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

Morten A. Karsdal,¹ Mette J. Nielsen,¹ Jannie M. Sand,¹ Kim Henriksen,¹ Federica Genovese,¹ Anne-Christine Bay-Jensen,¹ Victoria Smith,^{1,2} Joanne I. Adamkewicz,² Claus Christiansen,¹ and Diana J. Leeming¹

¹Nordic Bioscience A/S, Herlev Hovedgade, Herlev, Denmark.

²Department of Biology, Gilead Sciences, Inc., Foster City, California.

ABSTRACT

Increased attention is paid to the structural components of tissues. These components are mostly collagens and various proteoglycans. Emerging evidence suggests that altered components and noncoded modifications of the matrix may be both initiators and drivers of disease, exemplified by excessive tissue remodeling leading to tissue stiffness, as well as by changes in the signaling potential of both intact matrix and fragments thereof. Although tissue structure until recently was viewed as a simple architecture anchoring cells and proteins, this complex grid may contain essential information enabling the maintenance of the structure and normal functioning of tissue. The aims of this review are to (1) discuss the structural components of the matrix and the relevance of their mutations to the pathology of diseases such as fibrosis and cancer, (2) introduce the possibility that post-translational modifications (PTMs), such as protease cleavage, citrullination, cross-linking, nitrosylation, glycosylation, and isomerization, generated during pathology, may be unique, disease-specific biochemical markers, (3) list and review the range of simple enzyme-linked immunosorbent assays (ELISAs) that have been

developed for assessing the extracellular matrix (ECM) and detecting abnormal ECM remodeling, and (4) discuss whether some PTMs are the cause or consequence of disease. New evidence clearly suggests that the ECM at some point in the pathogenesis becomes a driver of disease. These pathological modified ECM proteins may allow insights into complicated pathologies in which the end stage is excessive tissue remodeling, and provide unique and more pathology-specific biochemical markers.

INTRODUCTION

The extracellular matrix (ECM) is of paramount importance for tissue function, and controls cell phenotype and function. That was initially illustrated by Mintz and colleagues who showed that the normal mouse embryonic tissue microenvironment could repress expression of the tumor phenotype;^{1,2} thus, the ECM was able to control genotype/phenotype relationships. These interactions between cells and the ECM components are mediated through receptors, such as integrins and the discoidin receptors.³ To maintain healthy tissue, the ECM must regenerate itself by normal remodeling, in which old or damaged proteins are broken down in a specific sequence of proteolytic events and replaced by new proteins. However, during pathological conditions, such as cancer, fibrosis, and inflammation, the delicate repair-response balance is disturbed.^{4,5} The original proteins of the ECM are replaced by different matrix constituents, and consequently, the composition and quality of the matrix are altered. During cancer and fibrosis propagation, the ECM may be stiffened, and this can actually enhance tumor cell migration, myofibroblast activation, and collagen deposition,⁶⁻¹⁴ thereby linking the actual matrix quality to disease progression.

ABBREVIATIONS: 2-D, two-dimensional; 3-D, three-dimensional; AASLD, American Association for the Study of Liver Disease; AGE, advanced glycation/glycosylation end product; BM, basement membrane; BSAP, bone-specific alkaline phosphatase; CIA, collagen-induced arthritis; CL, cutis laxa; COMP, cartilage oligomeric matrix protein; CRP, C-reactive protein; CTX-I, cross-linked C-terminal telopeptide of type I collagen; CTX-II, cross-linked C-terminal telopeptide of type II collagen; DDR, discoidin domain receptors; EAE, experimental autoimmune encephalomyelitis; ECM, extracellular matrix; ECMR, ECM remodeling; ELISA, enzyme-linked immunosorbent assay; EMT, epithelial-to-mesenchymal transition; GAG, glycosaminoglycan; hMFB, hepatic myofibroblast-like cells; HDAC1, histone deacetylase 1; HSCs, hepatic stellate cells; ICTP, C-terminal telopeptide of type I collagen; LN, laminin N-terminal; LOX, lysyl oxidase; MED, multiple epiphyseal dysplasia; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; MS, multiple sclerosis; NO, nitrogen oxide; NTX, N-telopeptide of type I collagen; PADs, peptidylarginine deiminases; PDGF, platelet-derived growth factor; PIIANP, type IIA procollagen N-terminal peptide; PRELP, proline arginine-rich end leucine-rich repeat protein; PSACH, pseudoachondroplasia; PTM, post-translational modifications; RA, rheumatoid arthritis; RAGE, receptor for AGEs; RNS, reactive nitrogen species; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SLRPs, small leucine-rich proteoglycans; SMCD, Schmid type metaphyseal chondrodysplasia; SMD, spondylometaphyseal dysplasia; SVAS, supraaortic stenosis; TG, transglutaminase; TGF, transforming growth factor; TNF, tumor necrosis factor; WBS, William-Beuren syndrome.

During this pathological remodeling of the ECM, excessive levels of tissue- and pathology-specific turnover products are released into the circulation. Turnover products holding post-translational modifications (PTMs) are defined as modifications made secondary to translation of the protein into the peptide sequence from mRNA. Thus, most PTMs are not directly DNA coded, and are a consequence of tissue physiology and pathophysiology.^{15,16} PTMs may be derived from processes, such as aging (in which amino acid isomerization occurs), citrullination (during inflammation), protease degradation (fibrosis and inflammation), and glycosylation (diabetes),^{15,16} as will be carefully discussed. Protease-generated neopeptides have, to date, received more attention than other PTMs. However, potentially important PTMs that are believed to be specific for cancer as well as fibrotic and other pathological conditions have recently been identified.^{15,17–19} The PTMs made to proteins result in unique protein fingerprints.²⁰ These modified structures are prime candidates for biochemical marker development, as they may be more related to the pathogenesis than unmodified proteins. Several lines of independent evidence suggest that PTMs to specific proteins contribute to abnormal cellular proliferation, adhesion characteristics, and morphology,²¹ and may cause many of the differences in cancer tissue compared to normal tissue.^{21–26} Furthermore, the generation of PTMs of key structural proteins, generated by protease cleavage, citrullination, nitrosylation, glycosylation, and isomerization, is emerging as a critical factor in tissue homeostasis and remodeling. Thus, PTM profiles may be used as biochemical fingerprints for detecting and verifying the function and activity of key cellular signaling pathways^{21–26} involved in tissue homeostasis and integrity. Additional lines of evidence highlight that the structural components of the matrix, after PTM, are central part of the pathogenesis itself,¹⁵ thus highlighting the matrix structural proteins as central and active participants rather than passive bystanders in disease pathogenesis.

The aims of this review are to discuss the structural components of the matrix, the potential applicability to pathology, and the measurement of structural molecules in serum. We review the PTMs, which may be both a consequence of disease and a part of the pathogenesis, as exemplified by the role of tissue stiffness in cancer and fibrosis. Lastly, we list the current methods for measuring post-translational modified matrix proteins in serum. These PTMs may serve as disease-specific biochemical markers and assist in the identification of key molecular pathways leading to enhanced connective tissue remodeling.

DISCUSSION

Function of the ECM

The ECM is a three-dimensional (3-D) structure that encapsulates cells and defines their microenvironment.²⁷ It consists of a meshwork of proteins to which soluble factors, such as growth factors and cytokines, can bind. There are two main types of ECM. The first is the basement membrane (BM), which interacts directly with the epithelium and endothelium, and it is composed of primarily of type IV collagen, laminins, entactin/nidogen, and heparan sulfate proteoglycans (e.g., perlecan) (Fig. 1).²⁸

Fig. 1. The molecular structure of a typical basal lamina. The basal lamina is formed by specific interactions between the proteins type IV collagen, laminin, and entactin plus the proteoglycan Perlecan. Adapted by S.H. Madsen, from Yurchenco and Schittny.²⁸

The second type is the interstitial matrix, which makes up the bulk of the ECM in the body. The interstitial matrix consists of many types of collagens, including types I and III, together with fibronectin. The interstitial matrix additionally consists of tenascin and proteoglycans that provide tissue hydration, enable binding of growth factors and cytokines to the tissue, and cross-link the matrix to enhance its integrity.²⁹

Although originally considered as merely a support system for the cells within the tissue, the ECM is now recognized as a central regulator of cell and tissue behavior via transmembrane signaling.^{1,30–33} While the basic characteristics and composition of the BM and interstitial matrix are constant across tissues, variations in ECM components, such as protein isoform expression, ratio between individual matrix components, and PTMs, contribute to differences in ECM organization and structure and ensure tissue specificity.¹⁵ PTMs, such as glycosylation and cross-linking, significantly affect the mechanical properties of the ECM, including its viscoelasticity or stiffness. Both the stiffness and topology (3-D appearance) of the ECM regulate the growth, remodeling, differentiation, migration, and phenotype of a wide variety of cell and tissue types.^{8–14,34}

Matrix Composition Affects Cell Phenotype

The importance of matrix stiffness in tissue-specific differentiation is exemplified by the fact that cells grown as monolayers (two-dimensional: 2-D) on top of either a plastic substrate or a glass coverslip, with or without ECM ligand, fail to assemble the same tissue-like structures as those growing in the normal ECM (3-D). Cells growing on plastic or glass are less likely to express differentiated proteins upon stimulation,³⁴ or respond to growth factors or protease inhibitors in the same way as cells growing in a 3-D setting.³⁵ These phenotypic disparities can be explained, in part, by the fact that

living tissues in 3-D emit biological signals that may be read by specific integrins, but this signaling is nonexistent in 2-D substrata such as tissue culture on plastic. The role of cell polarity versus non-polarity in cultures is receiving increased attention. Another illustration of this phenomenon is that when epithelial cells and melanocytes are grown in a 3-D ECM microenvironment, they assemble into tissue-like structures and express differentiated proteins when given the correct soluble stimuli.³⁶ Neither behavior is seen when the same cells are cultured on 2-D plastic substrata.

The architecture of the interstitial matrix *in vivo* also differs substantially from that found typically in tissues cultured on plastic, and this too can have dramatic effects on cell behavior.³⁵ For instance, osteoblasts grown on plastic in 2-D do not rely on matrix metalloproteinases (MMPs) for survival, whereas osteoblasts embedded in an interstitial matrix, such as 3-D type I collagen, are critically dependent for their survival on MMP activation of latent transforming growth factor (TGF)- β .³⁵ Thus, the matrix architecture is crucial to the phenotype and survival of cells. Interestingly, the orientation of collagen fibers can critically regulate cell and tissue behavior.^{37–39} This 3-D contextual information is lost when cells are grown in 2-D.

Varying components of the ECM also influence the ability of the matrix to regulate cell and tissue behavior. The ECM transmits signals through various specialized cell membrane receptors, including integrins, discoidin domain receptors (DDR), and syndecans.^{40–44} Integrins provide an excellent model of how an altered ECM could promote tumor progression. Integrins consist of 24 distinct transmembrane heterodimers that relay cues from the surrounding ECM to regulate cell growth, survival, motility, invasion, and differentiation.^{40–44} They are able to interact with the ECM externally, and with cytoplasmic adhesion plaque proteins and the cytoskeleton intracellularly to influence cell behavior. Integrin–ECM interactions regulate the cell fate by activating multiple biochemical signaling circuits and altering the cell shape.^{45,46} This occurs either through direct interactions between ECM receptors and actin-linked proteins or cytoskeletal reorganization induced by activating cytoskeletal-remodeling enzymes, such as RhoGTPases.^{45,46}

This section highlights that the composition of the ECM affects the phenotype of cells through specific receptor-mediated interactions. Certain ECM compositions and structures result in a context-dependent response to a given stimulus, which is absent in other experimental settings.

ECM Proteins

The ECM mainly consists of collagens and proteoglycans, each with their unique function. In the following section, the most important and well-investigated collagens and proteoglycans are discussed, together with other important structural components of the ECM.

Collagens. Collagens are a family of proteins made up of three α -chains supercoiled around each other completely or partially in a triple helix with a characteristic Gly-X-Y repeat. Intra- and intermolecular cross-links bring stability to the collagen molecules, contributing to the characteristically high tensile strength and min-

imal extensibility of collagen. Type I, II, III, and V collagens belong to the group of fibrillar collagens, which are the most abundant collagen group in the body. In addition to the triple-helical domain, they also contain N- and C-terminal propeptide domains that are cleaved off by N- and C-procollagenases, respectively, before fibril assembly.⁴⁷ Type I–VI collagens are the most well described at present and are the focus of this section.

Type I collagen is composed of the heterotrimer $\alpha_1\alpha_1\alpha_2(I)$ and is the most abundant type of collagen that is ubiquitously expressed. It provides tensile stiffness in bone and has important load-bearing, tensile strength, and stress-carrying properties in other tissues as well. In tendons, type I collagen fibrils are arranged in parallel to form bundles, whereas in skin, the arrangement is more random, forming a complex network of interlaced fibrils. These different arrangements contribute to the different properties of the tissues. Type I collagen is often incorporated into fibrils with either type III⁴⁸ or type V collagen.⁴⁹ The synthesis, concentration, and circulating levels (serum concentration) of degradation products of type I collagen have been proven to be increased during breast, bone, lung, ovarian, prostate, and skin malignancy.^{50–55}

Type II collagen is the major component of hyaline cartilage, but is also found in the vitreous body of the eye, the corneal epithelium, the notochord, the nucleus pulposus of intervertebral discs, and embryonic epithelial-to-mesenchymal transitions (EMTs).⁴⁷ Type II collagen is a homotrimer consisting of three $\alpha_1(II)$ chains, and the primary sequence has a high content of hydroxylysine and glycosyl residues, which mediate interactions with proteoglycans, another important component of hyaline cartilage. Type II collagen degradation is mainly associated with rheumatological diseases such as osteoarthritis and rheumatoid arthritis.⁵⁶

Type III collagen is mainly present in association with type I collagen and is an important component of the interstitial tissues of the lung, liver, dermis, spleen, and vessels. Type III collagen is a homotrimer consisting of three $\alpha_1(III)$ chains. A characteristic feature of type III collagen is that it is correlated to extensibility of tissues, and that it may contribute to elasticity, a property that is uniquely connected to this type of collagen.⁵⁷ Type III collagen has been mostly associated with various fibrotic diseases.^{58–61}

Type IV collagen is the main component of the BM, a specialized type of ECM that separates the epithelium from the stroma in all tissues in the body. It consists of three domains: N-terminal 7S domain, a central triple helix, and a large C-terminal NC1 globular domain. Its triple helix is $\sim 25\%$ longer than those seen in the fibrillar collagens, and the Gly-X-Y repeat is frequently interrupted, accounting for the relatively high flexibility of this type of collagen.²⁸ Instead of fibrils, type IV collagen molecules assemble into a flexible 3-D network. The most abundant isoform of type IV collagen is $\alpha_1\alpha_1\alpha_2(IV)$, but tissue-specific isoforms also exist: $\alpha_3\alpha_4\alpha_5(IV)$ heterotrimers are found in the lung, glomeruli of the kidney, cochlea, eyes, and testis, whereas $\alpha_5\alpha_5\alpha_6(IV)$ is found in skin, Bowman's capsule of the kidney, the esophagus, and the knee joint.⁶² Turnover of the basement membrane is associated with a range of diseases.

Type V collagen is expressed in tissues containing type I collagen, but is a quantitatively minor component.⁶³ The most common structure of type V collagen is $\alpha_1\alpha_1\alpha_2(V)$, although homotrimers of three $\alpha_1(V)$ chains and heterotrimers of the $\alpha_1\alpha_2\alpha_3(V)$ isoforms have also been detected.⁶⁴ It typically forms heterofibrils with type I collagen,^{49,63} where it makes up the core structure of these heterotypic fibrils. Type V collagen is of special importance for the structure of tissues. It has been shown to be essential for the correct assembly of collagen fibrils and to regulate their size and organization.⁶⁵ This characteristic makes type V collagen especially unique and interesting to study. The N-terminal domain contains a high level of tyrosine sulfated residues that contribute to the strong interactions that type V collagen has with triple-helical domains of other collagen types. This enhances the stability of fibrils.⁶⁶

Type VI collagen is a heterotrimeric molecule with the isoform $\alpha_1\alpha_2\alpha_3(VI)$ and consists of a short triple helix flanked by two extended globular domains, and it is expressed, albeit variably, in virtually all tissues. The primary fibrils are arranged in overlapping dimers in an antiparallel manner and form parallel tetramers that are stabilized by intermolecular disulfide bonds. They aggregate to form filaments and an independent microfibrillar network. Type VI collagen molecules have a uniquely beaded appearance and interact with several ECM components such as type I collagen and fibronectin.⁶⁷

Proteoglycans. Proteoglycans are ECM macromolecules formed by a protein core with one or more glycosaminoglycans (GAGs) bound covalently. Due to the negative charge and structural conformation of GAGs, proteoglycans can interact with a large variety of macromolecules.⁶⁸ Proteoglycans can be divided into five families according to the structural properties of their core protein.^{69,70}

The small leucine-rich proteoglycan (SLRP) family is formed by proteoglycans that bind specifically to other ECM constituents and contribute to the structural framework of connective tissues. SLRPs are small molecules, with core proteins of 40 kDa, and possess characteristic 6–10 leucine residues at conserved locations between the flanking cysteine-rich disulfide-bonded domains at the N- and C-terminus that participate in protein–protein interactions with collagens, matrix glycoproteins, and cell membrane components.^{70,71} Based on several parameters, including gene organization and amino acid homologies, SLRPs are further divided into five classes: class I includes decorin, biglycan, and asporin; class II includes fibromodulin, lumican, keratocan, proline arginine-rich end leucine-rich repeat protein (PRELP), and osteoadherin; class III includes epiphykan, mimican, and opticin; class IV includes chondroadherin and nyctalopin; and class V includes podocan.^{69,72}

Decorin, fibromodulin, asporin, lumican, PRELP, and chondroadherin can interact with collagen and influence collagen fibril formation and interaction.⁶⁹ In addition to their ECM functions in tissue hydration and collagen fibrillogenesis, proteoglycans are able to influence tissue repair and tumor growth, to facilitate cellular adhesion, proliferation, and migration, and to modulate growth factors and cytokine activities. For this reason, they are referred to as

matricellular protein, with the ability to modulate cell–matrix interactions and cell functions.⁷² In particular, decorin, biglycan, and lumican exert many modulation roles in different biological processes. These functions highlight the important effect of ECM components in the cellular phenotype by influencing cell communication through, that is, signal transduction, cytokine modulation, adhesion, and migration.⁷² All the different matricellular functions exerted by these three important SLRPs are detailed in *Table 1*.^{3,73–118}

Some of the most important proteoglycans expressed in the ECM are briefly described in the following paragraphs.

Aggrecan is the major proteoglycan of the cartilage, and it is the most highly glycosylated, with 150 chondroitin sulfate and keratan sulfate GAGs bound to a large central core protein. Through its specific binding with hyaluronan and link protein, it forms a supramolecular structure whose characteristics make it able to retain water molecules in the cartilage, providing the tissue with the property of resisting compressional forces with minimal deformation.⁶⁹

Versican is a large interstitial chondroitin sulfate proteoglycan. It is present in many tissues, and it is one of the principal ECM components of normal blood vessels where it influences the assembly of ECM and controls elastic fiber fibrillogenesis.¹¹⁹ It is present in the intima and adventitia of most arteries and veins, and it is synthesized by vascular smooth muscle cells as well as endothelial cells, myofibroblasts, and macrophages.¹²⁰ Versican interacts with hyaluronan and link protein to form high molecular weight stable aggregates. These complexes create a reversibly compressive compartment and provide a swelling pressure within the ECM that is compensated by collagen and elastic fibers. Dramatically increased levels of versican have been observed in atherosclerosis and restenosis, implying that this proteoglycan is a specific component of developing lesions and contributes to their progression in atherosclerosis and restenosis.¹¹⁹

Perlecan is a heparan sulfate proteoglycan widely distributed in BMs, and it has the largest core protein found in proteoglycans. It is able to self-associate or interact with several other BM macromolecules, including laminin and type IV collagen.⁶⁸

Decorin is the most abundant SLRP in cartilage. It contains one GAG chain, often dermatan sulfate, which can adopt complex secondary structures and form specific interactions with matrix molecules. Its level increases with age. Its main function is to regulate collagen fibrillogenesis and to maintain tissue integrity by its binding with fibronectin and thrombospondin.⁶⁹ However, decorin also exerts important matricellular functions, favoring the cell–matrix interactions and influencing cell phenotype (see *Table 1*). Decorin is an important antifibrotic agent: it influences fibrogenesis in different organs by inhibiting TGF- β ; it regulates ECM synthesis and turnover, and it is involved in regulation of cell death, adhesion, and migration.⁷²

Biglycan is a small SLRP. It is found in many connective tissues, such as skin, bones, and blood vessels. Within the hyaline cartilage tissue, biglycan is localized mainly pericellularly.⁷⁰ Together with decorin, biglycan is a key regulator of the lateral assembly of collagen fibres, and it interacts primarily with type VI collagen.⁶⁹

Table 1. The Matricellular Effects of Extracellular Matrix Components

Protein or PTM	Cellular phenotype	Responsible receptor	Reference
Elastin-derived peptides	Chemotaxis of monocytes, fibroblasts, and endothelial cells	Elastin-binding protein in complex with protective protein/cathepsin A and neuraminidase-1	73,74
	Proliferation of fibroblasts and smooth muscle cells		
	Protease release from fibroblasts and leukocytes		
Thrombospondin	Inhibition of angiogenesis	CD36 and CD47	75–77
Type I collagen	Fibroblast migration	DDR2; integrins α_1 , α_2 , α_{10} , α_{11} , and β_1	3,78
Acetylated Proline–Glycine–Proline (acPGP; fragment of type I collagen)	Neutrophil chemotaxis	CXCR1 and CXCR2	79,80
Arresten, canstatin, and tumstatin (fragments of type IV collagen)	Inhibition of angiogenesis, tumor growth, and endothelial cell proliferation and migration.	Various integrins	81,82
	Induction of apoptosis		
Endostatin (fragment of collagen type XVIII)	Inhibition of endothelial proliferation, angiogenesis, and tumor growth	Glypicans, nucleolin	83–86
	Induction of endothelial cell apoptosis		
RGD motif (present in collagens, laminin, and fibronectin)	Cell adhesion, angiogenesis, and apoptosis	Various integrins	87,88
Fibromodulin	Proliferation, migration, and chemotaxis of HSCs	Unknown	89
Laminin-332 (elastase-generated fragment of γ_2)	Neutrophil chemotaxis	Unknown	90
SIKVAV and ASKVAV (sequences in linker regions between coiled-coil and globular domains of laminin α_1 and α_5 chains)	Neutrophil and macrophage chemotaxis	Unknown receptors; SIKVAV interacts with integrins α_1 , α_6 , and β_1 in the salivary gland carcinoma cell line	91,92
Laminin	Chemotactic migration of malignant cells toward laminin	67LR (LamR)	93,94
Lumican	Regulation of inflammation and innate immunity	CD14, FasL, CXCL1	95–98
	Apoptosis induction	Fas	
Biglycan	Regulation of inflammation and innate immunity	TLR2, TLR4, P2X4/P2X7, selectin L/CD44, C1q	99–110
	Cytokine modulation (PDGF, TGF- β , TNF- α , WISP-1, BMP-4)		
	Adhesion and migration	RhoA, Rac1	
Decorin	Signal transduction	LRP-1, c-MET	102–106, 110–118
	Cytokine modulation (PDGF, TGF- β , TNF- α , VWF, and WISP-1)		
	Regulation of inflammation and innate immunity	TGB- β , C1q	
	Antiapoptotic effect.	IGF-IR	
	Antioncogenic effect.	EGF-R, VEGF-R2,	
	Adhesion and migration	IGF-IR, integrin $\alpha_2\beta_1$, RhoA, Rac1	

PTMs, post-translational modifications; HSC, hepatic stellate cell; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Biglycan is thought to have a role also in fibrogenesis and in assembly of elastin fibers.¹²¹ Moreover, this proteoglycan is able to bind to the membrane-bound proteoglycan, dystroglycan, and to a wide variety of proteins. It is involved, for instance, in cell signal transduction during cell growth and differentiation and in regulating cytokine activity through its capacity to bind TGF- β and tumor necrosis factor (TNF) α (see *Table 1*).⁹⁹

Mimecan is a keratan sulfate proteoglycan belonging to the SLRP family, and it is the product of the gene that encodes for osteoglycin.¹²² Its main role consists in regulating the collagen fibril diameter.¹²³ Apart from corneal tissue, where it has been first identified, mimecan is expressed also in other tissues, such as medullary bone,¹²⁴ amniotic membrane,¹²⁵ cartilage¹²⁶ and pituitary.¹²⁷ In the lung, mimecan mRNA expression is correlated to nonsmall-cell lung cancers,¹²⁸ and in arteries, there is an indication that it can be involved in arterial remodeling during atherosclerosis.¹²⁹

Fibromodulin is one predominant SLRP in cartilage. It contains up to four keratan sulfate chains, and it is able to influence collagen fibril formation and maintain a sustained interaction with the formed fibrils.^{69,130}

Lumican is a highly biologically active SLRP. It can exist as proteoglycan (with GAG chains) and as glycoprotein (with mono- or polysaccharide chains). In the human adult cornea, it is present in the first form, whereas it is in a glycoprotein form in embryonic cornea and in skin. Lumican expression in cornea has been widely studied. In this tissue, it exerts its main role in controlling the polymerization of collagen into small-diameter fibrils.¹³¹ Lumican is also highly present in skeletal muscle, kidneys, placenta, heart, intervertebral discs, blood vessels, intestine, uterus, and pancreas,⁷¹ and it has a widespread distribution in connective tissues, including cartilage, where it modulates collagen fibrillogenesis and regulates the assembly and diameter of collagen fibers and interfibrillar spacing, enhancing collagen fibril stability.¹³² Together with decorin and biglycan, it is an important component of the ECM exerting matricellular functions (see *Table 1*).

Other proteins: The glycoproteins fibronectin and tenascin C modulate the integrin-mediated adhesion of cells to other ECM proteins, such as collagens, and as such play a key role in cancer invasion. A single gene encodes fibronectin, but alternative splicing allows formation of multiple isoforms from which some are tumor specific.¹³³ The fibulins, Galectin-1 and Fibulin-1, function as intramolecular bridges in the organization of ECM supramolecular structures, such as elastic fibres and BMs.¹³⁴ Galectin-1 and Fibulin-1 can bind ECM components, that is, laminin and fibronectin, and therefore modifies the adhesive properties of cancer cells.^{134–136}

Effect of structural proteins on cellular phenotype: selected examples. There is growing evidence that ECM molecules have functions other than structural roles, but as integrated players in the structure and functional homeostasis of tissue. A nonexhaustive list of these proteins is given in *Table 1*. This highlights that ECM proteins are beginning to be recognized as paracrine-signaling molecules, with profound effects on cellular phenotypes that until recently was

restricted to cytokines, growth factors, and hormones.¹³⁷ Of particular relevance, which will be discussed later in this review, some proteins do not change cellular phenotypes in their native conformation, whereas subsequent to a specific PTM, a highly potent and novel function of that protein is revealed. A well-thought example of such cryptic sites is RGD sequences that are either exposed by protease digestion in most collagen species⁸⁷ or even more scholarly exemplified by endostatin, which is a fragment of collagen type XVIII that by cleavage becomes possibly the most powerful anti-angiogenic molecule to date.⁸³

Table 1 lists examples from outstanding research groups which serve to highlight that the matrix encompasses strong signaling motifs that may be revealed during the pathological process. Consequently, the matrix molecules themselves, in addition to cytokines, growth factors, and hormones, become essential players in tissue homeostasis. As the ECM molecules both anchor cells in the right spatial distribution and cell orientation, these structural components may have a dual effect due to their emerging signaling roles.

ECM Remodeling in Cancer and Fibrosis

Cancer and fibrosis share a number of abnormal characteristics of the ECM structure and function, including constitutively high matrix degradation, formation, and turnover. Interestingly, both diseases involve aspects of inflammation and matrix assembly, destruction, and disorganization.^{24,138,139} As illustrated in *Figure 2*, cancer cell metastasis results in extensive ECM remodeling (ECMR), resulting in the release of matrix components, including neoepitopes, into the circulation. ECM components and remodeling enzymes are known to be elevated in the circulation of cancer patients.^{140,141}

The architecture of the tumor-associated ECM is fundamentally different from that of the normal tissue stroma.¹⁴² As an example, type I collagen is situated parallel to the epithelial cells in healthy tissue, but is less organized in the stroma surrounding metastases.¹⁴³ These stromal changes to the ECM promote transformation, tumor growth, motility, and invasion; enhance cancer cell survival; enable metastatic dissemination; and facilitate the establishment of tumor cells at distant sites.¹⁴³ Cancer is caused when the essential rules governing how cells should be organized in a stable manner within all living tissue are disregarded. Uncontrolled cell growth is necessary for cancer formation. Such growth becomes self-directed, leading to a disorganization of the normal tissue architecture, which is known as neoplastic transformation. More than 90% of malignant tumors are epithelial tumors,⁴ occurring where there is a collapse in the boundary between the epithelial and connective tissues that encompass a given organ. Interruption of these tissue boundaries promotes cancer cell migration to nearby blood vessels or the lymph node system, enabling the cells to metastasize to remote organs resulting in multiorgan failure and death.

Fibrosis is an end-stage representation of a repair-response process after an injury. Like cancer, it may lead to serious organ damage.

Fig. 2. Schematic representation of the high extracellular matrix remodeling in fibrosis. All steps involve extracellular matrix (ECM) remodeling that generates unique protein degradation fingerprints. These enzymes degrade the ECM, releasing smaller fragments of protein from the ECM into the circulation. Interestingly, many of the same processes occur in both fibrosis and cancer.

The development of liver fibrosis resembles the process of wound healing, including the three essential phases after tissue injury: inflammation, synthesis of collagenous and noncollagenous ECM components, and tissue remodeling. Fibrosis may begin in response to various acute or chronic stimuli, including infections, autoimmune reactions, toxins, radiation, and mechanical injury.¹⁴⁴ The pathogenic process driving fibrogenesis is believed to be a dynamic series of events involving complex cellular and molecular mechanisms evolving from the acute or chronic activation of tissue repair that follows repeated tissue injury.⁵ In the case of liver fibrosis, these stimuli give rise to a series of events that involve several cell types working in synergy toward irreversible damage of the liver.¹⁴⁵

Identification and characterization of the cell types and the different mediators involved in liver fibrogenesis have expanded significantly during recent years.^{146–148} Hepatic stellate cells (HSCs) have been identified as the driving force of liver fibrosis. When HSCs are activated by inflammatory mediators,¹⁴⁹ they differentiate into hepatic myofibroblast-like cells (hMFB) capable of expression and secretion of several connective tissue components (for example, collagens, elastin, proteoglycans, and hyaluronan).^{149,150} HSCs are believed to be the main source of ECM proteins accumulated in the liver during chronic liver disease. Recent research has clearly demonstrated that other cell types contribute to the hMFB-pool.^{151–153} These cells can come from local sources such as portal myofibroblasts¹⁵⁴ or, may be, newly formed HSCs that originate from a process called EMT, in which biliary epithelial cells or hepatocytes transform into fibroblasts.¹⁵⁵ In addition, contributions to the hMFB-pool come from outside the liver from cells like bone marrow¹⁵⁶ and circulating

fibrocytes.¹⁵⁷ The bone marrow-derived myofibroblasts have been shown to be of a surprisingly large importance, as they can transdifferentiate into epithelial cells.^{158–161} The accumulation of fibrous tissue and myofibroblast contraction in the liver leads to mechanical increase of hepatic vascular resistance to portal vein blood.^{159,162} This in turn leads to loss of oxygen to the surrounding tissue, facilitating neoangiogenesis as HSCs and Kupffer cells begin overexpressing proangiogenic growth factors and cytokines.¹⁶³

The activation of HSCs involves multiple intracellular pathways and gene regulation. Regulation of growth factors plays an important role in HSC activation, with platelet-derived growth factor (PDGF) signaling is the best-characterized pathway leading to HSC activation. Binding of PDGF results in dimerization and phosphorylation of the tyrosine residues in the intracellular domain of the receptor. This activates the Ras/MAPK and the PI3K-AKT/PKB pathways, leading to cellular proliferation.¹⁶⁴ The increased matrix

production by HSCs is controlled by TGF- β , which is the most potent fibrogenic cytokine in the liver, by signaling via Smad proteins.¹⁶⁵ Chemokines induce the NF κ B signaling pathway, leading to further migration and proliferation of HSCs. Continued deposition of matrix proteins is controlled by a positive feedback loop that sustains the inflammatory response and proliferation and migration of HSCs as chemokines interact with immune cells.^{166,167}

Disregulation of ECM homeostasis is also central in the development of fibrosis of the lung, although the origin of fibrogenic precursors remains a subject of debate, and is potentially multifactorial in nature. Activation of resident fibroblasts, recruitment of circulating progenitors such as fibrocytes or other candidate progenitors, and EMT of alveolar epithelia have all been implicated in the formation of activated myofibroblasts^{168–172} in the lung. Consistent with findings in fibrotic liver disease, these activated myofibroblasts produce fibrillar collagens such as type I collagen and other matrix proteins, which apart from promoting remodeling and ultimately scarring the lung parenchyma, drive a sustained cycle of ongoing fibrogenesis, even in the absence of ongoing inflammatory insult. As with liver fibrosis, studies in disease models indicate that TGF- β is a key fibrogenic cytokine. Together with other cytokines, signaling pathways, and matrix proteins, TGF- β contributes to the ongoing disease cascade and destructive remodeling of the lung.^{172–178}

As a biomechanically sensitive organ, the lung could be considered as particularly dependent on the composition and architectural organization of ECM components, including BM collagens such as type IV collagen, structural fibrillar collagens (type I and III), and elastin.¹⁷⁹ The importance of collagen remodeling in resolution of

fibrosis has been demonstrated in models in which inhibition of the lysyl oxidase (LOX) family member, LOXL2, which catalyzes the cross-linking of fibrillar collagen, and thereby increases tissue tension, was sufficient to reverse established fibroblast activation and reduce TGF- β signaling, cytokine production, inflammation, and other markers of profibrogenic imbalance.¹⁴ These findings are consistent with previous data showing that ongoing myofibroblast activation and TGF- β signaling from the latency-associated complex can be driven by altered mechanical tension in a feed-forward loop.^{180,181} Selective inhibition of LOXL2, which is overexpressed in both human fibrotic disease and disease models, may also constitute a therapeutic target. Inhibition of aberrant fibrogenesis, while avoiding inhibition of other LOX family members, such as LOX and LOXL1, may play a critical role in elastin homeostasis in the lung.^{182,183}

Studies in humans and in animal models have suggested that some elements of fibrosis are reversible, and in specific circumstances, restoration to near-normal organ architecture can be achieved.^{184–189} Consequent to these findings is an emerging interest in the fibrosis field with focus on the ECM components. Measurement of the individual molecules gives a deeper understanding of fibrosis and attenuates pathological processes.

Noninvasive biomarkers of liver fibrosis have been sought for decades, and the FibroTest multimarker panel is approved for clinical usage in Europe. However, all of the current markers and panels have limitations,¹⁹⁰ and none have been recommended by the American Association for the Study of Liver Disease (AASLD) to replace liver biopsy.¹⁹¹ Clearly, novel markers are still needed, and measuring neopeptides of ECM proteins composes a snapshot of matrix dynamics that may be of diagnostic and prognostic value.¹⁹² Examples of well-studied liver ECM markers include collagen propeptides, notably PIIINP,¹⁹³ and caspase fragments of cytokeratin 18.¹⁹⁴ A recent mass spectrometry study of the ECM in two rodent models identified 16 different collagens in the liver, and profiled changes in the abundance of collagens and integrins in tumors compared with healthy livers and precancerous fibrotic livers.¹⁹⁵ Neopeptides of these proteins may serve as valuable markers of liver ECMR. Promising candidates have been reported, including those derived from type IV collagen,^{196,197} type I collagen,¹⁹⁸ type V collagen,¹⁹⁹ and type VI collagen.²⁰⁰ Systemic approaches, such as global profiling of serum glycoproteins, have also been utilized,²⁰¹ and this technique is now being validated in rodent models (*e.g.*, Fang *et al.*,²⁰² Blomme *et al.*,²⁰³) and in additional cohorts of liver disease patients.^{204,205}

A range of diseases involve excessive matrix remodeling in specific matrices. For example, in rheumatoid arthritis the turnover of type I, II, and III collagens are highly upregulated in the cartilage and synovium.²⁰⁶ The high turnover of ECM proteins are also found in other diseases, such as:

- osteoarthritis affecting the articular cartilage (type II collagen and aggrecan)⁵⁶
- metabolic bone diseases (type I collagen)^{138,207–210}
- sarcopenia (type VI collagen)^{211–213}
- cancer (basement membrane and desmoplasia)^{4,33,214–216}

- atherosclerosis (type I and III collagens, titin and versican)²¹⁷
- various fibrotic diseases including liver (type I, III, IV, V, VI collagens and biglycan),^{59–61,144,196,197,199,200,218} lung (elastin, type I, III, and V collagen),^{74,220–232} and kidney.²³³

Key lessons on the importance of the structural components of the matrix may be harvested from the genetic mutations that lead to pathologies. Table 2 contains a summary of key structural proteins and their known mutations leading to matrix and tissue failure.^{224,233–271} These disease phenotypes provide pivotal information on proteins important for tissue function, and thus how they are involved in some pathologies of nongenomic disorders, and subsequently how treatments that affect these proteins may counter disease progression.

PTMs in the ECM

PTMs are non-DNA-coded modifications to the composition or structure of proteins, which generate unique parts of a molecule known as neopeptides.¹⁷ Pathologically relevant protein modifications are not restricted to protease activity, although the subpopulation of neopeptides generated through this mechanism may be of paramount importance. Figure 3 depicts a handful of different types of PTMs. Some have been identified and used as biochemical markers as a measure of the disease activity,²⁷² but also as contributions to disease process,¹⁷ as they change the functionality of the proteins.

One gene, 1,000 protein subtypes. The importance of PTMs is best described by the fact that one gene may result in 1,000 different and unique proteins with different functional implications. This is illustrated in Figure 3. Here, modifications to amino acids by specific PTMs or degradation of the protein result in both immunologically different as well as functionally different proteins. Measurement of the same protein may provide highly different information, such as either protein formation or protein degradation, which obviously entails opposite information. Some pathologies may further modify the protein specifically, and thus give a specific protein fingerprint of pathology such as glycosylation of hemoglobin resulting in HbA1c type I diabetes.²⁷³ A long line of evidence suggests that measurement of the intact protein provides some information, and measurement of one of these subforms of a protein provides different and more pathologically-relevant information. These have been carefully described for C-reactive protein (CRP),²⁷⁴ type I and XVI collagens,^{275,276} osteocalcin,²⁷⁷ and a selection of other analytes. Thus, it is becoming increasingly clear that measurement of a given pathologically-modified protein enables refinement of clinical chemistry and diagnostic procedures. Most likely the best example is hemoglobin, in which the modification of glycosylation is the gold standard marker of diabetes; thus, the intact protein is a necessity of life, whereas the PTM-modified protein is a pathology-specific marker.

As will later be described in larger molecular detail, many amino acids are amenable to specific modifications (citrullinations,

Table 2. Genetic Mutations in Structural Proteins Leading to Distinct Pathologies

Protein	Disease	Reference
Type I collagen	Osteogenesis imperfecta, Ehlers-Danlos syndrome type VII	234,235
Type II collagen	Several chondrodysplasias, osteoarthritis	236–239
Type III collagen	Ehlers-Danlos syndrome type IV, aortic aneurysms	240,241
Type IV collagen	Kidney fibrosis, Alport syndrome	233,242–244
Type V collagen	Ehlers-Danlos syndrome type I and II	245,246
Type VI collagen	Bethlem myopathy, Ullrich congenital muscular dystrophy	247
Type VII collagen	Epidermolysis bullosa dystrophica	248
Type IX collagen	MED	249
Type X collagen	SMCD and Japanese-type SMD	250,251
Type XV collagen	Cardiac and muscle phenotypes	243
Type XVII collagen	Growth retardation	243
Type XVIII collagen	Renal filtration defects	243
Elastin	Lung, skin and arterial defects, SVAS, WBS, CL	224,252,253
Laminin	Alport syndrome	233
Biglycan	Cardiovascular disease, osteoporosis	254–256
Biglycan/decorin	Osteopenia and skin fragility	257
Biglycan/fibromodulin	Osteoarthritis	258
Perlecan	Multiple developmental defects and myotonia. Schwartz-Jampel syndrome	243
Nidogen 1 and 2	Lung and kidney development	243
Fibromodulin	Osteoarthritis	259
Lumican/fibromodulin	Joint laxity and impaired tendon integrity	260,261
Lumican	Reduced corneal transparency and skin fragility	262
Decorin	Intestinal tumor; skin fragility; Ehlers-Danlos syndrome-like.	263–265
Mimecan	Colorectal cancer early formation	266
Fibrillin	Marfan syndrome	267
COMP	PSACH and MED	268–270
Matrillin-3	MED	271

MED, multiple epiphyseal dysplasia; SMCD, Schmid-type metaphyseal chondrodysplasia; SMD, spondylometaphyseal dysplasia; SVAS, supraaortic stenosis; WBS, Williams-Beuren syndrome; CL, cutis laxa; COMP, Cartilage oligomeric matrix protein; PSACH, pseudoachondroplasia.

phosphorylations, acetylations, methylations, nitrosylations, and glycosylations¹⁷). These modifications can have both positive and negative impacts on the function of the protein, and even target the protein for degradation. In addition, many proteins are born with a propeptide that needs to be cleaved before the protein is in the active configuration that being enzymatic activity or structural enablement. Both N- and C-terminal propeptides are present, which may be further modified, and thus is a waste underestimation of the complexity of these peptides. The degradation products of proteins may be the specific action of pathological-specific enzymes, and there is an accumulating amount of evidence suggesting that different fragments of the same protein may have different physiological and pathophysiological meanings.²⁷⁸ Lastly, polymerization may both be understood as aggregates of the same protein such as hyperphosphorylated Tau or cross-linked collagens, but also pentameric CRP. Each of these subpools obviously holds unique information.

Cross-linking. Cross-linking plays an important role in the ECM meshwork and thereby in tissue integrity. Cross-linking between different ECM components or between different protein chains can result from enzymatic and nonenzymatic pathways. Enzymatic cross-linking is often processed by members of the LOX enzyme family, whose members have been shown to promote the linearization of interstitial collagens which stiffen the tissues, thus leading to neoplastic progression of tumor cells.^{280–283} Interestingly, this matrix stiffness was associated with different phenotypes and enhanced mechanoresponsiveness of the epithelium.^{280,281} Therefore, cross-linking plays an important part in both the initiation and progression of metastasis. Similarly, in fibrotic disease, increases in tissue tension mediated by cross-linking can lead to activation of TGF- β signaling from the latency-associated complex and other signaling changes, driving a fibrogenic feed-forward loop.

Valuable assays for evaluation of bone- and cartilage-related diseases have been developed using antibodies highly specific for protease-cleaved sites in type I collagen²⁷⁶ and type II collagen,²⁸⁴ respectively. The antibodies in these assays also assess the cross-linking between the lysines in the epitopes. C-terminal telopeptide of type I collagen (CTX-I) is an 8-amino acid fragment from the C-telopeptide of type I collagen generated by cathepsin K activity, and the rate of its release from bone is a useful reflection of the resorbing activity of osteoclasts.²⁸⁵ Measuring this fragment is useful for the evaluation of treatment efficacy in bone diseases such as osteoporosis.²⁸⁶ The CTX epitope contains an aspartylglycine motif (DG) that is prone to spontaneous isomerization. In other words, EKAHD(α)GGR epitopes

Fig. 3. Schematic figure of the modifications made to a protein that causes unique subpools to be generated, which each may entail specific pathological or physiological information.

are released during degradation of newly synthesized type I collagen, whereas EKAHD(β)GGR epitopes are released from matured type I collagen. It has been established that the α/β ratio is a useful measure of the age of bone tissue;^{287–289} the lower the ratio, the older the bone tissue.²⁹⁰ Further, the lysine residue of the CTX residue is cross-linked. *Figure 4* outlines schematically how assessment of both a cross-linked and cathepsin K-degraded epitope may be undertaken through the use of sandwich enzyme-linked immunosorbent assay (ELISA) technology. Additional ECM assays may be constructed by a similar approach, to include as much possible information of protein subtype as possible. Resorption rates of newly synthesized collagen type I can be assessed by specific immunoassays targeting the detection of α CTX in urine samples.²⁹¹ Degradation rate of matured, isomerized collagen can be estimated by another specific assay targeting β CTX in both urine and serum samples.²⁷⁶

Another way of cross-linking is through the actions of tissue transglutaminases (TGs). They play a fundamental role in tissue stabilization by transamidation of glutamine residues of one protein chain to the amino group of a lysine residue in a second protein chain. This results in the formation of the covalent N- γ -glutamyl- ϵ -lysyl-isopeptide bond, which is resistant to proteolytic degradation.²⁹² As several ECM proteins, such as collagens, fibronectin, laminin, and vimentin, act as substrates for TGs, they are involved in physiologic tissue integrity while being associated with various pathologies, including neurodegenerative diseases, cancer, inflammation, and fibrosis. In fibrosis, TGF- β promotes activation of TG cross-linking, thereby reducing the ECM turnover, leading to deposition and accumulation of ECM proteins, and thus stabilizing the ECM network and facilitating proteolytic resistance. In cancer, intracellular cross-linking by TG2 has been shown to be both pro- and antiapoptotic, and favoring cell survival, invasion, and motility by the close association with surface integrins.^{293,294}

Oxidations and hydroxylations. Oxidative damage to proteins is often caused by the action of free radicals, reactive oxygen species

Fig. 4. Development of an assay to detect a cancer-specific double neoepitope. (A) An enzyme, most likely an MMP, cleaves collagen molecules. This produces a cut in the peptide sequence, exposing an N- and C-terminal-truncated molecule. (B) Lysyl oxidase family members are highly upregulated in many cancers. This family of enzymes enzymatically cross-links the lysines in the collagen chains, resulting in stiffened tissue. In the local area of cancer metastasis and growth, these processes are occurring at a more rapid pace than in other parts of the body, resulting in increased expression of a range of collagen, proteases, and other enzymes. (C) The processes of protease generation and lysine cross-linking are combined. (D) Design and generation of a sandwich assay to detect both the lysine cross-link and the protease-generated degradation product. Thus, this type of ELISA contains more information than traditional assays (*i.e.*, both degradation and cross-link information).

(ROS) and reactive nitrogen species such as hydrogen peroxide and nitric oxide, generated in cells by the mitochondrial respiratory chain.²⁹⁵ Oxidizing PTMs have been implicated in several pathological and healthy tissue turnover processes. Although many amino acids can be attacked by ROS, some seem more likely to undergo oxidation than others. For example, lysine and proline are readily oxidized to aldehydes; methionine is sulfoxidized; and tyrosines are nitrosylated.²⁹⁶ Under normal conditions, these ROS are strictly regulated by antioxidants, such as peroxidases and dimutases among others.²⁹⁷ However, under pathological conditions, oxidation may be

implicated in tissue destruction. The role of ROS in almost all aspects of cancer initiation and development^{139,295,298–303} is still debated. Measurement of specific components of the ECM that hold these oxidized PTMs may be useful for both early diagnosis and prognosis of cancer.

Protease-generated neoepitopes. Matrix remodeling at specific disease stages results in both elevated levels of, and uniquely modified, proteins. Endopeptidases, such as MMPs and cysteine proteases, play major roles in the degradation of extracellular macromolecules such as collagens and proteoglycans. Specific proteolytic activities are a prerequisite for a range of cellular functions and interactions with the ECM, resulting in the generation of specific cleavage fragments. Even though many components of the ECM, as well as enzymes responsible for remodeling, are present in different tissues, the combination of a specific peptidase and specific ECM protein may provide a unique combination that elucidates activity in a particular tissue or a specific disease mechanism.

One often-taught example of protease degradation of a given tissue is that of joint degenerative diseases. Joint degenerative diseases lead to alterations in the metabolism of the articular cartilage and subchondral bone.^{278,304–309} Cartilage is for the most part composed of collagen type II, which accounts for 60%–70% of the dry weight of cartilage, and proteoglycans accounting for 10% of the dry weight, of which aggrecan is the most abundant.³¹⁰ Since type II collagen is the most abundant protein in cartilage, several different degradation fragments of collagen type II have been indicated as useful for monitoring degenerative diseases of the cartilage.^{272,311} C-terminal telopeptide of type II collagen (CTX-II) is an MMP-generated neoepitope derived from the C-terminal part of type II collagen,³¹⁰ and measurement of CTX-II is highly useful for monitoring degradation of type II collagen in experimental setups assessing cartilage degradation.^{278,312} Examples of a range of protease-generated neoepitopes have already been described in the literature, but they have not been utilized by applied science to produce quantifiable methods of disease assessment. Assays detecting a few neoepitopes that have been developed and that are used in both clinical and preclinical studies were reviewed recently.³¹³

To some extent, C-terminal telopeptide of type I collagen (ICTP) and MMP-derived fragments of type I collagen assays^{53,54,314–316} as an indicator of cancer progression have been developed and used in prognosis of lung and ovarian cancers. A range of biochemical markers based on degradation products of the ECM, particularly collagen, may be identified and used in cancer. The collagen composition of the BM and interstitial matrix may be relevant for the development of the given marker for the ECMR associated with soft tissue metastasis.

Isomerization: Advanced glycation end product of ECM proteins. Proteins containing aspartate (D), asparagine (N), glutamate (E), or glutamine (Q) residues linked to a low-molecular-weight amino acid, such as glycine (G), can undergo spontaneous nonenzymatic isomerization.¹⁵ This isomerization introduces a kink in the conformation

of the molecule, as the peptide backbone is redirected from the γ -carboxyl group in the native newly synthesized form to the side chain γ -carboxyl.²⁹⁰ Peptides that contain amino acid isomerizations are often resistant to proteolysis.^{317,318} This feature affects the processing of antigens for presentation on the major histocompatibility complex II during the immune response signaling for the production of T-cells and antibodies.¹⁵ In preclinical studies, it has been shown that various known autoantigens contain sites prone to deamidation and isomerization. These autoantigens are involved in type I diabetes, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and experimental autoimmune encephalomyelitis.^{317,319–322}

The C-telopeptide of type I collagen marker CTX-I is a marker of bone resorption. It has been shown that assessment of the non-isomerized epitope (α CTX-I) is more sensitive as a marker for bone metastases secondary to breast and prostate cancer than the isomerized epitope (β CTX-I).²⁰⁷ This is due to the high ECMR of type I collagen in the bone area invaded by cancer cells, and thus a high amount of newly formed nonisomerized collagen type I undergoes resorption by osteoclasts.

Nonenzymatic glycosylation. Nonenzymatic glycosylation is also called the Maillard reaction, and leads to PTMs of proteins, nucleic acids, and lipids.²⁷³ A common cause of nonenzymatic glycosylation is increased blood glucose levels, and accordingly, most knowledge about nonenzymatic glycosylation arises from studies performed in diabetics.²⁷³ The marker HbA1c is an established PTM marker in type II diabetes. Recently, advanced glycation end products (AGEs) have been implicated in cancers. The nicotine-induced accumulation of AGEs is a cause of cancer.³²³ The receptor for AGEs, called RAGE, is currently under intense investigation as both a marker and an inducer of cancer³²⁴ and to assess whether there is a link between chronic inflammation and cancer, since inflammatory mediators can both be pro- and antitumorigenic.^{139,216,324,325}

Citrullination. Citrullination or deimination is the term used for the PTM of the amino acid arginine, which can transform into the amino acid citrulline. The change is facilitated by peptidylarginine deiminases (PADs).^{326,327} The conversion of arginine into citrulline can have important consequences for the structure and function of proteins, since arginine is positively charged at a neutral pH, whereas citrulline is uncharged. The positive charge increases the hydrophobicity of the protein, leading to changes in protein folding.

Histone deacetylase 1 (HDAC1) inhibitors are currently under development for the treatment of certain cancers, particularly breast cancer.²¹ Histone lysine and arginine residues contain a wide array of PTM-producing processes, including methylation, citrullination, acetylation, ubiquitination, and sumoylation. The combined action of these modifications regulates critical DNA processes, including replication, repair, and transcription. In addition, enzymes that modify histone lysine and arginine residues have been correlated with not only cancer but also arthritis, heart disease, diabetes, and neurodegenerative disorders.^{328,329}

Histone methylation plays a key role in regulating the chromatin structure and function. The recent identification of enzymes that antagonize or remove histone methylation offers new insights into histone methylation plasticity in the regulation of epigenetic pathways. Peptidylarginine deiminase 4 (PADI4; also known as PAD4) was the first enzyme shown to antagonize histone methylation. PADI4 functions as a histone deiminase, converting a methylarginine residue to citrulline at specific sites on the tails of histones H3 and H4. PADI4 associates with HDAC1.^{328–330}

Novel Techniques Currently Available for Assessing the Structure of the ECM

Clinical biochemistry provides a battery of assessments for profiling tissue turnover profiles. A range of serological assessments have been developed to investigate some of the key structural proteins of the ECM (Table 3).^{56,60,129,144,196–200,217,274,276,278,311,314,331–347} Measurement of these proteins may provide key information in clinical settings on the tissue turnover profile, and thereby assists in patient diagnosis, in identification of those patients in most need of treatment, and finally, in monitoring of clinical efficacy of interventions. These technologies may also be used in preclinical settings, in *ex vivo* and *in vitro* cultures, to determine the molecular mode of action in the assembly and maintenance of the matrix.³¹¹

An example of a combined aged, cross-linked, and cleaved neoepitope for the evaluation of bone metastases. The relationship between skeletal tumor load and elevations in serum or urine levels of α CTX and seven other biomarkers related to bone turnover has been investigated in a pooled group of breast and prostate cancer patients.²⁰⁷ Patients were stratified according to the Soloway score:

- Score 0: 0 bone metastases
- Score 1: <6 bone metastases
- Score 2: 6–20 bone metastases
- Score 3: >20 bone metastases
- Score 4: superscan showing that >75% ribs, vertebrae, and pelvic bone are affected.

In breast cancer patients, a strong linear association was observed between bone metastases and all biomarkers, except osteoprotegerin and receptor activator of nuclear factor κ B ligand (Figure 5). All six remaining markers were significantly elevated in patients with a Soloway score of 1. The relative

Table 3. Currently Available Serological Markers Assessing the Structure of the Extracellular Matrix

Name of protein fragment	ECM component	Reference
C1M	MMP-mediated type I collagen degradation	198
C2M	MMP-mediated type II collagen degradation	56
C3M	MMP-mediated type III collagen degradation	60
C4M	MMP-mediated type IV collagen degradation	197
C5M	MMP-mediated type V collagen degradation	331
C6M	MMP-mediated type VI collagen degradation	200
P1NP	Type I collagen formation in tissues other than bone	144
P4NP 7S	Type IV collagen formation	196
P5CP	Type V collagen formation	199
PIIANP	Type II collagen formation	332,333
PIIINP	Type III collagen formation	334
VICM	MMP-mediated citrullinated vimentin degradation	335
CRPM	MMP-mediated CRP degradation	274
ELM	MMP-mediated elastin degradation	217
BGM	MMP-mediated biglycan degradation	336
MIM	MMP-mediated mimican degradation	129
VCANM	MMP-mediated versican degradation	337
TIM	MMP-mediated titin degradation	338
Aggrecan	MMP- and aggrenase-cleaved aggrecan	278
COMP	Intact COMP	339
Osteocalcin	Intact osteocalcin bone formation	340
HA	Hyalonic acid	334
ICTP	MMP-mediated type I collagen destruction	314
CTX-I	Cathepsin K degraded type I collagen	276,311,341,342
CTX-II	MMP-degraded type II collagen	343,344
C2C	MMP-degraded type II collagen	345
TELO-I	Citrullinated carboxyterminal telopeptides of type I collagen	346
TELO-II	Citrullinated carboxyterminal telopeptides of type II collagen	346
MCV	Mutated citrullinated vimentin	347
ECM, extracellular matrix; MMP, matrix metalloproteinase; CRP, C-reactive protein.		

percent increases in biomarker levels in the presence of bone metastases were most pronounced for α CTX-I, which was elevated by more than 600% in patients with Soloway score of 3. The next highest increases were in bone-specific alkaline phosphatase and N-telopeptide of type I collagen (NTX), which were elevated by 470% and 440% at Soloway score 3, respectively. These findings were supported by observations in prostate cancer patients, which showed that of the seven biomarkers, α CTX-I was the most sensitive for bone metastases.³⁴⁸ The higher sensitivity of α CTX-I could be explained by the fact that this epitope is released from sites of high bone remodeling, where collagen fibrils do not have time to mature and undergo β -isomerization. The α CTX-I epitope was located by immunostaining adjacent sections of bones invaded by breast cancer or prostate cancer,²⁰⁸ and at the sites of high bone remodeling.

These data support that careful selection of matrix constituents and, in particular, those that carry one or more PTMs such as isomerization in a type I collagen fragment generated by cathepsin K as described in this example may be superior markers reflecting pathological, including malignant, events in the ECM.

An example of MMP-degraded type III collagen for the assessment of liver fibrosis. The central pathological change in fibrosis is uncontrolled ECMR.^{349,350} During fibrogenesis, the quantity and composition of matrix proteins in the liver change, resulting in excessive

accumulation of fibrous (scar) tissue and an overall increase in the ECM density.³⁵¹ ECM matrix proteins in a normal liver are distributed mainly in the portal tracts, whereas a BM-like matrix is located in the perisinusoidal space of Disse. The most abundant collagens in the liver are type I and III collagens, which by immunohistochemistry are found predominantly in the perisinusoidal spaces, in portal tracts, and in subcapsular areas.^{5,352} The ECM of the cirrhotic liver contains approximately six times as much matrix as the normal liver,³⁵³ which is a result of increased levels of type I, III, and IV collagens.³⁵⁴ However, levels of MMPs such as MMP-9 also increase in cirrhosis.^{349,355} The combination of active and overexpressed MMP-9 with the accumulation of type III collagen poses the interesting hypothesis that an MMP-9-generated fragment of type III collagen could be used as a biochemical marker of liver fibrosis.

Type III collagen degradation by MMPs, and even MMP-9 exclusively, may result in many unique fragments, such as those derived from type II collagen and previously published.⁸¹ The CO3-610 (C3M) fragment (KNGETGPQGP) is one of those, and is exclusively derived from MMP-9. When this C3M fragment was assessed in two separate animal models of liver fibrosis, the BDL and CCl₄ animal models, a >200% fold upregulation was observed, as well as a highly significant correlation to portal pressure.^{60,356,357} These data strongly suggest that liver fibrosis is not merely an accumulation of ECM proteins, but a dynamic condition with accelerated ECM turnover, in which both tissue formation and tissue degradation are highly up-regulated. In the case of liver fibrosis, ECM tissue formation outstrips tissue degradation, leading to a net accumulation of scar tissue over time. This example also suggests that PTMs released by protease degradation of proteins may in some cases be more sensitive markers for pathology than intact proteins. This idea is receiving increased attention.¹⁷ This approach has been recently been described as the protein fingerprint technology, in which the different subpools of the total pool of information about one protein during formation or degradation provide distinct and important data.²⁰

PTMs: The Cause or Consequence of the Disease?

Proteins are complex molecules susceptible to numerous PTMs occurring spontaneously during aging or as a consequence of physiologic or pathologic processes. Today, it is well established that PTMs can uncover cryptic epitopes and/or create novel epitopes, to which no tolerance exists.¹⁵ Antigenicity and interactions of proteins with components of the immune system may be profoundly affected by PTMs. Thus, modified self-antigens may be absent (indicating they are not tolerated) during early T-cell selection, and trigger reactions by the immune system as they arise later in life. In turn, this may play a role in the initiation and pathogenesis of autoimmune diseases.¹⁵ Several studies have shown that various types of PTMs are hallmarks of aging and are associated with autoimmune diseases, such as RA, SLE, and type I diabetes.^{15,16,358–375}

The presence of PTMs in several known autoantigens has been reported. Many of these modifications have been implicated in the antigenicity of the proteins, as outlined in Table 4 (modified from Cloos and Christgau¹⁵).^{296,311,319–322,346,347,376–389} These observations

Fig. 5. Relative increases in bone resorption, bone formation, and osteoclastogenesis marker levels as a function of the extent of skeletal metastasis, assessed in 132 patients with breast or prostate cancer. Relative increases are expressed as a percentage of levels in patients with a Soloway score 0.²⁰⁷

Table 4. List of Post-Translational Modifications Involved in Immune Responses in Different Autoimmune Diseases

Autoantigen	Relevant disease/ animal model	Modification	Reference
MBP	MS/EAE	Acetylation	376
		Citrullination	377
		Isomerization	296
		Phosphorylation	378
aB-crystallin	MS/EAE	Citrullination	379,380
		Isomerization	321
		Phosphorylation	381
Type I collagen	RA	Citrullination	346
Type II collagen	RA/CIA	Glycosylation	382
		Protease degradation	311
		Hydroxylation	382
		Citrullination	346
Fibrin	RA	Citrullination	383
Fillagrin	RA	Citrullination	384
Vimentin	RA	Citrullination	347,385
IgG	RA	Isomerization	296
		Glycation	386
Insulin	Type I diabetes	Deamidation	319
		Isomerization	319
GAD	Type I diabetes	Oxidative damage	387
Histone H2B	SLE	Isomerization	322
		Deamidation	388
Transglutamination			388
SnRNP D	SLE	Isomerization	320
SnRNP 70k	SLE	Phosphorylation	389

MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; SLE, systemic lupus erythematosus.

have sparked a growing interest in the role and assessment of PTMs in autoimmune diseases as well as other pathological conditions associated with aging. Whether the presence of PTMs is merely a secondary phenomenon accompanying the disease or a primary event in disease initiation remains to be resolved.

It is noteworthy that T-cell responses to modified antigens in general are very specific.^{390,391} In contrast, autoantibodies recognizing modified proteins tend to be more nonspecific and often cross-react with the native antigen. This B-cell promiscuity may play an important role in the phenomenon of epitope-spreading characteristics of many autoimmune diseases,³⁹² which in part may be the disease driver in illnesses such as RA. These examples serve to highlight that in the immune system, PTMs, in various ways, may initiate, play parts in the pathogenesis, or even constitute the central events in some diseases. Regardless of whether PTMs are the chicken or the egg, these examples further emphasize that PTMs are relevant markers of diseases. Tools developed to measure specific monoclonal antibodies may aid the understanding of the temporal events leading to PTMs, and their role in disease mechanisms.

FUTURE DIRECTIONS

In this review, we have described the key components of the ECM and highlighted recent developments in the identification and measurement of PTMs. There is a growing body of evidence that modifications made to the structural proteins of the matrix may both be a consequence of the disease as well as drivers of disease progression. Thus, PTMs to specific ECM proteins may be more integrated in pathogenesis than previously thought. Indeed, the matrix serves as much more than just a structural framework for tissues.

Fibrosis and cancer involve signature proteins and enzymes. These enzymes degrade the ECM and create a range of other PTMs, releasing smaller fragments of ECM proteins into the circulation. An optimal biochemical marker may be designed by identifying the common denominator of specific pathophysiological processes to determine the marker's tissue specificity and sensitivity. Different cells of a particular tissue predominately express given proteases that in combination with different signature proteins from different host tissues, which may provide optimal selective markers for connective tissue diseases. Biochemical markers based on the advanced disease/tissue neopeptide approach could become an important tool to be used in combination with others for diagnosing and staging disease as well as assessing efficacy and safety of new therapeutic interventions.

ACKNOWLEDGMENTS

We acknowledge funding from the Danish Ministry of Science, Technology, and Science, and the Danish Science Foundation (Den Danske Forskningsfond).

DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Dolberg DS, Bissell MJ: Inability of Rous sarcoma virus to cause sarcomas in the avian embryo. *Nature* 1984;309:552-556.

2. Mintz B, Illmensee K: Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc Natl Acad Sci USA* 1975;72:3585–3589.
3. Ruiz PA, Jarai G: Discoidin domain receptors regulate the migration of primary human lung fibroblasts through collagen matrices. *Fibrogenesis Tissue Repair* 2012;5:3.
4. Ingber DE: Can cancer be reversed by engineering the tumor microenvironment? *Semin Cancer Biol* 2008;18:356–364.
5. Schuppan D, Ruehl M, Somasundaram R, Hahn EG: Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis* 2001;21:351–372.
6. Condeelis J, Pollard JW: Macrophages: obligate partners for tumor cell migration, invasion, metastasis. *Cell* 2006;124:263–266.
7. Ingman WV, Wyckoff J, Gouon-Evans V, Condeelis J, Pollard JW: Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. *Dev Dyn* 2006;235:3222–3229.
8. Barker HE, Erler JT: The potential for LOXL2 as a target for future cancer treatment. *Future Oncol* 2011;7:707–710.
9. Moreno-Bueno G, Salvador F, Martin A, et al.: Lysyl oxidase-like 2 (LOXL2), a new regulator of cell polarity required for metastatic dissemination of basal-like breast carcinomas. *EMBO Mol Med* 2011;3:528–544.
10. Bignon M, Pichol-Thievent C, Hardouin J, et al.: Lysyl oxidase-like protein-2 regulates sprouting angiogenesis and type IV collagen assembly in the endothelial basement membrane. *Blood* 2011;118:3979–3989.
11. Rodríguez HM, Vaysberg M, Mikels A, et al.: Modulation of lysyl oxidase-like 2 enzymatic activity by an allosteric antibody inhibitor. *J Biol Chem* 2010;285:20964–20974.
12. Kim YM, Kim EC, Kim Y: The human lysyl oxidase-like 2 protein functions as an amine oxidase toward collagen and elastin. *Mol Biol Rep* 2011;38:145–149.
13. Peng L, Ran YL, Hu H, et al.: Secreted LOXL2 is a novel therapeutic target that promotes gastric cancer metastasis via the Src/FAK pathway. *Carcinogenesis* 2009;30:1660–1669.
14. Barry-Hamilton V, Spangler R, Marshall D, et al.: Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat Med* 2010;16:1009–1017.
15. Cloos PA, Christgau S: Post-translational modifications of proteins: implications for aging, antigen recognition, and autoimmunity. *Biogerontology* 2004;5:139–158.
16. Cloos PA, Jensen AL: Age-related de-phosphorylation of proteins in dentin: a biological tool for assessment of protein age. *Biogerontology* 2000;1:341–356.
17. Karsdal MA, Henriksen K, Leeming DJ, et al.: Novel combinations of Post-Translational Modification (PTM) neo-epitopes provide tissue-specific biochemical markers—are they the cause or the consequence of the disease? *Clin Biochem* 2010;43:793–804.
18. Colado MI, Ormazabal MJ, Goicoechea C, et al.: Involvement of central serotonergic pathways in analgesia elicited by salmon calcitonin in the mouse. *Eur J Pharmacol* 1994;252:291–297.
19. Skjot-Arkil H, Barascuk N, Larsen L, et al.: Tumor necrosis factor- α and receptor activator of nuclear factor- κ B ligand augment human macrophage foam-cell destruction of extracellular matrix through protease-mediated processes. *Assay Drug Dev Technol* 2012;10:69–77.
20. Karsdal MA, Delvin E, Christiansen C: Protein fingerprints—relying on and understanding the information of serological protein measurements. *Clin Biochem* 2011;44:1278–1279.
21. Krueger KE, Srivastava S: Posttranslational protein modifications: current implications for cancer detection, prevention, and therapeutics. *Mol Cell Proteomics* 2006;5:1799–1810.
22. Bosques CJ, Raguram S, Sasisekharan R: The sweet side of biomarker discovery. *Nat Biotechnol* 2006;24:1100–1101.
23. Hanash SM, Pitteri SJ, Faca VM: Mining the plasma proteome for cancer biomarkers. *Nature* 2008;452:571–579.
24. Marx J: Cancer research. Inflammation and cancer: the link grows stronger. *Science* 2004;306:966–968.
25. Sawyers CL: The cancer biomarker problem. *Nature* 2008;452:548–552.
26. Spickett CM, Pitt AR, Morrice N, Kolch W: Proteomic analysis of phosphorylation, oxidation and nitrosylation in signal transduction. *Biochim Biophys Acta* 2006;1764:1823–1841.
27. Aumailley M, Gayraud B: Structure and biological activity of the extracellular matrix. *J Mol Med* 1998;76:253–265.
28. Yurchenco PD, Schittny JC: Molecular architecture of basement membranes. *FASEB J* 1990;4:1577–1590.
29. Bosman FT, Stamenkovic I: Functional structure and composition of the extracellular matrix. *J Pathol* 2003;200:423–428.
30. Bissell MJ, Aggeler J: Dynamic reciprocity: how do extracellular matrix and hormones direct gene expression? *Prog Clin Biol Res* 1987;249:251–262.
31. Bissell MJ, Radisky D: Putting tumours in context. *Nat Rev Cancer* 2001;1:46–54.
32. Lochter A, Bissell MJ: An odyssey from breast to bone: multi-step control of mammary metastases and osteolysis by matrix metalloproteinases. *APMIS* 1999;107:128–136.
33. Radisky DC, Bissell MJ: Cancer. Respect thy neighbor! *Science* 2004;303:775–777.
34. Paszek MJ, Zahir N, Johnson KR, et al.: Tensional homeostasis and the malignant phenotype. *Cancer Cell* 2005;8:241–254.
35. Karsdal MA, Larsen L, Engsig MT, et al.: Matrix metalloproteinase-dependent activation of latent transforming growth factor- β controls the conversion of osteoblasts into osteocytes by blocking osteoblast apoptosis. *J Biol Chem* 2002;277:44061–44067.
36. Haass NK, Smalley KS, Herlyn M: The role of altered cell-cell communication in melanoma progression. *J Mol Histol* 2004;35:309–318.
37. O'Brien LE, Jou TS, Pollack AL, et al.: Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. *Nat Cell Biol* 2001;3:831–838.
38. Pedersen JA, Lichter S, Swartz MA: Cells in 3D matrices under interstitial flow: effects of extracellular matrix alignment on cell shear stress and drag forces. *J Biomech* 2010;43:900–905.
39. Pedersen JA, Swartz MA: Mechanobiology in the third dimension. *Ann Biomed Eng* 2005;33:1469–1490.
40. Hynes RO: Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;110:673–687.
41. Hynes RO: Structural biology. Changing partners. *Science* 2003;300:755–756.
42. Hynes RO: The emergence of integrins: a personal and historical perspective. *Matrix Biol* 2004;23:333–340.
43. Hynes RO: Cell-matrix adhesion in vascular development. *J Thromb Haemost* 2007;5 Suppl 1:32–40.
44. Hynes RO: The extracellular matrix: not just pretty fibrils. *Science* 2009;326:1216–1219.
45. Clark EA, King WG, Brugge JS, Symons M, Hynes RO: Integrin-mediated signals regulated by members of the rho family of GTPases. *J Cell Biol* 1998;142:573–586.
46. Clark EA, Brugge JS: Integrins and signal transduction pathways: the road taken. *Science* 1995;268:233–239.
47. Gelse K, Poschl E, Aigner T: Collagens—structure, function, and biosynthesis. *Adv Drug Deliv Rev* 2003;55:1531–1546.
48. Fleischmajer R, MacDonald ED, Perlsh JS, Burgeson RE, Fisher LW: Dermal collagen fibrils are hybrids of type I and type III collagen molecules. *J Struct Biol* 1990;105:162–169.
49. Birk DE, Fitch JM, Babiarz JP, Linsenmayer TF: Collagen type I and type V are present in the same fibril in the avian corneal stroma. *J Cell Biol* 1988;106:999–1008.
50. Jussila T, Kaupilla S, Bode M, et al.: Synthesis and maturation of type I and type III collagens in endometrial adenocarcinoma. *Eur J Obstet Gynecol Reprod Biol* 2004;115:66–74.
51. Parikka V, Vaananen A, Risteli J, et al.: Human mesenchymal stem cell derived osteoblasts degrade organic bone matrix *in vitro* by matrix metalloproteinases. *Matrix Biol* 2005;24:438–447.

52. Santala M, Simojoki M, Risteli J, Risteli L, Kauppila A: Type I and III collagen metabolites as predictors of clinical outcome in epithelial ovarian cancer. *Clin Cancer Res* 1999;5:4091-4096.
53. Ylisirnio S, Sassi ML, Risteli J, Turpeenniemi-Hujanen T, Jukkola A: Serum type I collagen degradation markers, ICTP and CrossLaps, are factors for poor survival in lung cancer. *Anticancer Res* 1999;19:5577-5581.
54. Ylisirnio S, Hoyhtya M, Makitaro R, Paaakko P, Risteli J, Kinnula VL, Turpeenniemi-Hujanen T, Jukkola A: Elevated serum levels of type I collagen degradation marker ICTP and tissue inhibitor of metalloproteinase (TIMP) 1 are associated with poor prognosis in lung cancer. *Clin Cancer Res* 2001;7:1633-1637.
55. Zhu GG, Risteli L, Makinen M, Risteli J, Kauppila A, Stenback F: Immunohistochemical study of type I collagen and type I pN-collagen in benign and malignant ovarian neoplasms. *Cancer* 1995;75:1010-1017.
56. Bay-Jensen AC, Liu Q, Byrjalsen I, Li Y, Wang J, Pedersen C, Leeming DJ, Dam EB, Zheng Q, Qvist P, et al.: Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neopeptide, CIIM—increased serum CIIM in subjects with severe radiographic osteoarthritis. *Clin Biochem* 2011;44:423-429.
57. Kadler KE, Holmes DF, Trotter JA, Chapman JA: Collagen fibril formation. *Biochem J* 1996;316 (Pt 1):1-11.
58. Barascuk N, Vassiliadis E, Larsen L, et al.: Development and validation of an enzyme-linked immunosorbent assay for the quantification of a specific MMP-9 mediated degradation fragment of type III collagen—a novel biomarker of atherosclerotic plaque remodeling. *Clin Biochem* 2011;44:900-906.
59. Vassiliadis E, Larsen DV, Clausen RE, et al.: Measurement of CO3-610, a potential liver biomarker derived from matrix metalloproteinase-9 degradation of collagen type III, in a rat model of reversible carbon-tetrachloride-induced fibrosis. *Biomark Insights* 2011;6:49-58.
60. Barascuk N, Veidal SS, Larsen L, et al.: A novel assay for extracellular matrix remodeling associated with liver fibrosis: an enzyme-linked immunosorbent assay (ELISA) for a MMP-9 proteolytically revealed neo-epitope of type III collagen. *Clin Biochem* 2010;43:899-904.
61. Attallah AM, Mosa TE, Omran MM, et al.: Immunodetection of collagen types I, II, III, and IV for differentiation of liver fibrosis stages in patients with chronic HCV. *J Immunoassay Immunochem* 2007;28:155-168.
62. Kruegel J, Miosge N: Basement membrane components are key players in specialized extracellular matrices. *Cell Mol Life Sci* 2010;67:2879-2895.
63. Birk DE: Type V collagen: heterotypic type I/IV collagen interactions in the regulation of fibril assembly. *Micron* 2001;32:223-237.
64. Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE: Collagen: an overview. *Implant Dent* 2002;11:280-285.
65. Wenstrup RJ, Florer JB, Brunskill EW, et al.: Type V collagen controls the initiation of collagen fibril assembly. *J Biol Chem* 2004;279:53331-53337.
66. Fessler LI, Brosh S, Chapin S, Fessler JH: Tyrosine sulfation in precursors of collagen V. *J Biol Chem* 1986;261:5034-5040.
67. Engvall E, Hessler H, Klier G: Molecular assembly, secretion, and matrix deposition of type VI collagen. *J Cell Biol* 1986;102:703-710.
68. Yanagishita M: Function of proteoglycans in the extracellular matrix. *Acta Pathol Jpn* 1993;43:283-293.
69. Monfort J, Tardif G, Reboul P, et al.: Degradation of small leucine-rich repeat proteoglycans by matrix metalloprotease-13: identification of a new biglycan cleavage site. *Arthritis Res Ther* 2006;8:R26.
70. Bock HC, Michaeli P, Bode C, et al.: The small proteoglycans decorin and biglycan in human articular cartilage of late-stage osteoarthritis. *Osteoarthritis Cartilage* 2001;9:654-663.
71. Lu YP, Ishiwa T, Kawahara K, et al.: Expression of lumican in human colorectal cancer cells. *Pathol Int* 2002;52:519-526.
72. Merline R, Schaefer RM, Schaefer L: The matricellular functions of small leucine-rich proteoglycans (SLRPs). *J Cell Commun Signal* 2009;3:323-335.
73. Duca L, Floquet N, Alix AJ, Haye B, Debelle L: Elastin as a matrikine. *Crit Rev Oncol Hematol* 2004;49:235-244.
74. Senior RM, Griffin GL, Mecham RP, et al.: Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes. *J Cell Biol* 1984;99:870-874.
75. Good DJ, Polverini PJ, Rastinejad F, et al.: A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. *Proc Natl Acad Sci USA* 1990;87:6624-6628.
76. Lowin T, Straub RH: Integrins and their ligands in rheumatoid arthritis. *Arthritis Res Ther* 2011;13:244.
77. Asch AS, Barnwell J, Silverstein RL, Nachman RL: Isolation of the thrombospondin membrane receptor. *J Clin Invest* 1987;79:1054-1061.
78. Wang XQ, Frazier WA: The thrombospondin receptor CD47 (IAP) modulates and associates with alpha2 beta1 integrin in vascular smooth muscle cells. *Mol Biol Cell* 1998;9:865-874.
79. Pfister RR, Haddox JL, Sommers CI, Lam KW: Identification and synthesis of chemotactic tripeptides from alkali-degraded whole cornea. A study of N-acetyl-proline-glycine-proline and N-methyl-proline-glycine-proline. *Invest Ophthalmol Vis Sci* 1995;36:1306-1316.
80. Weathington NM, van Houwelingen AH, Noerager BD, et al.: A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. *Nat Med* 2006;12:317-323.
81. Mundel TM, Kalluri R: Type IV collagen-derived angiogenesis inhibitors. *Microvasc Res* 2007;74:85-89.
82. Hamano Y, Zeisberg M, Sugimoto H, et al.: Physiological levels of tumstatin, a fragment of collagen IV alpha3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via alphaV beta3 integrin. *Cancer Cell* 2003;3:589-601.
83. O'Reilly MS, Boehm T, Shing Y, et al.: Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997;88:277-285.
84. Dhanabal M, Ramchandran R, Waterman MJ, et al.: Endostatin induces endothelial cell apoptosis. *J Biol Chem* 1999;274:11721-11726.
85. Karumanchi SA, Jha V, Ramchandran R, et al.: Cell surface glypicans are low-affinity endostatin receptors. *Mol Cell* 2001;7:811-822.
86. Shi H, Huang Y, Zhou H, et al.: Nucleolin is a receptor that mediates antiangiogenic and antitumor activity of endostatin. *Blood* 2007;110:2899-2906.
87. Ruoslahti E: RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol* 1996;12:697-715.
88. Buckley CD, Pilling D, Henriquez NV, et al.: RGD peptides induce apoptosis by direct caspase-3 activation. *Nature* 1999;397:534-539.
89. Mormone E, Lu Y, Ge X, Fiel MI, Nieto N: Fibromodulin, an oxidative stress-sensitive proteoglycan, regulates the fibrogenic response to liver injury in mice. *Gastroenterology* 2012;142:612-621.
90. Mydel P, Shipley JM, ir-Kirk TL, et al.: Neutrophil elastase cleaves laminin-332 (laminin-5) generating peptides that are chemotactic for neutrophils. *J Biol Chem* 2008;283:9513-9522.
91. ir-Kirk TL, Atkinson JJ, Broekelmann TJ, et al.: A site on laminin alpha 5, AQARSAASKVKVSMKF, induces inflammatory cell production of matrix metalloproteinase-9 and chemotaxis. *J Immunol* 2003;171:398-406.
92. Freitas VM, Vilas-Boas VF, Pimenta DC, et al.: SIKVAV, a laminin alpha1-derived peptide, interacts with integrins and increases protease activity of a human salivary gland adenoid cystic carcinoma cell line through the ERK 1/2 signaling pathway. *Am J Pathol* 2007;171:124-138.
93. Song T, Choi CH, Cho YJ, et al.: Expression of 67-kDa laminin receptor was associated with tumor progression and poor prognosis in epithelial ovarian cancer. *Gynecol Oncol* 2012;125:427-432.
94. Di GC, Grottesi A, Lavecchia A: Conformational switch of a flexible loop in human laminin receptor determines laminin-1 interaction. *Eur Biophys J* 2012;41:353-358.
95. Wu F, Vij N, Roberts L, et al.: A novel role of the lumican core protein in bacterial lipopolysaccharide-induced innate immune response. *J Biol Chem* 2007;282:26409-26417.
96. Funderburgh JL, Mitschler RR, Funderburgh ML, et al.: Macrophage receptors for lumican. A corneal keratan sulfate proteoglycan. *Invest Ophthalmol Vis Sci* 1997;38:1159-1167.

97. Carlson EC, Lin M, Liu CY, et al.: Keratocan and lumican regulate neutrophil infiltration and corneal clarity in lipopolysaccharide-induced keratitis by direct interaction with CXCL1. *J Biol Chem* 2007;282:35502–35509.
98. Vij N, Roberts L, Joyce S, Chakravarti S: Lumican suppresses cell proliferation and aids Fas-Fas ligand mediated apoptosis: implications in the cornea. *Exp Eye Res* 2004;78:957–971.
99. Schaefer L, Babelova A, Kiss E, et al.: The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. *J Clin Invest* 2005;115:2223–2233.
100. Babelova A, Moreth K, Tsalas-Greul W, et al.: Biglycan, a danger signal that activates the NLRP3 inflammasome via toll-like and P2 $\times$ receptors. *J Biol Chem* 2009;284:24035–24048.
101. Kitaya K, Yasuo T: Dermatan sulfate proteoglycan biglycan as a potential selectin L/CD44 ligand involved in selective recruitment of peripheral blood CD16(–) natural killer cells into human endometrium. *J Leukoc Biol* 2009; 85:391–400.
102. Sjoberg AP, Manderson GA, Morgelin M, et al.: Short leucine-rich glycoproteins of the extracellular matrix display diverse patterns of complement interaction and activation. *Mol Immunol* 2009;46:830–839.
103. Nili N, Cheema AN, Giordano FJ, et al.: Decorin inhibition of PDGF-stimulated vascular smooth muscle cell function: potential mechanism for inhibition of intimal hyperplasia after balloon angioplasty. *Am J Pathol* 2003;163:869–878.
104. Kolb M, Margetts PJ, Sime PJ, Gauldie J: Proteoglycans decorin and biglycan differentially modulate TGF- β -mediated fibrotic responses in the lung. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1327–L1334.
105. Tufvesson E, Westergren-Thorsson G: Tumour necrosis factor- α interacts with biglycan and decorin. *FEBS Lett* 2002;530:124–128.
106. Guidetti GF, Bartolini B, Bernardi B, et al.: Binding of von Willebrand factor to the small proteoglycan decorin. *FEBS Lett* 2004;574:95–100.
107. Inkson CA, Ono M, Bi Y, et al.: The potential functional interaction of biglycan and WISP-1 in controlling differentiation and proliferation of osteogenic cells. *Cells Tissues Organs* 2009;189:153–157.
108. Moreno M, Munoz R, Aroca F, et al.: Biglycan is a new extracellular component of the Chordin-BMP4 signaling pathway. *EMBO J* 2005;24:1397–1405.
109. Chen XD, Fisher LW, Robey PG, Young MF: The small leucine-rich proteoglycan biglycan modulates BMP-4-induced osteoblast differentiation. *FASEB J* 2004; 18:948–958.
110. Tufvesson E, Westergren-Thorsson G: Biglycan and decorin induce morphological and cytoskeletal changes involving signalling by the small GTPases RhoA and Rac1 resulting in lung fibroblast migration. *J Cell Sci* 2003; 116:4857–4864.
111. Brandon E, Retamal C, Cabello-Verrugio C, Marzolo MP: The low density lipoprotein receptor-related protein functions as an endocytic receptor for decorin. *J Biol Chem* 2006;281:31562–31571.
112. Goldoni S, Humphries A, Nystrom A, et al.: Decorin is a novel antagonistic ligand of the Met receptor. *J Cell Biol* 2009;185:743–754.
113. Desnoyers L, Arnott D, Pennica D: WISP-1 binds to decorin and biglycan. *J Biol Chem* 2001;276:47599–47607.
114. Comalada M, Cardo M, Xaus J, et al.: Decorin reverses the repressive effect of autocrine-produced TGF- β on mouse macrophage activation. *J Immunol* 2003;170:4450–4456.
115. Schaefer L, Tsalas W, Babelova A, et al.: Decorin-mediated regulation of fibrillin-1 in the kidney involves the insulin-like growth factor-I receptor and Mammalian target of rapamycin. *Am J Pathol* 2007;170:301–315.
116. Seidler DG, Goldoni S, Agnew C, et al.: Decorin protein core inhibits *in vivo* cancer growth and metabolism by hindering epidermal growth factor receptor function and triggering apoptosis via caspase-3 activation. *J Biol Chem* 2006;281:26408–26418.
117. Iacob D, Cai J, Tsonis M, et al.: Decorin-mediated inhibition of proliferation and migration of the human trophoblast via different tyrosine kinase receptors. *Endocrinology* 2008;149:6187–6197.
118. Fiedler LR, Schonherr E, Waddington R, et al.: Decorin regulates endothelial cell motility on collagen I through activation of insulin-like growth factor I receptor and modulation of α 2 β 1 integrin activity. *J Biol Chem* 2008; 283:17406–17415.
119. Wight TN, Merrilees MJ: Proteoglycans in atherosclerosis and restenosis: key roles for versican. *Circ Res* 2004;94:1158–1167.
120. Asplund A, Friden V, Stillemark-Billton P, Camejo G, Bondjers G: Macrophages exposed to hypoxia secrete proteoglycans for which LDL has higher affinity. *Atherosclerosis* 2011;215:77–81.
121. Melchior-Becker A, Dai G, Ding Z, et al.: Deficiency of biglycan causes cardiac fibroblasts to differentiate into a myofibroblast phenotype. *J Biol Chem* 2011;286:17365–17375.
122. Funderburgh JL, Corpuz LM, Roth MR, et al.: Mimecan, the 25-kDa corneal keratan sulfate proteoglycan, is a product of the gene producing osteoglycin. *J Biol Chem* 1997;272:28089–28095.
123. Michelacci YM: Collagens and proteoglycans of the corneal extracellular matrix. *Braz J Med Biol Res* 2003;36:1037–1046.
124. Wang X, Ford BC, Praul CA, Leach RM Jr: Characterization of the non-collagenous proteins in avian cortical and medullary bone. *Comp Biochem Physiol B Biochem Mol Biol* 2005;140:665–672.
125. Hopkinson A, McIntosh RS, Shanmuganathan V, Tighe PJ, Dua HS: Proteomic analysis of amniotic membrane prepared for human transplantation: characterization of proteins and clinical implications. *J Proteome Res* 2006;5: 2226–2235.
126. Zhen EY, Brittain IJ, Laska DA, et al.: Characterization of metalloprotease cleavage products of human articular cartilage. *Arthritis Rheum* 2008;58: 2420–2431.
127. Ma QY, Zuo CL, Ma JH, et al.: Glucocorticoid up-regulates mimecan expression in corticotroph cells. *Mol Cell Endocrinol* 2010;321:239–244.
128. Zheng CX, Zhao SX, Wang P, et al.: Different expression of mimecan as a marker for differential diagnosis between NSCLC and SCLC. *Oncol Rep* 2009;22:1057–1061.
129. Barascuk N, Vassiliadis E, Zheng Q, et al.: Levels of circulating MMCN-151, a degradation product of mimecan, reflect pathological extracellular matrix remodeling in apolipoprotein E knockout mice. *Biomark Insights* 2011;6: 97–106.
130. Heathfield TF, Onnerfjord P, Dahlberg L, Heinegard D: Cleavage of fibromodulin in cartilage explants involves removal of the N-terminal tyrosine sulfate-rich region by proteolysis at a site that is sensitive to matrix metalloproteinase-13. *J Biol Chem* 2004;279:6286–6295.
131. Chakravarti S, Magnuson T, Lass JH, et al.: Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. *J Cell Biol* 1998;141:1277–1286.
132. Naito Z: Role of the small leucine-rich proteoglycan (SLRP) family in pathological lesions and cancer cell growth. *J Nihon Med Sch* 2005;72: 137–145.
133. Kaspar M, Zardi D, Neri D: Fibronectin as target for tumor therapy. *Int J Cancer* 2006;118:1331–1339.
134. Gallagher WM, Currid CA, Whelan LC: Fibulins and cancer: friend or foe? *Trends Mol Med* 2005;11:336–340.
135. Camby I, Le MM, Lefranc F, Kiss R: Galectin-1: a small protein with major functions. *Glycobiology* 2006;16:137R–157R.
136. Rabinovich GA: Galectin-1 as a potential cancer target. *Br J Cancer* 2005; 92:1188–1192.
137. ir-Kirk TL, Senior RM: Fragments of extracellular matrix as mediators of inflammation. *Int J Biochem Cell Biol* 2008;40:1101–1110.
138. Leeming DJ, Bay-Jensen AC, Vassiliadis E, et al.: Post-translational modifications of the extracellular matrix are key events in cancer progression: opportunities for biochemical marker development. *Biomarkers* 2011;16:193–205.
139. Schetter AJ, Heegaard NH, Harris CC: Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis* 2010;31: 37–49.
140. Winding B, NicAmhlaoibh R, Misander H, et al.: Synthetic matrix metalloproteinase inhibitors inhibit growth of established breast cancer

- osteolytic lesions and prolong survival in mice. *Clin Cancer Res* 2002;8:1932–1939.
141. Yu AE, Hewitt RE, Connor EW, Stetler-Stevenson WG: Matrix metalloproteinases. Novel targets for directed cancer therapy. *Drugs Aging* 1997;11:229–244.
142. Clarijs R, Ruiter DJ, De Waal RM: Pathophysiological implications of stroma pattern formation in uveal melanoma. *J Cell Physiol* 2003;194:267–271.
143. Ruiter D, Bogenrieder T, Elder D, Herlyn M: Melanoma-stroma interactions: structural and functional aspects. *Lancet Oncol* 2002;3:35–43.
144. Veidal SS, Vassiliadis E, Bay-Jensen AC, et al.: Procollagen type I N-terminal propeptide (PINP) is a marker for fibrogenesis in bile duct ligation-induced fibrosis in rats. *Fibrogenesis Tissue Repair* 2010;3:5.
145. Copple BL, Allen K, Welch TP: Mechanisms of Liver Fibrosis. *Compr Toxicol* 2010;9:263–274.
146. Borkham-Kamphorst E, van Roeyen CR, Ostendorf T, et al.: Pro-fibrogenic potential of PDGF-D in liver fibrosis. *J Hepatol* 2007;46:1064–1074.
147. Guyot C, Lepreux S, Combe C, et al.: Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved. *Int J Biochem Cell Biol* 2006;38:135–151.
148. Moreira RK: Hepatic stellate cells and liver fibrosis. *Arch Pathol Lab Med* 2007;131:1728–1734.
149. Gressner OA, Weiskirchen R, Gressner AM: Biomarkers of hepatic fibrosis, fibrogenesis and genetic pre-disposition pending between fiction and reality. *J Cell Mol Med* 2007;11:1031–1051.
150. Lin WR, Brittan M, Alison MR: The role of bone marrow-derived cells in fibrosis. *Cells Tissues Organs* 2008;188:178–188.
151. Wells RG: Cellular sources of extracellular matrix in hepatic fibrosis. *Clin Liver Dis* 2008;12:759–768, viii.
152. Wallace K, Burt AD, Wright MC: Liver fibrosis. *Biochem J* 2008;411:1–18.
153. Friedman SL: Hepatic fibrosis—overview. *Toxicology* 2008;254:120–129.
154. Kinnman N, Francoz C, Barbu V, et al.: The myofibroblastic conversion of peribiliary fibrogenic cells distinct from hepatic stellate cells is stimulated by platelet-derived growth factor during liver fibrogenesis. *Lab Invest* 2003;83:163–173.
155. Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 2003;112:1776–1784.
156. Forbes SJ, Russo FP, Rey V, et al.: A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. *Gastroenterology* 2004;126:955–963.
157. Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R: Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol* 2004;36:598–606.
158. Wynn TA: Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007;117:524–529.
159. Wynn TA: Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008;214:199–210.
160. Wynn TA: Integrating mechanisms of pulmonary fibrosis. *J Exp Med* 2011;208:1339–1350.
161. Wynn TA, Barron L: Macrophages: master regulators of inflammation and fibrosis. *Semin Liver Dis* 2010;30:245–257.
162. Saile B, Ramadori G: Inflammation, damage repair and liver fibrosis—role of cytokines and different cell types. *Z Gastroenterol* 2007;45:77–86.
163. Fernandez M, Semela D, Bruix J, et al.: Angiogenesis in liver disease. *J Hepatol* 2009;50:604–620.
164. Choi JH, Hwang YP, Park BH, et al.: Anthocyanins isolated from the purple-fleshed sweet potato attenuate the proliferation of hepatic stellate cells by blocking the PDGF receptor. *Environ Toxicol Pharmacol* 2011;31:212–219.
165. Inagaki Y, Okazaki I: Emerging insights into Transforming growth factor beta Smad signal in hepatic fibrogenesis. *Gut* 2007;56:284–292.
166. Schwabe RF, Bataller R, Brenner DA: Human hepatic stellate cells express CCR5 and RANTES to induce proliferation and migration. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G949–G958.
167. Friedman SL: Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008;88:125–172.
168. Maharaj SS, Baroke E, Gauldie J, Kolb MR: Fibrocytes in chronic lung disease—facts and controversies. *Pulm Pharmacol Ther* 2012;25:263–267.
169. Kim KK, Wei Y, Szekeres C, et al.: Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. *J Clin Invest* 2009;119:213–224.
170. Collard HR, Calfee CS, Wolters PJ, et al.: Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L3–L7.
171. Rock JR, Barkauskas CE, Counce MJ, et al.: Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci USA* 2011;108:E1475–E1483.
172. Li Y, Jiang D, Liang J, et al.: Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med* 2011;208:1459–1471.
173. Gauldie J, Bonniaud P, Sime P, Ask K, Kolb M: TGF-beta, Smad3 and the process of progressive fibrosis. *Biochem Soc Trans* 2007;35:661–664.
174. Moeller A, Gilpin SE, Ask K, et al.: Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:588–594.
175. Park SH, Saleh D, Gaiad A, Michel RP: Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. *Am J Respir Crit Care Med* 1997;156:600–608.
176. Xu J, Mora A, Shim H, et al.: Role of the SDF-1/CXCR4 axis in the pathogenesis of lung injury and fibrosis. *Am J Respir Cell Mol Biol* 2007;37:291–299.
177. Pulichino AM, Wang IM, Caron A, et al.: Identification of transforming growth factor beta1-driven genetic programs of acute lung fibrosis. *Am J Respir Cell Mol Biol* 2008;39:324–336.
178. Tager AM, LaCamera P, Shea BS, et al.: The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat Med* 2008;14:45–54.
179. Suki B, Ito S, Stamenovic D, Lutchen KR, Ingenito EP: Biomechanics of the lung parenchyma: critical roles of collagen and mechanical forces. *J Appl Physiol* 2005;98:1892–1899.
180. Hinz B, Phan SH, Thannickal VJ, et al.: The myofibroblast: one function, multiple origins. *Am J Pathol* 2007;170:1807–1816.
181. Wipff PJ, Rifkin DB, Meister JJ, Hinz B: Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol* 2007;179:1311–1323.
182. Liu X, Zhao Y, Gao J, et al.: Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nat Genet* 2004;36:178–182.
183. Maki JM, Sormunen R, Lippo S, et al.: Lysyl oxidase is essential for normal development and function of the respiratory system and for the integrity of elastic and collagen fibers in various tissues. *Am J Pathol* 2005;167:927–936.
184. Iredale JP: Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. *J Clin Invest* 2007;117:539–548.
185. Desmet VJ, Roskams T: Cirrhosis reversal: a duel between dogma and myth. *J Hepatol* 2004;40:860–867.
186. Dienstag JL, Goldin RD, Heathcote EJ, et al.: Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003;124:105–117.
187. Hammel P, Couvelard A, O'Toole D, et al.: Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. *N Engl J Med* 2001;344:418–423.
188. Issa R, Zhou X, Constandinou CM, et al.: Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* 2004;126:1795–1808.
189. Poynard T, McHutchison J, Manns M, et al.: Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002;122:1303–1313.
190. Castera L: Invasive and non-invasive methods for the assessment of fibrosis and disease progression in chronic liver disease. *Best Pract Res Clin Gastroenterol* 2011;25:291–303.

191. Ghany MG, Strader DB, Thomas DL, Seeff LB: Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335–1374.
192. Schuppan D: Connective tissue polypeptides in serum as parameters to monitor antifibrotic treatment in hepatic fibrogenesis. *J Hepatol* 1991;13 Suppl 3:S17–S25.
193. Jensen LT: The aminoterminal propeptide of type III procollagen. Studies on physiology and pathophysiology. *Dan Med Bull* 1997;44:70–78.
194. Yilmaz Y: Systematic review: caspase-cleaved fragments of cytokeratin 18—the promises and challenges of a biomarker for chronic liver disease. *Aliment Pharmacol Ther* 2009;30:1103–1109.
195. Lai KK, Shang S, Lohia N, et al.: Extracellular matrix dynamics in hepatocarcinogenesis: a comparative proteomics study of PDGFC transgenic and Pten null mouse models. *PLoS Genet* 2011;7:e1002147.
196. Leeming DJ, Nielsen MJ, Dai Y, et al.: An Enzyme-linked Immunosorbent Serum Assay ELISA specific for the 7S domain of Collagen Type IV (P4NP 7S)—a marker related to the extracellular matrix remodeling during liver fibrogenesis. *Hepatal Res* 2012;42:482–493.
197. Veidal SS, Karsdal MA, Nawrocki A, et al.: Assessment of proteolytic degradation of the basement membrane: a fragment of type IV collagen as a biochemical marker for liver fibrosis. *Fibrogenesis Tissue Repair* 2011;4:22.
198. Leeming D, He Y, Veidal S, et al.: A novel marker for assessment of liver matrix remodeling: an enzyme-linked immunosorbent assay (ELISA) detecting a MMP generated type I collagen neo-epitope (C1M). *Biomarkers* 2011;16:616–628.
199. Vassiliadis E, Veidal SS, Simonsen H, et al.: Immunological detection of the type V collagen propeptide fragment, PVCP-1230, in connective tissue remodeling associated with liver fibrosis. *Biomarkers* 2011;16:426–433.
200. 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:e24753.
201. Callewaert N, Van VH, Van HA, et al.: Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. *Nat Med* 2004;10:429–434.
202. Fang M, Dewaele S, Zhao YP, et al.: Serum N-glycome biomarker for monitoring development of DENA-induced hepatocellular carcinoma in rat. *Mol Cancer* 2010;9:215.
203. Blomme B, Van SC, Grassi P, et al.: Alterations of serum protein N-glycosylation in two mouse models of chronic liver disease are hepatocyte and not B cell driven. *Am J Physiol Gastrointest Liver Physiol* 2011;300:G833–G842.
204. Vanderschaeghe D, Laroy W, Sablon E, et al.: GlycoFibroTest is a highly performant liver fibrosis biomarker derived from DNA sequencer-based serum protein glycomics. *Mol Cell Proteomics* 2009;8:986–994.
205. Klein A, Carre Y, Louvet A, Michalski JC, Morelle W: Immunoglobulins are the major glycoproteins involved in the modifications of total serum N-glycome in cirrhotic patients. *Proteomics Clin Appl* 2010;4:379–393.
206. Karsdal MA, Woodworth T, Henriksen K, et al.: Biochemical markers of ongoing joint damage in rheumatoid arthritis—current and future applications, limitations and opportunities. *Arthritis Res Ther* 2011;13:215.
207. Leeming DJ, Koizumi M, Byrjalsen I, et al.: The relative use of eight collagenous and noncollagenous markers for diagnosis of skeletal metastases in breast, prostate, or lung cancer patients. *Cancer Epidemiol Biomarkers Prev* 2006;15:32–38.
208. Leeming DJ, Delling G, Koizumi M, et al.: Alpha CTX as a biomarker of skeletal invasion of breast cancer: immunolocalization and the load dependency of urinary excretion. *Cancer Epidemiol Biomarkers Prev* 2006;15:1392–1395.
209. Leeming DJ, Byrjalsen I, Qvist P, et al.: Does increased local bone resorption secondary to breast and prostate cancer result in increased cartilage degradation? *BMC Cancer* 2008;8:180.
210. Jensen VK, Nosjean O, Dziegiel MH, et al.: A quantitative assay for lysosomal acidification rates in human osteoclasts. *Assay Drug Dev Technol* 2011;9:157–164.
211. O'Connell K, Posthumus M, Collins M: COL6A1 gene and Ironman triathlon performance. *Int J Sports Med* 2011;32:896–901.
212. Allamand V, Brinas L, Richard P, et al.: ColVI myopathies: where do we stand, where do we go? *Skelet Muscle* 2011;1:30.
213. Bonnemant CG: The collagen VI-related myopathies: muscle meets its matrix. *Nat Rev Neurol* 2011;7:379–390.
214. Egeblad M, Werb Z: New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002;2:161–174.
215. Kerkela E, Saarialho-Kere U: Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer. *Exp Dermatol* 2003;12:109–125.
216. Tlsty TD, Coussens LM: Tumor stroma and regulation of cancer development. *Annu Rev Pathol* 2006;1:119–150.
217. Skjot-Arkil H, Barascuk N, Register T, Karsdal MA: Macrophage-mediated proteolytic remodeling of the extracellular matrix in atherosclerosis results in neoepitopes: a potential new class of biochemical markers. *Assay Drug Dev Technol* 2010;8:542–552.
218. Veidal SS, Bay-Jensen AC, Tougas G, Karsdal MA, Vainer B: Serum markers of liver fibrosis: combining the BIPED classification and the neo-epitope approach in the development of new biomarkers. *Dis Markers* 2010;28:15–28.
219. Walsh KM, Fletcher A, MacSween RN, Morris AJ: Basement membrane peptides as markers of liver disease in chronic hepatitis C. *J Hepatol* 2000;32:325–330.
220. Dillon TJ, Walsh RL, Scicchitano R, et al.: Plasma elastin-derived peptide levels in normal adults, children, and emphysematous subjects. Physiologic and computed tomographic scan correlates. *Am Rev Respir Dis* 1992;146:1143–1148.
221. Heinz A, Jung MC, Jahreis G, et al.: The action of neutrophil serine proteases on elastin and its precursor. *Biochimie* 2011.
222. Heinz A, Jung MC, Duca L, et al.: Degradation of tropoelastin by matrix metalloproteinases—cleavage site specificities and release of matrikines. *FEBS J* 2010;277:1939–1956.
223. Houghton AM, Quintero PA, Perkins DL, et al.: Elastin fragments drive disease progression in a murine model of emphysema. *J Clin Invest* 2006;116:753–759.
224. Jakob A, Unger S, Arnold R, et al.: A family with a new elastin gene mutation: broad clinical spectrum, including sudden cardiac death. *Cardiol Young* 2011;21:62–65.
225. Kielty CM, Sherratt MJ, Shuttleworth CA: Elastic fibres. *J Cell Sci* 2002;115:2817–2828.
226. Luisetti M, Ma S, Iadarola P, et al.: Desmosine as a biomarker of elastin degradation in COPD: current status and future directions. *Eur Respir J* 2008;32:1146–1157.
227. Ma S, Lieberman S, Turino GM, Lin YY: The detection and quantitation of free desmosine and isodesmosine in human urine and their peptide-bound forms in sputum. *Proc Natl Acad Sci USA* 2003;100:12941–12943.
228. Marciniak SJ, Lomas DA: What can naturally occurring mutations tell us about the pathogenesis of COPD? *Thorax* 2009;64:359–364.
229. Mecham RP, Broekmann TJ, Fliszar CJ, et al.: Elastin degradation by matrix metalloproteinases. Cleavage site specificity and mechanisms of elastolysis. *J Biol Chem* 1997;272:18071–18076.
230. Petersen E, Gineitis A, Wagberg F, Angquist KA: Serum levels of elastin-derived peptides in patients with ruptured and asymptomatic abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2001;22:48–52.
231. Taddese S, Weiss AS, Jahreis G, Neubert RH, Schmelzer CE: *In vitro* degradation of human tropoelastin by MMP-12 and the generation of matrikines from domain 24. *Matrix Biol* 2009;28:84–91.
232. Wendel DP, Taylor DG, Albertine KH, Keating MT, Li DY: Impaired distal airway development in mice lacking elastin. *Am J Respir Cell Mol Biol* 2000;23:320–326.
233. Barker DF, Hostikka SL, Zhou J, et al.: Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* 1990;248:1224–1227.
234. Sykes B, Ogilvie D, Wordsworth P, et al.: Consistent linkage of dominantly inherited osteogenesis imperfecta to the type I collagen loci: COL1A1 and COL1A2. *Am J Hum Genet* 1990;46:293–307.

235. Weil D, D'Alessio M, Ramirez F, Eyre DR: Structural and functional characterization of a splicing mutation in the pro- α 2(I) collagen gene of an Ehlers-Danlos type VII patient. *J Biol Chem* 1990;265:16007-16011.
236. Fernandes RJ, Wilkin DJ, Weis MA, et al.: Incorporation of structurally defective type II collagen into cartilage matrix in kniest chondrodysplasia. *Arch Biochem Biophys* 1998;355:282-290.
237. Ahmad NN, Donald-McGinn DM, Zackai EH, et al.: A second mutation in the type II procollagen gene (COL2A1) causing stickler syndrome (arthroophthalmopathy) is also a premature termination codon. *Am J Hum Genet* 1993;52:39-45.
238. Palotie A, Vaisanen P, Ott J, et al.: Predisposition to familial osteoarthritis linked to type II collagen gene. *Lancet* 1989;1:924-927.
239. Ala-Kokko L, Baldwin CT, Moskowitz RW, Prockop DJ: Single base mutation in the type II procollagen gene (COL2A1) as a cause of primary osteoarthritis associated with a mild chondrodysplasia. *Proc Natl Acad Sci USA* 1990;87:6565-6568.
240. Tromp G, Kuivaniemi H, Stolle C, Pope FM, Prockop DJ: Single base mutation in the type III procollagen gene that converts the codon for glycine 883 to aspartate in a mild variant of Ehlers-Danlos syndrome IV. *J Biol Chem* 1989;264:19313-19317.
241. Kontusaari S, Tromp G, Kuivaniemi H, Romanic AM, Prockop DJ: A mutation in the gene for type III procollagen (COL3A1) in a family with aortic aneurysms. *J Clin Invest* 1990;86:1465-1473.
242. Van AT, Bailey MA, Schlotzer-Schrehardt U, et al.: Col4a1 mutation in mice causes defects in vascular function and low blood pressure associated with reduced red blood cell volume. *Hum Mol Genet* 2010;19:1119-1128.
243. Van AT, Bruckner-Tuderman L: Basement membranes and human disease. *Cell Tissue Res* 2010;339:167-188.
244. Taylor SH, Al-Youha S, Van AT, et al.: Tendon is covered by a basement membrane epithelium that is required for cell retention and the prevention of adhesion formation. *PLoS One* 2011;6:e16337.
245. Wenstrup RJ, Florer JB, Willing MC, et al.: COL5A1 haploinsufficiency is a common molecular mechanism underlying the classical form of EDS. *Am J Hum Genet* 2000;66:1766-1776.
246. Richards AJ, Martin S, Nicholls AC, et al.: A single base mutation in COL5A2 causes Ehlers-Danlos syndrome type II. *J Med Genet* 1998;35:846-848.
247. Lampe AK, Bushby KM: Collagen VI related muscle disorders. *J Med Genet* 2005;42:673-685.
248. Dang N, Murrell DF: Mutation analysis and characterization of COL7A1 mutations in dystrophic epidermolysis bullosa. *Exp Dermatol* 2008;17:553-568.
249. Czarny-Ratajczak M, Lohiniva J, Rogala P, et al.: A mutation in COL9A1 causes multiple epiphyseal dysplasia: further evidence for locus heterogeneity. *Am J Hum Genet* 2001;69:969-980.
250. Woelfle JV, Brenner RE, Zabel B, Reichel H, Nelitz M: Schmid-type metaphyseal chondrodysplasia as the result of a collagen type X defect due to a novel COL10A1 nonsense mutation: a case report of a novel COL10A1 mutation. *J Orthop Sci* 2011;16:245-249.
251. Makitie O, Susic M, Cole WG: Early-onset metaphyseal chondrodysplasia type Schmid associated with a COL10A1 frame-shift mutation and impaired trimerization of wild-type α 1(X) protein chains. *J Orthop Res* 2010;28:1497-1501.
252. Milewicz DM, Urban Z, Boyd C: Genetic disorders of the elastic fiber system. *Matrix Biol* 2000;19:471-480.
253. Kielty CM: Elastic fibres in health and disease. *Expert Rev Mol Med* 2006;8:1-23.
254. Heegaard AM, Corsi A, Danielsen CC, et al.: Biglycan deficiency causes spontaneous aortic dissection and rupture in mice. *Circulation* 2007;115:2731-2738.
255. Chen XD, Shi S, Xu T, Robey PG, Young MF: Age-related osteoporosis in biglycan-deficient mice is related to defects in bone marrow stromal cells. *J Bone Miner Res* 2002;17:331-340.
256. Xu T, Bianco P, Fisher LW, et al.: Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nat Genet* 1998;20:78-82.
257. Young MF, Bi Y, Ameye L, Chen XD: Biglycan knockout mice: new models for musculoskeletal diseases. *Glycoconj J* 2002;19:257-262.
258. Ameye L, Aria D, Jepsen K, et al.: Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. *FASEB J* 2002;16:673-680.
259. Gill MR, Oldberg A, Reinholt FP: Fibromodulin-null murine knee joints display increased incidences of osteoarthritis and alterations in tissue biochemistry. *Osteoarthritis Cartilage* 2002;10:751-757.
260. Jepsen KJ, Wu F, Peragallo JH, et al.: A syndrome of joint laxity and impaired tendon integrity in lumican- and fibromodulin-deficient mice. *J Biol Chem* 2002;277:35532-35540.
261. Svensson L, Aszodi A, Reinholt FP, et al.: Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. *J Biol Chem* 1999;274:9636-9647.
262. Chakravarti S: Functions of lumican and fibromodulin: lessons from knockout mice. *Glycoconj J* 2002;19:287-293.
263. Bi X, Tong C, Dockendorff A, et al.: Genetic deficiency of decorin causes intestinal tumor formation through disruption of intestinal cell maturation. *Carcinogenesis* 2008;29:1435-1440.
264. Danielson KG, Baribault H, Holmes DF, et al.: Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol* 1997;136:729-743.
265. Corsi A, Xu T, Chen XD, Boyde A, et al.: Phenotypic effects of biglycan deficiency are linked to collagen fibril abnormalities, are synergized by decorin deficiency, and mimic Ehlers-Danlos-like changes in bone and other connective tissues. *J Bone Miner Res* 2002;17:1180-1189.
266. Wang Y, Ma Y, Lu B, et al.: Differential expression of mimecan and thioredoxin domain-containing protein 5 in colorectal adenoma and cancer: a proteomic study. *Exp Biol Med (Maywood)* 2007;232:1152-1159.
267. Hayward C, Brock DJ: Fibrillin-1 mutations in Marfan syndrome and other type-1 fibrillinopathies. *Hum Mutat* 1997;10:415-423.
268. Paulsson M, Heinegard D: Purification and structural characterization of a cartilage matrix protein. *Biochem J* 1981;197:367-375.
269. Briggs MD, Rasmussen IM, Weber JL, et al.: Genetic linkage of mild pseudoachondroplasia (PSACH) to markers in the pericentromeric region of chromosome 19. *Genomics* 1993;18:656-660.
270. Jackson GC, Mittaz-Crettol L, Taylor JA, et al.: Pseudoachondroplasia and multiple epiphyseal dysplasia: a 7-year comprehensive analysis of the known disease genes identify novel and recurrent mutations and provides an accurate assessment of their relative contribution. *Hum Mutat* 2012;33:144-157.
271. Briggs MD, Wright MJ, Mortier GR: Multiple epiphyseal dysplasia, dominant. In: *GeneReviews™* (Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, eds.) [Internet]. University of Washington, Seattle, WA, 2003 Jan 8 [updated 2011 Feb 1]. Available online at www.ncbi.nlm.nih.gov/books/NBK1123
272. Karsdal MA, Henriksen K, Leeming DJ, et al.: Biochemical markers and the FDA Critical Path: how biomarkers may contribute to the understanding of pathophysiology and provide unique and necessary tools for drug development. *Biomarkers* 2009;14:181-202.
273. Lapolla A, Traldi P, Fedele D: Importance of measuring products of non-enzymatic glycation of proteins. *Clin Biochem* 2005;38:103-115.
274. Skjot-Arkil H, Schett G, Zhang C, et al.: Investigation of two novel biochemical markers of inflammation, matrix metalloproteinase and cathepsin generated fragments of C-reactive protein, in patients with ankylosing spondylitis. *Clin Exp Rheumatol* 2012;30:371-379.
275. Lin HC, Chang JH, Jain S, et al.: Matrilysin cleavage of corneal collagen type XVIII NC1 domain and generation of a 28-kDa fragment. *Invest Ophthalmol Vis Sci* 2001;42:2517-2524.

276. Rosenquist C, Fledelius C, Christgau S, et al.: Serum CrossLaps one step ELISA. First application of monoclonal antibodies for measurement in serum of bone-related degradation products from C-terminal telopeptides of type I collagen. *Clin Chem* 1998;44:2281–2289.
277. Cloos PA, Christgau S: Characterization of aged osteocalcin fragments derived from bone resorption. *Clin Lab* 2004;50:585–598.
278. Karsdal MA, Madsen SH, Christiansen C, et al.: Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity. *Arthritis Res Ther* 2008;10:R63.
279. Reference deleted.
280. Erler JT, Bennewith KL, Nicolau M, et al.: Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* 2006;440:1222–1226.
281. Erler JT, Weaver VM: Three-dimensional context regulation of metastasis. *Clin Exp Metastasis* 2009;26:35–49.
282. Kass L, Erler JT, Dembo M, Weaver VM: Mammary epithelial cell: influence of extracellular matrix composition and organization during development and tumorigenesis. *Int J Biochem Cell Biol* 2007;39:1987–1994.
283. Levental KR, Yu H, Kass L, et al.: Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 2009;139:891–906.
284. Oestergaard S, Chouinard L, Doyle N, et al.: The utility of measuring C-terminal telopeptides of collagen type II (CTX-II) in serum and synovial fluid samples for estimation of articular cartilage status in experimental models of destructive joint diseases. *Osteoarthritis Cartilage* 2006;14:670–679.
285. Bone HG: The future of osteoporosis diagnosis and therapy. *Ann Ital Med Int* 1992;7:166S–170S.
286. Leeming DJ, Alexandersen P, Karsdal MA, et al.: An update on biomarkers of bone turnover and their utility in biomedical research and clinical practice. *Eur J Clin Pharmacol* 2006;62:781–792.
287. Byrjalsen I, Leeming DJ, Qvist P, Christiansen C, Karsdal MA: Bone turnover and bone collagen maturation in osteoporosis: effects of antiresorptive therapies. *Osteoporos Int* 2008;19:339–348.
288. Karsdal MA, Byrjalsen I, Leeming DJ, Delmas PD, Christiansen C: The effects of oral calcitonin on bone collagen maturation: implications for bone turnover and quality. *Osteoporos Int* 2008;19:1355–1361.
289. Leeming DJ, Henriksen K, Byrjalsen I, et al.: Is bone quality associated with collagen age? *Osteoporos Int* 2009;20:1461–1470.
290. Fledelius C, Johnsen AH, Cloos PA, Bonde M, Qvist P: Characterization of urinary degradation products derived from type I collagen. Identification of a beta-isomerized Asp-Gly sequence within the C-terminal telopeptide (alpha1) region. *J Biol Chem* 1997;272:9755–9763.
291. Cloos PA, Lyubimova N, Solberg H, et al.: An immunoassay for measuring fragments of newly synthesized collagen type I produced during metastatic invasion of bone. *Clin Lab* 2004;50:279–289.
292. Grenard P, Bresson-Hadni S, El AS, et al.: Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. *J Hepatol* 2001;35:367–375.
293. Elli L, Bergamini CM, Bardella MT, Schuppan D: Transglutaminases in inflammation and fibrosis of the gastrointestinal tract and the liver. *Dig Liver Dis* 2009;41:541–550.
294. Collighan RJ, Griffin M: Transglutaminase 2 cross-linking of matrix proteins: biological significance and medical applications. *Amino Acids* 2009;36:659–670.
295. Poyton RO, Ball KA, Castello PR: Mitochondrial generation of free radicals and hypoxic signaling. *Trends Endocrinol Metab* 2009;20:332–340.
296. Cloos PA, Christgau S: Non-enzymatic covalent modifications of proteins: mechanisms, physiological consequences and clinical applications. *Matrix Biol* 2002;21:39–52.
297. Kowluru RA, Atasi L, Ho YS: Role of Mitochondrial Superoxide Dismutase in the Development of Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2006;47:1594–1599.
298. Chaiswing L, Oberley TD: Extracellular/microenvironmental redox state. *Antioxid Redox Signal* 2010;13:449–465.
299. Colell A, Green DR, Ricci JE: Novel roles for GAPDH in cell death and carcinogenesis. *Cell Death Differ* 2009;16:1573–1581.
300. DeNicola GM, Tuveson DA: RAS in cellular transformation and senescence. *Eur J Cancer* 2009;45 Suppl 1:211–216.
301. Faux SP, Tai T, Thorne D, et al.: The role of oxidative stress in the biological responses of lung epithelial cells to cigarette smoke. *Biomarkers* 2009;14 Suppl 1:90–96.
302. Lu JM, Lin PH, Yao Q, Chen C: Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J Cell Mol Med* 2010;14:840–860.
303. Weinberg F, Chandel NS: Mitochondrial metabolism and cancer. *Ann NY Acad Sci* 2009;1177:66–73.
304. Karsdal MA, Leeming DJ, Dam EB, et al.: Should subchondral bone turnover be targeted when treating osteoarthritis? *Osteoarthritis Cartilage* 2008;16:638–646.
305. Anderson-MacKenzie JM, Quasnicka HL, Starr RL, et al.: Fundamental subchondral bone changes in spontaneous knee osteoarthritis. *Int J Biochem Cell Biol* 2005;37:224–236.
306. Bailey AJ, Mansell JP: Do subchondral bone changes exacerbate or precede articular cartilage destruction in osteoarthritis of the elderly? *Gerontology* 1997;43:296–304.
307. Dieppe P: Subchondral bone should be the main target for the treatment of pain and disease progression in osteoarthritis. *Osteoarthritis Cartilage* 1999;7:325–326.
308. Felson DT, Neogi T: Osteoarthritis: is it a disease of cartilage or of bone? *Arthritis Rheum* 2004;50:341–344.
309. Bay-Jensen AC, Sondergaard BC, Christiansen C, et al.: Biochemical markers of joint tissue turnover. *Assay Drug Dev Technol* 2010;8:118–124.
310. Sondergaard BC, Henriksen K, Wulf H, et al.: Relative contribution of matrix metalloprotease and cysteine protease activities to cytokine-stimulated articular cartilage degradation. *Osteoarthritis Cartilage* 2006;14:738–748.
311. Schaller S, Henriksen K, Hoegh-Andersen P, et al.: In vitro, ex vivo, and in vivo methodological approaches for studying therapeutic targets of osteoporosis and degenerative joint diseases: how biomarkers can assist? *Assay Drug Dev Technol* 2005;3:553–580.
312. Karsdal MA, Sumer EU, Wulf H, et al.: Induction of increased cAMP levels in articular chondrocytes blocks matrix metalloproteinase-mediated cartilage degradation, but not aggrecanase-mediated cartilage degradation. *Arthritis Rheum* 2007;56:1549–1558.
313. Qvist P, Christiansen C, Karsdal MA, et al.: Application of biochemical markers in development of drugs for treatment of osteoarthritis. *Biomarkers* 2010;15:1–19.
314. Garnero P, Ferreras M, Karsdal MA, et al.: The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. *J Bone Miner Res* 2003;18:859–867.
315. Santala M, Risteli J, Kaupilla A: Comparison of carboxyterminal telopeptide of type I collagen (ICTP) and CA 125 as predictors of prognosis in ovarian cancer. *Anticancer Res* 2004;24:1057–1062.
316. Simojoki M, Santala M, Risteli J, Kaupilla A: Carboxyterminal telopeptide of type I collagen (ICTP) in predicting prognosis in epithelial ovarian cancer. *Gynecol Oncol* 2001;82:110–115.
317. Cloos PA, Fledelius C: Collagen fragments in urine derived from bone resorption are highly racemized and isomerized: a biological clock of protein aging with clinical potential. *Biochem J* 2000;345 Pt 3:473–480.
318. Johnson BA, Aswad DW: Fragmentation of isoaspartyl peptides and proteins by carboxypeptidase Y: release of isoaspartyl dipeptides as a result of internal and external cleavage. *Biochemistry* 1990;29:4373–4380.
319. Brange J, Langkjaer L, Havelund S, Volund A: Chemical stability of insulin. 1. Hydrolytic degradation during storage of pharmaceutical preparations. *Pharm Res* 1992;9:715–726.
320. Mamula MJ, Gee RJ, Elliott JL, et al.: Isoaspartyl post-translational modification triggers autoimmune responses to self-proteins. *J Biol Chem* 1999;274:22321–22327.
321. Voorter CE, de Haard-Hoekman WA, van den Oetelaar PJ, Bloemendaal H, de Jong WW: Spontaneous peptide bond cleavage in aging alpha-crystallin through a succinimide intermediate. *J Biol Chem* 1988;263:19020–19023.

322. Young AL, Carter WG, Doyle HA, Mamula MJ, Aswad DW: Structural integrity of histone H2B *in vivo* requires the activity of protein L-isoaspartate O-methyltransferase, a putative protein repair enzyme. *J Biol Chem* 2001;276:37161–37165.
323. Treweek JB, Dickerson TJ, Janda KD: Drugs of abuse that mediate advanced glycation end product formation: a chemical link to disease pathology. *Acc Chem Res* 2009;42:659–669.
324. Riehl A, Nemeth J, Angel P, Hess J: The receptor RAGE: Bridging inflammation and cancer. *Cell Commun Signal* 2009;7:12.
325. Johansen JS: Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibrosis and cancer. *Dan Med Bull* 2006;53:172–209.
326. Anzilotti C, Pratesi F, Tommasi C, Migliorini P: Peptidylarginine deiminase 4 and citrullination in health and disease. *Autoimmun Rev* 2010;9:158–160.
327. Gyorgy B, Toth E, Tarcsa E, Falus A, Buzas EI: Citrullination: a posttranslational modification in health and disease. *Int J Biochem Cell Biol* 2006;38:1662–1677.
328. Chang X, Han J, Pang L, et al.: Increased PAD14 expression in blood and tissues of patients with malignant tumors. *BMC Cancer* 2009;9:40.
329. Chang X, Han J: Expression of peptidylarginine deiminase type 4 (PAD4) in various tumors. *Mol Carcinog* 2006;45:183–196.
330. Smith BC, Denu JM: Chemical mechanisms of histone lysine and arginine modifications. *Biochim Biophys Acta* 2009;1789:45–57.
331. Veidal SS, Larsen DV, Chen X, et al.: MMP mediated type V collagen degradation (C5M) is elevated in ankylosing spondylitis. *Clin Biochem* 45:541–546.
332. Quintana DJ, Garnero P, Huebner JL, Charni-Ben TN, Kraus VB: PIIANP and HELIXII diurnal variation. *Osteoarthritis Cartilage* 2008;16:1192–1195.
333. Rousseau JC, Zhu Y, Miossec P, et al.: Serum levels of type IIA procollagen amino terminal propeptide (PIANP) are decreased in patients with knee osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage* 2004;12:440–447.
334. Trocme C, Leroy V, Sturm N, et al.: Longitudinal evaluation of a fibrosis index combining MMP-1 and PIIINP compared with MMP-9, TIMP-1 and hyaluronic acid in patients with chronic hepatitis C treated by interferon-alpha and ribavirin. *J Viral Hepat* 2006;13:643–651.
335. Vassiliadis E, Oliveira CP, Alvares-da-Silva MR, et al.: Circulating levels of citrullinated and MMP-degraded vimentin (VICM) in liver fibrosis related pathology. *Am J Transl Res* 2012;4:403–414.
336. Barascuk N, Genovese F, Larsen L, et al.: Quantification of a specific matrix metalloproteinase-mediated degradation product of biglycan during extracellular matrix remodeling in liver fibrosis. *Hepato Res* 2012. (In press)
337. Barascuk N, Larsen L, Byrjalsen I, et al.: A MMP derived versican neoepitope is elevated in plasma from patients with atherosclerotic heart disease. *Atherosclerosis* 2012. (In press)
338. Vassiliadis E, Rasmussen LM, Byrjalsen I, et al.: Clinical evaluation of a matrix metalloproteinase-12 cleaved fragment of titin as a cardiovascular-specific serological biomarker. *J Translational Med* 2012;10:140. DOI: 10.1186/1479-5876-10-140.
339. Crnkic M, Mansson B, Larsson L, et al.: Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept. *Arthritis Res Ther* 2003;5:R181–R185.
340. Christiansen C, Tanko LB, Warming L, et al.: Dose dependent effects on bone resorption and formation of intermittently administered intravenous ibandronate. *Osteoporos Int* 2003;14:609–613.
341. Christgau S, Bitsch-Jensen O, Hanover BN, et al.: Serum Crosslaps for monitoring the response in individuals undergoing antiresorptive therapy. *Bone* 2000;26:505–511.
342. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C: Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. *Bone* 2002;31:57–61.
343. Alexandersen P, Karsdal MA, Qvist P, Reginster JY, Christiansen C: Strontium ranelate reduces the urinary level of cartilage degradation biomarker CTX-II in postmenopausal women. *Bone* 2007;40:218–222.
344. Dam EB, Byrjalsen I, Karsdal MA, Qvist P, Christiansen C: Increased urinary excretion of C-telopeptides of type II collagen (CTX-II) predicts cartilage loss over 21 months by MRI. *Osteoarthritis Cartilage* 2009;17:384–389.
345. Conrozier T, Poole AR, Ferrand F, et al.: Serum concentrations of type II collagen biomarkers (C2C, C1, 2C and CPII) suggest different pathophysiologies in patients with hip osteoarthritis. *Clin Exp Rheumatol* 2008;26:430–435.
346. Koivula MK, Heliovaara M, Rissanen H, et al.: Antibodies binding to citrullinated telopeptides of type I and type II collagens and to mutated citrullinated vimentin synergistically predict the development of seropositive rheumatoid arthritis. *Ann Rheum Dis* 2012;71:1666–1670.
347. Wagner E, Skoumal M, Bayer PM, Klaushofer K: Antibody against mutated citrullinated vimentin: a new sensitive marker in the diagnosis of rheumatoid arthritis. *Rheumatol Int* 2009;29:1315–1321.
348. Garnero P, Buchs N, Zekri J, et al.: Markers of bone turnover for the management of patients with bone metastases from prostate cancer. *Br J Cancer* 2000;82:858–864.
349. Arthur MJ: Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G245–G249.
350. Friedman SL: Liver fibrosis—from bench to bedside. *J Hepatol* 2003;38 Suppl 1:S38–S53.
351. Martinez-Hernandez A, Amenta PS: The hepatic extracellular matrix. II. Ontogenesis, regeneration and cirrhosis. *Virchows Arch A Pathol Anat Histopathol* 1993;423:77–84.
352. Martinez-Hernandez A, Amenta PS: The hepatic extracellular matrix. I. Components and distribution in normal liver. *Virchows Arch A Pathol Anat Histopathol* 1993;423:1–11.
353. Schuppan D: Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. *Semin Liver Dis* 1990;10:1–10.
354. Rojkind M, Giambrone MA, Biempica L: Collagen types in normal and cirrhotic liver. *Gastroenterology* 1979;76:710–719.
355. Hemmann S, Graf J, Roderfeld M, Roeb E: Expression of MMPs and TIMPs in liver fibrosis—a systematic review with special emphasis on anti-fibrotic strategies. *J Hepatol* 2007;46:955–975.
356. Segovia-Silvestre T, Reichenbach V, Fernandez-Varo G, et al.: Circulating CO3-610, a degradation product of collagen III, closely reflects liver collagen and portal pressure in rats with fibrosis. *Fibrogenesis Tissue Repair* 2011;4:19.
357. Veidal SS, Vassiliadis E, Barascuk N, et al.: Matrix metalloproteinase-9-mediated type III collagen degradation as a novel serological biochemical marker for liver fibrogenesis. *Liver Int* 2010;30:1293–1304.
358. Takahashi M, Suzuki M, Kushida K, Miyamoto S, Inoue T: Relationship between pentosidine levels in serum and urine and activity in rheumatoid arthritis. *Br J Rheumatol* 1997;36:637–642.
359. Senolt L, Braun M, Olejarova M, et al.: Increased pentosidine, an advanced glycation end product, in serum and synovial fluid from patients with knee osteoarthritis and its relation with cartilage oligomeric matrix protein. *Ann Rheum Dis* 2005;64:886–890.
360. Saudek DM, Kay J: Advanced glycation endproducts and osteoarthritis. *Curr Rheumatol Rep* 2003;5:33–40.
361. DeGroot J, Verzijl N, Wenting-Van Wijk MJ, et al.: Age-related decrease in susceptibility of human articular cartilage to matrix metalloproteinase-mediated degradation: the role of advanced glycation end products. *Arthritis Rheum* 2001;44:2562–2571.
362. Schwartz AV, Garnero P, Hillier TA, et al.: Pentosidine and increased fracture risk in older adults with type 2 diabetes. *J Clin Endocrinol Metab* 2009;94:2380–2386.
363. Chen JR, Takahashi M, Suzuki M, et al.: Pentosidine in synovial fluid in osteoarthritis and rheumatoid arthritis: relationship with disease activity in rheumatoid arthritis. *J Rheumatol* 1998;25:2440–2444.
364. Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T: Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab* 2008;93:1013–1019.

365. Miyata T, Ishiguro N, Yasuda Y, et al.: Increased pentosidine, an advanced glycation end product, in plasma and synovial fluid from patients with rheumatoid arthritis and its relation with inflammatory markers. *Biochem Biophys Res Commun* 1998;244:45–49.
366. Yamamoto M, Yamaguchi T, Yamauchi M, Sugimoto T: Low serum level of the endogenous secretory receptor for advanced glycation end products (esRAGE) is a risk factor for prevalent vertebral fractures independent of bone mineral density in patients with type 2 diabetes. *Diabetes Care* 2009; 32:2263–2268.
367. Kurien BT, Scofield RH: Autoimmunity and oxidatively modified autoantigens. *Autoimmun Rev* 2008;7:567–573.
368. Yoshida N, Okumura K, Aso Y: High serum pentosidine concentrations are associated with increased arterial stiffness and thickness in patients with type 2 diabetes. *Metabolism* 2005;54:345–350.
369. Choi YG, Lim S: Characterization of anti-advanced glycation end product antibodies to nonenzymatically lysine-derived and arginine-derived glycated products. *J Immunoassay Immunochem* 2009;30:386–399.
370. Griffiths HR: Is the generation of neo-antigenic determinants by free radicals central to the development of autoimmune rheumatoid disease? *Autoimmun Rev* 2008;7:544–549.
371. Sheikh Z, Ahmad R, Sheikh N, Ali R: Enhanced recognition of reactive oxygen species damaged human serum albumin by circulating systemic lupus erythematosus autoantibodies. *Autoimmunity* 2007;40:512–520.
372. Kralev S, Zimmerer E, Brueckmann M, et al.: Elevation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in patients presenting with acute myocardial infarction. *Clin Chem Lab Med* 2009;47:446–451.
373. Kurien BT, Scofield RH: Lipid peroxidation in systemic lupus erythematosus. *Indian J Exp Biol* 2006;44:349–356.
374. Ahsan H, Ali A, Ali R: Oxygen free radicals and systemic autoimmunity. *Clin Exp Immunol* 2003;131:398–404.
375. Sjöberg JS, Bulterijs S: Characteristics, formation, and pathophysiology of glucosamine: a major protein cross-link. *Rejuvenation Res* 2009;12:137–148.
376. Zamvil SS, Mitchell DJ, Moore AC, et al.: T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature* 1986;324: 258–260.
377. Moscarello MA, Wood DD, Ackerley C, Boulias C: Myelin in multiple sclerosis is developmentally immature. *J Clin Invest* 1994;94:146–154.
378. Pritzker LB, Joshi S, Harauz G, Moscarello MA: Deimination of myelin basic protein. 2. Effect of methylation of MBP on its deimination by peptidylarginine deiminase. *Biochemistry* 2000;39:5382–5388.
379. Fujii N, Momose Y, Ishii N, et al.: The mechanisms of simultaneous stereoinversion, racemization, and isomerization at specific aspartyl residues of aged lens proteins. *Mech Ageing Dev* 1999;107:347–358.
380. Fujii N, Momose Y, Ishibashi Y, et al.: Specific racemization and isomerization of the aspartyl residue of alphaA-crystallin due to UV-B irradiation. *Exp Eye Res* 1997;65:99–104.
381. van Stipdonk MJ, Willems AA, Amor S, et al.: T cells discriminate between differentially phosphorylated forms of alphaB-crystallin, a major central nervous system myelin antigen. *Int Immunol* 1998;10:943–950.
382. Corthay A, Backlund J, Broddefalk J, et al.: Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis. *Eur J Immunol* 1998;28:2580–2590.
383. Masson-Bessiere C, Sebbag M, Girbal-Neuhausser E, et al.: The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. *J Immunol* 2001; 166:4177–4184.
384. Girbal-Neuhausser E, Durieux JJ, Arnaud M, et al.: The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. *J Immunol* 1999;162:585–594.
385. Asaga H, Yamada M, Senshu T: Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. *Biochem Biophys Res Commun* 1998;243:641–646.
386. Newkirk MM, Goldbach-Mansky R, Lee J, et al.: Advanced glycation end-product (AGE)-damaged IgG and IgM autoantibodies to IgG-AGE in patients with early synovitis. *Arthritis Res Ther* 2003;5:R82–R90.
387. Trigwell SM, Radford PM, Page SR, et al.: Islet glutamic acid decarboxylase modified by reactive oxygen species is recognized by antibodies from patients with type 1 diabetes mellitus. *Clin Exp Immunol* 2001;126:242–249.
388. Kim JH, Nam KH, Kwon OS, et al.: Histone cross-linking by transglutaminase. *Biochem Biophys Res Commun* 2002;293:1453–1457.
389. Monneaux F, Lozano JM, Patarroyo ME, Briand JP, Muller S: T cell recognition and therapeutic effect of a phosphorylated synthetic peptide of the 70K snRNP protein administered in MR/lpr mice. *Eur J Immunol* 2003;33:287–296.
390. Doyle HA, Mamula MJ: Post-translational protein modifications in antigen recognition and autoimmunity. *Trends Immunol* 2001;22:443–449.
391. Doyle HA, Yan J, Liang B, Mamula MJ: Lupus autoantigens: their origins, forms, and presentation. *Immunol Res* 2001;24:131–147.
392. Mamula MJ: Epitope spreading: the role of self peptides and autoantigen processing by B lymphocytes. *Immunol Rev* 1998;164:231–239.

Address correspondence to:
Morten A. Karsdal, MSc, PhD
 Nordic Bioscience A/S
 Herlev Hovedgade 207
 Herlev 2730
 Denmark

E-mail: mk@nordicbioscience.com