Type VI Collagen: Biological Functions and Its Neo-epitope as Hepatic Fibrosis Biomarker

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Type VI Collagen: Biological Functions and Its Neo-epitope as Hepatic Fibrosis Biomarker

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Abstract

Collagen VI forms a filamentous network in connective tissue, linking matrix macromolecules and cells. It is composed of three genetically distinct chains, α1(VI), α2(VI), and α3(VI), with a globular domain at each end. Additionally, three novel chains α4, α5, and α6 have been identified. Intracellularly, collagen VI monomers dimerize and form tetramers, which are secreted and then associate into filaments extracellularly. Gene expression for collagen VI is regulated differently than collagen I or III. Collagen VI interacts with collagen V and fibronectin, contributing to the structural integrity of tissue scaffolds. Moreover, it mediates cell adhesion and promotes migration. Soluble collagen VI acts as a sensor for tissue damage, modulating mesenchymal cell proliferation and survival, matrix homeostasis, and wound healing. Collagen VI-deficient mouse models have been generated, which have been used to investigate collagen VI-related myopathies, mammary carcinogenesis, and skeletal muscle satellite cell homeostasis. Collagen VI expression is upregulated in fibrosis of the adipose tissue and liver. Elevated collagen VI in the circulation is considered an indicator of early alcoholic liver fibrosis. Hepatic stellate cells (HSCs) are likely the source of perisinusoidal collagen VI. Collagen VI immunostaining is enhanced in fibrotic foci, codistributing with collagens I, III, and V. The α2(VI) chain sequesters hepatic matrix metalloproteinase (MMP)-1, MMP-3, and MMP-8 and blocks enzyme activation, preventing fibrolysis. The collagen VI receptor on HSCs offers selective targets for antifibrotic agents. CO6-MMP, a collagen VI neo-epitope generated by the proteolytic actions of MMP-2 and MMP-9, serves as a noninvasive biomarker in experimental liver fibrogenesis.

Keywords

Type VI collagen • Filamentous collagen • Soluble collagen VI • Collagen VI assembly • Matrix metalloproteinase • Hepatic stellate cells • Immunohistochemistry • Liver fibrosis biomarker • Collagen VI neo-epitope • Protein fingerprint

List of Abbreviations

BM	Bethlem myopathy
CCl_4	Carbon tetrachloride
ECM	Extracellular matrix
ELISA	Enzyme-linked immunosorbent assay
HSA	Human serum albumin
HSC	Hepatic stellate cell
MMP	Matrix metalloproteinase
MMTV-PyMT	Mammary tumor virus-polyoma middle T antigen
TGF-β	Transforming growth factor-β

Key Facts of Liver Fibrosis

- Liver fibrosis is a chronic liver disease that results from wound healing in response to liver injury caused mainly by viral infections (hepatitis B and C), alcohol abuse, cholestasis, steatosis, toxins, and drugs. Cirrhosis is the end stage of liver fibrosis and is also a risk factor for developing hepatocellular carcinoma.
- Liver cirrhosis is a global health problem causing over one million deaths annually. In the USA, the prevalence of cirrhosis is estimated at 270 per 100,000 of population or approximately 675,000 patients. Cirrhosis is the 11th leading cause of death in the USA.
- Orthotopic liver transplantation is the only therapy that improves the survival rate in patients with cirrhosis, but the availability of liver transplants is limited by the shortage of donor livers. In the USA, there are 17,000 adults and children awaiting liver transplantation, and every year more than 1,500 people die waiting for a donor liver to be available. As an alternative, transplantation of fetal human liver stem cells or adult human liver stem/progenitor cells is being explored.
- Nevertheless, fibrosis and even cirrhosis can be reversed by the elimination of the underlying cause of liver disease. Thus, eradication of HBV or HCV has been shown to improve cirrhosis. Studies of animal models in reversibility have provided clues to underlying mechanisms.
- Liver biopsy is the gold standard for the clinical assessment of liver fibrosis; however, the biopsy is invasive with possible risks of injury to the patient. Moreover, the histological diagnosis is hampered by biopsy sample size and variations and a lack of consensus between pathologists.
- Currently, a single noninvasive marker for assessing the amount of fibrosis in a patient is unavailable. Very few molecules have been satisfactorily validated as fibrosis biomarkers. Combining measurement of several markers could help discriminate minimal fibrosis from advanced fibrosis, but this approach cannot entirely replace liver biopsy.

Definition of Words and Terms

Collagen	A macromolecule composed of three polypeptides arranged in helical chains. The collagen polypeptides contain two unique amino acids, hydroxyproline and hydroxylysine. To date, there are 28 types of collagen. Morphologically, types I, II, and III collagens occur as banded fibrils with the characteristic 64-nm periodicity and are called fibrillar collagens, while other collagens lack banding and are called non-fibrillar collagens. Collagen fibers are found in the

	connective tissue, providing support and tensile strength to the tissue.
Extracellular matrix (ECM)	Connective tissue consists of cells and tissue; the latter is known as ECM that surrounds the cells. It is composed of ground substances, fibers, and structural proteins. It is the medium through which nutrients and metabolites are transported between the blood circulation and organs.
Filaments	Threadlike protein molecules, ranging from 5–6 to 16 nm in diameter. The three types of filaments are actin, a thin filament; cytokeratin, an intermediate filament; and myosin, a thick filament. Filaments are found within the cells mainly for providing structural support and contraction.
Filamentous collagen	A type of collagen that exhibits the diameters of filaments and lacks the banding pattern as seen in fibrillar collagens. Type VI collagen is a filamentous collagen.
Immunohistochemistry	An immunological-based histochemical technique for detecting antigens (proteins) in cells or tissues using antibodies binding specifically to the antigens. Visualization of the antibody and antigen reaction in the tissue with the microscope (fluorescent or light) is achieved by labeling the antibody with a fluorescent molecule or an enzyme. Immunohistochemistry is widely used for diagnostic purposes as well as for research.
Liver fibrosis	Chronic liver disease characterized by excess deposition of ECM proteins particularly collagens in the extracellular space of the liver, namely, space of Disse, stroma of portal tracts, and wall of central veins.
Liver cirrhosis	End stage of liver fibrosis characterized by extensive scarring of the liver parenchyma causing obstruction to blood flow with complications including portal hypertension, jaundice, esophageal varices, ascites, caput medusae, and

	encephalopathy. The histological hallmark of cirrhosis is the formation of fibrotic nodules.
Liver fibrosis biomarkers	Molecules used for monitoring the development of fibrosis. These are related to liver function, extracellular matrix synthesis and degradation, or fibrogenic-related cytokines.
Matrix metalloproteinase (MMP)	Endopeptidase (proteolytic enzyme) capable of degrading extracellular matrix macromolecules in particular collagens and some other matrix proteins.
Neo-epitopes	Cleavage peptide fragments generated by the proteolytic action of an enzyme such as matrix metalloproteinase (MMP). The fragments are used as immunogen to generate antibodies that specifically react with the epitopes, which can be measured by enzyme-linked immunosorbent assay. Neo-epitopes are used in the development of noninvasive biochemical biomarkers for monitoring fibrosis development.

Introduction

The filamentous type VI collagen is present in most connective tissue matrices where it forms a flexible filamentous network, linking matrix macromolecules and cells. This chapter begins with an introduction to the nomenclature of type VI collagen and an overview of its molecular structure. The biosynthesis, assembly, degradation, and biological functions of collagen VI, as well as mouse models of collagen VI deficiency, are highlighted. This chapter also covers the role of soluble collagen VI as a stimulator of cell growth, promoter of cell survival, sensor molecule for tissue damage, modulator of connective tissue matrix homeostasis, mediator of mouse mammary tumorigenesis, and regulator of the self-renewal capacity of skeletal muscle satellite cells and muscle regeneration. In addition, the contribution of collagen VI to adipose tissue fibrosis is discussed. Finally, this chapter specifically reviews the involvement of collagen VI in liver fibrogenesis, the value of collagen VI as an indicator of early liver fibrosis, and the technological development of collagen VI neo-epitope as a noninvasive biomarker of liver fibrosis.

Nomenclature and Structure

Type VI collagen – designated by Furthmayr et al. (1983) – is classified as a non-fibrillar collagen, as opposed to interstitial fibrillar collagens I, II, and III. Along with type IV collagen of the basement membrane, collagen VI is grouped under the network-forming collagens (Knupp and Squire 2005). It is widely distributed in most connective tissue matrices (von der Mark et al. 1984; Keene et al. 1988; Marcelino and McDevitt 1995; Kuo et al. 1997). Chemically, the collagen VI molecule is a heterotrimeric collagenous glycoprotein made of three genetically distinct α-chains, α1(VI), α2(VI), and α3(VI). These chains differ in molecular mass: 140 kDa for α1 and α2 chains and 250 kDa for α3 chain (Weil et al. 1988). The monomer consists of two globular domains at the N- and C-terminals that are connected by a 105-nm-long triple helix (Chu et al. 1988; Keene et al. 1988; Baldock et al. 2003; Knupp and Squire et al 2005). Uniquely, the triple helical domains are extensively linked by intra- and interchain disulfide bonds that likely endow the collagen VI molecules with a high thermal stability and protease resistance. The cDNAs of the three constituent chains of human collagen VI have been cloned and a large portion of the amino acids has been sequenced (Chu et al. 1988). Of note, there are several Gly-Y-X triplet interruptions of the amino acid sequence that are thought to provide flexibility to the collagen VI molecules. This feature differs from the non-interrupted Gly-Y-X repeats in the fibrillar collagens that confer rigidity to the molecules and mechanical strength to the fibers. Another unique structural feature of collagen VI is that it contains the sequence Arg-Gly-Asp (RGD)-dependent cell attachment sites that are likely involved in interaction with specific cell receptors of the integrin family proteins. The genes for α1(VI) and α2(VI) chains are located on chromosome 21, while the α3(VI) gene is located on chromosome 2 (Weil et al. 1988). The major mRNA species encoding the chains of collagen VI have sizes of 4.2 kb (α1), 3.5 kb (α2), and 8.5 kb (α3).

More recently, three novel collagen VI genes (*COL6A4*, *COL6A5*, *and COL6A6* located at a single locus on human chromosome 3q22.1) that encode the α4(VI), α5(VI), and α6(VI) chains have been identified (Gara et al. 2008; Fitzgerald et al. 2008). These chains may substitute for the α3 chains, probably forming α1α2α4, α1α2α5, and α1α2α6 heterotrimers. Unlike the α1(V), α2(V), and α3(V) subunits, these collagen VI chains display a highly restricted tissue distribution pattern (Gara et al. 2011; Sabatelli et al. 2011), raising the possibility of the tissue-specific role for the chains in collagen VI assembly and function.

Synthesis, Assembly, and Secretion

The biosynthesis of type VI collagen was studied in cultured human fibroblasts (Engvall et al. 1986) and chick embryo fibroblasts (Colombatti et al. 1987) using [^{35}S]methionine metabolic labeling of cells. Two labeled polypeptides of 140 and 260 kDa were identified in the cell layer lysates, matrices, and media of the human fibroblast culture, while three polypeptides of 150 kDa, 140 kDa, and 260 kDa were

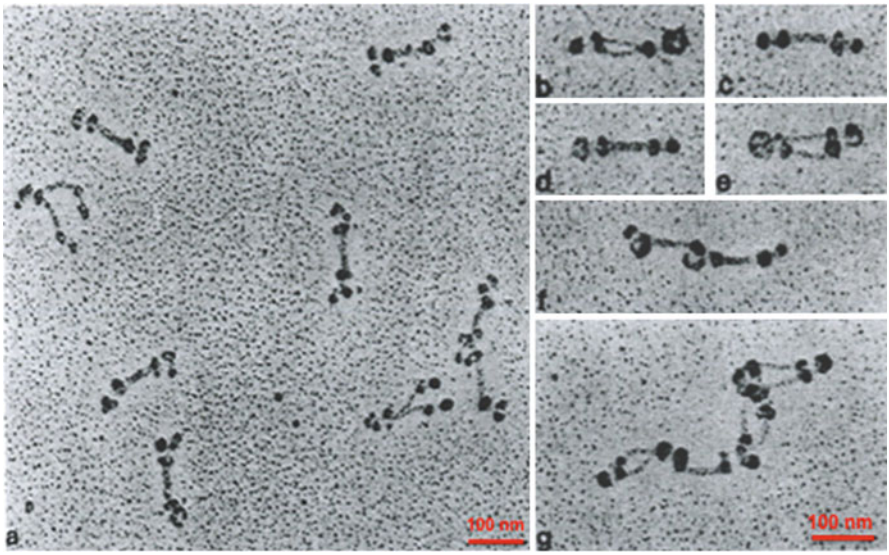

Fig. 1 Rotary-shadowed preparation of type Vl collagen. Electron microscopic images of type VI collagen after rotary shadowing. Collagen VI was isolated from the medium and or extracellular matrix extracts of cultured human lung fibroblasts. (**a**) shows a field of representative molecules in the medium. Most of the structures are ~150 nm long and composed of a central thin rod, 60 nm long, and two flanking globules. Extending from each of these globules is a thin strand with another globule at its outer end. These structures likely represent the tetramers secreted from the cells. (**b**–**g**) show selected collagen VI molecules isolated from the culture matrix extracts. They appear to be tetramers or oligomers of collagen VI. The structure in (**g**) probably represents four tetramers with end-to-end association forming a short beaded filament (©1986 Engvall et al. Journal of Cell Biology 102:703-710. doi:► 10.1083/jcb.102.3703)

identified in the chick embryo fibroblast culture media. These give rise, after pepsin digestion, to α1(VI), α2(VI), and α3(VI), respectively. Pulse-chase experiments in the embryo chick cells indicated that more than 60 % of the labeled type VI collagen was present in the culture medium after a 4-h chase duration. In both cell systems, the amounts of polypeptides deposited extracellularly were dependent on the presence of ascorbic acid and hydroxylation of prolines and lysines in the collagenous domains, as observed in fibrillar collagens (Engvall et al. 1986; Colombatti and Bonaldo 1987). But, unlike the fibrillar collagens, no proteolytic processing of the N- and C-terminal domains of the polypeptide chains occurred in collagen VI biosynthesis. Another study has shown that recombinant chicken α1(VI), α2(VI), and α3(VI) collagen chains can form monomers, dimers, and tetramers in NIH/3T3 cell lines. These molecules were secreted into the culture matrix, forming fibrillar meshwork (Colombatti et al. 1995). This model may offer a tool for the analysis of type VI collagen assembly and deposition.

The collagen VI polypeptide structures from the human fibroblast culture have been examined by electron microscopy after rotary shadowing (Engvall et al. 1986). The images in Fig. 1 revealed that the cell layer extracts contain monomers, dimers,

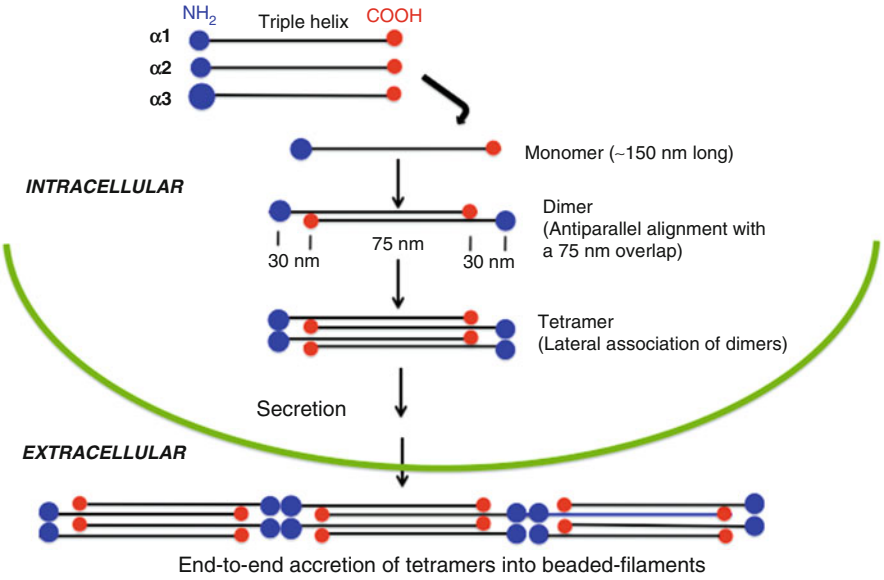

Fig. 2 Type Vl collagen assembly. This schema illustrates collagen VI formation in the cell and its secretion into the extracellular space. The assembly of monomers into dimers and then into tetramers occurs intracellularly. Following secretion, the tetramers associate end-to-end into beaded microfilaments. Note that the C-terminal globular domains contact the adjacent helices in the inner regions of the dimers that are stabilized by disulfide bonds (From Mak et al. 2014, Austin Biomarkers and Diagnosis 1(2): id1012, 2014)

and tetramers of collagen VI, the culture matrices contain both tetramers and oligomers, and the culture media contain predominantly tetramers. The distribution of these molecules in various compartments of the culture likely reflects the various stages of collagen VI assembly in vivo as described below. Based on the data of rotary shadowing electron microscopy, physical and biochemical analyses, the sequence of events of collagen VI's intracellular assembly has been established (Furthmayr et al. 1983; Engel et al. 1985; Engvall et al. 1986; Colombatti et al. 1987; Baldock et al. 2003; Knupp and Squire 2005). In this model, as illustrated in Fig. 2 (Mak et al. 2014), two triple helical monomers of 105 nm in length form a dimer in an antiparallel manner with a 75-nm overlap. Two dimers associate into a tetramer, with the chains stabilized by disulfide bonds (Furthmayr et al. 1983; Chu et al. 1988; Weil et al. 1988). Following secretion into the extracellular matrix (ECM), the tetramers assemble into filaments by end-to-end accretion, forming thin fibrils with prominent knobs at a periodicity of about 110 nm—so-called beaded filaments (Bruns et al. 1986; Engvall et al. 1986; Keene et al. 1988). The fibrils display a width of 6–10 nm; hence, collagen VI is described as filamentous (Amenta et al. 1988) or microfibrillar (Baldock et al. 2003).

Gene Expression

Collagen VI is abundantly expressed by cultured fibroblasts. Expression of collagen VI mRNA and its protein production were assessed in the human skin fibroblast culture, and the changes were compared with those of collagens I and III and fibronectin, which are known to be regulated in a coordinated fashion (Hatamochi et al. 1989). When the fibroblasts were grown at high densities or in a contracting collagen gel (conditions that reduce the proliferative capacity of the cells), a two- to threefold upregulation of the α1(VI), α2(VI), and α3(VI) mRNA chains was observed along with an increase in the corresponding proteins. Concurrently, there were only minimal changes for the mRNA levels of collagens I and III and fibronectin. Transformation of mouse 3T3 fibroblasts with tumor-promoting phorbol esters did not change the collagen VI mRNA level, but it did cause a three- to fivefold reduction in the mRNA levels of other matrix proteins. These data indicate that expression of α1(VI), α2(VI), and α3(VI) subunits is differently regulated in cultured fibroblasts than interstitial fibrillar collagens I and III and fibronectin. Moreover, in response to the pro-fibrotic mediator transforming growth factor-β (TGF-β), human skin fibroblasts selectively expressed the α3(VI) subunit mRNA (227 % of control), while the levels of α1(VI) and α2(VI) chains were not changed (Heckmann et al. 1992). Additionally, collagen VI protein was increased in the culture medium and cell layer extracts (170 % of control). Therefore, the regulation of α3(VI) gene expression by TGF-β is critical for the control of collagen VI synthesis and may affect the deposition of the collagen VI molecule in the ECM. These notions are compatible with a study that used recombinant type VI collagen (Colombatti et al. 1995).

Enzymatic Degradation

Collagen VI is made and deposited in the ECM where it becomes associated with fibrillar collagens I, III, and V, and together these collagens contribute to the structural integrity of the tissue scaffold of the ECM. However, the ECM is constantly remodeled even in normal conditions with a balanced production and degradation of the ECM proteins so as to achieve a balanced state of homeostasis for proper functioning of the tissue. An imbalanced remodeling of the ECM will cause pathological changes. Consequently, like other collagen types, collagen VI is subject to enzymatic digestion, contributing to ECM remodeling in normal and pathological conditions. Several matrix-degrading enzymes, namely, serine proteinases, matrix metalloproteinases (MMPs), and lysosomal enzymes, which act on collagen VI, are described below.

Intact collagen VI filaments are susceptible to degradation by serine proteinases, which are enzymes typically secreted by neutrophils and mast cells, but are resistant to both degradation by MMP-1, MMP-2, MMP-3, and MMP-9 that commonly

degrade other collagens, and to bacterial collagenase (Kielty et al. 1993). These properties of collagen VI suggest that it is a relatively stable molecule of the ECM, consistent with its role in matrix organization. The susceptibility of collagen VI to digestion by serine proteases suggests that collagen VI may be targeted for degradation primarily during physiological tissue turnover with inflammatory cell involvement and in early inflammatory lesions. However, Myint et al. (1996) demonstrated that activated MMP-2 cleaves collagen VI extracts from normal human cornea into lower molecular weight fragments. Additionally, recent data indicate that type VI collagen can be cleaved by MMP-2 and MMP-9 in vitro with the generation of neo-epitope peptide fragments that can be used as markers for assessing collagen VI turnover during hepatic fibrogenesis (Veidal et al. 2011). Furthermore, degradation of collagen VI has been reported for fibroblasts of periosteal explants via phagocytosis and subsequent digestion by lysosomal enzymes (Everts et al. 1995).

Biological Functions

Interaction with ECM Components

In most connective tissue matrices, collagen VI forms a flexible, branching filamentous network that surrounds the fibers of the major fibrillar collagens I, II and III, and V; hence, collagen VI is sometimes called fibril-associated collagen. It anchors nerves, blood vessels, and mesenchymal cells into place, partly through interconnections with collagen IV in endothelial cell basement membranes (Amenta et al. 1988; Kenne et al.1988; Kuo et al. 1997). More notably, it connects the fibrils of fibronectin in the ECM and binds matrix components, including hyaluronan, decorin, syndecan, von Willebrand factor, MMPs, and growth factors (Mak et al. 2014). Hence, collagen VI has been called a connecting protein, linking components of the ECM (Amenta et al. 1988; Keene et al. 1988), as schematized in Fig. 3. Strikingly, in the skin dermis, collagen VI filaments (marked by immunogold particles) could be seen interweaving with collagen V fibrils that in turn link other fibrillar collagens, elastic fibers, and proteoglycans, forming a network resembling wickerwork (Kobayasi and Karismark 2006). It is conceivable that these ECM structural proteins serve to provide support for the collagenous scaffold of the dermal tissue matrix.

Interaction with Fibronectin

Fibronectin is a multifunctional matrix glycoprotein with multiple domains that plays an important role in the interaction between cells and the surrounding ECM (Schuppan 1990). Electron microscopic study of replicas of whole-mounted cultured cells and matrix revealed that the filaments of collagen VI interconnect with the fibrils of fibronectin at some discrete sites (Sabatelli et al. 2001). The interaction is thought to render the three-dimensional configuration of the fibronectin fibrils. This

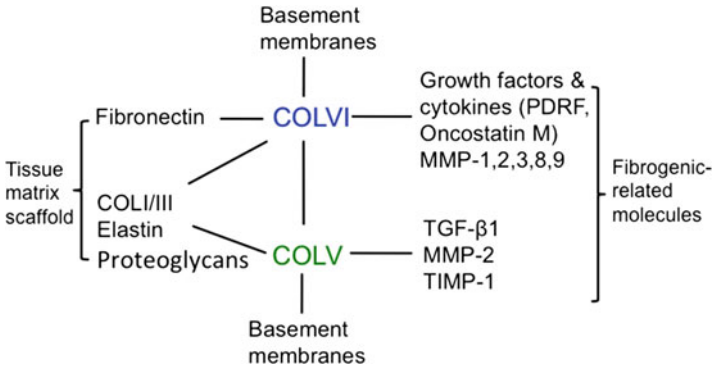

Fig. 3 Overview of type Vl collagen and matrix molecule interactions. Collagen VI (COLVI) interacts with collagen V (COLV), and in turn, both collagens are linked to matrix fibrillar collagens (COLI and III) and structural proteins, contributing to tissue matrix scaffold. Collagen VI as well as collagen V serves as reservoir for fibrogenic-related molecules, thus regulating their availability for fibrogenesis and fibrolysis. Both collagens VI and V have contacts with basement membranes, but are not integral basement membrane components. Collagen VI, but not collagen V, shows structural connection with fibronectin, a prominent matrix macromolecule. *PDGF* platelet-derived growth factor, *TIMP-1* tissue inhibitor of metalloproteinase-1

notion is corroborated by a study that used cultured fibroblasts obtained from *Col6a1* null mutant mice that lack the assembly of collagen VI and the capacity to secrete collagen VI into the ECM (Bonaldo et al. 1998). Consequently, the absence of collagen VI in the matrix of cultured fibroblasts resulted in a loss of the three-dimensional organization of the fibronectin fibrils. This effect could compromise various cellular functions. Additionally, patients affected by Bethlem myopathy (BM) present with an abnormal organization of fibronectin, possibly due to a drastically reduced secretion of collagen VI by fibroblasts. Thus, immunofluorescent labeling of collagen VI in skin fibroblast cultures derived from BM patients has been considered a useful addition to current diagnostic services for BM (Hicks et al. 2008).

Stimulation of Cell Growth

Soluble collagen VI, which is the pepsin-solubilized triple-helical core fragment of the native collagen VI, is released from the filamentous collagen VI in response to tissue damage and inflammation (Atkinson et al. 1996; Rühl et al. 1999a, b; Schuppan et al. 2001). In contrast to other collagens, soluble collagen VI stimulates proliferation of normal 3T3 fibroblasts and transformed fibrosarcoma cells in culture in the absence of growth factors (Atkinson et al. 1996). The cell growth effect of collagen VI is mediated by signal transduction cascades that involve induction of tyrosine phosphorylation of proteins, including paxillin, focal adhesion kinase, and the mitogen-activated protein kinase erk2 (Rühl et al. 1999a). Furthermore, these

signaling cascades appear to be independent of the integrin receptor protein α2ß, which mediates cell adhesion. The signaling transduction appears to require an aggregation of the collagen VI receptors or occupancy of the receptors by the native helical structure of collagen VI; interestingly, the effects on cell growth can be inhibited by single chains of collagen VI—prepared from the native collagen VI by reduction and alkylation with methylene imine (Rühl et al. 1999a; Schuppan et al. 2001).

Promotion of Cell Survival

Soluble collagen VI promotes survival of fibroblasts cultured in a serum-free medium through an anti-apoptotic mechanism involving downregulation of the proapoptotic Bax and upregulation of cyclins A, B, and D1 (Rühl et al. 1999b), whereas collagen I tested under the same experimental conditions had no anti-apoptotic action. The pro-survival action of collagen VI has also been seen in hepatic stellate cells (HSCs), the principal fibrogenic cells of the liver (Ruehl et al. 1999). These events are mediated, in part, by the activity of the transmembrane receptor NG2/chondroitin sulfate proteoglycan (Schuppan et al. 2001), which binds collagen VI (Tillet et al. 1997). This cellular interaction was examined by electron microscopy after rotary shadowing of a mixture of native NG2 and collagen VI, which revealed an alignment of collagen VI tetramers with the central region between the two N- and C-terminal globular regions of NG2. Furthermore, binding of collagen VI to the integrins α1β1and α2β1 facilitates cell adhesion, spreading, and migration of smooth muscle cells and corneal fibroblasts, as well as invasion of various tumor cell lines in primary culture (Howell and Doane 1998).

Sensor for Tissue Damage and Modulator of Matrix Homeostasis

While the filamentous collagen VI is important in maintaining the integrity of ECM, the soluble form of collagen VI has been proposed as a sensor molecule for tissue damage, stimulating surrounding mesenchymal cell growth and promoting cell survival and wound healing. Collagen VI, along with collagens I, III, IV, and V, serves as a reservoir for cell receptors, platelet-derived growth factor, oncostatin M, and MMP-1, MMP-3, MMP-8, MMP-2, and MMP-9 (Somasundaram and Schuppan 1996; Somasundaram et al. 2002; Freise et al. 2009) and therefore regulates their availability and activity in normal tissue turnover, in wound healing, and in the disease. In response to needs, growth factors are released and act on nearby fibrogenic cells in the matrix, initiating cell proliferation and mediating fibrogenic activity, while MMPs act on their collagen and protein substrates, facilitating tissue turnover. For these reasons, type VI collagen is regarded as a key modulator of matrix homeostasis.

Animal Models of Collagen VI Deficiency

Murine models of collagen VI deficiency have been described, namely, *Col6a1* (Donaldo et al. 1998), *Col6a3* (Pan et al. 2013), *and* $Col6a3^{+/d16}$ (heterozygous exon 16 deletion) (Pan et al. 2014). These animal models have been employed to investigate the molecular pathogenesis of collagen VI-related congenital Bethlem and Ullrich myopathies and skeletal muscle satellite cell homeostasis. Additionally, the collagen VI knockout ($Col6a1^{-/-}$) mice—in the background of the mammary tumor virus-polyoma middle T antigen (MMTV-PyMT) (Guy et al. 1992; Iyengar et al. 2005; Park and Scherer 2012)—have been used as a mammary cancer model.

Specialized Roles

Mammary Gland Tumorigenesis

Expression of collagen VI is upregulated during the progression of murine mammary tumors. Studies using $Col6a1^{-/-}$ mice (MMTV-PyMT mice) (Guy et al. 1992; Iyengar et al. 2005) have provided evidence indicating that adipocyte-derived soluble collagen VI exerts a stimulatory effect on the hyperplasia of mammary ductal epithelial cells, leading to primary tumor growth at the early stage of mammary tumorigenesis (Iyengar et al. 2005). Additionally, the lack of collagen VI in the knockout mice promotes apoptotic cell death of mammary epithelial cells, thereby reducing the likelihood of tumor expansion (Khan et al. 2009). Moreover, the carboxy-terminal fragment of collagen α3(VI) chain, a proteolytic product of the full-length molecule, was found to exert pro-mitogenic and pro-survival actions in part by signaling through the collagen VI binding proteoglycan NG2 receptor on the surface of malignant ductal epithelial cells (Iyengar et al. 2005; Khan et al. 2009). The action leads to the activation of Akt and β-catenin signaling pathways, resulting in the mitogenic response. Therefore, collagen VI secreted by adipocytes, acting as a paracrine factor, appears to mediate a critical interaction between adipocytes and tumor cells in the tumor-stroma microenvironment. In line with these data, Park and Scherer (2012) showed that endotrophin, the C-terminal cleavage product of the α3(VI) chain derived from adipose tissue, serves as a major mediator of collagen VI-stimulated mammary tumorigenesis by augmenting both mammary tumor growth and metastasis in PyMT/endotrophin mice. These effects are associated with the induction of adipose tissue fibrosis, angiogenesis, inflammation, and epithelial-mesenchymal transition of tumor cells, which are mediated in part through the upregulated signaling pathway of TGF-β, a pro-fibrotic factor.

Skeletal Muscle Regeneration

As an ECM protein of the skeletal muscle, collagen VI is a critical component of the satellite cell niche (Urciuolo et al. 2013). Deficiency of collagen VI in the skeletal muscle of mice is associated with muscular disorder resembling BM (Bonaldo et al. 1998). Investigation in collagen VI*a*1 null mice has shown that the lack of collagen VI causes impaired muscle regeneration accompanied by reduced capability of satellite cell to undergo self-renewal after injury to the skeletal muscle. When collagen VI is reinstated in vivo by grafting wild-type fibroblasts, the muscle stiffness associated with *Col6a1*$^{-/-}$ mice is ameliorated, and the satellite cell defects in self-renewal are corrected. Thus, it was proposed that collagen VI plays a regulatory role for satellite cell homeostasis.

Adipose Tissue Fibrosis

Collagen VI is abundantly produced and secreted by adipocytes (Iyengar et al. 2005; Scherer et al. 1998; Pasarica et al. 2009). In fact, adipose tissue is the single most abundant source of collagen VI systemically (Scherer et al. 1998). In adipose tissue, collagen VI forms an integral component of the extracellular scaffold for adipocytes and has a fibrogenic role in the development of obesity. Fibrosis of adipose tissue increases adipose tissue rigidity and constraints adipocyte expansion, thereby contributing to metabolic dysfunction. Conversely, the absence of collagen VI associated with collagen VI knockout in ob/ob mice appears to cause an unlimited expansion of individual adipocytes, resulting in metabolic benefits with a substantial improvement of body energy homeostasis (Khan et al. 2009). Clinically, obese human adipose tissue exhibits large areas of fibrosis containing increased deposition of collagen VI coincident with enhanced gene expression of α3(VI) (Pasarica et al. 2009) or α1(VI) (Spencer et al. 2010). Additionally, the fibrotic change correlates with increased inflammatory infiltrate of activated pro-fibrotic macrophages, body mass index (Pasarica et al. 2009), and insulin resistance (Spencer et al. 2010). However, in another study of human subjects, McCulloch et al. (2015) found that α3(VI) gene expression is not increased in obesity and does not correlate with impaired metabolic parameters or respond to variations in insulin. Also, these findings are at variance with the previous observations in obese, diabetic (ob/ob) mice (Khan et al. 2009). Instead, it was found that leptin, a pro-fibrotic and pro-inflammatory cytokine, downregulates *COL6A3* expression, suggesting that the cytokine can regulate ECM composition of adipose tissue.

In addition to displaying tumor-promoting effects (Park and Scherer 2012) as discussed above, endotrophin stimulates adipose tissue fibrosis. In endotrophin-overexpressing transgenic mice that were fed a high-fat diet, endotrophin was found to exert a local effect on the histogenesis of fibrosis in adipose tissue, leading to a systemic elevation of pro-inflammatory cytokines and insulin resistance in many other tissues (Sun et al. 2014). Blocking endotrophin with a neutralizing antibody reduces these adverse effects, emphasizing that endotrophin is a potential therapeutic target.

Hepatic Fibrosis

Immunohistochemistry

Immunohistological studies of the human liver revealed that type VI collagen is present in the liver lobules, stroma of portal tracts, wall of intralobular veins and Glisson's capsule (Griffiths et al. 1992; Loreal et al. 1992). Within the lobules, collagen VI immunostaining showed two distribution patterns: either uniform in the perisinusoidal space of Disse (Loreal et al. 1992) or stronger in the centrilobular and mid-lobular areas compared to the periportal area (Griffiths et al. 1992). By light immunohistochemistry, collagen VI immunoreactivity was detected in perisinusoidal HSCs (Griffiths et al. 1992), and by immunoelectron microscopy, the immunostain was localized to the HSC endoplasmic reticulum (Loreal et al. 1992), thereby disclosing the cellular source of collagen VI. Figure 4 illustrates positive collagen VI staining of human HSCs (Mak et al. 2014). In the space of Disse, amorphous or microfibrillar materials containing collagen VI immunostain were observed around and between banded fibrils, suggesting that this collagen interconnects collagens I and/or III fibers (Griffiths et al. 1992; Loreal et al. 1992). It might be presumed that collagen VI determines the organization of the fibrillar collagens in fibrogenesis of space of Disse.

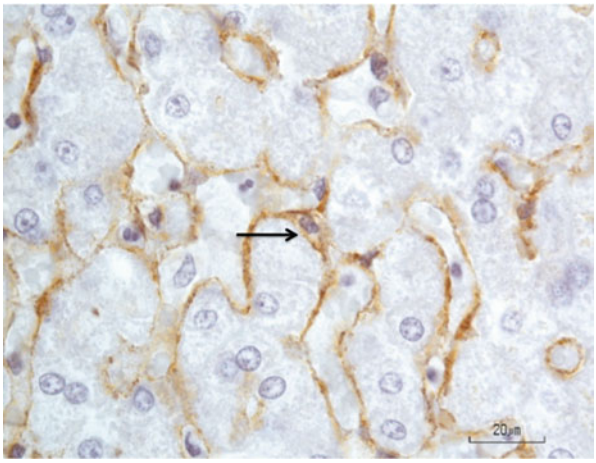

Fig. 4 Human hepatic stellate cells. Immunoperoxidase staining of human hepatic stellate cells for collagen VI. Liver tissue was fixed in formalin and embedded in paraffin. For immunohistochemistry, formalin-fixed and deparaffinized liver section was treated sequentially with a rabbit polyclonal collagen VI antibody (Novus Biologicals, Littleton, CO), anti-rabbit polymer-horseradish peroxidase (Dako Carpinteria, CA), and the chromogen diaminobenzidine tetrahydrochloride to yield a brown reaction product, with buffer washes between steps. Nuclei were counterstained with hematoxylin. The *arrow* points to a positively stained perisinusoidal stellate cell. The immune deposits (*brown*) of collagen VI could be seen in the cell body and its cell process along the sinusoidal border. The unstained clear space in the cytoplasm represents lipid droplets, characteristic of hepatic stellate cells (From Mak et al. 2014, Austin Biomarkers and Diagnosis 1(2): id1012, 2014)

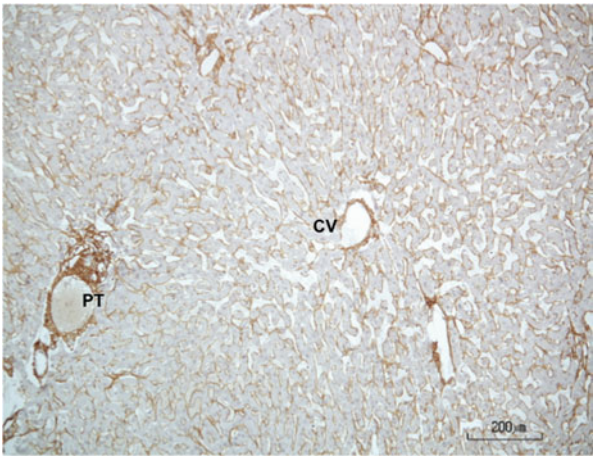

Fig. 5 Lobular distribution of type Vl collagen in the liver. Immunoperoxidase staining showing distribution of collagen VI in the liver lobule of human cadaver. The tissue from embalmed elderly cadavers was processed for paraffin embedment as previously described (Mak et al. 2012), and immunoperoxidase staining was performed as indicated in Fig. 4. This liver with a nearly normal histology shows a uniform distribution of collagen VI immunostain (*brown*) along the sinusoidal lining of the liver lobule. Immunostaining is also seen in the stroma of portal tracts and rims of central veins. Hematoxylin counterstained. *PT* portal tract, *CV* central vein

In alcoholic fibrosis and cirrhosis, intense collagen VI staining was present in the developing fibrous septa and bridging septa of cirrhotic nodules (Loreal et al. 1992). In biliary cirrhosis, strong staining for collagen VI was noted around proliferating bile ductules within the developing fibrous septa or the established septa of the cirrhotic liver (Griffiths et al. 1992). Little data, however, is available on the distribution of collagen VI in progressive stages of liver fibrosis and its codistribution with fibrillar collagens I, III, and V in the human liver. Fibrosis of the liver is prevalent in elderly cadavers, even when liver disease is not indicated as the cause of death (Mak et al. 2012). In cadaveric livers with progressive stages of fibrosis, there is an enhanced immunostaining for collagen VI in the parenchyma showing severe perisinusoidal/pericellular fibrosis, which appears to co-distribute with the increased staining for fibrillar collagens I, III, and V in the fibrotic foci (Figs. 5, 6, and 7). In the fibrous septa of septal fibrosis, bridging fibrosis, and cirrhosis, the fibrous matrices show strong immunostaining for collagen VI along with collagens I, III, and V (Fig. 8). These immunohistological data point to a role for collagen VI in the integration of the fibrillar collagens in the histogenesis of fibrotic lesions, thereby contributing to the progression of hepatic fibrosis.

In experimental fibrosis, gene expression of collagen VI was examined by in situ hybridization in conjunction with immunohistochemical detection of the protein in the liver of rats after acute CCl_4 injury (Takahara et al. 1995). The α2(VI) collagen mRNA levels were elevated three days after the CCl_4 treatment accompanied by

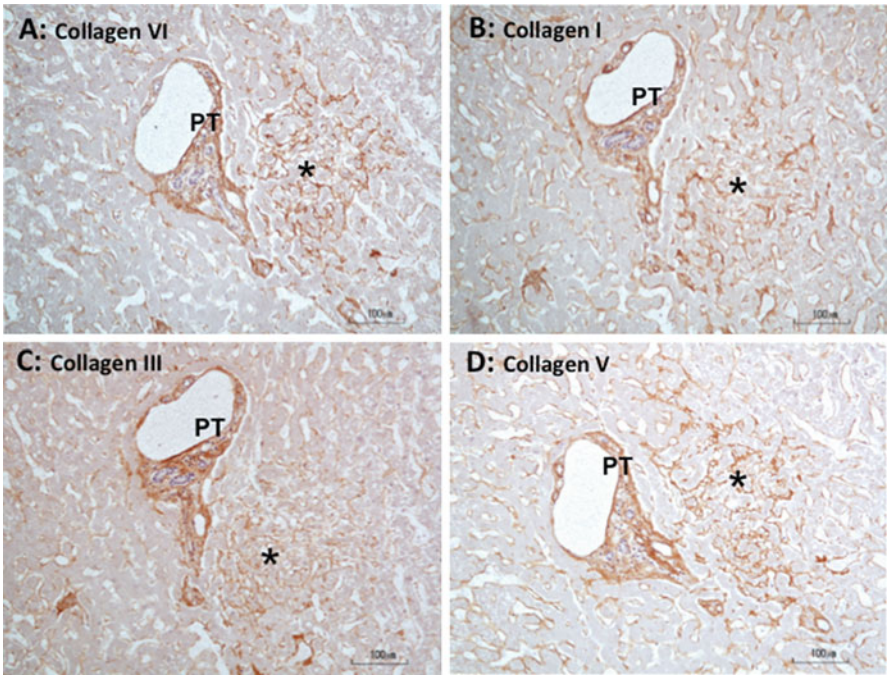

Fig. 6 Codistribution of collagens VI with collagens I, III, V in liver fibrosis. Codistribution of collagens VI, I, III, and V in fibrotic lesion of cadaveric liver. The tissue from embalmed elderly cadavers was processed for paraffin embedment cadavers as previously described (Mak et al. 2012), and immunoperoxidase staining was performed as indicated in Fig. 4. Collagen I antibody was rabbit polyclonal obtained from Rockland Immunochemicals (Gilbertsville, PA), and rabbit polyclonal collagen VI antibody was from Novus Biologicals (Littleton, CO). (**a**–**d**) are serial sections (5 μm thick) stained for collagens VI, I, III, and V, respectively. (**a**) The *asterisk* marks an area with perisinusoidal fibrosis in the periportal parenchyma, showing increased collagen VI immunostaining (*brown*) compared to the staining reaction surrounding the lesion. The increased collagen VI staining is coincident with enhanced staining for collagen I (**b**), collagen III (**c**), and collagen V (**d**) in the same fibrotic lesion, demonstrating co-distribution of these collagens. Hematoxylin counterstained. *PT* portal tract

upregulation of the mRNA for collagen I. The mRNA signals for collagens VI and I were concentrated around the perivenous area with a corresponding increased staining for the protein of collagen VI. With longer duration of treatment of 14 weeks, collagen VI mRNA levels did not change, while collagen VI protein was detected in the developing fibrous septa. It was concluded that the collagen VI gene is activated early in the fibrotic process, resulting in production of collagen VI protein. Along this line, others have described an increased deposition of collagen VI, along with collagens I, III, and V, in the developing fibrous septa and fibrotic bands of cirrhotic livers of rats caused by CCl_4 (Martinez-Hernandez and Amenta 1993).

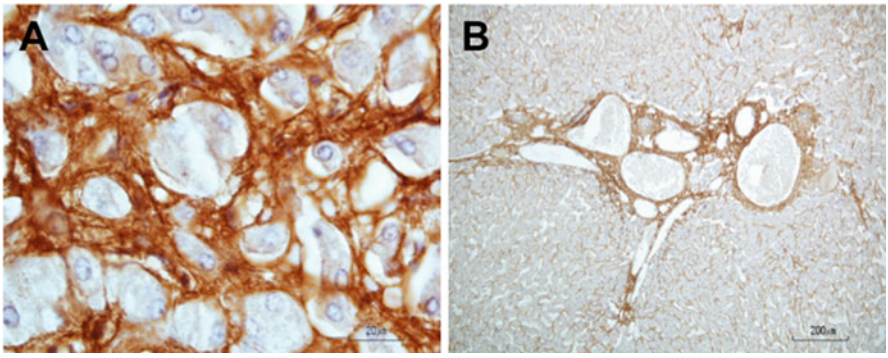

Fig. 7 Immunoperoxidase staining for type Vl collagen in liver fibrosis. (**a**) This high magnification image illustrates immunostaining for collagen VI in a severe perisinusoidal/pericellular fibrosis, described as chicken-wire fibrosis. The collagen VI immunostain (*brown*) in the lesion is robust and could be discerned as fibrils, forming a mesh around the hepatocytes. (**b**) Portal tract fibrosis. The fibrotic portal tract, characterized by irregular border with emerging short septa, is strongly stained for collagen V in the stroma. Hematoxylin counterstained

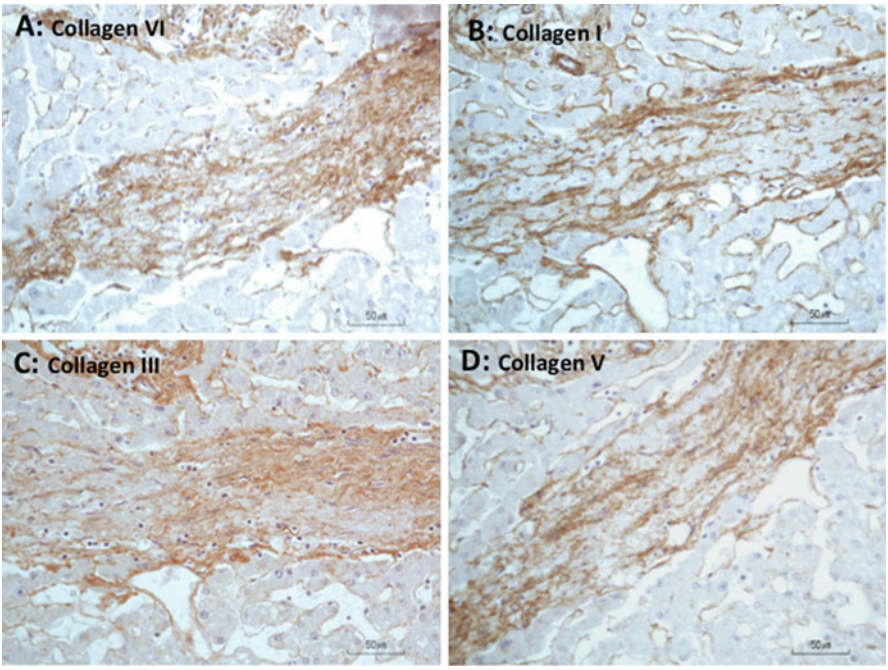

Fig. 8 Codistribution of collagens VI, I, III, and V in fibrous septum. Immunoperoxidase staining of fibrous septum in elderly cadaveric liver with bridging fibrosis. (**a**–**d**) are serial sections (5 μm thick) of a septum stained for collagens VI, I, III, and V, respectively. The matrix of the septum is positively stained (*brown*) for collagens VI, I, III, and V, thus demonstrating co-distribution of these proteins. Hematoxylin counterstained (From Mak et al. 2014, Austin Biomarkers and Diagnosis 1 (2): id1012, 2014)

Interactions with MMPs

MMPs are critical modulators of hepatic fibrogenesis (Arthur 2000). Their functions are to degrade interstitial fibrillar native collagens (MMP-1, MMP-8, and MMP-13), basement membrane type IV collagen, denatured fibrillar collagens (gelatinases MMP-2 and MMP-9), non-collagenous matrix proteins, and proteoglycans (stromelysin-1/MMP-3). Apart from being substrates for MMPs, collagens themselves also sequester and modulate the availability of MMPs, particularly of the catalytic inactive proforms. As shown by immunohistochemistry, the alcoholic cirrhotic liver displays an enhanced immunostaining for collagen VI in the matrix of the fibrous septa, which appears to co-distribute with the immunoreactivity of MMP-1 and MMP-3, suggesting MMP binding to collagen VI (Freise et al. 2009). Indeed, in vitro assays revealed that the degrees of MMP binding to the α2(VI) chain correlate with the inhibition of enzymatic activities of the MMPs. The binding of MMPs to collagen VI involves specifically the α2(VI) chain. Thus, it was proposed that collagen VI, which is upregulated in liver fibrosis, serves as a reservoir for the latent proMMPs and that the α2(VI) chain, as a binding molecule of proMMP-1, proMMP-3, and proMMP-8, modulates the availability and activities of the MMPs by sequestering the proteinases in the ECM of the fibrotic liver. Collagen VI binding of MMPs likely conserves the proform configuration of MMPs and protects these enzymes from activation, thereby diminishing matrix turnover and fibrolysis. Consequently, this biological action may perpetuate fibrous tissue deposition in the liver matrix, resulting in the progression of fibrogenesis.

HSC Collagen VI Receptor as Antifibrotic Drug Target

The perisinusoidal HSCs are the principal ECM-producing cells of the liver. HSCs become activated in response to fibrogenic stimuli and produce increased amounts of ECM, particularly fibrillar collagens and possibly the filamentous collagen VI. Therefore, collagen VI cell surface receptors expressed on HSCs are attractive targets for antifibrotic agents. Because the cyclic octapeptide (C*GRGDSPC*) containing the RGD sequence Arg-Gly-Asp specifically binds to mesenchymal cells via type VI collagen receptors (Marcelino and McDevitt 1995), it was used to design a specific carrier targeting HSCs in the liver. To that effect, the cyclic peptide was covalently coupled to human serum albumin (HSA), yielding pCVI-HSA (Beljaars et al. 2000). Accordingly, the distribution of pCVI-HSA in normal and in bile duct ligation-induced fibrotic rat livers was evaluated. There was a preferential distribution of pCVI-HSA to the control normal livers and the fibrotic livers (62–75 % of the total dose) at 10 min after an intravenous injection. Immunohistochemical analysis, however, revealed that 73 % of the injected dose of pCVI-HSA predominantly localized to HSCs in the fibrotic liver. Importantly, in vitro studies showed that pCVI-HSA specifically bound to culture-activated HSCs and was internalized by these cells. Therefore, pCVI-HSA targeting activation-induced cell receptors may be employed as a carrier to deliver antifibrotic agents or drugs to

HSCs to enhance the effectiveness and tissue selectivity of these factors against fibrogenesis. These findings highlight the involvement of HSC-associated collagen VI receptors in the pathogenesis of liver fibrosis.

Indicator of Early Liver Fibrogenesis

In the normal human liver, the interstitial fibrillar collagens I and III represent the most abundant collagens in the ECM, while the amount of filamentous collagen VI is low, accounting for less than 0.1 % of total hepatic collagens (Schuppan 1990; Schuppan et al. 1985). Elevated serum concentrations of collagen VI occur in chronic liver fibrotic disease irrespective of the underlying causes of liver damage, including viral hepatitis, schistosomiasis infection, children with cystic fibrosis, and alcoholic cirrhosis (Shahin et al. 1992; Gerling et al. 1997; Stickel et al. 2001). It was proposed that collagen VI serves as a predictor of liver fibrosis. Strikingly, circulating levels of collagen VI are already raised in the early stages of alcoholic liver injury (Stickel et al. 2001). Because serum collagen VI levels may reflect the activity of fibrolysis, its increase in the circulation likely represents an enhanced tissue turnover of collagen VI in the early events of hepatic fibrotic transformation and therefore is a good indicator of early fibrogenesis. In cirrhosis, tissue collagen VI levels rose tenfold compared to the control levels (Schuppan et al. 1985), while the serum concentrations of collagen VI almost doubled that of the control (Shahin et al. 1992; Gerling et al. 1997; Stickel et al. 2001). One hypothesis is that during the histogenesis of advanced fibrosis, degradation of collagen VI is impaired (Schuppan et al. 1985), resulting in a higher tissue concentration of collagen VI, possibly sustaining fibrogenesis by stimulation of activated HSCs or myofibroblasts for ECM production.

Biomarker for Hepatic Fibrosis

Despite advances made in the understanding of collagen VI's involvement in liver fibrosis and progression to cirrhosis, the issue of collagen VI serving as a liver fibrosis biomarker has yet to be addressed. In a number of authoritative reviews of liver fibrosis biomarkers, collagen VI has not been included in the discussion as a fibrosis marker (Gressner et al. 2007; Fallatah 2014). This may have been overlooked due to the lesser abundance of collagen VI relative to collagens I, III, and IV in the liver ECM.

Neo-epitope and Protein Fingerprinting Technology

The development of collagen VI as a biomarker of hepatic fibrosis largely stemmed from the notion of neo-epitope generation in conjunction with protein fingerprinting technology. It is known that a highly regulated equilibrium between the synthesis and degradation of ECM proteins—particularly the collagen types—is required to achieve tissue homeostasis. A disruption of this equilibrium is regarded as the basis

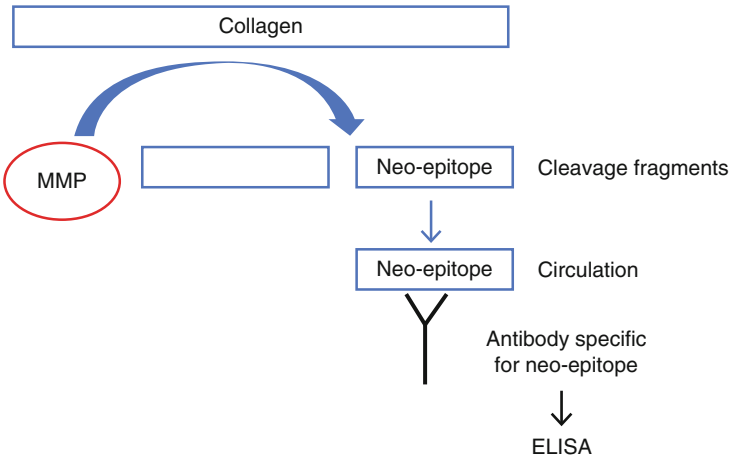

Fig. 9 Schema of enzyme-generated neo-epitope for ELISA. This diagram illustrates a specific matrix metalloproteinase (MMP) that acts on a selective collagen substrate, generating a peptide fragment – neo-epitope – which is released into the systemic circulation where it is detected by antibody specific for the neo-epitope. The antibody and neo-epitope reaction is quantified by ELISA

of pathological processes, fibrosis included. The degradation products of ECM proteins can be measured in biological fluids, and such measurement can give an indication of the disease activity and progression. MMPs and cysteine proteases are capable of degrading collagens and proteoglycans of the ECM, respectively, which result in the generation of specific cleavage peptide fragments, called neo-epitopes (Karsdal et al. 2009). Because the generation of neo-epitopes occurs locally in the pathologically affected areas and involves a specific disease, it may carry a unique disease peptide fingerprint. This approach has been given the term "protein fingerprinting technology" (Karsdal et al. 2011; Leeming et al. 2013; Schierwagen et al. 2013; Vassiliadis et al. 2013). Therefore, compared to measurement of the intact/whole proteins, quantifying neo-epitopes—or protein fingerprint—is likely to provide a more sensitive indicator of a pathological change. In particular, these neo-epitope fragments are small enough to be released into the circulation or urine, thereby allowing their detection by antibodies raised specifically to react against the neo-epitopes. This is commonly measured by ELISA—as schematized in Fig. 9. Thus, measurement of neo-epitopes in the serum may indicate the degree of remodeling of ECM collagens and proteins that are involved in the development of liver fibrosis. Notably, neo-epitopes have successfully been used as noninvasive serum markers in osteoporosis and arthritis (Karsdal et al. 2009), which are both characterized by extensive ECM remodeling.

Collagen VI Neo-epitope as a Biomarker of Experimental Liver Fibrosis

Collagen VI constitutes a minor component of the total hepatic collagen, but its level is significantly elevated in the event of liver fibrogenesis, as discussed above. To that

effect, neo-epitopes derived from the cleavage peptide fragments of collagen VI generated by the proteolytic action of MMP-2 or MMP-9 in vitro were used to raise antibodies that specifically react with the neo-epitopes, which can be quantified by ELISA (Veidal et al. 2011). Among the peptide fragments, the neo-epitope containing the sequence 573′.YRGPEGPQGPPG′584 in the α1 chain of collagen VI was selected based on the ability of the antibody to distinguish between the cleaved and uncleaved collagen VI. Also, this sequence was found to be 100 % homologous to human, rat, and mouse and is designated as CO6-MMP. Accordingly, the value of CO6-MMP as a biomarker of liver fibrosis was evaluated in two rat models of hepatic fibrosis: bile duct ligation and carbon tetrachloride (CCl_4) treatment (Veidal et al. 2011). It was demonstrated that CO6-MMP serum concentrations were significantly elevated and were highly associated with the histological severity of liver fibrosis in these animals. Importantly, because the CO6-MMP antibody is capable of quantifying collagen VI degradation by MMP-2 and MMP-9, it can be employed to assess collagen VI turnover in early stages of fibrogenesis, serving as an early marker for fibrosis, which is consistent with the conclusion of previous studies that collagen VI is a good indicator of early fibrogenesis (Stickel et al. 2001). It has yet to be determined whether or not MMP-2 and MMP-9 degraded collagen VI represents a useful biomarker for the clinical assessment of liver fibrogenesis.

In another study by Leeming et al. (2013), hepatic collagen in CCl_4-treated rats was quantified by histomorphometry of Sirius red staining for collagens, and the values were expressed as four quartiles Q1, Q2, Q3, and Q4, representing early, moderate, severe fibrosis, and cirrhosis, respectively. Levels of serum CO6-MMP neo-epitope—also designated as C6M—were significantly elevated in all collagen quartiles in CCl_4-treated rats compared to controls. When evaluated as a single collagen VI degradation marker, CO6-MMP cannot distinguish early, moderate, or severe fibrosis; however, when CO6-MMP was used in conjunction with P5CP, which is a collagen V formation marker, the combination of the two markers showed the highest and best correlation with total hepatic collagens in all quartiles than any single marker, including that of collagen I, III, or IV. The combination of scores generally enabled separation of early fibrosis, severe fibrosis, and cirrhosis from the respective controls. Moreover, the combined scores differentiate early and moderate fibrosis, as well as severe fibrosis and cirrhosis. Hence, the combined use of collagen VI and collagen V biomarkers is the most reliable indicator of both early- and late-stage fibrosis.

Conclusions

The molecular structure of type VI collagen has largely been determined since its discovery 32 years ago, and significant advances are being made in the fields of collagen VI-related muscular disorders, mammary carcinogenesis, and fibrotic diseases such as adipose tissue and liver fibrosis. There are clinical data pointing to collagen VI as a marker indicative of early hepatic fibrotic changes in alcoholic patients. Experimental data indicate that the collagen VI receptor expressed on HSCs

offers a selective target for antifibrotic agents, but this area has so far been understudied. Studies using collagen VI knockout mice in conjunction with induction of fibrosis—CCl_4 treatment or bile duct ligation—could help determine whether collagen VI plays a specific role in liver fibrogenesis. Collagen VI-derived neo-epitope (CO6-MMP), which can be quantified by ELISA, is useful in assessing tissue turnover in early fibrogenesis. Furthermore, the combined use of neo-epitopes of collagen VI and collagen V offers the most reliable indicator of both early- and late-stage fibrosis; however, its application as a noninvasive serum biomarker in patients has yet to be determined because the pathogenesis of fibrosis in humans is likely to be more variable than in the CCl_4 fibrosis model.

Potential Applications to Prognosis, Other Diseases, or Conditions

C06-MMP, the neo-epitope of collagen VI generated by the proteolytic degradation of MMP-2 and MMP-9-during liver fibrogenesis, serves as a useful noninvasive biomarker in two models of experimental liver fibrosis. Its increased presence in the serum is highly associated with liver fibrogenesis, reflecting the central role of VI collagen turnover in ECM remodeling in fibrogenesis. It is, however, regarded only as an investigative marker in accordance with the BIPED (burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic) system (Liu et al. 2012). Accordingly, further studies are needed to evaluate whether CO6-MMP has any potential applications to prognosis of hepatic fibrotic disease. It is equally important to assess whether CO6-MMP—either alone or in combination with other collagen type neo-epitopes–can be employed to monitor the efficacy of antifibrotic agents in the therapy of liver fibrosis or intervention of fibrosis progression. Because liver fibrosis and even cirrhosis can be reversed by eliminating the underlying cause of the disease, or upon cessation of the fibrogenic stimulant in the CCl_4 reversible model of fibrosis, it is clinically relevant to investigate whether CO6-MMP is useful for monitoring fibrosis regression. Finally, because collagen VI is involved and upregulated in other fibrotic diseases such as adipose tissue, heart, kidneys, lungs, and skin (Mak et al. 2014), CO6-MMP may serve as a potential biomarker for monitoring fibrotic changes in these organs.

Summary Points

- Type VI collagen is a filamentous collagen present in most connective tissue matrices where it forms a flexible network, linking matrix molecules and cells.
- Type VI collagen is composed of three genetically distinct chains, α1(VI), α2(VI), and α3(VI), with a globular domain at each end.
- In the cells, collagen VI monomers dimerize and form tetramers, which are secreted and associate into filaments in the extracellular matrix.

- Collagen VI gene expression is regulated differently than collagen I or III. Collagen VI interacts with collagen V and fibronectin, contributing to the structurally integrity of tissue matrix scaffolds.
- Collagen VI mediates cell adhesion and promotes migration, and soluble collagen VI acts as a sensor for tissue damage, modulating mesenchymal cell proliferation and survival, matrix homeostasis, and wound healing.
- Three collagen VI-deficient mouse models have been generated, which have been used to investigate collagen VI-related myopathies, mammary tumorigenesis, and skeletal muscle satellite cell homeostasis.
- Collagen VI expression is upregulated in fibrosis of adipose tissue and liver.
- Elevated collagen VI in circulation is considered an early biomarker of alcoholic liver fibrosis.
- Collagen VI immunostaining is enhanced in fibrotic foci, codistributing with collagens I, III, and V. Hepatic stellate cells (HSCs) are likely the source of perisinusoidal collagen VI.
- The α2(VI) chain sequesters hepatic matrix metalloproteinase (MMP)-1, MMP-3, and MMP-8 and prevents the enzymes' activation, diminishing fibrolysis.
- The collagen VI receptor on HSCs offers selective targets for antifibrotic agents.
- CO6-MMP, a collagen VI neo-epitope generated by the proteolytic actions of MMP-2 or MMP-9, serves as a specific biomarker of collagen VI degradation in experimental liver fibrogenesis.

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