Endocrine Reviews, 2024, **45**, 361–378
https://doi.org/10.1210/endrev/bnad036
Advance access publication 13 December 2023
Review

Endotrophin, a Key Marker and Driver for Fibroinflammatory Disease

Kim Henriksen,[1] Federica Genovese,[1] Alexander Reese-Petersen,[1] Laurent P. Audoly,[2] Kai Sun,[3] Morten A. Karsdal,[1] and Philipp E. Scherer[4]

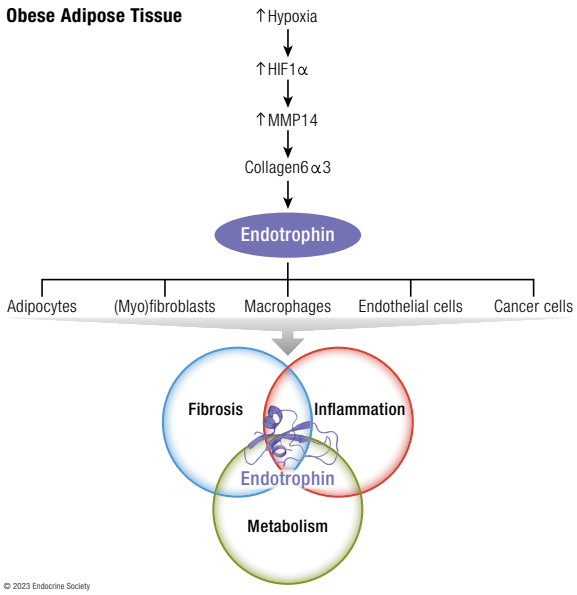
[1]Department of Cardiovascular Disease, Nordic Bioscience A/S, DK-2730 Herlev, Denmark
[2]Privebio Inc., Boston, MA 02445, USA
[3]Center for Metabolic and Degenerative Diseases, Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030, USA
[4]Touchstone Diabetes Center, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Correspondence: Philipp E. Scherer, PhD, Touchstone Diabetes Center, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-8549, USA. Email: Philipp.Scherer@utsouthwestern.edu.

Abstract

Our overview covers several key areas related to recent results obtained for collagen type VI and endotrophin (ETP). (1) An introduction to the history of ETP, including how it was identified, how it is released, and its function and potential receptors. (2) An introduction to the collagen family, with a focus on what differentiates collagen type VI from an evolutionary standpoint. (3) An overview of collagen type VI, the 6 individual chains (COL6A1, A2, A3, A4, A5, and A6), their differences and similarities, as well as their expression profiles and function. (4) A detailed analysis of COL6A3, including the cleaved product endotrophin, and what separates it from the other 5 collagen 6 molecules, including its suggested function based on insights gained from knockout and gain of function mouse models. (5) The pathology of ETP. What leads to its presence and release and what are the consequences thereof? (6) Functional implications of circulating ETP. Here we review the data with the functional roles of ETP in mind. (7) We propose that ETP is a mediator for fibrotic (or fibroinflammatory) disorders. Based on what we know about ETP, we have to consider it as a target for the treatment of fibrotic (or fibroinflammatory) disorders. What segment(s) of the patient population would most dramatically respond to an ETP-targeted intervention? How can we find the population that would profit most from an intervention? We aim to present a broad overview over the ETP field at large, providing an assessment of where the future research efforts need to be placed to tap into the vast potential of ETP, both as a marker and as a target in different diseases.

Graphical Abstract



Received: 8 August 2023. **Editorial Decision:** 8 December 2023. **Corrected and Typeset:** 3 January 2024

Key Words: endotrophin, ColVIα3, extracellular matrix, fibroinflammatory diseases

Abbreviations: ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; ETP, endotrophin; HCC, hepatocellular carcinoma; MMP, matrix metalloproteinase; NAFLD, nonalcoholic fatty liver disease; SVF, stromal vascular fraction; TGF, transforming growth factor; vWF, von Willebrand factor.

ESSENTIAL POINTS

- Endotrophin is a carboxy-terminal cleavage product of collagen VI alpha 3
- Endotrophin has emerged as an exciting new biomarker for a variety of fibroinflammatory diseases and for a number of cancer settings
- Kidney disease, liver disease, cardiovascular disease, and heart failure as well a pulmonary dysfunction are all associated with increased endotrophin levels
- Preclinical studies indicate that endotrophin is not just a biomarker, but a major driver for many of the fibroinflammatory and cancer disease states mentioned above

Introduction to Endotrophin

The History of Endotrophin

Endotrophin (ETP) was initially identified as a pathological signal promoting breast cancer growth by Park and Scherer in 2012 (1). Earlier work highlighted the relevance of the Col VI parent chain for similar functions (2). The idea derived from studies Iyengar et al showing that Col VI from adipocytes promotes tumor growth and that Col VI–deficient mice display reduced tumor growth (1, 2). Furthermore, the C5 domain of α3 (VI) was known to be released during fibril formation and to be both stable and enriched in breast cancer specimens (2), pointing in the direction of a potential pathological function.

Spurred by these intriguing findings, Park and Scherer (1) demonstrated that overexpression of ETP resulted in tumor growth in mammary glands and resulted in enhanced lung metastasis, through a mechanism involving enhanced epithelial–mesenchymal transition (EMT) (1, 3), thereby setting the stage for a host of studies of the pathological relevance and therapeutic potential of blocking ETP.

How Is ETP Generated and Released?

The original hypothesis for the release of ETP involved matrix metalloproteinase (MMP)–mediated release from the mature type VI collagen as these enzymes are known to be upregulated in fibrotic adipose tissue, which was the first source of ETP identified (1, 3). Furthermore, studies investigating cleavage of Col VI, in particular the native α3 (VI) chain, showing alterations in Col VI structure when MMP11 was present at the adipocyte–cancer cell interface, potentially indicating a relevance here. An interesting finding related to the release was that, at least in some cell systems, ETP remains attached to the matrix following cleavage, before ultimately being released (4), a finding correlating well with the presence of ETP in different pathological tissues, such as fibrotic kidneys and livers from patients with liver failure due to hepatitis (5, 6). This suggests that ETP's major function could be to signal locally rather than systemically.

ETP is generated as a cleavage product from ColVIα3. Recent results have revealed that MMP14 as well as other MMPs are the key processing enzyme responsible for cleaving ColVI α3 chain and releases ETP (7). The cleavage site is located at a consensus-cleavage site for MMP14, and the resulting fragment has been confirmed using an ETP-specific antibody (7). Other proteolytic enzymes, such as bone morphogenetic protein 1, have also been implicated in the generation of ETP (4). Moreover, the cleavage site of human ETP has also been reported, and downstream MMPs of Mmp14 have been found to be involved in the cleavage process (8, 9). Once cleaved, ETP is stable and can be released into the bloodstream, exerting local function within the tissue it originates from via its paracrine/autocrine functions or have systemic effects on distal organs via its endocrine roles (1, 3-5, 10-16)

Introduction to Collagens

The Physiological Roles of Collagens and Connection to Endotrophin

The extracellular matrix (ECM) is a vast, noncellular, component present in all tissues and organs. It is a structural support network made up from a range of different proteins, regulating numerous biological functions. The ECM not only provides important physical tissue support and scaffolding, but also regulates biomechanical signaling to cells, and regulating cell proliferation, differentiation, and migration (17-19). Research into the ECM and how the ECM impacts systemwide responses, regulating individual organ function all the way to the behavior of individual cells, has been subject to in-depth analysis for decades.

There are 28 different types of collagens which have been described to this date. Collagen is the most abundant type of protein within the body and is widely expressed (20, 21). The different types of collagen make up the bulk of the ECM, but tissue expression and localization of the different types of collagen can vary dramatically. Type I, II, and III collagen make up more than 80% of the total collagen mass in the human body. Collagens are characteristically assembled in triple helixes consisting of homotrimeric or heterotrimeric chains (22-24).

The ECM can be divided into 2 compartments, the basement membrane and the interstitial matrix, each comprising different types of collagens (25). The basement membrane is special, since the collagens residing in this space form intricate networks interlinked with adjacent collagen proteins, which provide structural integrity. The most abundant type of collagen in the basement membrane is type IV collagen, and the basement membrane collagens are mainly produced by epithelial cells. Conversely, the interstitial matrix provides the bulk of the ECM, and consists predominantly of type I and III collagens, which are produced by fibroblasts (26).

Proteolytic processing of the ECM is known to release a range of signaling fragments, thus serving as a repertoire for bioactive molecules referred to as *matricryptins*, which are involved in both physiological and pathological processes.

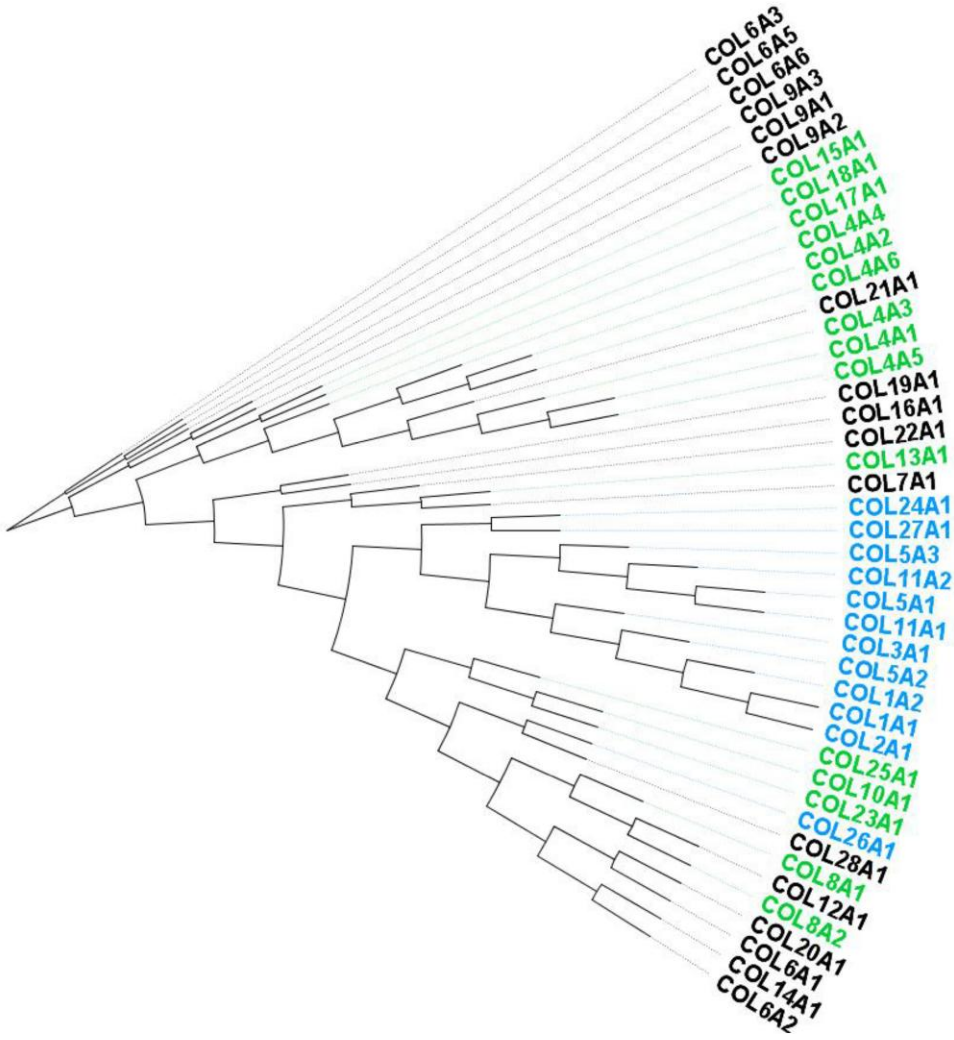


Figure 1. Phylogenetic tree of the 28 different collagen chains. The phylogenetic tree was generated by finding reviewed, human, collagen sequences (no isoforms were included) on the UniProt website (www.uniprot.org). The sequences were downloaded in the Fasta format and uploaded to the Clustal Omega website (https://www.ebi.ac.UK/Tools/msa/clustalo/, version 1.2.4) to generate a multiple sequence alignment file. The generated phylogenetic tree data was downloaded, and the phylogenetic tree was displayed by using FigTree software version 1.4.3 (http://tree.bio.ed.ac.UK/software/figtree/). The tree is presented as a midpoint rooted tree and proportionally transformed branches. The classic basement membrane collagens are dark green, while the newer basement membrane collagens are light green. Likewise, the classic fibrillar collagens are dark blue, and the newer fibrillar collagens light blue.

Phylogenetic Background

The ECM is important for guiding cell behavior and providing structural support. The different compartments of the ECM, the basement membrane and interstitial matrix, do so by directing migration, morphology, differentiation, and proliferation. Importantly, there is a need for the proper assembly of the ECM in relation to the development of organs and substructures within organs that requires a highly organized configuration provided by the basement membrane, as well as extracellular scaffolds to facilitate tissue expansion, provided by the interstitial matrix collagens (27).

Based on this model, it is likely that the collagens of the basement membrane evolved and developed prior to the collagens of the interstitial matrix. The evolutionary relationship between collagens can be studied by phylogenetic approaches, which reveal interesting results (Fig. 1). When aligning the data published for the 28 different types of collagens, the best described collagens of the basement membrane (type IV, XV, and XVIII) come up earlier than many of the other types of collagens (basement membrane collagens highlighted in green), which could indicate that these types of collagens developed before others. In line with this model, many of the interstitial collagens develop at a later stage, including collagen types I, III, and V (interstitial matrix collagens highlighted in blue) (Fig. 1).

It is also interesting that the collagens are divided into 2 main categories: 1 containing the classical basement membrane collagens, the other containing the interstitial matrix collagens. Furthermore, the length of the different nodes indicates "age," which further adds to the argument of the development of basement membrane collagens occurred before interstitial matrix collagens. Whereas the higher number of branch splits for the interstitial matrix collagens can indicate a more specified function driven by evolutionary needs.

However, above all else, the type VI collagen α3 chain is the collagen chain among all the others that developed first in relation to the other chains. This could indicate that type VI collagen, and the ETP molecule it derives from, have a highly

conserved biological and structural function in the ECM that is not like any of the other collagens. This is consistent with the biological roles of ETP, as well as the structure of type VI collagen which is somewhat different from all of the other types of collagens (28).

Collagen Type VI

Collagen VI (Col VI) is special in multiple aspects among the members of the collagen family. It has both unique structural features and a unique function. Over the recent past, studies have shed light on this remarkable collagen. It is not only a critical structural component for a number of tissues, but also dysregulated under a variety of pathophysiological conditions. It also gives rise to a carboxy-terminal cleavage fragment referred to as ETP, which serves as both a biomarker and a potentially relevant therapeutic target. Col VI expression is broad, and, accordingly, studies have demonstrated functions of Col VI across many tissues and cells. Specifically, Col VI plays a vital role as the predominant collagen in shaping the ECM architecture within adipose tissue.

The Different Fiber Members of Type VI Collagen

In total, there are 6 genes encoding collagen type VI chains. These are *COL6A1* through *COL6A6* (29-31), with *COL6A4-COL6A6* members being among the most recently identified components, all 3 of which are highly homologous to *COL6A3* (32, 33).

At the chromosomal level, *COL6A1* and *COL6A2* are located on chromosome 21 in a tandem arrangement, *COL6A3* is on chromosome 2, while the 3 newest members are located in tandem on chromosome 3, with a highly conserved location; however, detailed studies have revealed that the *COL6A4* gene at some time point during development has been pericentrically inverted, rendering the gene dysfunctional in humans, chimpanzees, and gorillas, whereas other mammals have an intact gene (34).

As the focus of this review is mainly on human and studies relating to function of Col VI in humans, COL6A4 will not be described in further detail.

Synthesis and Structure of Type VI

Collagen type VI is a special molecule when it comes to biosynthesis and structure. A series of important studies have illuminated this highly interesting configuration and its unique position among the collagens. The early characterization steps leading to insights into the biochemistry and structure of Col VI are presented in a series of reviews (35-37).

Studies have shed light on the composition of the collagen type VI trimers. The most prominent is the trimer consisting of 1 of each of the Col VI α1(VI):α2(VI):α3(VI) chains. The α3(VI) chain can be substituted with either the α5(VI) or the α6(VI) chain; however, here very little is known about assembly, structure, and function, although based on homology to the α3(VI) chain, it appears likely to work in a similar manner (33). However, we focus here on Col VI α1(VI):α2(VI):α3(VI) (36-38) (Table 1).

What are the key aspects of the development of the beaded filament structure for which Col VI is well known for? This unique structure of the filaments has been elucidated through electron microscopy (36, 37). As with other collagens, Col VI trimers have a central triple helical domain, defined by the classical Gly-X-Y repeats, and stretching approximately 335 amino acid residues in each chain. The triple helical domain is bordered by large N- and C-terminal regions (see Fig. 2), with the N-terminal part consisting mainly of 10 von Willebrand factor (vWF) type A domains (of approximately 200 amino acids each), and the C-terminal of COL6A1 and COL6A2 containing an additional 2 vWF domains, each of approximately 200 amino acids in length. In contrast, the COL6A3 chain is unique and contains 3 additional domains, 1 called the unique region or "C3" (approximately 200 amino acids), a type III fibronectin repeat called "C4" (approximately 100 amino acids), and a small C-terminal Kunitz-like domain, called "C5," which is released upon extracellularization and is referred to as ETP (77 amino acids) (1). In terms of domain structure, the 2 newer human members α5(VI) and α6(VI) are similar to α3(VI); however, they have fewer N-terminal vWF domains (7 instead 10), as well as a different C-terminal structure, with the α5(VI) having 3 C-terminal domains and the α6(VI) having 5 domains. Importantly, the C5 domain is clearly distinct from the one found at the α3(VI) chain, and does not appear to harbor any signaling capacity (36-38).

The intracellular assembly of Col VI sets it apart from the other collagens, and an understanding of the structure has been critical in terms of shedding light on the impact that mutations within the chains have on structure and function, thereby delineating the pathological consequences.

The assembly of the most prevalent Col VI trimer is illustrated in Fig. 2. It starts with the 3 chains interacting at the C1 globular domain, which then triggers the generation of the triple helix from the C-terminal to the N-terminal (55, 74), resulting in a Col VI monomer with a stoichiometry of 1:1:1 (28). Further interactions between the triple helical domain and the C2 domain lead to formation of Col VI dimers, which are connected by cysteine bridges between the triple helix and the C-terminal globular domains, a process resulting in a stabilized dimer, with the monomers in opposing directions (Fig. 2) (75). Following the formation of the antiparallel dimer, the triple helices undergo a twist in the overlapping regions resulting in a supercoil, and, finally, when 2 of these supercoiled dimers interact, they assemble into a tetramer (76, 77). The tetrameric form is stabilized by interactions between the N-terminal regions, which cross over, and by disulfide bonds between the α3(VI) triple helical cysteine residues (76). These tetramers are now the final building blocks for the Col VI microfibrils. The tetramers are released outside the cell, in a process requiring the C5 domain from the α3(VI) chain. Upon exiting the cells, the tetramers connect end-to-end resulting in the well-established beaded microfibrillar structure (55).

The structure of the microfibrils is equally complicated, and electron microscopy (EM) analysis has revealed that the microfibrils are shorter than the individual tetramers, strongly suggesting an overlap between the fibrillar domains and the N- and C-terminal globular domains of the neighboring tetramer (30, 76). This results in a junctional complex with a series of close contacts between the adjacent tetramers (30). The beads are generated through interactions between the N- and C-terminal domains. In the vWF domains, there are sequences/units allowing the folding into the globular structures located between each fibrillar domain, ultimately resulting in the beads on a string arrangement observed using EM (78-80).

Table 1. Summary of type VI collagen properties and mutations

Type VI collagen	Description	Reference
Gene name and number	*COL6A1*, Location 21q22.3 (gene ID 1291) *COL6A2*, Location 21q22.3 (gene ID 1292) *COL6A3*, Location 2q37 (gene ID 1293) *COL6A4* (pseudogenes due to a chromosome break) *COL6A5*, Location 3q22.1 (gene ID 256076) *COL6A6*, Location 3q22.1 (gene ID 131873)	NCBI NCBI NCBI NCBI NCBI
Mutations with diseases in humans	Bethlem myopathy Ullrich congenital muscular dystrophy Limb-girdle muscular dystrophy phenotype Autosomal recessive myosclerosis	(39-41)
Null mutation in mice	In Col6a1, Col6a2, and Col6a3 knockout mice, skeletal muscle and adipocyte size were abnormal. Accelerated development of osteoarthritis was found. However, type VI collagen knockout had protective roles after myocardial infarction	(42-46)
Tissue distribution in healthy states	Dermis, skeletal muscle, lung, kidney, blood vessels, cornea, tendon, skin, cartilage, intervertebral discs, adipose tissue	(2, 47-51)
Tissue distribution in pathological affected states	Upregulated in tissue fibrosis	(5, 52-54)
Special domains	Soluble cleaved fragment of the C5 domain, also called ETP	(3, 55-57)
Special neoepitopes	A cleavage in the C-terminal portion of the α3 chain can release the soluble fragment ETP. Furin-like proprotein convertase and bone morphogenetic protein 1 (BMP-1) are involved in the cleavage. The possible cleavage sites are between the C1 and C2 domain and between the C4 and C5 domain. Cleavage by MMP-2 and MMP-9 and MMP-14 also observed	(4, 55, 58)
Protein structure and function	Type VI collagen is composed of 3 different chains, α1, α2, and α3. The protein contains a short collagenous region and globular regions at both the N- and C-terminus. Dimers are formed by 2 monomers in antiparallel manner, while tetramers are formed in a parallel manner. After secretion into the ECM, tetramers are assembled into a microfibrillar network Three additional chains (α4, α5, and α6) have been identified with a structure similar to the α3 chain and specific localization Type VI collagen is expressed in many tissues and connects related tissues to the matrix	(28, 33, 47, 48, 59-61)
Binding proteins	Type IV collagen, biglycan, decorin, perlecan, NG2 proteoglycan, fibronectin, tenascin, integrin	(28, 62-67)
Known central function	Helps cell attachment and connection to the surrounding matrix Involvement in a series of pathologies, where it induces fibrotic processes through release of ETP	(1, 3, 28, 48, 63, 68)
Animals models with protein affected	Col6a1, Col6a2, Col6a3 knockout mouse models, AnxA2−/− mice	(42, 44-46, 69)
Biomarkers derived from col VI	ETP (PRO-C6), C6M, C6Ma3	(58, 70-72)

Finally, a series of studies used high resolution X-ray scattering and EM to resolve the interactions in the actual bead region, and as illustrated in Fig. 2, they confirmed that among the overlapping regions between the neighboring tetramers, there are numerous interactions clustering them together in the end to end arranged beads (36, 73, 81), which maintain regions extending from the beads, which are likely involved in interactions with the surrounding milieu/matrix (81).

While the Col VI structure in itself is rather complex and unique, it is important to remember that it interacts with a host of other proteins. Additional ECM molecules feature prominently among them (28). While we only scratch the surface in terms of a full understanding of both function and arrangements of these interactions, some of these structures are highly relevant in terms of functionality. This is illustrated by the interactions between Col VI, Col IV, and perlecan (a large basement membrane proteoglycan) in the basal lamina. It ensures a proper link between the muscle and the ECM. This also is likely to explain the muscle dysfunction observed in subjects with Col VI mutations (36). Further members of the Collagen type VI interactome include interactions with collagen type I, type II and type XIV (62, 68, 82, 83), as well as a series of noncollagenous matrix proteins, such as biglycan, WARP, fibronectin, microfibril-associated MAGP1 glycoprotein, matrilin-1, fibulin-2, lumican, heparin, hyaluronan, and decorin, as reviewed by Cescon et al (28).

However, the functional importance of all of these interactions remains to be fully elucidated. Suffice it to say that Col VI is a central player in the organization of the matrix of some tissues. Col VI has also been shown to bind to vWF (84) and to induce vWF-dependent platelet adhesion/aggregation (85), a function that likely resides in the N-terminal globular regions of the α3 chain of Col VI tetramers (86).

Expression Profile of Collagen Type VI Chains

Col VI is a beaded filament collagen that forms a unique microfibrillar network and is expressed in the ECM of many tissues, including the dermis, skeletal muscle, kidney, lung, blood vessels, cornea, tendon, skin, cartilage, intervertebral

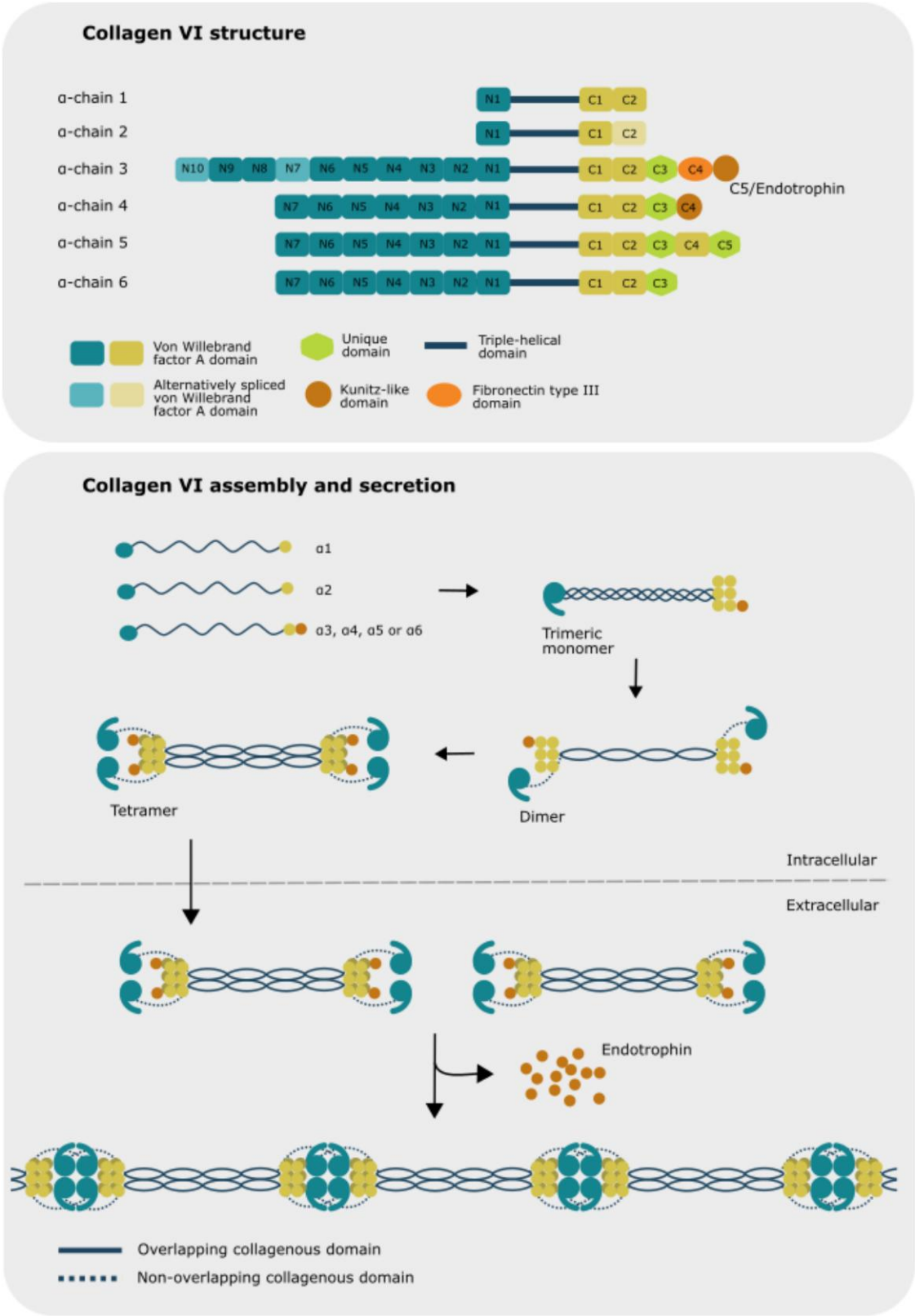


Figure 2. Assembly of the type VI collagen beaded microfilaments. The process includes the release of the C5/ETP domain upon formation of the microfibrils. Inspired by (73).

discs, and, most prominently, adipose tissue (2, 47-50). At the cellular level, myofibers, chondrocytes, neurons, fibroblasts, chondrocytes, adipocytes, and cardiomyocytes all express Col VI (35-37).

As Col VI entails a total of 5 different chains in humans, 6 in most other animals, much work has gone into elucidating whether any of these are tissue specific, or at least somewhat selective in terms of distribution (28). While the α1(VI),

α2(VI) and α3(VI) chains are broadly expressed, the newer chains α5(VI) and α6(VI) have a far more restricted tissue profile. α5(VI) is found in skeletal muscle at the neuromuscular junctions, in kidney glomeruli, ovaries, and testis. Expression of α6(VI) is seen in the endomysial and perimysial regions of skeletal muscle and in the pericardium (87). Furthermore, both of these chains have been detected in skin (in the papillary dermis), and they appear to have some sort of compensatory expression/function in the event of mutations rendering α3(VI) inactive or absent (52). An interesting finding regarding the expression of the Col VI chains is that most, if not all, of the chains are upregulated by fibrotic drivers, and as described later, particularly the upregulation of the α3(VI) appears to have pathological consequences (88).

These pieces of evidence go far towards suggesting that Col VI does have tissue specific functions, which are then highly dependent on the composition of the triple helix; however, they could also provide an explanation for why mutations in this very central and ubiquitous collagen lead to restricted phenotypes primarily in muscle and skin (36, 37).

Function of Type VI Collagen

Much can be learned about type VI collagen function by both its physiology and pathophysiology. The physiological function of Col VI has been carefully elucidated through studies on subjects with mutations in the Col VI chains.

Col VI mutations are known to lead to 2 separate types of muscle dystrophy, Bethlem myopathy, and Ullrich congenital muscular dystrophy, which are inherited disorders caused by mutations in the genes encoding isoforms *COL6A1*, *COL6A2*, and *COL6A3* (36, 89-91). The diagnosis entails DNA sequencing of the Col VI genes (92).

The mutations can be either recessive or dominant, and the phenotypes observed as a function of these are highly variable, ranging from mild to severe. Importantly, there appears to be a connection between the position of the mutation in the collagen type VI chains and the phenotype, although the direct relationship still has to be explored for many of the observed mutations, especially those in the N- and C-terminal regions (36, 37). Numerous types of mutations, including point mutations, premature stop codons and frameshifts, have been identified. For a thorough overview, see (36, 37). Interestingly, mutations in the G-X-Y repeats have been identified in the 3 most common Col VI chains and these lead to the more severe forms of muscle dystrophies underscoring the importance of a functional triple helix for assembly of the entire beaded microfibril structure (36, 37, 89-91). While a detailed clinical description of the phenotypes is beyond the scope of this review, it is important to notice that similar phenotypes arise from mutations in the 3 different chains, underscoring that the main functional unit of Col VI consists of the α1(VI), α2(VI) and α3(VI) chains (36, 37). This is further supported by studies showing overall reductions in Col VI expression in fibroblasts from patients with various mutations (93-95). Analyses of the Col VI fibrillar structure also underscore that the fibrils formed can be of inferior quality, number, and length, and that these changes are directly related to the severity of the phenotype (93-95). In addition to the observed muscle dystrophy, some patients also present with skin phenotypes, including keratosis pilaris and keloids, underscoring that Col VI also has a normal physiological role in the skin, albeit a less prominent one than in muscle (92).

Importantly, the function of Col VI, at least as far as the α1(VI), α2(VI), and α3(VI) chains are concerned, is similar among mammals. Both mouse models and dogs deficient in any of these 3 chains also present with muscle deficiencies, some very similar to the human conditions and some less severe (42, 96-99), data which support a general evolutionarily conserved function, but also hint at some changes in the function of the Col VI chains, potentially involving the less known chains, including the chain not active in humans, the α4(VI) chain. Surprisingly, obese mice deficient in Col VI have an improved metabolic read out systemically (100) due to mechanically stress-free expansion of their adipose issue. While metabolically beneficial, this is also associated with reduced mechanical stability of the adipose tissue (101).

Interestingly, not much is known about mutations in the α5(VI) and α6(VI) chains, although a study showing chronic itching in subjects with mutations in *COL6A5* has been published, and mutations in *COL6A6* have been observed in autosomal dominant retinitis pigmentosa, and genetic variants were found in atopic dermatitis (102-105).

At the pathological level, the data supporting that an upregulation of Col VI, irrespective of the chain, is associated with a host a pathological conditions, such as cancer, various types of fibrosis, and a series of other diseases, many of which have the activation of a fibrotic program as a common denominator (88).

Processing of Type VI Collagen

While processing of type VI collagen during synthesis of the beaded microfibrils has been described and is mentioned in the previous section, the specific release of the C5 (the "ETP domain") will be described later. The mature collagen type VI is also processed. Studies by Heumuller et al have demonstrated the presence of fragments matching the size of the full-length C-terminal sequence (C1-C5), as well as shorter fragments, containing fewer of the C-domains, all the way down to the C5 domain alone (4). An interesting observation was a differential expression of these across the various tissues, as well as their presence in circulation, data which clearly demonstrate the processing and ultimately the release of the C-terminal domain from at least the α3 chain (1, 4, 56, 59, 73).

Furthermore, a series of studies have looked into the fragments generated enzymatically upon turnover of the matrix in both normal and pathological conditions. Although this is still not fully understood, fragments originating from the different chains of Col VI have been measured in circulation across a span of diseases, such as liver fibrosis, chronic obstructive pulmonary disease, cancer, and Crohn diseases (58, 70-72, 106, 107). Further studies using these proteomic tools will likely shed further light on the processing and the relevance of these fragments in relation to pathologies (see "Function of Type VI Collagen").

Collagen Type VIα α3

Special Domains of Type VIα3

As illustrated in Fig. 2, the α3 chain is a unique chain in terms of its composition. Rather than mainly consisting of a triple helical domain, the majority of the COL VIα3 chain is actually a series of vWF domains (37). In total, the α3(VI) chain harbors 12 domains, 10 of which reside upstream of the triple

helical domain and 2 of which are downstream. While the understanding of the individual domains in the α3(VI) chain is complicated by its interactions with the other Col VI chains, there are indications for an involvement in platelet aggregation (79, 86). Structural information about the N-terminal vWF domain array was in general lacking until a study by Solomon-Degafa et al (80) provided high-resolution data on these domains. Interestingly, a high level of flexibility of these domains was observed, visualized by the presence of multiple confirmations. This finding could be related to the multitude of interactions in the ECM due to its role in maintaining the stiffness of the individual tissues (80).

With respect to the individual N-terminal domains, the N2 is rich in pathogenic point mutations (108-110), while the N6 to N3 domain appear central in the aforementioned flexibility and are involved in both the formation of the Col VI microfibrils and in their interactions with many ECM molecules, including biglycan, decorin, heparin, and hyaluronan (79, 83, 111).

Another interesting finding illustrating the special nature of the α3 (VI) chain is that the linkers between the N-terminal vWF domains are different from the otherwise quite similar α4 (VI), α5 (VI), and α6 (VI) chains, as the α3 (VI) linkers do not contain cysteines and therefore are unable to form disulfide bridges, and thus possess a larger structural flexibility (78). This flexible structure combined with the tissue specific distribution of the minor chains likely plays an important role in the tissue specific functions of the different chains as described previously.

The triple helical domain is home to many of the pathology causing mutations, underscoring that the interactions formed here are critical for the fully functional Col VI microfibril; however, as these mutations also are frequent in the other chains of Col VI, this is not a unique feature of the α3 (VI) chain (see "Function of Type VI Collagen").

With respect to the C-terminal domain, it is constructed of 5 separate domains (see Fig. 2), including 2 vWF domains called C1 and C2, C3 (which is a unique and poorly understood domain), C4, which is a fibronectin type III repeat, and finally the Kunitz type domain referred to as C5/ETP (4). These domains have been shown to undergo proteolytic cleavage, leading to the release of fragments of various sizes, ranging from fragments including C2-C5 through smaller fragments containing only the C5 domain (4).

Interestingly, this cleavage is essential for the formation of the microfibrils and the elimination of the C5 domain may lead to an arrest of the full generation of the microfibrils resulting in only tetramers (55). This is however all based on in vitro data. Furthermore, underscoring processing of the C-terminal of the α3 (VI) chain as an important step in the maturation of the fibril, none of the C2 to C5 domains are found in mature fibril (73).

The release the C-terminal domains was shown to be mediated by 2 enzymes, a furin type preproconvertase releasing the C2-C5 sequence, and bone morphogenetic protein 1 specifically releasing the C5 domain (4). Also, MMP14 has been implicated as a critical factor releasing C5 (ETP) (7).

Functionality of the α3 (VI) chain under normal physiological conditions centers around its structural role, particularly in muscle tissue, but also for skin and potentially other tissues (37). Considering the widespread expression profile of the α3 (VI) chain, there could be other more discrete roles that have yet to be discovered.

On the other hand, the Col α3 (VI) chain has been shown to be upregulated in a large number of pathological conditions, and there is very little doubt about its function as a pathological driver of disease. The build-up of large amounts of Col VI in tissues, including the α3 (VI) chain, is associated with fibrosis of the tissues. A series of studies focused on the expression of the α3 (VI) chain demonstrated that COL6A3 was highly expressed in both primary and metastatic ovarian cancer tissues, such as in the omentum and in stromal cells (mesenchymal-like ovarian carcinoma stromal progenitor cells) within the tumor microenvironment, but exhibited much lower expression in benign tissues and epithelial-like ovarian carcinoma stromal progenitor cells. When the mesenchymal-like ovarian carcinoma stromal progenitor cells or type VI collagen was added to epithelial ovarian carcinoma, cell invasion and spheroid formation increased. The authors also observed that COL6A3-derived spheroids from epithelial ovarian carcinoma injected in a xenograft mouse model induced metastases in the lungs, and pinpointed the CDK4/6 and the p-Rb signaling pathway as the one stimulated by COL6A3 (112). In a human bladder cancer cell line, the silencing of COL6A3 caused a significant inhibition of cell proliferation, angiogenesis, a downregulation of proteins related to EMT, as well as proteins involved in the transforming growth factor (TGF)-β/Smads signaling pathway (113).

In patients with bladder cancer, the COL6α3 gene expression was higher in the tumor tissues than in the adjacent tissues, and patients with high COL6α3 expression had a lower survival rate (follow-up 800 days) than those with low COL6A3 expression (113). Expression data and clinical data from the Cancer Genome Atlas Program (TCGA dataset) and the Gene Expression Omnibus website showed that COL6α3 expression is significantly higher in stage III than in stage II ovarian cancer and that advanced ovarian cancer patients exhibit higher expression of COL6α3 and had a shorter overall survival rate than those with lower expression (HR for high vs low COL6α3: 2.0). The authors also reported that in the analysis of their patient cohorts with primary ovarian cancer (follow-up of 60 months), patients with higher expression of COL6α3 had shorter overall survival and shorter progression-free survival than did patients with lower expression of COL6α3. COL6α3 mRNA levels were associated with stage and debulking status, which are the most important prognostic factors in patients with ovarian cancer (112). A high expression of the COL6A3 gene was also linked to worse survival in metastatic clear cell renal carcinoma (114). Since these studies were relying on gene expression analyses, it was not possible to indicate whether the prognostic effects of the COL6α3 chain expression are directly attributable to ETP, which will be further discussed below.

In patients with soft-tissue sarcoma, COLVIα3 was more abundant in primary lesions of patients who manifested post-surgical metastatic disease. However, enhanced expression of COLVIα3 alone did not significantly impact the disease course, but high expression of both COLVIα3 and the proteoglycan chondroitin sulphate proteoglycan 4 (CSPG4)/NG2 correlated with the worst disease-free survival (115). In specimens from salivary gland cancers, the levels of type VI collagen staining were related to mortality: patients with a low expression of type VI collagen had a significantly better overall survival at 5 years than patients with a high expression. Interestingly, high type VI collagen expression was significantly associated with decreased survival in patients with

otherwise favorable prognostic factors and did not impact survival in the adverse prognostic subgroups (116). Would include some description of ETP expression in biopsies and cancer patients/expand on Table 3.

Finally, data from the GEPIA database (a web server for cancer and normal gene expression profiling and interactive analyses) indicated that patients with pancreatic cancer with high COL6α3 expression levels exhibited significantly decreased overall survival (follow-up 50 months) compared with those with low COLVIα3 expression. Even though this study did not focus specifically on the COLVIα3 gene, this data further confirms that COLVI gene expression is a crucial component associated with cancer-related mortality (117).

ETP and its Receptor

General Information About ETP

As discussed above, several of the C-terminal domains of Col VI are released during assembly into the microfibrils, and some of these are known to have signaling properties exacerbating the diseases (35, 36). Especially the C5 domain, also called ETP, can be detected in circulation during a host of pathologies (118). More importantly, ETP has been shown to drive pathological changes, and thereby can serve potentially as a highly relevant drug target (8, 88).

The Receptor for ETP

While the existence of an ETP receptor or receptors have not been conclusively demonstrated, a number of hypotheses have emerged. Upon considering potential ETP receptors, the list of proteins interacting with mature Col VI is of interest, as especially as the specific domains/chains for these interactions have not been elucidated fully. Potential binding partners include integrins α1β1, α2β1, α3β1, α10β1, and αvβ1 (67, 119, 120), the cell surface chondroitin sulfate proteoglycan, CSPG4 (also known as NG2) (121), anthrax receptor 1 (ANTXR1/TEM8) (122), and anthrax receptor 2 (ANTXR2/CMG2) (123). In this context, an early study hinted at an interaction between ETP and the anthrax toxin receptor 1 (ANTRX1), also called tumor endothelial marker 8 (TEM8) (122), potentially indicating that ANTRX1 could be the ETP receptor. However, a recent study addressing the ETP interactions with both ANTRX1 and its closely related (and Col VI) interacting relative ANTRX2 failed to confirm the interaction between ETP and these proteins (124). We conclude that to date, the ETP receptor remains unknown. Identifying the ETP receptor(s) will constitute important step towards a better understanding of ETP action on target cells.

Elevated Circulating Levels of ETP

ETP Measured by the PRO-C6 Assay

The vast majority of studies reporting data on ETP as a circulatory biomarker used the PRO-C6 assay targeting the last 10 amino acids of the α3 chain of collagen type VI, which are also the last 10 amino acids of the proposed sequence of ETP. Increased levels of PRO-C6 in circulation were reported in multiple disease states (Table 2). In most cases, levels were associated with disease severity. Several studies reported data on PRO-C6 measured at baseline and occurrence of adverse outcome or mortality in multiple diseases (Table 3).

Alternative Measurements of ETP

Some reports in the literature use other assays to measure ETP (16, 156-162). These reports showed elevated levels of ETP in populations with different disease states. This included fibrotic interstitial lung disease (16), polycystic ovary syndrome (156), nonalcoholic steatohepatitis (though in the latter case not related to liver fibrosis burden (162)) and in obesity in children and adolescents (160). On the opposite end of the spectrum, lower levels of ETP in circulation were observed in patients with bicuspid aortic valve defects than in controls (159). In these patients, ETP levels correlate with aortic strain and aortic distensibility and inversely correlate with the aortic stiffness index. Levels of circulating ETP ≤ 82 ng/mL could significantly predict ascending aorta dilatation (159). Levels of circulating ETP were also tested in heart failure and COVID-19 patients, but no elevation nor a decrease compared with healthy was observed (158, 161). However, many of these studies need to be revisited in light of more recent data (see below). Some evidence of a pharmacodynamic change in ETP was shown in individuals with type 2 diabetes following a medical nutrition treatment and an exercise program on the background of metformin, where ETP levels and urinary albumin to creatinine ratio decreased significantly with a decrease in HbA1c (157). Patients with heart failure with midrange ejection fraction treated with renin angiotensin–aldosterone system inhibitors had lower levels of ETP than those not treated (158). However, ETP was not elevated in patients with heart failure compared with matched controls (158). Considering the multiple evidence of elevated levels of ETP measured by PRO-C6 assays in heart failure cohorts (147) may suggest that the assay used in these investigations did not measure ETP levels with sufficient specificity and accuracy.

Pathological Aspects of ETP

As a newly identified small adipokine with altered levels in circulation, the pathological functions of ETP have been broadly studied in recent years (reviewed in (163)). These studies have shown that ETP plays a significant role in stimulating adipogenesis, fibrosis, and inflammation within obese adipose tissue, leading to systemic insulin resistance and other unfavorable metabolic consequences, including lipotoxicity (3, 7, 164). Furthermore, ETP triggers profibrotic and proinflammatory reactions in other metabolic tissues, including the heart, liver, and kidney (10, 11, 158, 162, 165-167). It is worth noting that ETP is also enriched in many types of cancer lesions, where it can promote malignant tumor progression (1, 8, 12, 13, 168).

A host of clinical studies have revealed that circulating ETP levels are correlated with the development of nonalcoholic fatty liver disease (NAFLD), heart failure, and diabetes (154, 158, 162). Additionally, urinary or serum ETP levels correlate with local fibrosis, tubular atrophy, and immune cell infiltration in lupus nephritis disease, as well as the risk of delayed graft function after kidney transplantation (5, 10, 131, 148). Circulating levels of ETP are also closely linked to many other fibrotic diseases, such as fibrotic interstitial lung disease (16, 169). Collectively, both animal and clinical studies suggest that ETP may play significant pathological roles in different tissues and organs, making it a potential target for the treatment and prevention of various disease entities (8, 11, 88, 163, 170).

Table 2. Cross-sectional data on ETP measured by PRO-C6

Disease in which PRO-C6 was elevated	Control group	Association to disease severity	Reference
Cancer			
Biliary tract cancer	Healthy controls		(125)
Cardiovascular diseases			
Angina	Healthy controls		(126)
Kidney diseases			
Chronic kidney disease (CKD)		Highest levels in patients with underlying diabetes. Strong inverse correlation with estimated glomerular filtration rate (eGFR)	(10)
IgA nephropathy		Increased with increasing CKD stage and levels of tubulointerstitial fibrosis	(127)
Antineutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis		Highest in the sclerotic phenotype, which has the worst prognosis	(128)
Kidney transplant recipients		Associated with worse kidney function and interstitial fibrosis and tubular atrophy	(129, 130)
Lupus nephritis	Healthy controls	Associated with tubular atrophy and interstitial mononuclear cell infiltration. Higher in patients with high NIH activity index	(131)
Liver diseases			
Nonalcoholic steatohepatitis (NASH)		Separation between fibrosis stages 3-4 and 0-2	(132)
Nonalcoholic fatty liver disease (NAFLD)		Separation between fibrosis stage ≥2 and <2	(133)
Acute on chronic liver failure (ACLF)	Patients with stable cirrhosis, acute decompensated cirrhosis and healthy controls	Significantly higher in ACLF patients with non-hepatic (ie, kidney, brain, circulatory and respiratory) failure compared to those without, but not different between those with and without hepatic failure	(6)
Cirrhosis with cardiac fibrosis		Associated with both cardiac and liver extracellular volume	(134)
Lung diseases			
Chronic obstructive pulmonary disease (COPD)	Never-smoking control patients	Inversely correlated with forced expiratory volume in 1 second (FEV1), correlated with percent predicted forced vital capacity (FVC), and quality of life and blood eosinophil counts Highest in patients with previous exacerbations. Lower during acute exacerbation	(135-137)
Idiopathic pulmonary fibrosis (IPF)		Higher in patients that progressed (all-cause mortality or ≥10% decline in FVC at 12 months) baseline, and at 1, 3, and 6 months	(138)
Diabetes			
Type 1 diabetes		Increased in patients with arterial stiffness	(139)
Autoimmune/inflammatory diseases			
Psoriatic arthritis	Healthy controls		(140)
Systemic sclerosis (SSc)	Healthy controls	Higher in early diffuse SSc compared to early limited SSc and late diffuse SSc and correlated with the modified Rodnan skin score Higher in patients with concomitant pulmonary arterial hypertension	(141-143)
Crohn's disease in clinical remission	Crohn's mild disease and moderate to severe disease		(107)
Others			
Elderly women		Increased levels with increasing number of comorbidities	(118)

The Role of Endotrophin in Adipose Tissue Physiology

ETP is an important signaling peptide derived from adipose tissue. It is can be secreted by mature adipocytes within the adipose tissue, but other stromal vascular cells can also contribute to the local levels depending on the disease state (1, 3, 8, 164). Abundant levels of ETP have been detected in the differentiated 3T3-L1 adipocytes, and its production is dramatically increased in the adipose tissue of diet-induced and genetically induced *ob/ob* obese mice (1, 164). Human

Table 3. Association of baseline circulating ETP/PRO-C6 and adverse outcomes in longitudinal prospective studies

Disease	Outcome	Reference
Cancer		
Pancreatic ductal adenocarcinoma (PDAC)	Overall survival	(144)
Biliary tract cancer	Mortality	(125)
Hepatocellular carcinoma (HCC)	Overall survival	(145)
Metastatic colorectal cancer	Overall survival	(146)
Cardiovascular diseases		
Heart failure with preserved ejection fraction	Mortality and hospitalization for heart failure	(147)
Atherosclerosis	Mortality and cardiovascular outcome	(54)
Kidney diseases		
Acute to chronic kidney disease	Mortality and kidney disease progression (≥25% decline in eGFR combined with a decline in CKD stage)	(128)
Kidney transplant	Delayed graft function (PRO-C6 measured before transplant) and mortality and kidney graft failure (PRO-C6 measured after transplant)	(148, 149)
CKD	Mortality and end-stage kidney disease	(10)
Liver diseases		
Liver cirrhosis	Transplant-free survival	(150)
Lung diseases		
COPD	Mortality	(71, 137)
IPF	Mortality and combined endpoint of disease progression or death at 6 months	(151)
Diabetes		
Type 1 diabetes	Mortality and end-stage kidney disease	(152)
Type 2 diabetes	Mortality, cardiovascular outcome, and kidney disease progression	(54, 153-155)
Others		
Elderly women	Mortality	(118)

studies further reveal a significant upregulation of ETP in the adipose tissue of obese and diabetic patients, further underlining its association with obesity (1, 3, 8).

Proadipogenic Functions

ETP is highly enriched in adipose tissue of people living with obese and diabetes, and it has been found to promote adipogenesis (3). Adipogenesis refers to the formation of new fat cells from preadipocytes, but it also promotes the growth and expansion of existing adipocytes. Excessive adipogenesis and increased fat cell size can lead to profound disturbances in energy homeostasis, metabolic dysfunction, and the development of obesity-related conditions, such as insulin resistance, type 2 diabetes, and cardiovascular disease (1, 3, 7, 12). ETP stimulates adipogenesis by upregulating adipogenic genes, such as Pparγ, Fabp4, Srebp1, and Pref-1 in white adipose tissue (164). Increased levels of PPARγ, a key regulator of adipogenesis, have been observed in the adipose tissue of ETP transgenic mice (164). ETP overexpression also affects lipogenesis and lipolysis. Specifically, the level serine^{660} phosphorylation on hormone-sensitive lipase, a modification associated with enhanced lipolysis, was dramatically decreased in the adipose tissue of ETP transgenic mice (164). Additionally, ETP reduces the levels of CD36, a fatty acid transporter in adipose tissue (7, 164). These changes in adipogenesis and lipolysis contribute to increased lipid content in adipocytes isolated from ETP transgenic mice (164). The direct function of ETP on adipogenesis has been confirmed in 3T3-L1 cells treated with ETP-containing medium, and blocking ETP function with a specific neutralizing antibody reverses the adipocyte phenotype in diet-induced obese mice and in human adipocytes (3, 8, 164, 166). Importantly, all of these effects can be achieved by ETP alone, independent of the presence of the mature ColVI complex, emphasizing the independent roles of endotrophin from its parent molecule.

Profibrotic/Proinflammatory Functions

During the development of obesity, adipose tissue undergoes rapid expansion, creating a hypoxic microenvironment locally (171, 172). In response to hypoxia, HIF1α is induced and stimulates extensive fibrosis locally in adipose tissue, leading to local inflammation and systemic insulin resistance (171, 172). While it is widely accepted that fibrosis eventually leads to metabolic deficiencies, there has been limited focus on understanding the modifiers that regulate this pathological process in obese adipose tissue (173). In that context, we found that ETP produced in the obese adipose tissue serves as a powerful costimulator for fibrosis and local inflammation (3). Clinical studies have also shown a correlation between the levels of ETP and local fibrosis and inflammation in white adipose tissue of patients with obesity and diabetes (3). In ETP transgenic mouse models, ETP interacts with TGF-β signaling to initiate the profibrotic and proinflammatory pathways (1, 3).

To further investigate the mechanisms by which ETP stimulates fibrosis and inflammation, experiments were conducted using ETP-conditioned media to treat the stromal vascular fraction (SVF) isolated from white adipose tissue. The experiments revealed that the levels of multiple collagen proteins, including ColIα, ColVIα1, and ColVIα3, as well as the collagen cross-linking enzyme LOX, were significantly upregulated (164). Additionally, the levels of TGF-β1 and its receptor TGF-βR2 were upregulated in ETP-treated SVF cells and in mature adipocytes, consistent with the reported simulation of TGF-β signaling by ETP in the ETP transgenic mice (1, 3, 164). This suggests that ETP may be upstream of the TGF-β pathway. Under the same experimental conditions, ETP also stimulated upregulation of proinflammatory genes, such as Toll-like receptor 4 (Tlr4) and NLR family pyrin domain containing 3 (Nlrp3) in SVF cells and adipocytes (164). These in vitro findings suggest that ETP acts on SVF cells and mature adipocytes by triggering both profibrotic and proinflammatory programs, including an activation of the jun kinase pathway.

Fibrosis triggers activation and accumulation of macrophages in the obese adipose tissue, which further induces local inflammation and systemic insulin resistance (172, 174-178). In various mouse models, ETP has been reported to exhibit chemoattractant actions and promotes their accumulation and activation (1, 3, 7). Upon stimulation by ETP, macrophages polarize toward a proinflammatory state, initiating local proinflammatory reactions. Importantly, the profibrotic and proinflammatory reactions in obese adipose tissue can be efficiently prevented by blocking ETP using a neutralizing antibody, suggesting that this pathological process can be reversed pharmacologically (1, 3, 8). Although the profibrotic and proinflammatory effects of ETP have been observed primarily in the adipose tissue and mammary glands, similar pathological changes are expected in other metabolic tissues and tumors, as recent findings have demonstrated the enrichment of ETP in these tissues in response to different pathological stimuli (reviewed in (163)). Particularly, ETP neutralization through targeted antibody treatment protects the kidney from renal fibrosis in a podocyte ablation model (167).

Protumorigenic Function

There is increasing evidence supporting the notion that obesity is associated with an increased risk for a number of different cancer types. The pathophysiological changes in adipose tissue caused by obesity, such as chronic inflammation, angiogenesis, and enhanced fibrosis create an environment that is conducive to cancer development and progression (12, 13, 15, 168). ETP is enriched in tissues and in circulation of obese individuals, and presents at even higher levels in human cancer specimens. ETP has emerged as a potential candidate that links obesity-associated adipose tissue dysfunction with tumor promotion and metastatic spread (1, 2, 12). Multiple malignant cancer cells, including those derived from breast cancers, colon cancers, pancreatic ductal adenocarcinomas, and hepatocellular carcinomas (HCCs) have been found to express high levels of ColVIα3, and the levels of ETP increase dramatically during progression and invasion of these tumors (1, 57, 166, 168). ETP may be derived from both stromal adipocytes in adipose tissue and from the tumor cells themselves, and they may function cooperatively through paracrine and autocrine pathways (8, 12).

Studies using transgenic mouse models with selective overexpression of ETP in adipose tissue or mammary glands have demonstrated that ETP promotes aggressive growth and invasion of mammary tumor, together with increased infiltration of tumors with endothelial and macrophages, enhanced fibrosis and dramatically promoted metastatic growth (1). Mechanistically, ETP exerts profibrotic and proinflammatory functions in the mammalian tumor microenvironment through activation of TGF-β signaling pathways (1, 12). In other studies, it was found that ETP contributes to chemoresistance of HCC to cisplatin in tumor-bearing mouse models (168). However, this effect can be bypassed in the ColVI knockout mice, or by treating wild type mice with the PPARγ agonists, the thiazolidinediones, which downregulate ETP effectively (168). In vitro experiments have also shown that human recombinant ETP exerts similar effects on macrophages and endothelial cells as its rodent counterpart, enhancing EMT in human breast cancer cells and inducing chemoresistance (8).

Recent studies have further implicated ETP in tumor progression and metastasis in various other types of cancer (8, 11, 145, 166). During the development of NAFLD-induced HCC, ETP can act as a "second hit" during the progression of NAFLD, facilitating the development of nonalcoholic steatohepatitis and HCC (8, 11, 166). In agreement with this model, serum levels of ETP are positively associated with NAFLD (162). In the liver, ETP may exert its functions in a similar manner as in breast cancer cells, promoting angiogenesis, enhancing local fibrosis and enhancing a proinflammatory environment that supports tumor growth (8, 11, 166). Further research is needed to fully elucidate the underlying mechanisms by which ETP functions on other types of cancer. Importantly, based on the existing knowledge about the function of ETP, neutralizing monoclonal antibodies against human ETP have been developed and both in vitro and in vivo evidence supports effectiveness of these antibodies in preventing/curbing tumor growth and enhancing chemosensitivity in different types of cancer cells (8).

In summary, the studies conducted thus far have revealed divergent functions of ETP in different cell types (Fig. 3), thereby highlighting it as a potential target from a therapeutic perspective in the context of metabolism and cancer (8, 11, 170). Research in this area is rapidly advancing, and ongoing studies are focused on unraveling the precise mechanisms underlying the function of ETP on metabolic dysregulation and tumor development.

The Theragnostic Potential of ETP and Patient Selection

ETP levels have been demonstrated to be prognostic in clinical settings for a range of diseases, in which the heart and kidney outcomes have been the ones with biggest effects (5, 127, 128, 147, 148, 155, 158, 159, 163, 167, 169)

ETP has been suggest to be a disease driver in a range of metabolic models including liver, kidney, and cancer models (57, 163, 167, 169). Neutralization of ETP, with an antibody, has demonstrated efficacy in kidney, cancer, and liver fibrosis animal models (8, 11, 167).

This suggests that ETP both could be a good biomarker for outcome studies. In addition, given the preclinical data, it clearly serves as a drug target as well, and thereby enables a true theragnostic approach. This type of precision medicine approach, by identification of patients with high levels of

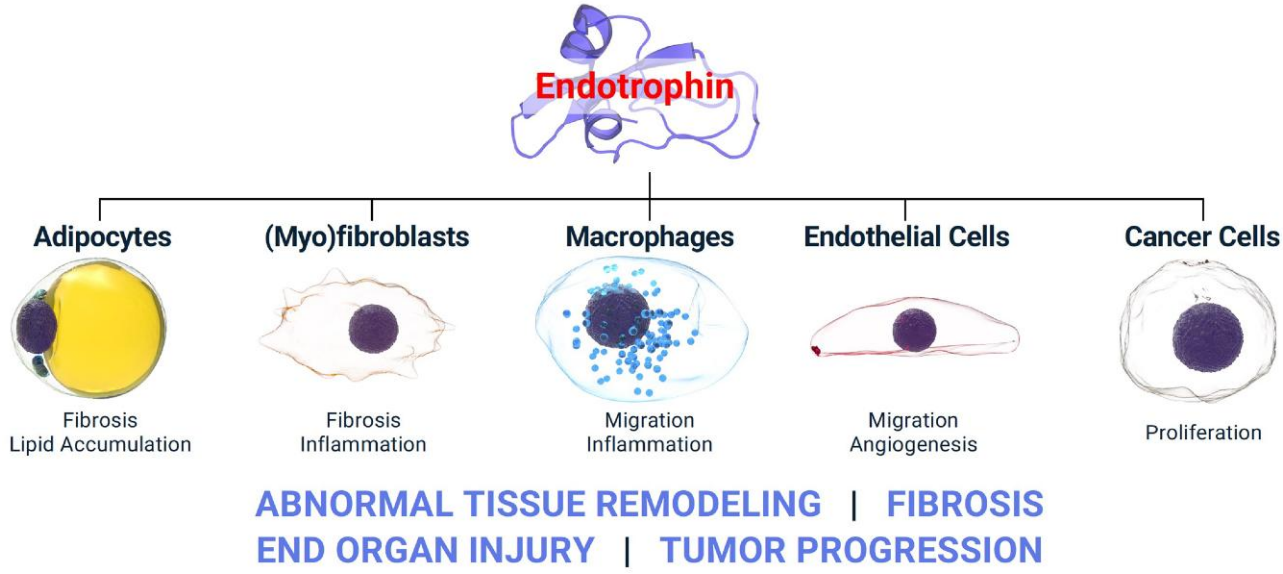


Figure 3. Diverse functions of ETP across various cell types. In this illustration, the multifaceted roles of ETP are depicted within different cellular environments. Obese adipocytes act as sources of EPT, influencing multiple cell types in various metabolic tissues/organs as well as tumor tissues. ETP exerts its effects through autocrine, paracrine, and endocrine pathways, triggering a range of responses including fibrosis, inflammation, lipid accumulation, angiogenesis, and proliferation of cancer cells.

ETP with a higher risk of a negative outcome, and treatment of this patient population may be a very cost effective and efficacious way of treating the right molecular endotype with the most appropriate treatment.

ETP may be used as a patient selection tool in clinical studies, for identification of patients at a higher risk of cardiovascular disease, heart failure with preserved ejection fraction, outcome, based on the recent publication by Chirinos et al (147). By subsequent analysis, the risk of having a heart failure with preserved ejection fraction outcome in the highest tertile compared with the lower tertile was 10-fold, suggesting this to be a true treatable endotype.

Originally, ETP was identified as a hormone associated with metabolic dysfunction and insulin resistance (3). Subsequently, it was identified to predict response to insulin sensitizers, such as PPARγ agonists (15, 165). ETP levels have been shown to be pharmacodynamically modulated by several metabolic interventions, including insulin sensitizers with a weight reduction (179), weight loss modulators such as GLP-1 receptor agonists, as well as compounds modulating fibroblast activities (88).

Conclusion and Perspectives

ETP is clearly associated with important pathological processes as outlined in this manuscript. Type VI collagen is a unique collagen, a beaded filament, that is very distinct from other collagens (31, 32). It holds very different functions from the standard fibrillar collagens, which mainly convey structural functions. In addition, the α3 chain of type VI collagen displays 10 vWF binding domains, which implicate the parent collagen chain in wound healing. In fact, this collagen chain may be the protein in the system with the most vWF binding domain. However, there is no evidence so far for a role of ETP in wound healing, subsequent to its release from the parent collagen A3 chain. However, it seems to be a central player in important biological processes, including cancer and fibrosis.

These data suggest that ETP is upregulated and released into circulation as a consequence of pathophysiological mechanisms that play a role in multiple diseases. Moreover, levels of circulating ETP are strongly associated with disease severity and with a worse prognosis across an ample spectrum of chronic diseases. Can important biological processes be modified by modulation of ETP for the benefit of patients or is ETP "only" a very potent biomarker for kidney, lung, cancer, and liver related pathologies? Given strong interventional data in preclinical models using genetic gain and loss of function models combined with neutralizing antibodies gives us high hope for successful pharmacological interventions in the clinic.

Acknowledgments

P.E.S. was supported by US National Institutes of Health grants R01-DK55758, R01-DK127274, R01-DK131537, R01-DK099110, P01-AG051459, and RC2-DK118620. K.S. was supported by R01-DK109001, R56-DK124419, and R01-DK129815.

Disclosures

K.S. and P.E.S. have no conflicts to declare. L.A. works for Privebio Inc.; K.H., F.G., A. R.-P., and M.A.K. are employees of Nordic Biosciences, own stock in Nordic Bioscience A/S and have patents on type VI collagen biomarkers.

References

1. Park J, Scherer PE. Adipocyte-derived endotrophin promotes malignant tumor progression. *J Clin Invest*. 2012;122(11):4243-4256.
2. Iyengar P, Espina V, Williams TW, *et al*. Adipocyte-derived collagen VI affects early mammary tumor progression in vivo, demonstrating a critical interaction in the tumor/stroma microenvironment. *J Clin Invest*. 2005;115(5):1163-1176.
3. Sun K, Park J, Gupta OT, *et al*. Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction. *Nat Commun*. 2014;5(1):3485.

4. Heumuller SE, Talantikite M, Napoli M, *et al.* C-terminal proteolysis of the collagen VI alpha3 chain by BMP-1 and proprotein convertase(s) releases endotrophin in fragments of different sizes. *J Biol Chem*. 2019;294(37):13769-13780.
5. Rasmussen DGK, Fenton A, Jesky M, *et al.* Urinary endotrophin predicts disease progression in patients with chronic kidney disease. *Sci Rep*. 2017;7(1):17328.
6. Kerbert AJC, Gupta S, Alabsawy E, *et al.* Biomarkers of extracellular matrix formation are associated with acute-on-chronic liver failure. *JHEP Rep*. 2021;3(6):100355.
7. Li X, Zhao Y, Chen C, *et al.* Critical role of matrix metalloproteinase 14 in adipose tissue remodeling during obesity. *Mol Cell Biol*. 2020;40(8):e00564-19.
8. Bu D, Crewe C, Kusminski CM, *et al.* Human endotrophin as a driver of malignant tumor growth. *JCI Insight*. 2019;5(9):e125094.
9. Jo W, Kim M, Oh J, *et al.* MicroRNA-29 ameliorates fibro-inflammation and insulin resistance in HIF1alpha-deficient obese adipose tissue by inhibiting endotrophin generation. *Diabetes*. 2022;71(8):1746-1762.
10. Fenton A, Jesky MD, Ferro CJ, *et al.* Serum endotrophin, a type VI collagen cleavage product, is associated with increased mortality in chronic kidney disease. *PLoS One*. 2017;12(4):e0175200.
11. Lee C, Kim M, Lee JH, *et al.* COL6A3-derived endotrophin links reciprocal interactions among hepatic cells in the pathology of chronic liver disease. *J Pathol*. 2019;247(1):99-109.
12. Park J, Scherer PE. Endotrophin—a novel factor linking obesity with aggressive tumor growth. *Oncotarget*. 2012;3(12):1487-1488.
13. Park J, Scherer PE. Endotrophin in the tumor stroma: a new therapeutic target for breast cancer? *Expert Rev Anticancer Ther*. 2013;13(2):111-113.
14. Ronnow SR, Langholm LL, Karsdal MA, *et al.* Endotrophin, an extracellular hormone, in combination with neoepitope markers of von willebrand factor improves prediction of mortality in the ECLIPSE COPD cohort. *Respir Res*. 2020;21(1):202.
15. Sun K, Park J, Kim M, Scherer PE. Endotrophin, a multifaceted player in metabolic dysregulation and cancer progression, is a predictive biomarker for the response to PPARgamma agonist treatment. *Diabetologia*. 2017;60(1):24-29.
16. Dasdemir Ilkhan G, Demirci Ucsular F, Celikhisar H, Arman Y, Yalniz E, Tukek T. Original article: clinical research. *Sarcoidosis Vasc Diffuse Lung Dis*. 2021;38(2):e2021020.
17. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science*. 2009;326(5957):1216-1219.
18. Discher DE, Janmey P, Wang Y-L. Tissue cells feel and respond to the stiffness of their substrate. *Science*. 2005;310(5751):1139-1143.
19. Kim S-H, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol*. 2011;209(2):139-151.
20. Karsdal MA, Nielsen SH, Leeming DJ, *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.
21. Karsdal MA, Anne-Christine B-J, Kim H, Leeming Diana J, Signe NH, Cecilie B. *Biochemistry of Collagens, Laminins and Elastin*. 2nd ed. Elservier; 2019.
22. Ricard-Blum S. The collagen family. *Cold Spring Harb Perspect Biol*. 2011;3(1):1-19.
23. Sorushanova A, Delgado LM, Wu Z, *et al.* The collagen suprafamily: from biosynthesis to advanced biomaterial development. *Adv Mater*. 2019;31(1):e1801651.
24. Kirkness MW, Lehmann K, Forde NR. Mechanics and structural stability of the collagen triple helix. *Curr Opin Chem Biol*. 2019;53:98-105.
25. Karsdal MA, Nielsen MJ, Sand JM, *et al.* Extracellular matrix remodeling: the common denominator in connective tissue diseases. Possibilities for evaluation and current understanding of the matrix as more than a passive architecture, but a key player in tissue failure. *Assay Drug Dev Technol*. 2013;11(2):70-92.
26. Dobaczewski M, De Haan JJ, Frangogiannis NG. The extracellular matrix modulates fibroblast phenotype and function in the infarcted myocardium. *J Cardiovasc Transl Res*. 2012;5(6):837-847.
27. Walma DAC, Yamada KM. The extracellular matrix in development. *Development*. 2020;147(10):dev175596.
28. Cescon M, Gattazzo F, Chen P, Bonaldo P. Collagen VI at a glance. *J Cell Sci*. 2015;128(19):3525-3531.
29. Chu ML, Mann K, Deutzmann R, *et al.* Characterization of three constituent chains of collagen type VI by peptide sequences and cDNA clones. *Eur J Biochem*. 1987;168(2):309-317.
30. Chu ML, Pan TC, Conway D, *et al.* Sequence analysis of alpha 1(VI) and alpha 2(VI) chains of human type VI collagen reveals internal triplication of globular domains similar to the A domains of von willebrand factor and two alpha 2(VI) chain variants that differ in the carboxy terminus. *EMBO J*. 1989;8(7):1939-1946.
31. Chu ML, Zhang RZ, Pan TC, *et al.* Mosaic structure of globular domains in the human type VI collagen alpha 3 chain: similarity to von willebrand factor, fibronectin, actin, salivary proteins and aprotinin type protease inhibitors. *EMBO J*. 1990;9(2):385-393.
32. Fitzgerald J, Rich C, Zhou FH, Hansen U. Three novel collagen VI chains, alpha4(VI), alpha5(VI), and alpha6(VI). *J Biol Chem*. 2008;283(29):20170-20180.
33. Gara SK, Grumati P, Urciuolo A, *et al.* Three novel collagen VI chains with high homology to the alpha3 chain. *J Biol Chem*. 2008;283(16):10658-10670.
34. Wagener R, Gara SK, Kobbe B, Paulsson M, Zaucke F. The knee osteoarthritis susceptibility locus DVWA on chromosome 3p24.3 is the 5' part of the split COL6A4 gene. *Matrix Biol*. 2009;28(6):307-310.
35. Di Martino A, Cescon M, D'Agostino C, *et al.* Collagen VI in the musculoskeletal system. *Int J Mol Sci*. 2023;24(6):5095.
36. Lamandé SR. Collagen VI muscle disorders: mutation types, pathogenic mechanisms and approaches to therapy. *Adv Exp Med Biol*. 2021;1348:311-323.
37. Lamandé SR, Bateman JF. Collagen VI disorders: insights on form and function in the extracellular matrix and beyond. *Matrix Biol*. 2018;71-72:348-367.
38. Baker NL, Mörgelin M, Pace RA, *et al.* Molecular consequences of dominant bethlem myopathy collagen VI mutations. *Ann Neurol*. 2007;62(4):390-405.
39. Bushby KMD, Collins J, Hicks D. Collagen type VI myopathies. *Adv Exp Med Biol*. 2014;802:185-199.
40. Lampe AK, Bushby KMD. Collagen VI related muscle disorders. *J Med Genet*. 2005;42(9):673-685.
41. Lampe AK, Zou Y, Sudano D, *et al.* Exon skipping mutations in collagen VI are common and are predictive for severity and inheritance. *Hum Mutat*. 2008;29(6):809-822.
42. Bonaldo P, Braghetta P, Zanetti M, Piccolo S, Volpin D, Bressan GM. Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for bethlem myopathy. *Hum Mol Genet*. 1998;7(13):2135-2140.
43. Alexopoulos LG, Youn I, Bonaldo P, Guilak F. Developmental and osteoarthritic changes in Col6a1-knockout mice: biomechanics of type VI collagen in the cartilage pericellular matrix. *Arthritis Rheum*. 2009;60(3):771-779.
44. Luther DJ, Thodeti CK, Shamhart PE, *et al.* Absence of type vi collagen paradoxically improves cardiac function, structure, and remodeling after myocardial infarction. *Circ Res*. 2012;110(6):851-856.
45. Mohassel P, Rooney J, Zou Y, Bönnemann C. Col6A2 null mice are a new mouse model of collagen-VI related dystrophies and relevant to the human disease. *Neuromuscul Dis*. 2015;25(2):S266.
46. Oh J, Kim CS, Kim M, Jo W, Sung YH, Park J. Type VI collagen and its cleavage product, endotrophin, cooperatively regulate the

adipogenic and lipolytic capacity of adipocytes. *Metabolism*. 2021;114:154430.
47. Gelse K, Pöschl E, Aigner T. Collagens—structure, function, and biosynthesis. *Adv Drug Deliv Rev*. 2003;55(12):1531-1546.
48. Keene DR, Engvall E, Glanville RW. Ultrastructure of type VI collagen in human skin and cartilage suggests an anchoring function for this filamentous network. *J Cell Biol*. 1988;107(5):1995-2006.
49. Zou Y, Zhang RZ, Sabatelli P, Chu ML, Bönnemann CG. Muscle interstitial fibroblasts are the main source of collagen VI synthesis in skeletal muscle: implications for congenital muscular dystrophy types Ullrich and Bethlem. *J Neuropathol Exp Neurol*. 2008;67(2):144-154.
50. Lennon R, Byron A, Humphries JD, *et al.* Global analysis reveals the complexity of the human glomerular extracellular matrix. *J Am Soc Nephrol*. 2014;25(5):939-951.
51. Burgstaller G, Oehrle B, Gerckens M, White ES, Schiller HB, Eickelberg O. The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. *Eur Respir J*. 2017;50(1):1601805.
52. Sabatelli P, Gualandi F, Gara SK, *et al.* Expression of collagen VI α5 and α6 chains in human muscle and in duchenne muscular dystrophy-related muscle fibrosis. *Matrix Biol*. 2012;31(3):187-196.
53. 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(6):1956-1964.
54. Holm Nielsen S, Edsfeldt A, Tengryd C, *et al.* The novel collagen matrikine, endotrophin, is associated with mortality and cardiovascular events in patients with atherosclerosis. *J Intern Med*. 2021;290(1):179-189.
55. Lamandé SR, Mörgelin M, Adams NE, Selan C, Allen JM. The C5 domain of the collagen VI alpha3(VI) chain is critical for extracellular microfibril formation and is present in the extracellular matrix of cultured cells. *J Biol Chem*. 2006;281(24):16607-16614.
56. Aigner T, Hambach L, Söder S, Schlötzer-Schrehardt U, Pöschl E. The C5 domain of Col6a3 is cleaved off from the Col6 fibrils immediately after secretion. *Biochem Biophys Res Commun*. 2002;290(2):743-748.
57. Chen P, Cescon M, Bonaldo P. Collagen VI in cancer and its biological mechanisms. *Trends Mol Med*. 2013;19(7):410-417.
58. Veidal SS, Karsdal MA, Vassiliadis E, *et al.* MMP mediated degradation of type VI collagen is highly associated with liver fibrosis—identification and validation of a novel biochemical marker assay. *PLoS One*. 2011;6(9):e24753.
59. Fitzgerald J, Holden P, Hansen U. The expanded collagen VI family: new chains and new questions. *Connect Tissue Res*. 2013;54(6):345-350.
60. Baldock C, Sherratt MJ, Shuttleworth CA, Kielty CM. The supramolecular organization of collagen VI microfibrils. *J Mol Biol*. 2003;330(2):297-307.
61. Sabatelli P, Gara SK, Grumati P, *et al.* Expression of the collagen VI alpha5 and alpha6 chains in normal human skin and in skin of patients with collagen VI-related myopathies. *J Invest Dermatol*. 2011;131(1):99-107.
62. Bidanset DJ, Guidry C, Rosenberg LC, Choi HU, Timpl R, Hook M. Binding of the proteoglycan decorin to collagen type VI. *J Biol Chem*. 1992;267(8):5250-5256.
63. Kuo HJ, Maslen CL, Keene DR, Glanville RW. Type VI collagen anchors endothelial basement membranes by interacting with type IV collagen. *J Biol Chem*. 1997;272(42):26522-26529.
64. Stallcup WB, Dahlin K, Healy P. Interaction of the NG2 chondroitin sulfate proteoglycan with type VI collagen. *J Cell Biol*. 1990;111(6):3177-3188.
65. Carter WG. Transformation-dependent alterations in glycoproteins of the extracellular matrix of human fibroblasts. Characterization of GP250 and the collagen-like GP140. *J Biol Chem*. 1982;257(22):13805-13815.
66. Minamitani T, Ariga H, Matsumoto KI. Deficiency of tenascin-X causes a decrease in the level of expression of type VI collagen. *Exp Cell Res*. 2004;297(1):49-60.
67. Pfaff M, Aumailley M, Specks U, Knolle J, Zerwes HG, Timpl R. Integrin and Arg-Gly-Asp dependence of cell adhesion to the native and unfolded triple helix of collagen type VI. *Exp Cell Res*. 1993;206(1):167-176.
68. Bonaldo P, Russo V, Bucciotti F, Doliana R, Colombatti A. Structural and functional features of the α3 chain indicate a bridging role for chicken collagen VI in connective tissues. *Biochemistry*. 1990;29(5):1245-1254.
69. Dassah M, Almeida D, Hahn R, Bonaldo P, Worgall S, Hajjar KA. Annexin A2 mediates secretion of collagen VI, pulmonary elasticity and apoptosis of bronchial epithelial cells. *J Cell Sci*. 2014;127(Pt 4):828-844.
70. Sand JMB, Knox AJ, Lange P, *et al.* Accelerated extracellular matrix turnover during exacerbations of COPD. *Respir Res*. 2015;16(1):69.
71. Sand JMB, Leeming DJ, Byrjalsen I, *et al.* High levels of biomarkers of collagen remodeling are associated with increased mortality in COPD—results from the ECLIPSE study. *Respir Res*. 2016;17(1):125.
72. Leeming DJ, Byrjalsen I, Sand JMB, *et al.* Biomarkers of collagen turnover are related to annual change in FEV1 in patients with chronic obstructive pulmonary disease within the ECLIPSE study. *BMC Pulm Med*. 2017;17(1):164.
73. Beecher N, Roseman AM, Jowitt TA, *et al.* Collagen VI, conformation of A-domain arrays and microfibril architecture. *J Biol Chem*. 2011;286(46):40266-40275.
74. Lamandé SR, Mörgelin M, Selan C, Jöbsis GJ, Baas F, Bateman JF. Kinked collagen VI tetramers and reduced microfibril formation as a result of bethlem myopathy and introduced triple helical glycine mutations. *J Biol Chem*. 2002;277(3):1949-1956.
75. Ball S, Bella J, Kielty C, Shuttleworth A. Structural basis of type VI collagen dimer formation. *J Biol Chem*. 2003;278(17):15326-15332.
76. Furthmayr H, Wiedemann H, Timpl R, Odermatt E, Engel J. Electron-microscopical approach to a structural model of intima collagen. *Biochem J*. 1983;211(2):303-311.
77. Knupp C, Squire JM. A new twist in the collagen story–the type VI segmented supercoil. *EMBO J*. 2001;20(3):372-376.
78. Becker A-KA, Mikolajek H, Paulsson M, Wagener R, Werner JM. A structure of a collagen VI VWA domain displays N and C termini at opposite sides of the protein. *Structure*. 2014;22(2):199-208.
79. Specks U, Mayer U, Nischt R, *et al.* Structure of recombinant N-terminal globule of type VI collagen alpha 3 chain and its binding to heparin and hyaluronan. *EMBO J*. 1992;11(12):4281-4290.
80. Solomon-Degefa H, Gebauer JM, Jeffries CM, *et al.* Structure of a collagen VI α3 chain VWA domain array: adaptability and functional implications of myopathy causing mutations. *J Biol Chem*. 2020;295(36):12755-12771.
81. Maaß T, Bayley CP, Mörgelin M, *et al.* Heterogeneity of collagen VI microfibrils: STRUCTURAL ANALYSIS OF NON-COLLAGENOUS REGIONS. *J Biol Chem*. 2016;291(10):5247-5258.
82. Brown JC, Golbik R, Mann K, Timpl R. Structure and stability of the triple-helical domains of human collagen XIV. *Matrix Biol*. 1994;14(4):287-295.
83. Wiberg C, Klatt AR, Wagener R, *et al.* Complexes of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. *J Biol Chem*. 2003;278(39):37698-37704.
84. Rand JH, Patel ND, Schwartz E, Zhou SL, Potter BJ. 150-kD von willebrand factor binding protein extracted from human vascular subendothelium is type VI collagen. *J Clin Invest*. 1991;88(1):253-259.

85. Ross JM, McIntire LV, Moake JL, Rand JH. Platelet adhesion and aggregation on human type VI collagen surfaces under physiological flow conditions. *Blood*. 1995;85(7):1826-1835.
86. Mazzucato M, Spessotto P, Masotti A, *et al*. Identification of domains responsible for von willebrand factor type VI collagen interaction mediating platelet adhesion under high flow. *J Biol Chem*. 1999;274(5):3033-3041.
87. Gara SK, Grumati P, Squarzoni S, *et al*. Differential and restricted expression of novel collagen VI chains in mouse. *Matrix Biol*. 2011;30(4):248-257.
88. Scherer PE, Gupta OT. Endotrophin: nominated for best supporting actor in the fibro-inflammatory saga. *EBioMedicine*. 2021;69:103447.
89. Jöbsis GJ, Keizers H, Vreijling JP, *et al*. Type VI collagen mutations in bethlem myopathy, an autosomal dominant myopathy with contractures. *Nat Genet*. 1996;14(1):113-115.
90. Camacho Vanegas O, Bertini E, Zhang RZ, *et al*. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. *Proc Natl Acad Sci U S A*. 2001;98(13):7516-7521.
91. Merlini L, Martoni E, Grumati P, *et al*. Autosomal recessive myosclerosis myopathy is a collagen VI disorder. *Neurology*. 2008;71(16):1245-1253.
92. Foley AR, Mohassel P, Donkervoort S, Bolduc V, Bönnemann CG. Collagen VI-Related Dystrophies. 2004 Jun 25 [updated 2021 Mar 11]. In: Adam MP, Feldman J, Mirzaa GM, *et al*. eds. *GeneReviews®* [Internet]. University of Washington Seattle; 1993-2023.
93. Schessl J, Goemans NM, Magold AI, *et al*. Predominant fiber atrophy and fiber type disproportion in early ullrich disease. *Muscle Nerve*. 2008;38(3):1184-1191.
94. Jimenez-Mallebrera C, Maioli MA, Kim J, *et al*. A comparative analysis of collagen VI production in muscle, skin and fibroblasts from 14 Ullrich congenital muscular dystrophy patients with dominant and recessive COL6A mutations. *Neuromuscul Disord*. 2006;16(9-10):571-582.
95. Almici E, Chiappini V, López-Márquez A, *et al*. Personalized in vitro extracellular matrix models of collagen VI-related muscular dystrophies. *Front Bioeng Biotechnol*. 2022;10:851825.
96. Pan T-C, Zhang R-Z, Arita M, *et al*. A mouse model for dominant collagen VI disorders: heterozygous deletion of Col6a3 exon 16. *J Biol Chem*. 2014;289(15):10293-10307.
97. Meehan TF, Conte N, West DB, *et al*. Disease model discovery from 3,328 gene knockouts by the international mouse phenotyping consortium. *Nat Genet*. 2017;49(8):1231-1238.
98. Steffen F, Bilzer T, Brands J, *et al*. A nonsense variant in COL6A1 in landseer dogs with muscular dystrophy. *G3 (Bethesda)*. 2015;5(12):2611-2617.
99. Marioni-Henry K, Haworth P, Scott H, Witte P, Guo LT, Shelton GD. Sarcolemmal specific collagen VI deficient myopathy in a labrador retriever. *J Vet Intern Med*. 2014;28(1):243-249.
100. Khan T, Muise ES, Iyengar P, *et al*. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Mol Cell Biol*. 2009;29(6):1575-1591.
101. Lackey DE, Burk DH, Ali MR, *et al*. Contributions of adipose tissue architectural and tensile properties toward defining healthy and unhealthy obesity. *Am J Physiol Endocrinol Metab*. 2014;306(3):E233-E246.
102. Martinelli-Boneschi F, Colombi M, Castori M, *et al*. COL6A5 variants in familial neuropathic chronic itch. *Brain*. 2017;140(3):555-567.
103. de Sousa Dias M, Hernan I, Delás B, *et al*. New COL6A6 variant detected by whole-exome sequencing is linked to break points in intron 4 and 3'-UTR, deleting exon 5 of RHO, and causing adRP. *Mol Vis*. 2015;21:857-870.
104. Vaclavik V, Tiab L, Sun YJ, *et al*. New COL6A6 variant causes autosomal dominant retinitis pigmentosa in a four-generation family. *Invest Ophthalmol Vis Sci*. 2022;63(3):23.
105. Heo WI, Park KY, Jin T, *et al*. Identification of novel candidate variants including COL6A6 polymorphisms in early-onset atopic dermatitis using whole-exome sequencing. *BMC Med Genet*. 2017;18(1):8.
106. Willumsen N, Bager C, Karsdal MA. Matrix metalloprotease generated fragments of type VI collagen have serum biomarker potential in cancer—a proof of concept study. *Transl Oncol*. 2019;12(5):693-698.
107. Lindholm M, Godskesen LE, Manon-Jensen T, *et al*. Endotrophin and C6Ma3, serological biomarkers of type VI collagen remodelling, reflect endoscopic and clinical disease activity in IBD. *Sci Rep*. 2021;11(1):14713.
108. Pan TC, Zhang RZ, Pericak-Vance MA, *et al*. Missense mutation in a von willebrand factor type A domain of the alpha 3(VI) collagen gene (COL6A3) in a family with bethlem myopathy. *Hum Mol Genet*. 1998;7(5):807-812.
109. Baker NL, Mörgelin M, Peat R, *et al*. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. *Hum Mol Genet*. 2005;14(2):279-293.
110. Marakhonov AV, Tabakov VY, Zernov NV, Dadali EL, Sharkova IV, Skoblov MY. Two novel COL6A3 mutations disrupt extracellular matrix formation and lead to myopathy from Ullrich congenital muscular dystrophy and Bethlem myopathy spectrum. *Gene*. 2018;672:165-171.
111. Fitzgerald J, Mörgelin M, Selan C, *et al*. The N-terminal N5 subdomain of the alpha 3(VI) chain is important for collagen VI microfibril formation. *J Biol Chem*. 2001;276(1):187-193.
112. Ho C-M, Chang T-H, Yen T-L, Hong K-J, Huang S-H. Collagen type VI regulates the CDK4/6-p-Rb signaling pathway and promotes ovarian cancer invasiveness, stemness, and metastasis. *Am J Cancer Res*. 2021;11:668-690.
113. Huang Y, Li G, Wang K, *et al*. Collagen type VI alpha 3 chain promotes epithelial-mesenchymal transition in bladder cancer cells via transforming growth factor β (Tgf-β)/smad pathway. *Med Sci Monit*. 2018;24:5346-5354.
114. Zhong T, Jiang Z, Wang X, *et al*. Key genes associated with prognosis and metastasis of clear cell renal cell carcinoma. *PeerJ*. 2022;9:e12493.
115. Cattaruzza S, Nicolosi PA, Braghetta P, *et al*. NG2/CSPG4-collagen type VI interplays putatively involved in the microenvironmental control of tumour engraftment and local expansion. *J Mol Cell Biol*. 2013;5(3):176-193.
116. Angenendt L, Mikesch JH, Görlich D, *et al*. Stromal collagen type VI associates with features of malignancy and predicts poor prognosis in salivary gland cancer. *Cell Oncol (Dordr)*. 2018;41(5):517-525.
117. Owusu-Ansah KG, Song G, Chen R, *et al*. COL6A1 promotes metastasis and predicts poor prognosis in patients with pancreatic cancer. *Int J Oncol*. 2019;55(2):391-404.
118. Staunstrup LM, Bager CL, Frederiksen P, *et al*. Endotrophin is associated with chronic multimorbidity and all-cause mortality in a cohort of elderly women. *EBioMedicine*. 2021;68:103391.
119. Doane KJ, Yang G, Birk DE. Corneal cell-matrix interactions: type VI collagen promotes adhesion and spreading of corneal fibroblasts. *Exp Cell research*. 1992;200(2):490-499.
120. Tulla M, Pentikäinen OT, Viitasalo T, *et al*. Selective binding of collagen subtypes by integrin alpha 1I, alpha 2I, and alpha 10I domains. *J Biol Chem*. 2001;276(51):48206-48212.
121. Doane KJ, Howell SJ, Birk DE. Identification and functional characterization of two type VI collagen receptors, alpha 3 beta 1 integrin and NG2, during avian corneal stromal development. *Invest Ophthalmol Vis Sci*. 1998;39(2):263-275.
122. Nanda A, Carson-Walter EB, Seaman S, *et al*. TEM8 interacts with the cleaved C5 domain of collagen alpha 3(VI). *Cancer Res*. 2004;64(3):817-820.
123. Bürgi J, Kunz B, Abrami L, *et al*. CMG2/ANTXR2 regulates extracellular collagen VI which accumulates in hyaline fibromatosis syndrome. *Nat Commun*. 2017;8(1):15861.
124. Przyklenk M, Heumüller SE, Freiburg C, *et al*. Lack of evidence for a role of anthrax toxin receptors as surface receptors for

collagen VI and for its cleaved-off C5 domain/endotrophin. *iScience*. 2022;25(10):105116.
125. Christensen TD, Jensen C, Larsen O, *et al.* Blood-based tumor fibrosis markers as diagnostic and prognostic biomarkers in patients with biliary tract cancer. *Int J Cancer*. 2023;152(5):1036-1049.
126. Nielsen SH, Mygind ND, Michelsen MM, *et al.* Accelerated collagen turnover in women with angina pectoris without obstructive coronary artery disease: an iPOWER substudy. *Eur J Prev Cardiol*. 2018;25(7):719-727.
127. Sparding N, Genovese F, Kring Rasmussen DG, *et al.* Endotrophin, a collagen type VI-derived matrikine, reflects the degree of renal fibrosis in patients with IgA nephropathy and in patients with ANCA-associated vasculitis. *Nephrol Dial Transplant*. 2022;37(6):1099-1108.
128. Sparding N, Guldager Kring Rasmussen D, Genovese F, *et al.* Circulating levels of endotrophin are prognostic for long-term mortality after AKI. *Kidney360*. 2022;3(5):809-817.
129. Stribos EGD, Nielsen SH, Brix S, *et al.* Non-invasive quantification of collagen turnover in renal transplant recipients. *PLoS One*. 2017;12(4):e0175898.
130. Yepes-Calderón M, Sotomayor CG, Rasmussen DGK, *et al.* Biopsy-controlled non-invasive quantification of collagen type VI in kidney transplant recipients: a post-hoc analysis of the MECANO trial. *J Clin Med*. 2020;9(10):3216.
131. Genovese F, Akhgar A, Lim SS, *et al.* Collagen type III and VI remodeling biomarkers are associated with kidney fibrosis in lupus nephritis. *Kidney360*. 2021;2(9):1473-1481.
132. Luo Y, Oseini A, Gagnon R, *et al.* An evaluation of the collagen fragments related to fibrogenesis and fibrolysis in nonalcoholic steatohepatitis. *Sci Rep*. 2018;8(1):12414.
133. Armandi A, Rosso C, Nicolosi A, *et al.* Crosstalk between irisin levels, liver fibrogenesis and liver damage in non-obese, non-diabetic individuals with non-alcoholic fatty liver disease. *J Clin Med*. 2022;11(3):635.
134. Wiese S, Voiosu A, Hove JD, *et al.* Fibrogenesis and inflammation contribute to the pathogenesis of cirrhotic cardiomyopathy. *Aliment Pharmacol Ther*. 2020;52(2):340-350.
135. Sand JMB, Martinez G, Midjord A-K, Karsdal MA, Leeming DJ, Lange P. Characterization of serological neo-epitope biomarkers reflecting collagen remodeling in clinically stable chronic obstructive pulmonary disease. *Clin Biochem*. 2016;49(15):1144-1151.
136. Bihlet AR, Karsdal MA, Sand JMB, *et al.* Biomarkers of extracellular matrix turnover are associated with emphysema and eosinophilic-bronchitis in COPD. *Respir Res*. 2017;18(1):22.
137. Stolz D, Leeming DJ, Kristensen JHE, *et al.* Systemic biomarkers of collagen and elastin turnover are associated with clinically relevant outcomes in COPD. *Chest*. 2017;151(1):47-59.
138. Organ LA, Duggan AMR, Oballa E, *et al.* Biomarkers of collagen synthesis predict progression in the PROFILE idiopathic pulmonary fibrosis cohort. *Respir Res*. 2019;20(1):148.
139. Frimodt-Møller M, Hansen TW, Rasmussen DGK, *et al.* A marker of type VI collagen formation (PRO-C6) is associated with higher arterial stiffness in type 1 diabetes. *Acta Diabetol*. 2019;56(6):711-712.
140. Schett G, Loza MJ, Palanichamy A, *et al.* Collagen turnover biomarkers associate with active psoriatic arthritis and decrease with guselkumab treatment in a phase 3 clinical trial (DISCOVER-2). *Rheumatol Ther*. 2022;9(4):1017-1030.
141. Juhl P, Bay-Jensen A-C, Karsdal M, Siebuhr AS, Franchimont N, Chavez J. Serum biomarkers of collagen turnover as potential diagnostic tools in diffuse systemic sclerosis: a cross-sectional study. *PLoS One*. 2018;13(12):e0207324.
142. Kubo S, Siebuhr AS, Bay-Jensen AC, *et al.* Correlation between serological biomarkers of extracellular matrix turnover and lung fibrosis and pulmonary artery hypertension in patients with systemic sclerosis. *Int J Rheum Dis*. 2020;23(4):532-539.
143. Juhl P, Vinderslev Iversen L, Karlsmark T, *et al.* Association of metabolites reflecting type III and VI collagen formation with modified Rodnan skin score in systemic sclerosis–a cross-sectional study. *Biomarkers*. 2019;24(4):373-378.
144. Nissen NI, Johansen AZ, Chen IM, *et al.* Collagen biomarkers quantify fibroblast activity in vitro and predict survival in patients with pancreatic ductal adenocarcinoma. *Cancers (Basel)*. 2022;14(3):819.
145. Leeming DJ, Nielsen SH, Vongsuvanh R, *et al.* Endotrophin, a pro-peptide of type VI collagen, is a biomarker of survival in cirrhotic patients with hepatocellular carcinoma. *Hepat Oncol*. 2020;8(2):HEP32.
146. Nissen NI, Kehlet S, Boisen MK, *et al.* Prognostic value of blood-based fibrosis biomarkers in patients with metastatic colorectal cancer receiving chemotherapy and bevacizumab. *Sci Rep*. 2021;11(1):865.
147. Chirinos JA, Zhao L, Reese-Petersen AL, *et al.* Endotrophin, a collagen VI formation–derived peptide, in heart failure. *NEJM Evid*. 2022;1(10):10.1056/evidoa2200091.
148. Tepel M, Alkaff FF, Kremer D, *et al.* Pretransplant endotrophin predicts delayed graft function after kidney transplantation. *Sci Rep*. 2022;12(1):4079.
149. Kremer D, Alkaff FF, Post A, *et al.* Plasma endotrophin, reflecting tissue fibrosis, is associated with graft failure and mortality in KTR: results from two prospective cohort studies. *Nephrol Dialysis Transplant*. 2023;38(4):1041-1052.
150. Nielsen MJ, Genovese F, Lehmann J, *et al.* The collagen hormone endotrophin, a biomarker of type VI collagen formation, is associated with severe decompensation and predicts transplant-free survival in patients with trans-jugular intrahepatic portosystemic shunt. *J Hepatol*. 2019;70(1):e668.
151. Hoyer N, Jessen H, Prior TS, *et al.* High turnover of types III and VI collagen in progressive idiopathic pulmonary fibrosis. *Respirology*. 2021;26(6):582-589.
152. Pilemann-Lyberg S, Rasmussen DGK, Hansen TW, *et al.* Markers of collagen formation and degradation reflect renal function and predict adverse outcomes in patients with type 1 diabetes. *Diabetes care*. 2019;42(9):1760-1768.
153. Rasmussen DGK, Hansen TW, von Scholten BJ, *et al.* Higher collagen VI formation is associated with all-cause mortality in patients with type 2 diabetes and microalbuminuria. *Diabetes Care*. 2018;41(7):1493-1500.
154. Tougaard NH, Moller AL, Ronn PF, *et al.* Endotrophin as a marker of complications in a type 2 diabetes cohort. *Diabetes Care*. 2022;45(11):2746-2748.
155. Rasmussen DGK, Hansen MK, Blair J, *et al.* Endotrophin is a risk marker of complications in CANagliflozin cardioVascular assessment study (CANVAS): a randomized controlled trial. *Cardiovasc Diabetol*. 2022;21(1):261.
156. Guney G, Taskin MI, Baykan O, *et al.* Endotrophin as a novel marker in PCOS and its relation with other adipokines and metabolic parameters: a pilot study. *Ther Adv Endocrinol Metab*. 2021;12:20420188211049607.
157. Yoldemir SA, Arman Y, Akarsu M, Altun O, Ozcan M, Tukek T. Correlation of glycemic regulation and endotrophin in patients with type 2 diabetes; pilot study. *Diabetol Metab Syndr*. 2021;13(1):9.
158. Eruzun H, Toprak ID, Arman Y, *et al.* Serum endotrophin levels in patients with heart failure with reduced and mid-range ejection fraction. *Eur J Intern Med*. 2019;64:29-32.
159. Baykiz D, Demirtakan ZG, Govdeli EA, *et al.* Endotrophin and matrix metalloproteinase-2 levels in bicuspid aortic valve and hypertension associated aortopathy and their relationship with strain parameters of the ascending aorta. *Eur Rev Med Pharmacol Sci*. 2023;27(6):2358-2376.
160. Ezzati-Mobaser S, Malekpour-Dehkordi Z, Nourbakhsh M, *et al.* The up-regulation of markers of adipose tissue fibrosis by visfatin

in pre-adipocytes as well as obese children and adolescents. *Cytokine*. 2020;134:155193.
161. Mohamed AA, Nour AA, Mosbah NM, *et al.* Evaluation of circulating insulin-like growth factor-1, heart-type fatty acid-binding protein, and endotrophin levels as prognostic markers of COVID-19 infection severity. *Virol J*. 2023;20(1):94.
162. Hagstrom H, Bu D, Nasr P, *et al.* Serum levels of endotrophin are associated with nonalcoholic steatohepatitis. *Scand J Gastroenterol*. 2021;56(4):437-442.
163. Sun K, Li X, Scherer PE. Extracellular matrix (ECM) and fibrosis in adipose tissue: overview and perspectives. *Compr Physiol*. 2023;13(1):4387-4407.
164. Zhao Y, Gu X, Zhang N, Kolonin MG, An Z, Sun K. Divergent functions of endotrophin on different cell populations in adipose tissue. *Am J Physiol Endocrinol Metab*. 2016;311(6): E952-E963.
165. Karsdal MA, Henriksen K, Genovese F, *et al.* Serum endotrophin identifies optimal responders to PPARgamma agonists in type 2 diabetes. *Diabetologia*. 2017;60(1):50-59.
166. Kim M, Lee C, Seo DY, *et al.* The impact of endotrophin on the progression of chronic liver disease. *Exp Mol Med*. 2020;52(10):1766-1776.
167. An YA, Xiong W, Chen S, *et al.* Endotrophin neutralization through targeted antibody treatment protects from renal fibrosis in a podocyte ablation model. *Mol Metab*. 2023;69:101680.
168. Park J, Morley TS, Scherer PE. Inhibition of endotrophin, a cleavage product of collagen VI, confers cisplatin sensitivity to tumours. *EMBO Mol Med*. 2013;5(6):935-948.
169. Williams L, Layton T, Yang N, Feldmann M, Nanchahal J. Collagen VI as a driver and disease biomarker in human fibrosis. *FEBS J*. 2022;289(13):3603-3629.
170. Funcke JB, Scherer PE. Beyond adiponectin and leptin: adipose tissue-derived mediators of inter-organ communication. *J Lipid Res*. 2019;60(10):1648-1697.
171. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest*. 2011;121(6):2094-2101.
172. Sun K, Tordjman J, Clement K, Scherer PE. Fibrosis and adipose tissue dysfunction. *Cell Metab*. 2013;18(4):470-477.
173. Lin CT, Kang L. Adipose extracellular matrix remodelling in obesity and insulin resistance. *Biochem Pharmacol*. 2016;119:8-16.
174. Cinti S, Mitchell G, Barbatelli G, *et al.* Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res*. 2005;46(11):2347-2355.
175. Divoux A, Moutel S, Poitou C, *et al.* Mast cells in human adipose tissue: link with morbid obesity, inflammatory status, and diabetes. *J Clin Endocrinol Metab*. 2012;97(9):E1677-E1685.
176. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol*. 2011;29(1):415-445.
177. Kim M, Neinast MD, Frank AP, *et al.* ERalpha upregulates Phd3 to ameliorate HIF-1 induced fibrosis and inflammation in adipose tissue. *Mol Metab*. 2014;3(6):642-651.
178. Lee YS, Kim JW, Osborne O, *et al.* Increased adipocyte O2 consumption triggers HIF-1alpha, causing inflammation and insulin resistance in obesity. *Cell*. 2014;157(6):1339-1352.
179. Karsdal MA, Henriksen K, Genovese F, *et al.* Serum endotrophin identifies optimal responders to PPARγ agonists in type 2 diabetes. *Diabetologia*. 2017;60(1):50-59.