure 4C) in the Y position of G-X-Y repeats in collagen chains (44). The most frequent collagen posttranslational modification (PTM) is 4-Hyp, which is known to contribute to the thermostability of collagen molecules. Care must be taken to distinguish collagen P4H enzymes from collagen P3H and prolyl hydroxylase domain proteins (PHD). All three prolyl hydroxylase types catalyze hydroxylation of Pro, but collagen P3Hs do so at the C3 position, generating 3-hydroxyproline (Figure 4B), a comparably rare collagen PTM. PHDs, in contrast, hydroxylate two highly conserved Pro residues of hypoxia-inducible factor 1α (HIF-1α), which leads to proteasomal degradation in normoxia. Decrease of PHD activity under hypoxic conditions induces HIF-mediated gene expression; PHDs act as molecular sensors for oxygen (141). Notably, PHDs are also potential pharmacological targets for fibrotic disease (142), but not discussed here, as they are not part of the collagen biosynthetic machinery.

Three different isoforms of human collagen P4HAs exist, namely the α(I), α(II), and α(III) isoforms encoded by *P4HA1*, *P4HA2*, and *P4HA3*, respectively. All three isoforms have been mentioned in the context of fibrotic disease (143–146), but the reported evidence is mostly circumstantial (143, 144, 146). Some studies have used the α-ketoglutarate analogue pyridine-2,4-dicarboxylate (24PDC) or an analogue thereof as a P4H inhibitor and observed antifibrotic effects (147, 148). These studies, however, need to be interpreted with caution, as 24PDC similarly inhibits other enzymes that use α-ketoglutarate as a cofactor (149, 150). Probably to date, the strongest evidence for a possible therapeutic benefit of P4HA as a drug target has been provided by Luo and colleagues (145). Here, the authors used pyridine-2,5-dicarboxylate (25PDC), which is considered selective for P4HA enzymes (172, 173), and demonstrated that 25PDC protected from bleomycin-induced lung fibrosis (145). Nevertheless, 25PDC has a relatively high inhibition constant in the micromolar range and may still not be specific considering the plethora of enzymes that use α-ketoglutarate as a cofactor, particularly when used at relatively high concentrations (145, 150). Other P4HA inhibitors are being developed for therapeutic application (149).

FKBP10

FKBP10, also termed FKBP65, is a procollagen chaperone (44, 74) expression which is developmentally regulated in many tissues, limited to bone and ligaments postnatally, but also reactivated in visceral tissues upon injury (72, 151, 152). *FKBP10* mutations cause recessive osteogenesis imperfecta and Bruck syndrome (153), both characterized by bone fragility and low bone mass. Mechanistically, FKBP10 deficiency leads to decreased collagen secretion and deposition as well as reduced lysyl hydroxylation of collagen telopeptides and crosslinking (72, 153, 154), the latter through mediating dimerization of lysyl hydroxylase 2 (66). To date, FKBP10 has been suggested as a drug target for IPF, and studies in patient-derived primary human lung fibroblasts have shown that downregulating FKBP10 inhibits collagen synthesis and secretion, attenuates myofibroblast differentiation, and reduces fibroblast migration, all representing important hallmarks of lung fibrogenesis (71, 72). In summary, targeting FKBP10 has the potential to act on multiple regulatory levels relevant for disease. Specifically targeting FKBP10 with small molecular inhibitors is, to date, not possible. FK506 (tacrolimus) only weakly binds to one of the four FK506-binding domains of FKBP10 and has little effect on its collagen chaperone activity (74), hence not providing a promising lead structure. In addition, the three-dimensional structure of FKBP10 is not resolved, preventing structure-based inhibitor design.

The establishment of an FKBP10 activity screening assay is hampered by our current lack of understanding of the molecular determinants of the FKBP10/procollagen interaction. Hence, antisense oligonucleotide strategies that do not require any knowledge of the target except for the transcript sequence (155) may provide the only current option to specifically target FKBP10.

HSP47

The potential of targeting HSP47 (encoded by *SERPINH1*) for the treatment of fibrotic disease has been comprehensively summarized very recently, and the interested reader is referred to this excellent review and references therein (156). Owing to its central role in collagen triple helix formation as a procollagen-specific chaperone, many studies have explored HSP47 as a target for treating fibrotic conditions. In contrast to FKBP10, the three-dimensional structure of HSP47 has been resolved, and the interaction with procollagen is elucidated in molecular detail (157). *In vitro* and *in silico* screening assays have been established by several groups, and a number of promising small-molecule HSP47 inhibitors have been identified (156). However, to date, none of the suggested small-molecule HSP47 inhibitors has been tested *in vivo*. Instead, efforts to target HSP47 have mostly relied on antisense strategies, and all clinical trials, closed and ongoing, have applied vitamin A-coupled lipid nanoparticles containing anti-HSP47 siRNA (156). This targeting strategy will be described and briefly discussed in a broader context below. To date, no results of these clinical trials have been reported in detail. A brief poster presentation abstract on the use of BMS-98626 in patients with advanced hepatic fibrosis indicated target engagement and good tolerance of the drug but moderate effects at best (158).

TANGO1

TANGO1 is ascribed a central role at the ER exit site, where procollagen destined for secretion is recruited for trafficking via the Golgi. Initially identified as facilitating secretion of type VII procollagen (86), subsequent studies have established a broader role for procollagen secretion involving many more types (159, 160), possibly via HSP47 as adapter protein (83). To the best of my knowledge, only one published study has addressed TANGO1 as a potential antifibrotic target. Using TANGO1 depletion in hepatic stellate cells and TANGO1 heterozygous knockout mice, the authors showed that TANGO1 deficiency attenuated procollagen secretion and TANGO1 heterozygosity led to a decreased extent of hepatic fibrosis in two independent mouse models (159).

Lysyl Oxidases (LOX/LOXL2)

LOX family members are secreted copper-dependent enzymes that catalyze the oxidative deamination of specific collagen lysines and hydroxylysines. The resulting highly reactive allysine and hydroxyallysine residues, respectively, trigger a number of succeeding condensation reactions resulting in divalent and trivalent crosslinks between collagen molecules (16). Increases in collagen crosslinking cause elevated tissue stiffness in fibrosis (161) and may impede collagen degradation, leading to an accumulation of extracellular collagen (162, 163). Of the five LOX family members (LOX, LOXL1–LOXL4), most studies have addressed the role of LOX and LOXL2 in collagen crosslinking and fibrogenesis (164), and, to date, there is little evidence for other LOXL members to directly act on collagen (98). Some reports, including a systematic comparison of all LOX family members in the context of pulmonary fibrosis, point toward an outstanding role of LOXL2 in fibrosis (100, 165). Therefore, this review focuses on LOX and LOXL2, even though there is evidence for contributions of other LOXL isoforms to fibrotic disease (164).

While studies in various animal models of fibrosis showed great potential for LOXL2 as a drug target in antifibrotic therapy (100, 166–169), results of clinical trials using a LOXL2-specific antibody (simtuzumab) for therapy of liver, lung, and bone marrow fibrosis were disappointing, showing lack of efficacy (170–172). Investigators currently discuss whether this may be a result of limited potency and insufficient target engagement of the antibody in fibrotic areas, hence not arguing against inhibition of LOXL2 *per se* (173). In addition, comparative studies *in vitro* and *ex vivo* indicate that a pan-LOX inhibitor may be more effective than specifically targeting LOXL2 alone (165, 174). Hence, novel small-molecule inhibitors are being developed, and, interestingly, two pan-LOX inhibitors have already shown efficacy in an animal model of myelofibrosis (168). The pan-LOX inhibitor PXS-5505 is currently being tested in a clinical trial (NCT04676529), and interim reports state an excellent safety profile (175).

MMPs

MMPs comprise 23 extracellular zinc-dependent endopeptidases, which initially were thought to mainly degrade ECM components (111). Collagenolytic activity has been described for many MMPs (108, 111), but enzymological evidence for MMP-mediated degradation of interstitial triple-helical collagens, the collagens considered most relevant for fibrosis, has been reported for the following members of the MMP family: MMP-1, MMP-2, MMP-8, MMP-9, MMP-13, and two membrane-type MMPs, MMP-14 (MT1-MMP), and MMP-16 (MT3-MMP) (176).

While one would anticipate collagenolytic activity to be beneficial in fibrotic disease, multiple studies in animal models, on the contrary, have demonstrated that some MMPs rather have profibrotic functions (108, 177, 178). This is probably a reflection of the fact that MMPs display moonlighting functions, cleaving a variety of non-ECM substrates, including intra- and extracellular representatives (108, 178). Hence, modulation of MMPs in animal models of fibrosis cannot be attributed to effects on collagen turnover only, and tissue-specific effects complicate the matter even more (177). For example, MMP-8 deficiency protects mice from bleomycin-induced lung fibrosis, and the proposed mechanisms are largely independent of ECM degradation (108, 179). In liver fibrosis, however, MMP-8 appears to serve an antifibrotic function, as overexpression of MMP-8 ameliorates CCl_4- and bile duct-ligation-induced liver cirrhosis (180). For some excellent and more comprehensive reviews on the complexity of this matter, the interested reader is referred to reviews by Craig and colleagues (108), Giannandrea and colleagues (177), and Roderfeld (178).

Specificity of Proposed Drug Targets for Disease-associated Collagen and Potential Adverse Effects

The brief chapter on MMPs above illustrates the importance of specificity for collagen as a substrate if the aim is to reduce deposited extracellular collagen in fibrosis. With evidence for many more moonlighting proteins accumulating in the literature (181), it is probably naive to assume 100% substrate specificity for any given chaperone or

enzyme. Accordingly, for most of the potential drug targets given in Table 1, there is evidence for other substrates than collagen.

LARP6 may be the most specific representative of the described drug targets in this review. To date, no high-affinity substrates other than the COL1A1, COL1A2, and COL3A1 transcripts have been identified. Furthermore, homo- and heterozygous mice with a mutation in the 5′-UTR stem-loop of Col1A1, the LARP6 binding site, display a normal phenotype, indicating that LARP6-independent baseline collagen synthesis is sufficient to maintain the normal type I collagen synthesis.

In contrast, miR-29, as is in the nature of regulation by microRNAs, has multiple substrates. Even if it can be argued that collagens are overrepresented within that list and that it may be beneficial to downregulate multiple profibrotic targets with miR-29 as a single regulatory hub, unintended side effects are not unlikely. Indeed, some studies cast some doubt on the applicability of hsa-miR-29 mimics as drugs for the treatment of fibrosis. For instance, a comprehensive study using genetic deficiency models, pharmacological inhibition, and a cell-specific gene therapy approach has shown that cardiomyocyte-specific deletion of miR-29 may be beneficial in the context of cardiac remodeling and fibrosis (182). This is in sharp contrast to most other studies reporting antifibrotic effects of miR-29 and emphasizes the need for studies addressing the relative contribution of the different isoforms and cell types to fibrogenesis (182, 183). Moreover, overexpression of miR-29 leads to muscular dystrophy in mice, indicating that systemic application of miR-29 mimics may lead to muscular abnormalities (184). Hence, organ-targeted delivery seems to be key for the successful translation of miR-29 into the clinics.

Collagen P4HA enzymes, even if they, to the best of my knowledge, can be considered as specifically acting on collagens, modulate cofactor availability for other α-ketoglutarate-dependent enzymes (for example, for HIF-1α) (185). Furthermore, as prolyl-4-hydroxylation is essential for collagen thermodynamic stability, it is highly likely that targeting P4HA affects baseline collagen expression, potentially leading to adverse connective tissue alterations if not delivered in a highly specific manner to fibrotic areas only.

For FKBP10, primarily interactions with collagens and effects on collagen expression have been described, but several reports equally support a chaperone activity for tropoelastin (151, 186). Given its multidomain structure, additional functions are likely, in particular during development and organogenesis, where FKBP10 is expressed in many different tissues (151, 152). A recent report on the function of FKBP10 in lung cancer even reports a more general role in lung cancer cells as a ribosome-binding protein, which regulates translation (187). In healthy adult tissues, however, it seems that FKBP10 expression is restricted to bone under normal conditions but reactivated in other tissues upon injury or in disease (72, 151, 187). In addition, FKBP10 has recently been put forward as a potential drug target in lung cancer, further justifying the development of FKBP10-targeting drugs (187). Given that FKBP10 deficiency results in connective tissue alterations and osteogenesis imperfecta (152, 153), again, organ-targeted delivery may be key for successful translation.

Similarly, mutations in *SERPINH1*, encoding HSP47, cause osteogenesis imperfecta (188). However, even though no detailed report of the clinical trials targeting HSP47 is publicly available, a conference abstract states no changes in bone parameters upon application of a vitamin A-coupled lipid nanoparticle containing anti-HSP47 siRNA for treatment of hepatic fibrosis (158). As preliminary as this is, it is an encouraging finding because it indicates the feasibility of targeting collagen-modifying proteins in fibrosis, even though some of these proteins are crucial for normal bone development. Furthermore, in contrast to other heat shock proteins, HSP47 displays remarkable specificity for collagen (156), and to date, only three other interacting proteins have been described. Among these, FKBP10 and TANGO equally play important roles in collagen folding and Golgi trafficking/secretion, respectively.

Interestingly, HSP47 has also been described to interact with inositol-requiring enzyme 1α (IRE1α), an important regulator of the unfolded protein response (UPR) (82) as a result of ER stress. The aspect of ER stress requires special attention because, in general, targeting chaperone proteins like FKBP10 and HSP47 for treatment of fibrotic disease raises the concern of whether such an approach would lead to the accumulation of misfolded proteins in the ER and thus cause ER stress. Importantly, ER stress-induced apoptosis of alveolar epithelial cells may be a key profibrotic event in IPF etiology (189–191), and ER stress is known to contribute to fibroblast to myofibroblast differentiation (192–194). Therapeutic strategies targeting the ER-resident collagen folding and modification machinery, therefore, must be assessed in terms of *1*) whether they induce ER stress in alveolar epithelial cells and *2*) whether they lead to intracellular procollagen accumulation, which, by inducing ER stress, may either counterbalance the initial antifibrotic effects or, in fact, a potentially beneficial scenario, lead to apoptosis of myofibroblasts. Indeed, due to accumulating misfolded procollagen in the ER, the UPR is activated in fibroblasts isolated from osteogenesis imperfecta patients with loss-of-function mutations in *SERPINH1* (encoding HSP47) (82, 195). Furthermore, silencing of HSP47 in cancer cells activates ER stress via accumulation of oxidative stress-altered proteins other than collagens (196), and HSP47 also localizes to alveolar type II epithelial cells where induction of ER stress may trigger the development of fibrosis (197). These aspects argue against the suitability of targeting HSP47 for antifibrotic therapy. In contrast, FKBP10 is little expressed in alveolar epithelial cells, and knockdown of FKBP10 in primary human lung fibroblasts, including such from IPF patients, did not result in intracellular accumulation of procollagen but even led to downregulation of FKBP10 transcription, by so far incompletely understood mechanisms that must go beyond a mere collagen chaperone function (72). Hence, considering the aspect of induction of ER stress in alveolar epithelial cells, FKBP10 may be a more suitable drug target than HSP47. For myofibroblasts, however, induction of apoptosis may be desirable if it sensitizes myofibroblasts to apoptosis.

Many studies support the idea of TANGO1 as a specific cargo receptor for procollagen in mammalian cells (86, 159, 160), and specificity for procollagen may in part be mediated via binding to HSP47 (83). Nonetheless, it has been observed that TANGO1 also facilitates the secretion of large lipid particles like pre-chylomicrons or pre-very-low-density lipoproteins (198), actually supporting a more general function in the secretion of bulky cargos. Interestingly, deficiency of TANGO1 has been shown to lead to accumulation of non-secreted intracellular protein and induce ER stress and apoptosis of fibroblasts (199), which

may, under fibrotic conditions, support the restoration of normal tissue structure. These interesting observations clearly warrant more studies on TANGO1 as an antifibrotic target.

In addition to collagen, LOX enzymes also catalyze the first step in elastin crosslinking (98). Arguing against its suitability as a drug target for fibrosis in general, all members of the LOX family are comparatively strongly expressed in lung, kidney, and other tissues, maybe except for LOXL4 and liver, where expression of LOX enzymes is generally low, but specifically upregulated in fibrosis (173). Therefore, it is likely that LOX enzymes fulfill important functions under healthy conditions in extra-hepatic tissues. In addition, LOX functions in cellular proliferation and differentiation independent of ECM crosslinking have been described, but the direct substrates remain poorly understood (200).

Future Directions: Strategies for Specific Targeting of Fibrotic Areas

With collagen being essential for tissue integrity, targeted delivery of collagen biosynthesis blocking agents to fibrotic areas may hold the key for therapeutic success. Unique for lung fibrosis, inhalation therapy provides a simple way of targeting the lung specifically. Whether the inhalative route is sufficient to engage the target in likely less ventilated fibrotic areas is not fully explored, but recent reports, which demonstrate *in vivo* engagement and efficacy of an inhaled small-molecule αvβ6 integrin inhibitor for the treatment of pulmonary fibrosis, are very encouraging (201, 202). Notably, inhalation has also been successfully applied to administer antisense therapeutics into the lung to treat animal models of cystic fibrosis (203). Inhalative antisense strategies hold great promise for those drug targets for which no safe and effective small-molecule inhibitors exist.

However, the inhalative route poses challenges for drug delivery to fibrotic regions in the lung. Cellular and non-cellular barriers like mucus, surfactant, the airway epithelium, and the basement membrane must be overcome and at the same time remain intact to avoid exacerbation of disease. For other types of organ fibrosis, different application routes need to be devised. Depending on whether the drug target is located in the intra- or extracellular compartment, targeting the culprit cells via surface receptors or targeting the fibrotic ECM via disease-specific ECM binding tools are plausible strategies. Several membrane surface proteins of myofibroblast have been proposed that may allow for specific drug uptake in ECM-secreting fibroblasts (204). One such strategy that has already entered clinical trials takes advantage of a unique property of hepatic stellate cells (HSCs), the progenitors for the main effector cells in liver fibrosis, which are responsible for storing up to 80% of total-body vitamin A (205). Conjugating drugs to vitamin A, therefore, represents a promising strategy to specifically target drugs to HSCs. As mentioned above, clinical trials targeting HSP47 already make use of vitamin-A coupled lipid nanoparticles containing HSP47 siRNA (156). The same strategy has been reported to be effective in an animal model of lung fibrosis (206) and recently entered a clinical trial for IPF (NCT03538301 [139]). Even though there is little evidence for the concept that lung myofibroblasts, similar to their hepatic counterparts derived from HSCs, are equally targeted by vitamin A-coupled liposomes, preclinical results are encouraging and emphasize the feasibility of taking advantage of specific functions of effector cells for targeted drug delivery. But other strategies along these lines are plausible and could be explored in future work; for instance, it may be possible to exploit the metabolic reprogramming of myofibroblasts and take advantage of the increased glutamine uptake for specific targeting (26). Finally, also targeting the platelet-derived growth factor receptor (PDGFR) using PDGFR-recognizing peptides has been demonstrated to be effective in delivering the antifibrotic cytokine IFN-γ to fibrotic areas in liver and kidney fibrosis and limit fibrogenesis (207, 208). Additional surface receptors being explored in that context are the mannose-6-phosphate/insulin-like growth factor-II receptor (M6P/IGF-IIR) and fibroblast activation protein (204), and currently emerging single-cell studies have the potential to identify additional specific surface receptors.

Strategies to specifically target fibrotic ECM have been less explored, but ECM-targeting approaches have gained considerable attention in the cancer field, where the tumor ECM microenvironment affects the invasiveness of tumors and penetration of drugs. Investigated drug delivery strategies range from ECM-antibody-drug conjugates to drug carriers composed of liposomes and nanoparticles coated with ECM-binding peptides. Currently explored ECM targets in the context of tumor ECM are tenascin-C, fibronectin, and collagens, but also less abundant ECM components (209). Such strategies can be translated into the fibrosis field, given that the composition and molecular properties of the fibrotic ECM relative to healthy ECM are known. Several studies in the past, using model systems of fibrosis and patient material, have given valuable insight into the composition of the fibrotic ECM (52, 210–212), providing critical knowledge to devise similar strategies to treat fibrogenesis. Notably, this includes small molecular changes like PTMs or crosslinks. For instance, allysine, the initial reactive aldehyde product of LOX-mediated crosslinking, is increased in fibrosis. This property has been used to generate gadolinium-coupled probes that react with allysine, allowing imaging areas of active fibrogenesis by magnetic resonance tomography (213). Other fibrosis-specific collagen PTMs may include prolyl-3-hydroxylations, glycosylations, or other types of crosslinks, and technology is emerging that enables the site-specific and comprehensive determination of those (16, 52, 214).

Further Future Directions

Some regulatory levels within the collagen biosynthesis and maturation pathway have remained largely unexplored in the quest for antifibrotic drug targets (Figure 3). For instance, collagen prolyl-3- and lysyl hydroxylases, as well as collagen glycosylating enzymes, may qualify as drug targets, too. Among these, targeting LH2 may be particularly promising as it, just as FKBP10, acts directly on procollagen in the ER as well as indirectly on the level and stability of extracellular collagen crosslinking (215). Furthermore, recent reports on three-dimensional structures of LH enzymes can guide the development of small-molecule inhibitors (67, 216). For reasons detailed above in the discussion about activating the UPR, it is likely not advisable to target general ER foldases, and to date, to the best of my knowledge, no collagen-specific chaperones for C- or N-terminal propeptide folding or formation of the triple helix

nucleus have been identified. Nevertheless, given that these processes have not been elucidated in full molecular detail, future studies may still reveal novel targets in that context.

Most of the approaches listed above aim to attenuate collagen deposition. While this should be effective to stop excessive extracellular collagen accumulation in fibrosis, abolish the profibrotic feedback loop exerted by fibrotic ECM (217), and stop disease progression, the challenge of restoring normal tissue architecture remains. The resolution of fibrosis in the context of normally controlled tissue repair is critically dependent on matrix degradation (218, 219). Unfortunately, activation of collagenolytic enzymes in the extracellular space may overshoot and cause tissue destruction, as has been demonstrated for induction of MMPs and cathepsins (220, 221). As to the intracellular collagen degradation pathway, there is strong evidence that its disruption promotes fibrosis *in vivo* (124, 222, 223). However, the underlying mechanisms are not fully understood, and targets to activate intracellular collagen degradation have so far not been identified. Future work is warranted to unravel strategies that enhance intracellular collagen degradation for antifibrotic therapy. Ultimately, combination therapies may be required that collectively result in cessation of excessive ECM production on the one hand and ECM degradation and tissue restoration on the other. Given that the current standard-of-care drugs nintedanib and pirfenidone do not allow for tissue restoration and also directly act on the level of collagen fibril formation (224), it can be anticipated that combination therapy with those (anti-collagen drugs with nintedanib and/or pirfenidone) may not provide a significant benefit. Instead, future work must consider more targeted strategies to cease ECM deposition in combination with novel regeneration approaches.

All processes described above and the thereof derived therapeutic strategies have focused on fibrillar collagen and the concept of excessive collagen deposition as the major cause of organ dysfunction in fibrosis. It should be mentioned in this context that this, however, clearly is an oversimplification. While it remains true that the fibrillar collagen types I and III are consistently increased in fibrosis, levels of other collagen types remain unchanged, are variably regulated dependent on disease stage or anatomic location, or are even downregulated during fibrogenesis (17). This is already evident in an *in vitro* model of lung fibrosis, where the profibrotic cytokine TGF-β1 specifically increases and decreases different types of collagens simultaneously, thus clearly affecting the relative composition of the deposited collagen far beyond a simple increase in overall collagen (52). Some of these collagens may be indispensable for physiological tissue regeneration. For instance, loss of basement membrane integrity marks a point of no return for physiological tissue repair (116). Blocking the expression of type IV collagen as a major basement membrane constituent may perpetuate tissue fibrosis instead of halting it. Ultimately, it again boils down to the concept that we need to target the pathological collagen only, which requires a deeper knowledge of collagen-type specific biosynthetic mechanisms or, as detailed above, specific strategies to target the culprit cells, which secrete the "bad" collagen or to directly target the "bad" collagen in the extracellular space.

Conclusions

In summary, targeting collagen biosynthesis and maturation holds great promise for novel future therapies for lung and other types of organ fibrosis. Numerous drug targets have been identified, and drugs targeting some of these (miR-29 mimic, HSP47 siRNA, LOXL2 antibody, pan-LOX inhibitor) have entered clinical trials already. While the LOXL2 antibody simtuzumab showed a lack of efficiency, results of other clinical trials are eagerly awaited. Targeting molecules with specific expression in fibrosis and/or strategies for targeted delivery to myofibroblasts or disease-associated collagen in combination with approaches activating resolution of fibrosis may hold the key for future therapeutic success. ■

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References

1. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; 214:199–210.
2. Zeisberg M, Kalluri R. Cellular mechanisms of tissue fibrosis. 1. Common and organ-specific mechanisms associated with tissue fibrosis. *Am J Physiol Cell Physiol* 2013;304:C216–C225.
3. Rockey DC, Bell PD, Hill JA. Fibrosis—a common pathway to organ injury and failure. *N Engl J Med* 2015;372:1138–1149.
4. Henderson NC, Rieder F, Wynn TA. Fibrosis: from mechanisms to medicines. *Nature* 2020;587:555–566.
5. Beachley VZ, Wolf MT, Sadtler K, Manda SS, Jacobs H, Blatchley MR, *et al.* Tissue matrix arrays for high-throughput screening and systems analysis of cell function. *Nat Methods* 2015;12:1197–1204.
6. Decaris ML, Gatmaitan M, FlorCruz S, Luo F, Li K, Holmes WE, *et al.* Proteomic analysis of altered extracellular matrix turnover in bleomycin-induced pulmonary fibrosis. *Mol Cell Proteomics* 2014;13:1741–1752.
7. Frangogiannis N. Transforming growth factor-β in tissue fibrosis. *J Exp Med* 2020;217:e20190103.
8. Lipson KE, Wong C, Teng Y, Spong S. CTGF is a central mediator of tissue remodeling and fibrosis and its inhibition can reverse the process of fibrosis. *Fibrogenesis Tissue Repair* 2012;5:S24.
9. Platé M, Guillotin D, Chambers RC. The promise of mTOR as a therapeutic target pathway in idiopathic pulmonary fibrosis. *Eur Respir Rev* 2020;29:200269.
10. Pallet N, Legendre C. Adverse events associated with mTOR inhibitors. *Expert Opin Drug Saf* 2013;12:177–186.
11. Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem* 2009;78:929–958.
12. Gordon MK, Hahn RA. Collagens. *Cell Tissue Res* 2010;339: 247–257.
13. Kadler KE, Baldock C, Bella J, Boot-Handford RP. Collagens at a glance. *J Cell Sci* 2007;120:1955–1958.
14. Sorushanova A, Delgado LM, Wu Z, Shologu N, Kshirsagar A, Raghunath R, *et al.* The collagen suprafamily: from biosynthesis to advanced biomaterial development. *Adv Mater* 2019;31: e1801651.
15. Veit G, Kobbe B, Keene DR, Paulsson M, Koch M, Wagener R. Collagen XXVIII, a novel von Willebrand factor A domain-containing protein with many imperfections in the collagenous domain. *J Biol Chem* 2006;281: 3494–3504.
16. Onursal C, Dick E, Angelidis I, Schiller HB, Staab-Weijnitz CA. Collagen biosynthesis, processing, and maturation in lung ageing. *Front Med (Lausanne)* 2021;8:593874.

17. Karsdal MA, Nielsen SH, Leeming DJ, Langholm LL, Nielsen MJ, Manon-Jensen T, *et al.* The good and the bad collagens of fibrosis—their role in signaling and organ function. *Adv Drug Deliv Rev* 2017;121:43–56.
18. Urushiyama H, Terasaki Y, Nagasaka S, Terasaki M, Kunugi S, Nagase T, *et al.* Role of α1 and α2 chains of type IV collagen in early fibrotic lesions of idiopathic interstitial pneumonias and migration of lung fibroblasts. *Lab Invest* 2015;95:872–885.
19. Specks U, Nerlich A, Colby TV, Wiest I, Timpl R. Increased expression of type VI collagen in lung fibrosis. *Am J Respir Crit Care Med* 1995;151: 1956–1964.
20. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K, *et al.* Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 2006;173: 188–198.
21. King JL, Jukes TH. Non-Darwinian evolution. *Science* 1969;164:788–798.
22. Lu KP, Finn G, Lee TH, Nicholson LK. Prolyl *cis-trans* isomerization as a molecular timer. *Nat Chem Biol* 2007;3:619–629.
23. Li P, Wu G. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino Acids* 2018;50:29–38.
24. Hamanaka RB, Mutlu GM. Metabolic requirements of pulmonary fibrosis: role of fibroblast metabolism. *FEBS J* 2021;288:6331–6352.
25. Karna E, Szoka L, Huynh TYL, Palka JA. Proline-dependent regulation of collagen metabolism. *Cell Mol Life Sci* 2020;77:1911–1918.
26. Hamanaka RB, O'Leary EM, Witt LJ, Tian Y, Gökalp GA, Meliton AY, *et al.* Glutamine metabolism is required for collagen protein synthesis in lung fibroblasts. *Am J Respir Cell Mol Biol* 2019;61:597–606.
27. Schwörer S, Berisa M, Violante S, Qin W, Zhu J, Hendrickson RC, *et al.* Proline biosynthesis is a vent for TGFβ-induced mitochondrial redox stress. *EMBO J* 2020;39:e103334.
28. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat Rev Cancer* 2016;16:749.
29. Hamanaka RB, Mutlu GM. The role of metabolic reprogramming and de novo amino acid synthesis in collagen protein production by myofibroblasts: implications for organ fibrosis and cancer. *Amino Acids* 2021;53: DOI: 10.1007/s00726-021-02996-8.
30. Endo M, Oyadomari S, Terasaki Y, Takeya M, Suga M, Mori M, *et al.* Induction of arginase I and II in bleomycin-induced fibrosis of mouse lung. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L313–L321.
31. Kitowska K, Zakrzewicz D, Königshoff M, Chrobak I, Grimminger F, Seeger W, *et al.* Functional role and species-specific contribution of arginases in pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L34–L45.
32. Alves A, Bassot A, Bulteau AL, Pirola L, Morio B. Glycine metabolism and its alterations in obesity and metabolic diseases. *Nutrients* 2019;11:1356.
33. Nigdelioglu R, Hamanaka RB, Meliton AY, O'Leary E, Witt LJ, Cho T, *et al.* Transforming growth factor (TGF)-β promotes de novo serine synthesis for collagen production. *J Biol Chem* 2016;291:27239–27251.
34. Hamanaka RB, Nigdelioglu R, Meliton AY, Tian Y, Witt LJ, O'Leary E, *et al.* Inhibition of phosphoglycerate dehydrogenase attenuates bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2018; 58:585–593.
35. Ge J, Cui H, Xie N, Banerjee S, Guo S, Dubey S, *et al.* Glutaminolysis promotes collagen translation and stability via α-ketoglutarate-mediated mTOR activation and proline hydroxylation. *Am J Respir Cell Mol Biol* 2018;58:378–390.
36. Bernard K, Logsdon NJ, Benavides GA, Sanders Y, Zhang J, Darley-Usmar VM, *et al.* Glutaminolysis is required for transforming growth factor-β1-induced myofibroblast differentiation and activation. *J Biol Chem* 2018;293:1218–1228.
37. Cui H, Xie N, Jiang D, Banerjee S, Ge J, Sanders YY, *et al.* Inhibition of glutaminase 1 attenuates experimental pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2019;61:492–500.
38. Song M, Kim SH, Im CY, Hwang HJ. Recent development of small molecule glutaminase inhibitors. *Curr Top Med Chem* 2018;18: 432–443.
39. McNamee MJ, Michod D, Niklison-Chirou MV. Can small molecular inhibitors that stop de novo serine synthesis be used in cancer treatment? *Cell Death Discov* 2021;7:87.
40. Cuthbertson CR, Arabzada Z, Bankhead A III, Kyani A, Neamati N. A review of small-molecule inhibitors of one-carbon enzymes: SHMT2 and MTHFD2 in the spotlight. *ACS Pharmacol Transl Sci* 2021;4:624–646.
41. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009; 19:92–105.
42. Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol Genomics* 2012;44:237–244.
43. Cushing L, Kuang P, Lü J. The role of miR-29 in pulmonary fibrosis. *Biochem Cell Biol* 2015;93:109–118.
44. Ishikawa Y, Bächinger HP. A molecular ensemble in the rER for procollagen maturation. *Biochim Biophys Acta* 2013;1833:2479–2491.
45. Ricard-Blum S. The collagen family. *Cold Spring Harb Perspect Biol* 2011;3:a004978.
46. Burjanadze TV. Thermodynamic substantiation of water-bridged collagen structure. *Biopolymers* 1992;32:941–949.
47. Holster T, Pakkanen O, Soininen R, Sormunen R, Nokelainen M, Kivirikko KI, *et al.* Loss of assembly of the main basement membrane collagen, type IV, but not fibril-forming collagens and embryonic death in collagen prolyl 4-hydroxylase I null mice. *J Biol Chem* 2007;282: 2512–2519.
48. Kobayashi Y, Sakai R, Kakiuchi K, Isemura T. Physicochemical analysis of (Pro-Pro-Gly)n with defined molecular weight—temperature dependence of molecular weight in aqueous solution. *Biopolymers* 1970;9:415–425.
49. Rappu P, Salo AM, Myllyharju J, Heino J. Role of prolyl hydroxylation in the molecular interactions of collagens. *Essays Biochem* 2019;63: 325–335.
50. Sakakibara S, Inouye K, Shudo K, Kishida Y, Kobayashi Y, Prockop DJ. Synthesis of (Pro-Hyp-Gly) n of defined molecular weights. Evidence for the stabilization of collagen triple helix by hydroxypyroline. *Biochim Biophys Acta* 1973;303:198–202.
51. Eyre DR, Weis MA, Wu JJ. Advances in collagen cross-link analysis. *Methods* 2008;45:65–74.
52. Merl-Pham J, Basak T, Knüppel L, Ramanujam D, Athanason M, Behr J, *et al.* Quantitative proteomic profiling of extracellular matrix and site-specific collagen post-translational modifications in an in vitro model of lung fibrosis. *Matrix Biol Plus* 2019;1:100005.
53. Morello R, Bertin TK, Chen Y, Hicks J, Tonachini L, Monticone M, *et al.* CRTAP is required for prolyl 3- hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell* 2006;127:291–304.
54. Vranka JA, Pokidysheva E, Hayashi L, Zientek K, Mizuno K, Ishikawa Y, *et al.* Prolyl 3-hydroxylase 1 null mice display abnormalities in fibrillar collagen-rich tissues such as tendons, skin, and bones. *J Biol Chem* 2010;285:17253–17262.
55. Weis MA, Hudson DM, Kim L, Scott M, Wu JJ, Eyre DR. Location of 3-hydroxyproline residues in collagen types I, II, III, and V/XI implies a role in fibril supramolecular assembly. *J Biol Chem* 2010;285:2580–2590.
56. Eyre DR, Weis M, Hudson DM, Wu JJ, Kim L. A novel 3-hydroxyproline (3Hyp)-rich motif marks the triple-helical C terminus of tendon type I collagen. *J Biol Chem* 2011;286:7732–7736.
57. Pokidysheva E, Zientek KD, Ishikawa Y, Mizuno K, Vranka JA, Montgomery NT, *et al.* Posttranslational modifications in type I collagen from different tissues extracted from wild type and prolyl 3-hydroxylase 1 null mice. *J Biol Chem* 2013;288:24742–24752.
58. Pokidysheva E, Boudko S, Vranka J, Zientek K, Maddox K, Moser M, *et al.* Biological role of prolyl 3-hydroxylation in type IV collagen. *Proc Natl Acad Sci USA* 2014;111:161–166.
59. Basak T, Vega-Montoto L, Zimmerman LJ, Tabb DL, Hudson BG, Vanacore RM. Comprehensive characterization of glycosylation and hydroxylation of basement membrane collagen iv by high-resolution mass spectrometry. *J Proteome Res* 2016;15:245–258.
60. Ishikawa Y, Wirz J, Vranka JA, Nagata K, Bächinger HP. Biochemical characterization of the prolyl 3-hydroxylase 1.cartilage-associated protein.cyclophilin B complex. *J Biol Chem* 2009;284:17641–17647.
61. Gruenwald K, Castagnola P, Besio R, Dimori M, Chen Y, Akel NS, *et al.* Sc65 is a novel endoplasmic reticulum protein that regulates bone mass homeostasis. *J Bone Miner Res* 2014;29:666–675.
62. Heard ME, Besio R, Weis M, Rai J, Hudson DM, Dimori M, *et al.* Sc65-null mice provide evidence for a novel endoplasmic reticulum complex regulating collagen lysyl hydroxylation. *PLoS Genet* 2016;12:e1006002.

63. Tiainen P, Pasanen A, Sormunen R, Myllyharju J. Characterization of recombinant human prolyl 3-hydroxylase isoenzyme 2, an enzyme modifying the basement membrane collagen IV. *J Biol Chem* 2008;283: 19432–19439.
64. Gjaltema RA, Bank RA. Molecular insights into prolyl and lysyl hydroxylation of fibrillar collagens in health and disease. *Crit Rev Biochem Mol Biol* 2017;52:74–95.
65. Yamauchi M, Sricholpech M. Lysine post-translational modifications of collagen. *Essays Biochem* 2012;52:113–133.
66. Gjaltema RA, van der Stoel MM, Boersema M, Bank RA. Disentangling mechanisms involved in collagen pyridinoline cross-linking: the immunophilin FKBP65 is critical for dimerization of lysyl hydroxylase 2. *Proc Natl Acad Sci USA* 2016;113:7142–7147.
67. Scietti L, Chiapparino A, De Giorgi F, Fumagalli M, Khoriauli L, Nergadze S, *et al.* Molecular architecture of the multifunctional collagen lysyl hydroxylase and glycosyltransferase LH3. *Nat Commun* 2018;9:3163.
68. Boudko SP, Sasaki T, Engel J, Lerch TF, Nix J, Chapman MS, *et al.* Crystal structure of human collagen XVIII trimerization domain: a novel collagen trimerization Fold. *J Mol Biol* 2009;392:787–802.
69. Khoshnoodi J, Cartailler JP, Alvares K, Veis A, Hudson BG. Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. *J Biol Chem* 2006;281:38117–38121.
70. Ishikawa Y, Boudko S, Bächinger HP. Ziploc-ing the structure: triple helix formation is coordinated by rough endoplasmic reticulum resident PPIases. *Biochim Biophys Acta* 2015;1850:1983–1993.
71. Knüppel L, Heinzelmann K, Lindner M, Hatz R, Behr J, Eickelberg O, *et al.* FK506-binding protein 10 (FKBP10) regulates lung fibroblast migration via collagen VI synthesis. *Respir Res* 2018;19:67.
72. Staab-Weijnitz CA, Fernandez IE, Knüppel L, Maul J, Heinzelmann K, Juan-Guardela BM, *et al.* FK506-binding protein 10, a potential novel drug target for idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2015;192:455–467.
73. Ishikawa Y, Bächinger HP. A substrate preference for the rough endoplasmic reticulum resident protein FKBP22 during collagen biosynthesis. *J Biol Chem* 2014;289:18189–18201.
74. Ishikawa Y, Vranka J, Wirz J, Nagata K, Bächinger HP. The rough endoplasmic reticulum-resident FK506-binding protein FKBP65 is a molecular chaperone that interacts with collagens. *J Biol Chem* 2008; 283:31584–31590.
75. Ishikawa Y, Holden P, Bächinger HP. Heat shock protein 47 and 65-kDa FK506-binding protein weakly but synergistically interact during collagen folding in the endoplasmic reticulum. *J Biol Chem* 2017;292: 17216–17224.
76. Duran I, Nevarez L, Sarukhanov A, Wu S, Lee K, Krejci P, *et al.* HSP47 and FKBP65 cooperate in the synthesis of type I procollagen. *Hum Mol Genet* 2015;24:1918–1928.
77. Duran I, Martin JH, Weis MA, Krejci P, Konik P, Li B, *et al.* A chaperone complex formed by hsp47, fkbp65, and bip modulates telopeptide lysyl hydroxylation of type i procollagen. *J Bone Miner Res* 2017;32:1309–1319.
78. Smith T, Ferreira LR, Hebert C, Norris K, Sauk JJ. Hsp47 and cyclophilin B traverse the endoplasmic reticulum with procollagen into pre-Golgi intermediate vesicles. A role for Hsp47 and cyclophilin B in the export of procollagen from the endoplasmic reticulum. *J Biol Chem* 1995;270: 18323–18328.
79. Ishida Y, Nagata K. Hsp47 as a collagen-specific molecular chaperone. *Methods Enzymol* 2011;499:167–182.
80. Drögemüller C, Becker D, Brunner A, Haase B, Kircher P, Seeliger F, *et al.* A missense mutation in the SERPINH1 gene in Dachshunds with osteogenesis imperfecta. *PLoS Genet* 2009;5:e1000579.
81. Syx D, Ishikawa Y, Gebauer J, Boudko SP, Guillemyn B, Van Damme T, *et al.* Aberrant binding of mutant HSP47 affects posttranslational modification of type I collagen and leads to osteogenesis imperfecta. *PLoS Genet* 2021;17:e1009339.
82. Ito S, Nagata K. Roles of the endoplasmic reticulum-resident, collagen-specific molecular chaperone Hsp47 in vertebrate cells and human disease. *J Biol Chem* 2019;294:2133–2141.
83. Ishikawa Y, Ito S, Nagata K, Sakai LY, Bächinger HP. Intracellular mechanisms of molecular recognition and sorting for transport of large extracellular matrix molecules. *Proc Natl Acad Sci USA* 2016;113: E6036–E6044.
84. McCaughey J, Stephens DJ. ER-to-Golgi transport: a sizeable problem. *Trends Cell Biol* 2019;29:940–953.
85. McCaughey J, Stevenson NL, Cross S, Stephens DJ. ER-to-Golgi trafficking of procollagen in the absence of large carriers. *J Cell Biol* 2019;218:929–948.
86. Saito K, Chen M, Bard F, Chen S, Zhou H, Woodley D, *et al.* TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. *Cell* 2009; 136:891–902.
87. Ma W, Goldberg J. TANGO1/cTAGE5 receptor as a polyvalent template for assembly of large COPII coats. *Proc Natl Acad Sci USA* 2016;113: 10061–10066.
88. Raote I, Ortega Bellido M, Pirozzi M, Zhang C, Melville D, Parashuraman S, *et al.* TANGO1 assembles into rings around COPII coats at ER exit sites. *J Cell Biol* 2017;216:901–909.
89. Raote I, Ortega-Bellido M, Santos AJ, Foresti O, Zhang C, Garcia-Parajo MF, *et al.* TANGO1 builds a machine for collagen export by recruiting and spatially organizing COPII, tethers and membranes. *eLife* 2018;7: e32723.
90. Venditti R, Scanu T, Santoro M, Di Tullio G, Spaar A, Gaibisso R, *et al.* Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. *Science* 2012;337:1668–1672.
91. Jin L, Pahuja KB, Wickliffe KE, Gorur A, Baumgärtel C, Schekman R, *et al.* Ubiquitin-dependent regulation of COPII coat size and function. *Nature* 2012;482:495–500.
92. Prox J, Arnold P, Becker-Pauly C. Meprin α and meprin β: procollagen proteinases in health and disease. *Matrix Biol* 2015;44-46:7–13.
93. Kadler KE, Holmes DF, Trotter JA, Chapman JA. Collagen fibril formation. *Biochem J* 1996;316:1–11.
94. Williams BR, Gelman RA, Poppke DC, Piez KA. Collagen fibril formation. Optimal in vitro conditions and preliminary kinetic results. *J Biol Chem* 1978;253:6578–6585.
95. Bowness JM, Folk JE, Timpl R. Identification of a substrate site for liver transglutaminase on the aminopropeptide of type III collagen. *J Biol Chem* 1987;262:1022–1024.
96. Bowness JM, Tarr AH, Wiebe RI. Transglutaminase-catalysed cross-linking: a potential mechanism for the interaction of fibrinogen, low density lipoprotein and arterial type III procollagen. *Thromb Res* 1989; 54:357–367.
97. Shweke N, Boulos N, Jouanneau C, Vandermeersch S, Melino G, Dussaule JC, *et al.* Tissue transglutaminase contributes to interstitial renal fibrosis by favoring accumulation of fibrillar collagen through TGF-beta activation and cell infiltration. *Am J Pathol* 2008;173:631–642.
98. Vallet SD, Ricard-Blum S. Lysyl oxidases: from enzyme activity to extracellular matrix cross-links. *Essays Biochem* 2019;63:349–364.
99. Lorand L, Graham RM. Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat Rev Mol Cell Biol* 2003;4:140–156.
100. Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M, *et al.* Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat Med* 2010;16: 1009–1017.
101. Kliment CR, Tobolewski JM, Manni ML, Tan RJ, Enghild J, Oury TD. Extracellular superoxide dismutase protects against matrix degradation of heparan sulfate in the lung. *Antioxid Redox Signal* 2008;10:261–268.
102. Schamberger AC, Schiller HB, Fernandez IE, Sterclova M, Heinzelmann K, Hennen E, *et al.* Glutathione peroxidase 3 localizes to the epithelial lining fluid and the extracellular matrix in interstitial lung disease. *Sci Rep* 2016;6:29952.
103. Kliment CR, Englert JM, Gochuico BR, Yu G, Kaminski N, Rosas I, *et al.* Oxidative stress alters syndecan-1 distribution in lungs with pulmonary fibrosis. *J Biol Chem* 2009;284:3537–3545.
104. Zelko IN, Folz RJ. Extracellular superoxide dismutase attenuates release of pulmonary hyaluronan from the extracellular matrix following bleomycin exposure. *FEBS Lett* 2010;584:2947–2952.
105. Bradley K, McConnell-Breul S, Crystal RG. Collagen in the human lung. Quantitation of rates of synthesis and partial characterization of composition. *J Clin Invest* 1975;55:543–550.
106. Sivan SS, Wachtel E, Tsitron E, Sakkee N, van der Ham F, Degroot J, *et al.* Collagen turnover in normal and degenerate human intervertebral discs as determined by the racemization of aspartic acid. *J Biol Chem* 2008;283:8796–8801.

107. Verzijl N, DeGroot J, Thorpe SR, Bank RA, Shaw JN, Lyons TJ, *et al.* Effect of collagen turnover on the accumulation of advanced glycation end products. *J Biol Chem* 2000;275:39027–39031.
108. Craig VJ, Zhang L, Hagood JS, Owen CA. Matrix metalloproteinases as therapeutic targets for idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2015;53:585–600.
109. Afratis NA, Selman M, Pardo A, Sagi I. Emerging insights into the role of matrix metalloproteases as therapeutic targets in fibrosis. *Matrix Biol* 2018;68-69:167–179.
110. Jobin PG, Butler GS, Overall CM. New intracellular activities of matrix metalloproteinases shine in the moonlight. *Biochim Biophys Acta Mol Cell Res* 2017;1864:2043–2055.
111. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003;92:827–839.
112. Ricard-Blum S, Salza R. Matricryptins and matrikines: biologically active fragments of the extracellular matrix. *Exp Dermatol* 2014;23: 457–463.
113. Ricard-Blum S, Vallet SD. Matricryptins network with matricellular receptors at the surface of endothelial and tumor cells. *Front Pharmacol* 2016;7:11.
114. Chung L, Dinakarpandian D, Yoshida N, Lauer-Fields JL, Fields GB, Visse R, *et al.* Collagenase unwinds triple-helical collagen prior to peptide bond hydrolysis. *EMBO J* 2004;23:3020–3030.
115. Manka SW, Carafoli F, Visse R, Bihan D, Raynal N, Farndale RW, *et al.* Structural insights into triple-helical collagen cleavage by matrix metalloproteinase 1. *Proc Natl Acad Sci USA* 2012;109: 12461–12466.
116. Stultz CM. Localized unfolding of collagen explains collagenase cleavage near imino-poor sites. *J Mol Biol* 2002;319:997–1003.
117. Aguda AH, Panwar P, Du X, Nguyen NT, Brayer GD, Brömme D. Structural basis of collagen fiber degradation by cathepsin K. *Proc Natl Acad Sci USA* 2014;111:17474–17479.
118. Panwar P, Du X, Sharma V, Lamour G, Castro M, Li H, *et al.* Effects of cysteine proteases on the structural and mechanical properties of collagen fibers. *J Biol Chem* 2013;288:5940–5950.
119. Everts V, van der Zee E, Creemers L, Beertsen W. Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. *Histochem J* 1996;28:229–245.
120. Lee W, Sodek J, McCulloch CA. Role of integrins in regulation of collagen phagocytosis by human fibroblasts. *J Cell Physiol* 1996;168: 695–704.
121. Kjøller L, Engelholm LH, Høyer-Hansen M, Danø K, Bugge TH, Behrendt N. uPARAP/endo180 directs lysosomal delivery and degradation of collagen IV. *Exp Cell Res* 2004;293:106–116.
122. Madsen DH, Engelholm LH, Ingvarsen S, Hillig T, Wagenaar-Miller RA, Kjøller L, *et al.* Extracellular collagenases and the endocytic receptor, urokinase plasminogen activator receptor-associated protein/Endo180, cooperate in fibroblast-mediated collagen degradation. *J Biol Chem* 2007;282:27037–27045.
123. Madsen DH, Ingvarsen S, Jürgensen HJ, Melander MC, Kjøller L, Moyer A, *et al.* The non-phagocytic route of collagen uptake: a distinct degradation pathway. *J Biol Chem* 2011;286:26996–27010.
124. Atabai K, Jame S, Azhar N, Kuo A, Lam M, McKleroy W, *et al.* Mfge8 diminishes the severity of tissue fibrosis in mice by binding and targeting collagen for uptake by macrophages. *J Clin Invest* 2009;119: 3713–3722.
125. Martinez-Pomares L, Wienke D, Stillion R, McKenzie EJ, Arnold JN, Harris J, *et al.* Carbohydrate-independent recognition of collagens by the macrophage mannose receptor. *Eur J Immunol* 2006;36: 1074–1082.
126. Fonović M, Turk B. Cysteine cathepsins and extracellular matrix degradation. *Biochim Biophys Acta* 2014;1840:2560–2570.
127. Kafienah W, Brömme D, Buttle DJ, Croucher LJ, Hollander AP. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. *Biochem J* 1998;331:727–732.
128. Song F, Wisithphrom K, Zhou J, Windsor LJ. Matrix metalloproteinase dependent and independent collagen degradation. *Front Biosci* 2006; 11:3100–3120.
129. Parsons CJ, Stefanovic B, Seki E, Aoyama T, Latour AM, Marzluff WF, *et al.* Mutation of the 5′-untranslated region stem-loop structure inhibits α1(I) collagen expression in vivo. *J Biol Chem* 2011;286: 8609–8619.
130. Stefanovic B, Manojlovic Z, Vied C, Badger CD, Stefanovic L. Discovery and evaluation of inhibitor of LARP6 as specific antifibrotic compound. *Sci Rep* 2019;9:326.
131. Stefanovic B, Michaels HA, Nefzi A. Discovery of a lead compound for specific inhibition of type I collagen production in fibrosis. *ACS Med Chem Lett* 2021;12:477–484.
132. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, *et al.* Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology* 2011;53:209–218.
133. Sekiya Y, Ogawa T, Yoshizato K, Ikeda K, Kawada N. Suppression of hepatic stellate cell activation by microRNA-29b. *Biochem Biophys Res Commun* 2011;412:74–79.
134. Matsumoto Y, Itami S, Kuroda M, Yoshizato K, Kawada N, Murakami Y. MiR-29a assists in preventing the activation of human stellate cells and promotes recovery from liver fibrosis in mice. *Mol Ther* 2016;24: 1848–1859.
135. Kumar V, Mondal G, Dutta R, Mahato RI. Co-delivery of small molecule hedgehog inhibitor and miRNA for treating liver fibrosis. *Biomaterials* 2016;76:144–156.
136. Gallant-Behm CL, Piper J, Lynch JM, Seto AG, Hong SJ, Mustoe TA, *et al.* A MicroRNA-29 mimic (remlarsen) represses extracellular matrix expression and fibroplasia in the skin. *J Invest Dermatol* 2019;139: 1073–1081.
137. Heller KN, Mendell JT, Mendell JR, Rodino-Klapac LR. MicroRNA-29 overexpression by adeno-associated virus suppresses fibrosis and restores muscle function in combination with micro-dystrophin. *JCI Insight* 2017;2:e93309.
138. Montgomery RL, Yu G, Latimer PA, Stack C, Robinson K, Dalby CM, *et al.* MicroRNA mimicry blocks pulmonary fibrosis. *EMBO Mol Med* 2014;6:1347–1356.
139. U.S. National Library of Medicine. ClinicalTrials.gov database of clinical studies. 2021 [created 2021 Sep 16; accessed 2021 Sep 16]. Available from: ClinicalTrials.gov.
140. miRagen Therapeutics, Inc. miRagen press release: miRagen announces encouraging preclinical safety and efficacy data for its next-generation miR-29 replacement product candidate intended for the treatment of idiopathic pulmonary fibrosis. 2020 [created 2020 Jun 22; accessed 2021 Oct 15]. Available from: https://www.globenewswire.com/news-release/2020/06/22/2051595/31683/en/miRagen-Announces-Encouraging-Preclinical-Safety-and-Efficacy-Data-for-its-Next-generation-miR-29-Replacement-Product-Candidate-Intended-for-the-Treatment-of-Idiopathic-Pulmonary-F.html.
141. Gorres KL, Raines RT. Prolyl 4-hydroxylase. *Crit Rev Biochem Mol Biol* 2010;45:106–124.
142. Strowitzki MJ, Ritter AS, Kimmer G, Schneider M. Hypoxia-adaptive pathways: a pharmacological target in fibrotic disease? *Pharmacol Res* 2019;147:104364.
143. Feng GX, Li J, Yang Z, Zhang SQ, Liu YX, Zhang WY, *et al.* Hepatitis B virus X protein promotes the development of liver fibrosis and hepatoma through downregulation of miR-30e targeting P4HA2 mRNA. *Oncogene* 2017;36:6895–6905.
144. Li J, Ghazwani M, Zhang Y, Lu J, Li J, Fan J, *et al.* miR-122 regulates collagen production via targeting hepatic stellate cells and suppressing P4HA1 expression. *J Hepatol* 2013;58:522–528.
145. Luo Y, Xu W, Chen H, Warburton D, Dong R, Qian B, *et al.* A novel profibrotic mechanism mediated by TGFβ-stimulated collagen prolyl hydroxylase expression in fibrotic lung mesenchymal cells. *J Pathol* 2015;236:384–394.
146. Xiao Z, Reddy DPK, Xue C, Liu X, Chen X, Li J, *et al.* Profiling of miR-205/P4HA3 following angiotensin II-induced atrial fibrosis: implications for atrial fibrillation. *Front Cardiovasc Med* 2021;8:609300.
147. Sakaida I, Matsumura Y, Kubota M, Kayano K, Takenaka K, Okita K. The prolyl 4-hydroxylase inhibitor HOE 077 prevents activation of Ito cells, reducing procollagen gene expression in rat liver fibrosis induced by choline-deficient L-amino acid-defined diet. *Hepatology* 1996;23: 755–763.
148. Chung JM, Jung MJ, Lee SJ, Lee SD. Effects of prolyl 4-hydroxylase inhibitor on bladder function, bladder hypertrophy and collagen subtypes in a rat model with partial bladder outlet obstruction. *Urology* 2012;80:1390. e1397–1312.

149. Vasta JD, Raines RT. Collagen prolyl 4-hydroxylase as a therapeutic target. *J Med Chem* 2018;61:10403–10411.
150. Rose NR, McDonough MA, King ON, Kawamura A, Schofield CJ. Inhibition of 2-oxoglutarate dependent oxygenases. *Chem Soc Rev* 2011;40:4364–4397.
151. Patterson CE, Abrams WR, Wolter NE, Rosenbloom J, Davis EC. Developmental regulation and coordinate reexpression of FKBP65 with extracellular matrix proteins after lung injury suggest a specialized function for this endoplasmic reticulum immunophilin. *Cell Stress Chaperones* 2005;10:285–295.
152. Lietman CD, Rajagopal A, Homan EP, Munivez E, Jiang MM, Bertin TK, *et al.* Connective tissue alterations in Fkbp10-/- mice. *Hum Mol Genet* 2014;23:4822–4831.
153. Schwarze U, Cundy T, Pyott SM, Christiansen HE, Hegde MR, Bank RA, *et al.* Mutations in FKBP10, which result in Bruck syndrome and recessive forms of osteogenesis imperfecta, inhibit the hydroxylation of telopeptide lysines in bone collagen. *Hum Mol Genet* 2013;22:1–17.
154. Barnes AM, Cabral WA, Weis M, Makareeva E, Mertz EL, Leikin S, *et al.* Absence of FKBP10 in recessive type XI osteogenesis imperfecta leads to diminished collagen cross-linking and reduced collagen deposition in extracellular matrix. *Hum Mutat* 2012;33:1589–1598.
155. Bennett CF. Therapeutic antisense oligonucleotides are coming of age. *Annu Rev Med* 2019;70:307–321.
156. Bellaye PS, Burgy O, Bonniaud P, Kolb M. HSP47: a potential target for fibrotic diseases and implications for therapy. *Expert Opin Ther Targets* 2021;25:49–62.
157. Widmer C, Gebauer JM, Brunstein E, Rosenbaum S, Zaucke F, Drögemüller C, *et al.* Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition. *Proc Natl Acad Sci USA* 2012;109:13243–13247.
158. Lawitz E, Shevell D, Revankar R, Gao S, Wadhawan S, Chen W, *et al.* BMS-986263 (novel targeted lipid nanoparticle delivering HSP47siRNA) safety and target engagement in patients with advanced hepatic fibrosis: week 36 results from a phase 2 randomized,double-blind, placebo-controlled trial. *J Hepatol* 2020;73:S123–S124.
159. Maiers JL, Kostallari E, Mushref M, deAssuncao TM, Li H, Jalan-Sakrikar N, *et al.* The unfolded protein response mediates fibrogenesis and collagen I secretion through regulating TANGO1 in mice. *Hepatology* 2017;65:983–998.
160. Wilson DG, Phamluong K, Li L, Sun M, Cao TC, Liu PS, *et al.* Global defects in collagen secretion in a Mia3/TANGO1 knockout mouse. *J Cell Biol* 2011;193:935–951.
161. Wells RG. Tissue mechanics and fibrosis. *Biochim Biophys Acta* 2013; 1832:884–890.
162. Klepfish M, Gross T, Vugman M, Afratis NA, Havusha-Laufer S, Brazowski E, *et al.* LOXL2 inhibition paves the way for macrophage-mediated collagen degradation in liver fibrosis. *Front Immunol* 2020; 11:480.
163. van der Slot-Verhoeven AJ, van Dura EA, Attema J, Blauw B, Degroot J, Huizinga TW, *et al.* The type of collagen cross-link determines the reversibility of experimental skin fibrosis. *Biochim Biophys Acta* 2005; 1740:60–67.
164. Trackman PC. Lysyl oxidase isoforms and potential therapeutic opportunities for fibrosis and cancer. *Expert Opin Ther Targets* 2016; 20:935–945.
165. Aumiller V, Strobel B, Romeike M, Schuler M, Stierstorfer BE, Kreuz S. Comparative analysis of lysyl oxidase (like) family members in pulmonary fibrosis. *Sci Rep* 2017;7:149.
166. Yang J, Savvatis K, Kang JS, Fan P, Zhong H, Schwartz K, *et al.* Targeting LOXL2 for cardiac interstitial fibrosis and heart failure treatment. *Nat Commun* 2016;7:13710.
167. Harlow CR, Wu X, van Deemter M, Gardiner F, Poland C, Green R, *et al.* Targeting lysyl oxidase reduces peritoneal fibrosis. *PLoS One* 2017;12:e0183013.
168. Leiva O, Ng SK, Matsuura S, Chitalia V, Lucero H, Findlay A, *et al.* Novel lysyl oxidase inhibitors attenuate hallmarks of primary myelofibrosis in mice. *Int J Hematol* 2019;110:699–708.
169. Piasecki A, Leiva O, Ravid K. Lysyl oxidase inhibition in primary myelofibrosis: a renewed strategy. *Arch Stem Cell Ther* 2020;1:23–27.
170. Harrison SA, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, *et al.*; GS-US-321-0105 and GS-US-321-0106 Investigators. Simtuzumab is ineffective for patients with bridging fibrosis or compensated cirrhosis caused by nonalcoholic steatohepatitis. *Gastroenterology* 2018;155:1140–1153.
171. Raghu G, Brown KK, Collard HR, Cottin V, Gibson KF, Kaner RJ, *et al.* Efficacy of simtuzumab versus placebo in patients with idiopathic pulmonary fibrosis: a randomised, double-blind, controlled, phase 2 trial. *Lancet Respir Med* 2017;5:22–32.
172. Verstovsek S, Savona MR, Mesa RA, Dong H, Maltzman JD, Sharma S, *et al.* A phase 2 study of simtuzumab in patients with primary, post-polycythaemia vera or post-essential thrombocythaemia myelofibrosis. *Br J Haematol* 2017;176:939–949.
173. Chen W, Yang A, Jia J, Popov YV, Schuppan D, You H. Lysyl oxidase (LOX) family members: rationale and their potential as therapeutic targets for liver fibrosis. *Hepatology* 2020;72:729–741.
174. Tjin G, White ES, Faiz A, Sicard D, Tschumperlin DJ, Mahar A, *et al.* Lysyl oxidases regulate fibrillar collagen remodelling in idiopathic pulmonary fibrosis. *Dis Model Mech* 2017;10:1301–1312.
175. How J, Liu Y, Story JL, Neuberg DS, Ravid K, Jarolimek W, *et al.* Evaluation of a pan-lysyl oxidase inhibitor, Pxs-5505, in myelofibrosis: a phase I, randomized, placebo controlled double blind study in healthy adults. *Blood* 2020;136:16.
176. Amar S, Smith L, Fields GB. Matrix metalloproteinase collagenolysis in health and disease. *Biochim Biophys Acta Mol Cell Res* 2017;1864: 1940–1951.
177. Giannandrea M, Parks WC. Diverse functions of matrix metalloproteinases during fibrosis. *Dis Model Mech* 2014;7:193–203.
178. Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. *Matrix Biol* 2018;68-69:452–462.
179. Mahalanobish S, Saha S, Dutta S, Sil PC. Matrix metalloproteinase: an upcoming therapeutic approach for idiopathic pulmonary fibrosis. *Pharmacol Res* 2020;152:104591.
180. Siller-López F, Sandoval A, Salgado S, Salazar A, Bueno M, Garcia J, *et al.* Treatment with human metalloproteinase-8 gene delivery ameliorates experimental rat liver cirrhosis. *Gastroenterology* 2004;126: 1122–1133, discussion 949.
181. Jeffery CJ. Protein moonlighting: what is it, and why is it important? *Philos Trans R Soc Lond B Biol Sci* 2018;373:20160523.
182. Sassi Y, Avramopoulos P, Ramanujam D, Grüter L, Werfel S, Giosele S, *et al.* Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling. *Nat Commun* 2017;8:1614.
183. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, *et al.* Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci USA* 2008;105:13027–13032.
184. Liu C, Li L, Ge M, Gu L, Wang M, Zhang K, *et al.* Overexpression of miR-29 leads to myopathy that resemble pathology of ullrich congenital muscular dystrophy. *Cells* 2019;8:459.
185. Xiong G, Stewart RL, Chen J, Gao T, Scott TL, Samayoa LM, *et al.* Collagen prolyl 4-hydroxylase 1 is essential for HIF-1α stabilization and TNBC chemoresistance. *Nat Commun* 2018;9:4456.
186. Miao M, Reichheld SE, Muiznieks LD, Huang Y, Keeley FW. Elastin binding protein and FKBP65 modulate in vitro self-assembly of human tropoelastin. *Biochemistry* 2013;52:7731–7741.
187. Ramadori G, Ioris RM, Villanyi Z, Firnkes R, Panasenko OO, Allen G, *et al.* FKBP10 regulates protein translation to sustain lung cancer growth. *Cell Rep* 2020;30:3851–3863.e6.
188. Christiansen HE, Schwarze U, Pyott SM, AlSwaid A, Al Balwi M, Alrasheed S, *et al.* Homozygosity for a missense mutation in SERPINH1, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. *Am J Hum Genet* 2010;86:389–398.
189. Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M, *et al.* Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178: 838–846.
190. Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, Lane KB, *et al.* Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. *Am J Physiol Lung Cell Mol Physiol* 2008; 294:L1119–L1126.
191. Tanjore H, Lawson WE, Blackwell TS. Endoplasmic reticulum stress as a pro-fibrotic stimulus. *Biochim Biophys Acta* 2013;1832:940–947.

192. Baek HA, Kim DS, Park HS, Jang KY, Kang MJ, Lee DG, *et al.* Involvement of endoplasmic reticulum stress in myofibroblastic differentiation of lung fibroblasts. *Am J Respir Cell Mol Biol* 2012;46:731–739.
193. Burman A, Tanjore H, Blackwell TS. Endoplasmic reticulum stress in pulmonary fibrosis. *Matrix Biol* 2018;68-69:355–365.
194. Kropski JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. *J Clin Invest* 2018;128:64–73.
195. Lindert U, Weis MA, Rai J, Seeliger F, Hausser I, Leeb T, *et al.* Molecular consequences of the SERPINH1/HSP47 mutation in the dachshund natural model of osteogenesis imperfecta. *J Biol Chem* 2015;290:17679–17689.
196. Yoneda A, Minomi K, Tamura Y. HSP47 promotes metastasis of breast cancer by interacting with myosin IIA via the unfolded protein response transducer IRE1α. *Oncogene* 2020;39:4519–4537.
197. Kakugawa T, Mukae H, Hayashi T, Ishii H, Nakayama S, Sakamoto N, *et al.* Expression of HSP47 in usual interstitial pneumonia and nonspecific interstitial pneumonia. *Respir Res* 2005;6:57.
198. Santos AJ, Nogueira C, Ortega-Bellido M, Malhotra V. TANGO1 and Mia2/cTAGE5 (TALI) cooperate to export bulky pre-chylomicrons/VLDLs from the endoplasmic reticulum. *J Cell Biol* 2016;213:343–354.
199. Ríos-Barrera LD, Sigurbjörnsdóttir S, Baer M, Leptin M. Dual function for Tango1 in secretion of bulky cargo and in ER-Golgi morphology. *Proc Natl Acad Sci USA* 2017;114:E10389–E10398.
200. Trackman PC. Enzymatic and non-enzymatic functions of the lysyl oxidase family in bone. *Matrix Biol* 2016;52-54:7–18.
201. John AE, Graves RH, Pun KT, Vitulli G, Forty EJ, Mercer PF, *et al.* Translational pharmacology of an inhaled small molecule αvβ6 integrin inhibitor for idiopathic pulmonary fibrosis. *Nat Commun* 2020;11:4659.
202. Maher TM, Simpson JK, Porter JC, Wilson FJ, Chan R, Eames R, *et al.* A positron emission tomography imaging study to confirm target engagement in the lungs of patients with idiopathic pulmonary fibrosis following a single dose of a novel inhaled αvβ6 integrin inhibitor. *Respir Res* 2020;21:75.
203. Crosby JR, Zhao C, Jiang C, Bai D, Katz M, Greenlee S, *et al.* Inhaled ENaC antisense oligonucleotide ameliorates cystic fibrosis-like lung disease in mice. *J Cyst Fibros* 2017;16:671–680.
204. Yazdani S, Bansal R, Prakash J. Drug targeting to myofibroblasts: implications for fibrosis and cancer. *Adv Drug Deliv Rev* 2017;121:101–116.
205. Haaker MW, Vaandrager AB, Helms JB. Retinoids in health and disease: a role for hepatic stellate cells in affecting retinoid levels. *Biochim Biophys Acta Mol Cell Biol Lipids* 2020;1865:158674.
206. Otsuka M, Shiratori M, Chiba H, Kuronuma K, Sato Y, Niitsu Y, *et al.* Treatment of pulmonary fibrosis with siRNA against a collagen-specific chaperone HSP47 in vitamin A-coupled liposomes. *Exp Lung Res* 2017;43:271–282.
207. Bansal R, Prakash J, De Ruiter M, Poelstra K. Targeted recombinant fusion proteins of IFNγ and mimetic IFNγ with PDGFβR bicyclic peptide inhibits liver fibrogenesis in vivo. *PLoS One* 2014;9:e89878.
208. Poosti F, Bansal R, Yazdani S, Prakash J, Post E, Klok P, *et al.* Selective delivery of IFN-γ to renal interstitial myofibroblasts: a novel strategy for the treatment of renal fibrosis. *FASEB J* 2015;29:1029–1042.
209. Raavé R, van Kuppevelt TH, Daamen WF. Chemotherapeutic drug delivery by tumoral extracellular matrix targeting. *J Control Release* 2018;274:1–8.
210. Schiller HB, Fernandez IE, Burgstaller G, Schaab C, Scheltema RA, Schwarzmayr T, *et al.* Time- and compartment-resolved proteome profiling of the extracellular niche in lung injury and repair. *Mol Syst Biol* 2015;11:819.
211. Schiller HB, Mayr CH, Leuschner G, Strunz M, Staab-Weijnitz C, Preisendörfer S, *et al.* Deep proteome profiling reveals common prevalence of MZB1-positive plasma B cells in human lung and skin fibrosis. *Am J Respir Crit Care Med* 2017;196:1298–1310.
212. Åhrman E, Hallgren O, Malmström L, Hedström U, Malmström A, Bjermer L, *et al.* Quantitative proteomic characterization of the lung extracellular matrix in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *J Proteomics* 2018;189:23–33.
213. Chen HH, Waghorn PA, Wei L, Tapias LF, Schühle DT, Rotile NJ, *et al.* Molecular imaging of oxidized collagen quantifies pulmonary and hepatic fibrogenesis. *JCI Insight* 2017;2:e91506.
214. Soboleva A, Schmidt R, Vikhnina M, Grishina T, Frolov A. Maillard proteomics: opening new pages. *Int J Mol Sci* 2017;18:2677.
215. Piersma B, Bank RA. Collagen cross-linking mediated by lysyl hydroxylase 2: an enzymatic battlefield to combat fibrosis. *Essays Biochem* 2019;63:377–387.
216. Guo HF, Tsai CL, Terajima M, Tan X, Banerjee P, Miller MD, *et al.* Pro-metastatic collagen lysyl hydroxylase dimer assemblies stabilized by Fe^{2+}-binding. *Nat Commun* 2018;9:512.
217. Parker MW, Rossi D, Peterson M, Smith K, Sikström K, White ES, *et al.* Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J Clin Invest* 2014;124:1622–1635.
218. Atabai K, Yang CD, Podolsky MJ. You say you want a resolution (of fibrosis). *Am J Respir Cell Mol Biol* 2020;63:424–435.
219. Horowitz JC, Thannickal VJ. Mechanisms for the resolution of organ fibrosis. *Physiology (Bethesda)* 2019;34:43–55.
220. Golovatch P, Mercer BA, Lemaître V, Wallace A, Foronjy RF, D'Armiento J. Role for cathepsin K in emphysema in smoke-exposed guinea pigs. *Exp Lung Res* 2009;35:631–645.
221. Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr, *et al.* Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000;106:1081–1093.
222. Bundesmann MM, Wagner TE, Chow YH, Altemeier WA, Steinbach T, Schnapp LM. Role of urokinase plasminogen activator receptor-associated protein in mouse lung. *Am J Respir Cell Mol Biol* 2012;46:233–239.
223. McKleroy W, Lee TH, Atabai K. Always cleave up your mess: targeting collagen degradation to treat tissue fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2013;304:L709–L721.
224. Knüppel L, Ishikawa Y, Aichler M, Heinzelmann K, Hatz R, Behr J, *et al.*. A novel antifibrotic mechanism of nintedanib and pirfenidone. Inhibition of Collagen Fibril Assembly. *Am J Respir Cell Mol Biol* 2017;57:77–90.
225. Sengupta S, den Boon JA, Chen IH, Newton MA, Stanhope SA, Cheng YJ, *et al.* MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. *Proc Natl Acad Sci USA* 2008;105:5874–5878.
226. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, *et al.* miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2011;45:287–294.
227. Fang Y, Yu X, Liu Y, Kriegel AJ, Heng Y, Xu X, *et al.* miR-29c is downregulated in renal interstitial fibrosis in humans and rats and restored by HIF-α activation. *Am J Physiol Renal Physiol* 2013;304:F1274–F1282.
228. Maurer B, Stanczyk J, Jüngel A, Akhmetshina A, Trenkmann M, Brock M, *et al.* MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum* 2010;62:1733–1743.
229. Kivirikko KI, Myllyharju J. Prolyl 4-hydroxylases and their protein disulfide isomerase subunit. *Matrix Biol* 1998;16:357–368.
230. Natsume T, Koide T, Yokota S, Hirayoshi K, Nagata K. Interactions between collagen-binding stress protein HSP47 and collagen. Analysis of kinetic parameters by surface plasmon resonance biosensor. *J Biol Chem* 1994;269:31224–31228.
231. López B, González A, Hermida N, Valencia F, de Teresa E, Díez J. Role of lysyl oxidase in myocardial fibrosis: from basic science to clinical aspects. *Am J Physiol Heart Circ Physiol* 2010;299:H1–H9.
232. Huang M, Liu Z, Baugh L, DeFuria J, Maione A, Smith A, *et al.* Lysyl oxidase enzymes mediate TGF-β1-induced fibrotic phenotypes in human skin-like tissues. *Lab Invest* 2019;99:514–527.
233. Berisio R, Vitagliano L, Mazzarella L, Zagari A. Crystal structure of the collagen triple helix model [(Pro-Pro-Gly)(10)](3). *Protein Sci* 2002;11:262–270.