Review Article

Atherofibrosis - a unique and common process of the disease pathogenesis of atherosclerosis and fibrosis - lessons for biomarker development

Efstathios Vassiliadis[1, 2], Natasha Barascuk[1, 2], Morten A Karsdal[1]

[1]Nordic Bioscience A/S, Herlev Hovedgade 207, 2730 Herlev, Denmark; [2]University of Southern Denmark, Odense, Denmark

Abstract: The hallmark of a variety of fibrotic diseases such as liver fibrosis, lung fibrosis, skin fibrosis and atherosclerosis is extensive extracellular matrix remodeling (ECMr) of the disease affected tissue. Inflammation often leads to tissue disruption and destruction, upon which locally released battery of proteases such as matrix metalloproteinases and cysteine proteases degrade the surrounding matrix. The degradation products of ECM proteins, the co-called neoepitopes, are released into the systemic circulation. By recent development of Enzyme-Linked Immunosorbent Assays (ELISAs) detecting the pathological tissue turnover in atherosclerosis and liver fibrosis, we have introduced a novel class of biomarkers into the field of fibrotic diseases, which have been proved efficient in the early diagnosis. This work has resulted in identification of common mechanisms involving specific cell types, proteins and proteases as well as pathways shared among the fibrotic diseases. In this analysis we seek to answer following questions: a) Are there common disease mechanisms and cell types involved in both atherosclerosis and fibrosis? b) Can the lessons learned in developing fibrosis biomarkers be used for the development of atherosclerosis biomarkers? Our hypothesis is that by answering the above questions, we may be able to improve general understanding of the early-stage disease initiation and progression of fibrotic diseases, which in turn may aid in early diagnosis, prognosis and ultimately patient management.

Keywords: Atherosclerosis, fibrosis, extracellular matrix, collagens, proteoglycans, matrix metalloproteinases, neoepitopes, biomarkers

Introduction

ECMr is a key process of tissue homeostasis [1]. Specific proteolytic activities are a prerequisite for a range of cellular functions and interactions with the extracellular matrix [1]. These specific activities are precisely coordinated under physiological situations, in which a specified sequence of events results in controlled tissue turnover. In pathological situations, including inflammation, atherosclerosis, fibrosis and cancer, the normal repair-response relationship is perturbed [2], leading to excessive remodeling, and tissue turnover.

ECM degradation is an important component of atherogenesis playing a central role in arterial remodeling important to clinical outcomes associated with coronary heart disease.

Changes in the composition of ECM are in part due to elevated levels of proteases produced by resident and migratory inflammatory cells, such as macrophages [3]. The extracellular and pericellular matrix of arteries is mainly composed of fibrous proteins such as collagens (type I and type III) and proteoglycans [4]. Endopeptidases such as matrix metalloproteinases (MMPs) play major roles in the degradation of extracellular macromolecules such as collagens and proteoglycans [5, 6].

Liver fibrosis pathology is related to increased deposition and abnormal distribution of ECM proteins, mainly collagens and proteoglycans. Liver fibrosis is a serious complication of chronic inflammatory liver disorders with a diversity of infectious, inflammatory and toxic causes [7]. Progression of fibrosis eventually leads to

liver cirrhosis, which poses a major global health problem. During fibrogenesis, the quantity and composition of ECM proteins in the liver changes, resulting in excessive accumulation of fibrous tissue and an overall increase of ECM density [8]. The ECM of the cirrhotic livers contains approximately six times as much matrix mass as the normal liver [9], which is a result of increased levels of mainly type I, type III, and type IV collagen [10]. However, levels of matrix metalloproteinases (MMPs), such as MMP-9, also increase [11, 12].

The degradation of the ECM components by MMPs in both atherosclerosis and in liver fibrosis is accompanied by the release of breakdown products, called neoepitopes, into the circulation. Thus these neoepitopes may be useful indicators of arterial and hepatic enzymatic activity and the local matrix turnover in the atherosclerotic plaques and in diseased livers, respectively. Furthermore, these novel biomarkers may serve as predictors of future clinical outcomes and complications as well as markers of efficacy of drugs.

Neoepitope based biochemical markers found in urine and serum are receiving increased attention for their diagnostic and prognostic potential [13]. In particular for slowly progressing diseases, such as osteoporosis and osteoarthritis, bone resorption and cartilage degradation markers have been used extensively [14]. However at present, the use of neo-epitope based biochemical markers is restricted to those pathologies.

The aim of the current review is to present parallel insights of two fibrotic processes that involve pathological tissue turnover. We believe that we hereby may be able to identify common pathology denominators. This knowledge may be of great value in order to transfer valuable lessons from successful fibrosis biomarker development into the field of atherosclerosis.

Atherosclerosis - vulnerable vs. stable lesions

Atherosclerotic plaque formation in the arterial intima, results from complex cellular interactions mainly between resident cells of the vessel wall, such as smooth muscle cells (SMCs) and endothelial cells (ECs), and cells of the immune system, mainly leukocytes [15]. Disturbances in blood flow and increased lipid infiltration are major contributors to this process. When the atherosclerotic plaque has formed, it displays a very characteristic architecture composed of a fibrous cap covering a central core which mainly is composed of extracellular lipids and debris – the so called atheroma, which is soft, weak and highly thrombogenic. Unlimited phagocytosis of oxidized LDL by macrophages through scavenger receptors results in formation of foam cells, another hallmark of atherosclerosis [16]. Death of foam cells plays a central role in the formation of atheroma, together with extracellular binding of lipids to collagen fibers and proteoglycans [17, 18]. Histopathologic studies of atherosclerotic lesions in humans have revealed substantial variations in the thickness of fibrous caps, in the size of atheromas n the extent of calcifications and in relative amounts of inflammatory cells [19, 20]. Such studies contain valuable information and have contributed to understanding that only specific types of lesions appear to be associated with acute events of atherosclerotic disease.

Key molecular mechanisms of the atherosclerotic plaque and fibrotic cap formation

Extensive pathological analysis of fatally occluded arteries has led to realization that such fatal cardiovascular events are more likely due to artery occlusion by thrombi rather that due to simple obliteration of arterial lumen by atheroma [21-23]. During the evolvement of atherosclerosis, the affected vessels undergo a continuous process of remodeling, during which they initially compensate for the lesion development in the intima by increasing overall diameter [15, 24, 25]. Later as the disease progresses, remodeling may continue toward destructive stages in which the tissue weakens and the plaque structure is compromised. Thrombi develop at the areas where the continuity of a plaque's fibrous cap is disrupted by fissures [23] or in places where the endothelial lining is eroded [26, 27]. The weakest areas of the plaque are plaque "shoulders", where the plaque meets the normal arterial wall [28]. Pathological determinants associated with plaque instability include high lipid content and increased number of macrophage-derived foam cells [23]. In addition, it is widely recognized that a dynamic process of weakening contributes to plaque rupture. Here the extensive arterial plaque remodeling by matrix
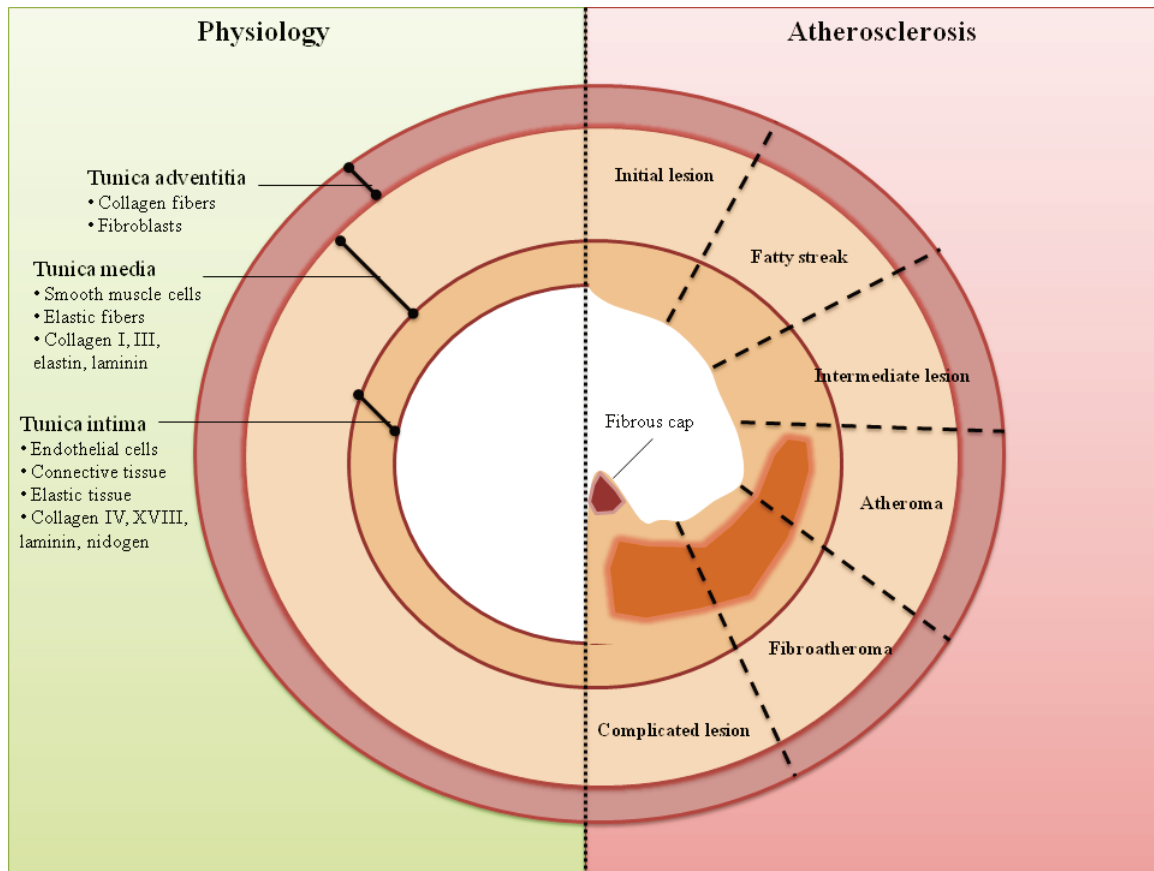
Figure 1. Cross section of an artery. Arterial structure is composed of 3 main layers, tunica adventitia which forms the outer layer of the structure, the tunica media which lie in the middle and the tunica intima which is in direct contact with blood circulation. During atherosclerosis progression, the arterial wall is gradually thickened and the lumen is narrowed. Complicated lesions are ultimately formed in an ongoing a process which can last for decades ultimately blocking blood flow through the artery.

degrading enzymes leading to active destruction of the extracellular matrix is a process identified in many advanced lesions.

Extracellular matrix remodeling in atherosclerosis

Cardiovascular disease is predicted to be the leading cause of death by 2020. Despite a lot of progress in earlier diagnosis and better treatment regimens, CVD remains one of the leading causes of death both in men and women in the western world [29]. Atherosclerosis is a systemic disease of the vessel wall and is considered responsible for ischemic heart disease, ischemic strokes, and critical limb ischemia [30]. These events almost always start with stable clinical manifestations of atherosclerosis result from progressive luminal narrowing by an atherosclerotic plaque development with inward remodeling (**Figure 1**).

The potential development of a thrombus over an underlying plaque is one of the major causes of acute coronary syndromes of unstable angina, myocardial infarction and ischemic sudden cardiac death [31, 32]. Thrombi forming near or on plaque may become incorporated into the plaque, where thrombin generation and the release of mediators by platelets may increase the rate of cell proliferation and thereby increase the production of extracellular matrix (ECM) proteins, further contributing to plaque growth [15, 33]. Many of the cell responses involved in plaque development have an inflammatory nature, further highlighting the pathophysiologic link between atherogenesis and thrombosis. The likehood of thrombosis increases with disruption of atherosclerotic plaques, the process where the fibrous cap sealing the plaque and preventing plaque material to come in contact with the interior of the blood vessel and the circulating blood. Recent

findings have indicated that inflammation influences the composition of the fibrous cap, increasing the risk of rupture [34, 35]. Various inflammatory cells, particularly monocytes infiltrate the atherosclerotic lesions and differentiate into macrophages and further into foam cells, thereby contributing to the development of the plaque [33]. Mast cells have also been identified in the atherosclerotic plaques, and may act in concert with macrophages promoting the inflammatory process [36]. Both macrophages/foam cells and mast cells contain a variety of enzymes such as serine proteases matrix-metalloproteinases, (MMPs), which are major participants in the degradation of ECM proteins of the atherosclerotic fibrous caps [37-39]. The biomechanical properties of blood vessels are largely dependent on ECM constituents and the absolute and relative quantities of these constituents. Thus the balance between tissue generation and degradation is a key modulator in the transition from physiology to pathology.

ECM Proteins: collagens and proteoglycans

Among many different collagen types described until now, type I and III collagen are the major fibrillar collagens detectable in vessels, representing 60% and 30% of vascular collagens, respectively [40, 41]. The remaining 10% include fibrillar type V collagen, fibril-associated collagens XII and XIV, microfibrillar collagen IV and type VIII collagen [40]. After secretion on the extracellular compartment and the removal of N- and C-terminal propeptides, collagen triple helices aggregate into fibrils [42]. During the formation of intermolecular cross-links, collagen fibers become increasingly insoluble, more refractory to the action of enzymes and show a progressive increase in tensile strength [40, 43]. The process of cross-linking is initiated by the lysyl oxidase (LOX) enzyme, through the oxidation of specific lysine or hydroxylysine residues in the N-terminal telopeptid regions. The resulting aldehydes undergo a series of reactions with adjacent reactive residues to give both inter- and intramolecular cross-links [44].

Proteoglycans, in particular versican, lumican, and biglycan have been identified in the ECM of the atherosclerotic arteries and implicated in facilitation of plaque rupture and thrombosis. However, at present, there is a complete lack of

understanding of the protease generated fragments of these molecules, and the relation to atherosclerosis progression and outcome. Distribution and expression of proteoglycans change during atherogenesis due to the matrix remodeling.

The chondroitin sulfate proteoglycan (CSPG) versican is present in the arterial wall of normal blood vessels. Versican interacts with hyaluronan, a long chain molecular weight glycosaminoglycan (GAG) that is also present in the ECM of blood vessels and increases as versican in vascular disease [45]. Versican is also present in the advanced lesions and is prominent at the edges of the necrotic core. Furthermore, versican is also present in close proximity to deposited lipoproteins, suggesting a role in the retention of lipoproteins in the vessel wall [46].

Lumican, a keratan sulfate proteoglycan, has been involved in wound healing and tissue response injuries. In atherosclerotic arteries lumican is localized in the inner layer of the media, suggesting a local synthesis by intimal and medial smooth muscle cells of the atherosclerotic arteries [47]. Rodent models of atherosclerosis suggest an increase in lumican content by app. 2 fold in the intima of atherosclerotic arteries [48], suggesting high turnover. However, the role of lumican in human atherosclerotic tissues is not clearly elucidated.

Biglycan – a member of small leucine rich family, has been implicated in lipoprotein retention in human atherosclerosis, through the interactions of its glycosaminoglycan side chains [49]. Immunohistochemical staining for biglycan in arteries reveal prominent biglycan localization to the proteoglycan rich layer of the elastic laminae, but the distribution of biglycan seems to shift during atherosclerosis, since biglycan was mostly localized at the plaque shoulders and underneath the lesion.

Proteases

The MMP family posses fairly homologous amino acid domains and share several important features. All cell-secreted MMPs are produced as latent pro-enzymes or zymogens, thus a common feature of MMP activation is necessary in order to convert the enzymes to an active form. Furthermore, all MMPs are

dependent on the zinc ion for their proteolytic action [50]. The mechanism of activation, also known as the cysteine switch mechanism [51] can be initiated either through a conformational modifications of the proenzyme molecule or through the proteolytic removal of its pro-domain. The conformational modification leads to partially active enzyme that can bind and digest substrate and also, it can also initiate autocatalysis of the partially active enzyme. Through a series of consecutive series of auto-lytic steps, the MMPs are shortened going through a fully activated stage, and later with continuous processing loose the catalytic domain and thus the enzymatic activity. This molecular processing is characteristic of MMPs, whose enzymatic activity is absent under normal conditions because the stored zymogens are inactive and subsequent initiation of activation results in a limited life span for the enzyme. Moreover, the cells can also secrete natural MMP inhibitors, called tissue inhibitors of metalloproteinases (TIMPs) which can interfere with zymogen activation and with substrate degradation by activated MMPs [52].

MMPs have been classified into several classes. Each class shares high degree of sequence homology and substrate specificity. Some of the major classes of MMPs are collagenases, stromelysins, gelatinases, matrilysines and membrane-associated (MT) MMPs.

Morphological and cell culture studies have contributed to gaining knowledge on MMP expression in healthy and diseased tissues. In culture, endothelial cells (ECs) synthesize several MMPs and their natural inhibitors [53-55]. All cultured ECs seem to constitutively secrete MMP-2, which is thought to be necessary for the normal turnover of all cellular basement membranes. Expression of MMP-1 and MMP-3 by ECs is induced or enhanced depending on the vascular source of EC, by exposure to cytokines [56, 57]. Cytokines have also been shown to stimulated in vitro expression of MT1-MMP in human saphenous vein EC and in smooth muscle cells (SMCs) [58]. Vascular SMC constitute most of the cells in the blood vessel wall and are therefore likely the main contributors to protein synthesis in general. In vitro, SMC isolated from different vascular sources of different species have been shown to produce several types of MMPs [59-64]. In vitro, MMP-2

was found to be one of the major MMPs secreted by the SMCs, thus likely to be abundant in the vascular wall, and may be one of the main vascular MMPs under normal physiological conditions. Expression of pro-MMP9, pro-MMP1, and pro-MMP3 by human SMCs is induced in vitro by different growth factors and cytokines, which are expressed in atherosclerotic arteries in areas of atheroma infiltrated with inflammatory cells [65]. Furthermore, other mechanisms of activation of MMP-2 production by SMCs have been shown to be mediated by reactive oxygen species (ROS) [66]. In vitro ROS have been shown to be able to activate latent forms of gelatinases secreted by cultured human SMCs. Production of ROS known to be present in the atherosclerotic lesions is attributed to inflammatory cells [67].

Blood-borne cells are not a common component of the healthy vessel. However, the mono-cyte-derived macrophages have been identified in all atherosclerotic lesions and seem to be prevalent among the several types of inflammatory cells such as, T-lymphocytes and mast cells. Activated inflammatory cells have natural ability to penetrate tissues, largely due to the matrix degrading action of MMPs [68].

Key molecular mechanisms of fibrogenesis-the example of liver

Fibrosis results from chronic tissue injury and results in the formation of scar tissue formation. Even though fibrotic mechanisms may vary depending from the tissue affected, in all cases it the transition from an ECM balanced remodeling state to an unbalanced ECM remodeling (ECMR) state which is the main driver of shift to pathology [69]. During this process, ECM undergoes significant changes which alter the distribution, quantity and ultimately the quality of ECM [70-72]. The paradigm of ECM altered composition in the space of Disse where the predominant expression of collagen type III, IV, laminin and fibronectin under physiological conditions, is distorted during fibrosis initiation towards a state in which collagen type I is greatly upregulated is indicative of the ECM quantitative and qualitative changes that occur during fibrosis related ECMr (**Figure 2**) [73].

Since ECMr imbalances are in the centre of pathology creation, it is of great value to highlight some of the mechanisms, proteins, cells
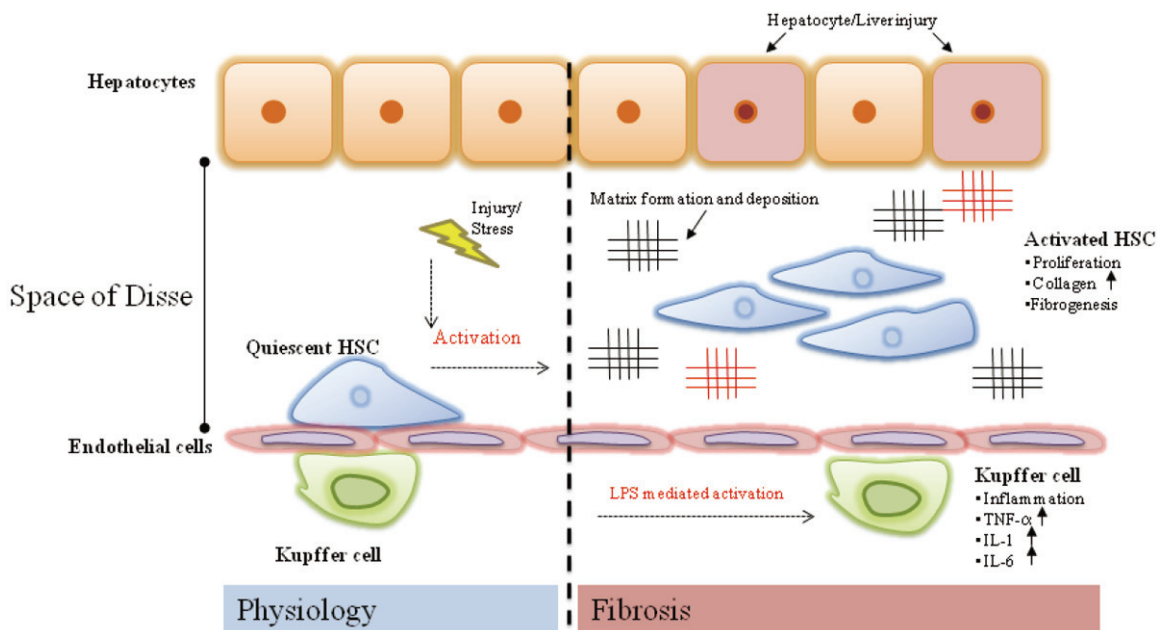
Figure 2. Quiescent HSC can be activated by a variety of chronic stress and/or signals such as alcohol, virus and auto-immune stimuli as well as by interaction from KC cytokines. When activated HSC can activate adjacent HSC and can produce MMPs and a number of different matrix constituents such as collagens and proteoglycans. These promote fibrogenesis and substantially alter tissue structure and ultimately function.

and proteases involved in different forms of fibrosis in order to highlight common mechanisms between atherofibrosis and other types of fibrotic events. For this reason, liver fibrosis mechanism will be used as an example that will facilitate the comparison with atherofibrotic events.

Liver extracellular matrix (ECM) consists of collagens, proteoglycans and glycoproteins, all of which play important, unique and interrelated functions in maintaining the physicochemical structure of liver tissue while they also posses signalling functions as both ligands and receptors [69, 70, 74]. Even though for years, the main focus was restricted on fibrillar collagens and the alterations in quantity and quality, the role of other proteins and proteoglycans such as fibronectin and laminin has become evident and brought additional attention to the important interplay of a wider variety of ECM constituents which collectively result in significantly increased, ECM derived protein in liver [75, 76]. An additional feature of this ECM centred interaction during liver fibrosis progression is the diverse post translational modification (PTM) and cross linking activities that concurrently have a direct impact on protein life cycle and pathology progression [77-80].

Myofibroblasts (MF) are the main matrix producing cells and due to this main function they are playing a key role in fibrosis processes in all tissues [69, 81, 82]. In liver, the MF precursor cells are the hepatic stellate cells (HSC) which are considered the main source of fibrotic processes, even though other mesenchymal cell types, also contribute to a lesser extend [83]. HSCs activation is a key event in liver fibrosis pathology as they are known to react to cellular injury and death as part of their constant interaction with their environment [84]. Activation is achieved by distinct stimuli, such as oxidative stress and apoptotic bodies, that can stimulate activation initiation and others that can ultimately result to fibrosis through contributing to HSC activation continuation, a process which include key events such as proliferation, fibrogenesis and ECM degradation that ultimately result in ECM accumulation and tissue fibrosis [85]. The multivariate role of HSCs in fibrotic processes and resulting inflammation is further highlighted by their role as antigen presenting cells (APCs) and containment of inflammasome components which can induce inflammatory responses as a reaction to cellular injury and death [84]. The involvement of the immune system in liver fibrosis processes is not limited to inflammatory events but is extended in ECMR
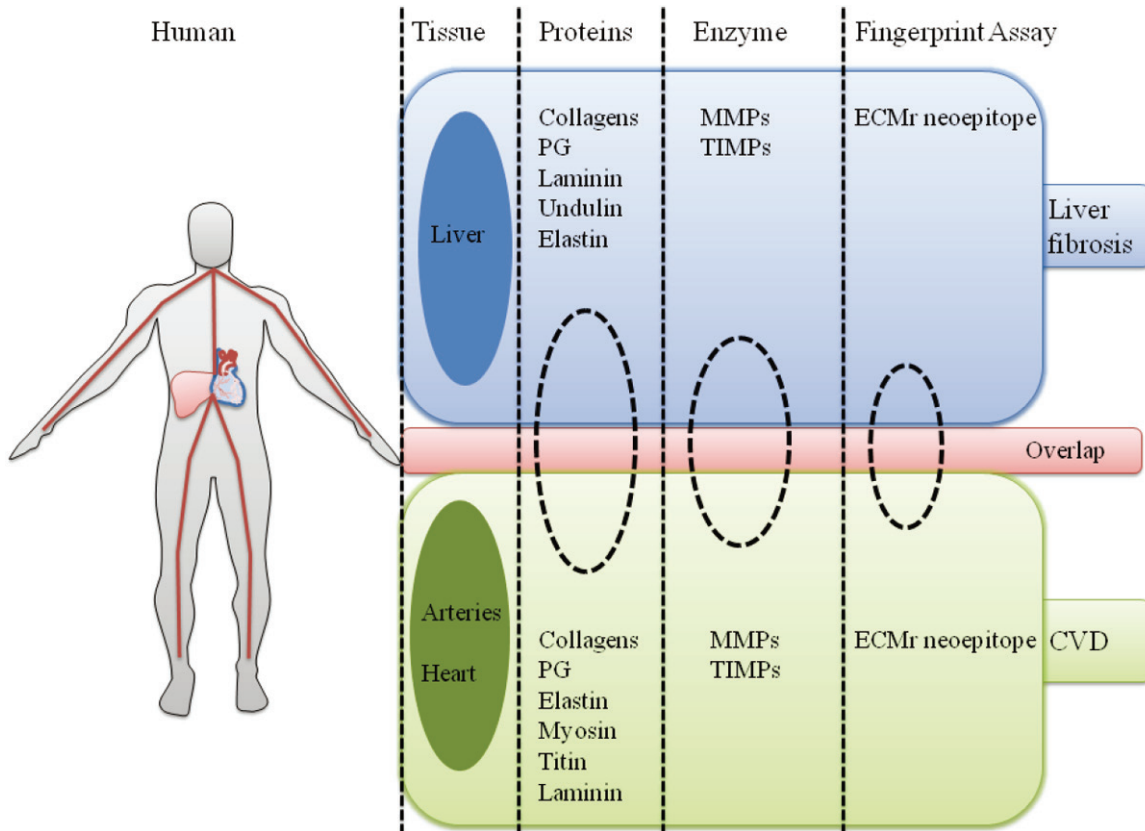
Figure 3. Tissue fingerprint technology relies on the selection and generation of pathology relevant neoepitopes. Among different tissues ECM may have both tissue specific as well as common macromolecules and proteases. This may result in ECMr neoepitope formation which can be either tissue specific or overlap among tissues. In turn, certain neoepitopes may be useful as disease predictors for several pathologies. The proteins and enzymes presented in the figure are only indicative.

through the contribution of macrophages, lymphocytes and monocytes in ECM degradation and interaction with myofibroblasts. Additional implication of immune cells to ECMR stems from information on the activity of cytokines such as Th1 and Th2 have been found to have antifibrogenic and fibrogenic properties respectively, adding to our knowledge of participating cells in liver fibrosis related pathology [86].

Kupffer cells (KCs) are another cell type which is also an active participant in the transition from balanced to unbalanced ECMR which ultimately leads to liver fibrosis. KCs are involved in livers response to various stresses as they are the local liver macrophages which upon LPS mediated activation can release a range of cytokines which can contribute to stellate cell activation and stimulate ECM synthesis [87]. KC activation via toll-like receptor 4 (TLR-4) was recently described, suggesting that macrophages may be serving as a link between fibrot-

ic and inflammatory processes [88]. KCs represent a vital source of chemoattractant molecules and they play a key role in HSC transformation to myofibroblasts, a feature which adds to their central part in activation of fibrotic processes and the transition from physiology to pathology [89]. KC cells have been described to be stimulated by apoptotic fragments, as in the case of HSC, and release fibrogenic mediators as a response while they have been found to also stimulate stellate cell apoptosis [90, 91].

An additional active participant in fibrotic progression is the sinusoidal endothelial cells (SEC), which are part of the endothelial wall and are interacting with HSCs during early liver fibrosis progression stages while also involved in sinusoidal capillarization through basement membrane proteins which is ultimately accompanying liver fibrosis development in central vein walls and is implicated to portal hypertension [92-95]. The morphological alterations

that SEC undergo after stress or injury it is believed to be an initial stimulus for fibrosis progression, since this is a process which precedes myofibroblast activation and ECM deposition [96].

Collagens, in terms of quantity, are the main ECM constituents. During fibrosis related pathology, increased collagen deposition is being counterbalanced by increased collagenolytic activity. This is mainly carried out by interstitial MMPs that degrade collagen fibers through TIMP regulation [97]. Proteases are active participants in both physiological and pathological related ECMr and their action directly affects the three dimensional structure of the matrix scaffold, the amount of fibrous and non-fibrous molecules as well as cell-matrix interactions. Alteration of the balance between TIMPs and MMPs contributes significantly to the progression of liver fibrosis [98]. More than 26 MMPs and 4 TIMPs have been identified so far and 11 of the MMPs have been found in the liver MMP levels are constantly changed during different stages of the progression of liver fibrosis [99].

Discussion

The first question that we seek to answer is whether there are common disease mechanisms and cell types involved in the pathologies of atherosclerosis and liver fibrosis. Both are ECMr related pathologies that involve and affect distinctly different tissues. However, both of these pathologies share some similar characteristics. Even though fibrosis in general can occur in a variety of tissues, the hallmark of the disease is the abnormal and unbalanced ECMr which is also the case in atherosclerosis. In order to highlight some of the common mechanisms that might be implicated in these two pathologies the example of liver fibrosis was used as a comparison to atherosclerosis. An overview of the mechanisms and key ECM participants of both pathologies was presented. From these it is evident that in both pathologies, a large number of ECM components are involved in the shift towards abnormal ECMr. Some of these active pathology participants seem to be common for both disease states since in both cases they form the ECM scaffold. An example of such an occurrence is collagen type I, III, IV and V as well as proteoglycans such as versican, biglycan, decorin and perlecan.

Concurrently, both pathologies seem to share a considerable amount of proteases such as MMP-1, -2, -3, -8, -9, -13 and -14. In both cases, the main aim of the macrophage derived increased protease levels is removal of the excessive ECM. The constant interaction of common ECM components across different tissues may create protein fingerprints that may overlap, while tissue specific interactions may create tissue specific fingerprints (**Figure 3**).

The second question we set to answer is whether lessons learned through the development of fibrosis markers can be used for atherosclerosis biomarker development. We have previously described and validated in pre-clinical and clinical studies a number of novel ECMr specific biomarkers for fibrotic diseases based on the tissue fingerprint technology [71, 72, 100-107]. This unique technology is based on precise measurements of specific protein fragments, neo-epitopes that are generated due to proteolytic action of proteases on tissue during pathology. The interaction of a specific ECM related protein with a particular protease could form a unique fragment that could be indicative of *in vivo* underlying ECMr.

The recognition that ECM constituent proteins found in both pathologies are interacting with a set of identical proteases leads to the probable suggestion that the valuable experience and positive results obtained so far in the field of fibrosis may also be applicable in the field of atherosclerosis. Early suggestions of neoepitope application in the field of atherosclerosis relied on the observation that macrophage mediated proteolysis of ECM constituents and their release into circulation is an important process in atherosclerogenesis [108]. The understanding of the above relationship lead to the suggestion that specific neo-epitope fragments may be of unique diagnostic and prognostic value for detailed atherosclerosis staging and biomarker development and maybe related with the cardiovascular continuum [15].

Early evidence of the possible opportunities for biomarker development indicates that the above approach may have some promising merits in the case of atherosclerosis. The examples of Mimecan and Collagen III markers are indicative of novel and promising ECM biomarkers that are related to atheromatic plaques and are utilising the concept of protein finger-

prints [104, 109]. The example of MMP-9 degraded Collagen III biomarker which is found to be upregulated in liver and skin fibrosis as well as in atherosclerosis is highly suggestive of the possible common mechanisms occurring in both diseases [72, 104, 105, 110]. Additionally, novel cardiovascular specific markers that are relying on the tissue fingerprint technology show some promising early results in clinical studies which should be investigated further [111].

The transition of ECMr from physiological to pathological processes is lengthy and complicated. In both fibrosis and atherosclerosis this transition involves a long period of transient state in which ECMr shift is asymptomatic and therefore almost impossible to diagnose. The above suggests that individuals that unknowingly are experiencing this transient underlying pathology of either fibrosis or atherosclerosis would await the first symptoms to appear before any diagnosis would be possible with the current diagnostic means. The vast majority of existing biomarkers utilised in clinics are unfortunately end stage disease markers, where few successful intervention options exist [15]. The requirement for new markers that can accurately describe the early and tissue specific shift to pathology is as important as ever. According to the world health organisation 17.3 million people have died from CVD related pathologies in 2008, and this number is expected to reach 23.6 million by the year 2030. Adding to the above numbers is individuals gradually developing fibrosis related pathology in any tissue (lung, liver etc.). The above numbers indicate that great numbers of individuals that are seemingly healthy today maybe already experiencing this transient ECMr shift to pathology which will eventually place them in high risk groups for either CVD or fibrotic diseases by 2030. It is therefore of great importance to characterise markers that can facilitate early diagnosis and can accurately monitor pathology related ECMr. Utilisation of protein fingerprint markers seems to have promising momentum towards this quest since they seem to be informative of protease specific protein cleavages that are related with pathology. Such fragments may become of great diagnostic and potentially prognostic value in the future. The example of Titin-12670 (TIM) marker is indicative of such promising potential, even though

additional work is required to further validate these findings [112].

Acknowledgements

Address correspondence to: Dr. Efstathios Vassiliadis, Nordic Bioscience A/S, Herlev Hovedgade 207, 2730 Herlev, Denmark. Phone: (45) 44547738; FAX: (45) 44525251; E-mail: eva@nordicbioscience.com

References

[1] Lochter A and Bissel MJ. An odyssey from breast to bone: multi-step control of mammary metastases and osteolysis by matrix metalloproteinases. APMIS 1999; 107: 128-136.

[2] Karsdal MA, Madsen SH, Christiansen C, Henriksen K, Fosang AJ, and Sondergaard BC. Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity. Arthritis Res Ther 2008; 10: R63

[3] Galis ZS, Sukhova GK, Kranzhofer R, Clark S, and Libby P. Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. Proc Natl Acad Sci U S A 1995; 92: 402-406.

[4] Katsuda S and Kaji T. Atherosclerosis and extracellular matrix. J Atheroscler Thromb 2003; 10: 267-274.

[5] Zhen EY, Brittain IJ, Laska DA, Mitchell PG, Sumer EU, Karsdal MA, and Duffin KL. Characterization of metalloprotease cleavage products of human articular cartilage. Arthritis Rheum 2008; 58: 2420-2431.

[6] Galis ZS and Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002; 90: 251-262.

[7] Friedman SL. Liver fibrosis -- from bench to bedside. J Hepatol 2003; 38 Suppl 1: S38-S53.

[8] Martinez-Hernandez A and Amenta PS. The hepatic extracellular matrix. II. Ontogenesis, regeneration and cirrhosis. Virchows Arch A Pathol Anat Histopathol 1993; 423: 77-84.

[9] Schuppan D. Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. Semin Liver Dis 1990; 10: 1-10.

[10] Rojkind M, Giambrone MA, and Biempica L. Collagen types in normal and cirrhotic liver. Gastroenterology 1979; 76: 710-719.

[11] Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2000; 279: G245-G249.

[12] Hemmann S, Graf J, Roderfeld M, and 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.

[13] Karsdal MA, Henriksen K, Leeming DJ, Mitchell P, Duffin K, Barascuk N, Klickstein L, Aggarwal P, Nemirovskiy O, Byrjalsen I, Qvist P, Bay-Jensen AC, Dam EB, Madsen SH, and Christiansen C. 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.

[14] Schaller S, Henriksen K, Hoegh-Andersen P, Sondergaard BC, Sumer EU, Tanko LB, Qvist P, and Karsdal MA. 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.

[15] Vassiliadis E, Barascuk N, Didangelos A, and Karsdal MA. Novel cardiac-specific biomarkers and the cardiovascular continuum. Biomark Insights 2012; 7: 45-57.

[16] Steinberg D and Witztum JL. Lipoproteins and atherogenesis. Current concepts. JAMA 1990; 264: 3047-3052.

[17] Wight TN. Cell biology of arterial proteoglycans. Arteriosclerosis 1989; 9: 1-20.

[18] Wight TN and Merrilees MJ. Proteoglycans in atherosclerosis and restenosis: key roles for versican. Circ Res 2004; 94: 1158-1167.

[19] Davies MJ, Richardson PD, Woolf N, Katz DR, and Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. Br Heart J 1993; 69: 377-381.

[20] van der Wal AC, Becker AE, van der Loos CM, Tigges AJ, and Das PK. Fibrous and lipid-rich atherosclerotic plaques are part of interchangeable morphologies related to inflammation: a concept. Coron Artery Dis 1994; 5: 463-469.

[21] Fuster V, Stein B, Ambrose JA, Badimon L, Badimon JJ, and Chesebro JH. Atherosclerotic plaque rupture and thrombosis. Evolving concepts. Circulation 1990; 82: II47-II59.

[22] Falk E. Dynamics in thrombus formation. Ann N Y Acad Sci 1992; 667: 204-223.

[23] Lendon C, Born GV, Davies MJ, and Richardson PD. Plaque fissure: the link between atherosclerosis and thrombosis. Nouv Rev Fr Hematol 1992; 34: 27-29.

[24] Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, and Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med 1987; 316: 1371-1375.

[25] Clarkson TB, Prichard RW, Morgan TM, Petrick GS, and Klein KP. Remodeling of coronary arteries in human and nonhuman primates. JAMA 1994; 271: 289-294.

[26] Fuster V. Elucidation of the role of plaque instability and rupture in acute coronary events. Am J Cardiol 1995; 76: 24C-33C.

[27] Fuster V, Stein B, Ambrose JA, Badimon L, Badimon JJ, and Chesebro JH. Atherosclerotic plaque rupture and thrombosis. Evolving concepts. Circulation 1990; 82: II47-II59.

[28] Fuster V, Stein B, Ambrose JA, Badimon L, Badimon JJ, and Chesebro JH. Atherosclerotic plaque rupture and thrombosis. Evolving concepts. Circulation 1990; 82: II47-II59.

[29] Murray CJ and Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 1997; 349: 1436-1442.

[30] Shah PK. Inflammation and plaque vulnerability. Cardiovasc Drugs Ther 2009; 23: 31-40.

[31] Shah PK. Molecular mechanisms of plaque instability. Curr Opin Lipidol 2007; 18: 492-499.

[32] DeWood MA, Stifter WF, Simpson CS, Spores J, Eugster GS, Judge TP, and Hinnen ML. Coronary arteriographic findings soon after non-Q-wave myocardial infarction. N Engl J Med 1986; 315: 417-423.

[33] Libby P. The interface of atherosclerosis and thrombosis: basic mechanisms. Vasc Med 1998; 3: 225-229.

[34] Biasucci LM, Leo M, and De Maria GL. Local and systemic mechanisms of plaque rupture. Angiology 2008; 59: 73S-76S.

[35] Finet G, Ohayon J, Rioufol G, Lefloch S, Tracqui P, Dubreuil O, and Tabib A. Morphological and biomechanical aspects of vulnerable coronary plaque. Arch Mal Coeur Vaiss 2007; 100: 547-553.

[36] Libby P. Inflammation in atherosclerosis. Nature 2002; 420: 868-874.

[37] Schroeder AP and Falk E. Pathophysiology and inflammatory aspects of plaque rupture. Cardiol Clin 1996; 14: 211-220.

[38] Finet G, Ohayon J, Rioufol G, Lefloch S, Tracqui P, Dubreuil O, and Tabib A. Morphological and biomechanical aspects of vulnerable coronary plaque. Arch Mal Coeur Vaiss 2007; 100: 547-553.

[39] Galis ZS, Sukhova GK, Kranzhofer R, Clark S, and Libby P. Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. Proc Natl Acad Sci U S A 1995; 92: 402-406.

[40] Prockop DJ and Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995; 64: 403-434.

[41] van der RM and Garrone R. Collagen family of proteins. FASEB J 1991; 5: 2814-2823.

[42] Bonnet J, Garderes PE, Aumailley M, Moreau C, Gouverneur G, Benchimol D, Crockett R, Larrue J, and Bricaud H. Serum type III procollagen peptide levels in coronary artery disease (a marker of atherosclerosis). Eur J Clin Invest 1988; 18: 18-21.

[43] Wu JJ and Eyre DR. Structural analysis of crosslinking domains in cartilage type XI collagen. Insights on polymeric assembly. J Biol Chem 1995; 270: 18865-18870.

[44] Wu JJ and Eyre DR. Structural analysis of crosslinking domains in cartilage type XI collagen. Insights on polymeric assembly. J Biol Chem 1995; 270: 18865-18870.

[45] Wight TN and Merrilees MJ. Proteoglycans in atherosclerosis and restenosis: key roles for versican. Circ Res 2004; 94: 1158-1167.

[46] Wight TN and Merrilees MJ. Proteoglycans in atherosclerosis and restenosis: key roles for versican. Circ Res 2004; 94: 1158-1167.

[47] Onda M, Ishiwata T, Kawahara K, Wang R, Naito Z, and Sugisaki Y. Expression of lumican in thickened intima and smooth muscle cells in human coronary atherosclerosis. Exp Mol Pathol 2002; 72: 142-149.

[48] Knauper V, Lopez-Otin C, Smith B, Knight G, and Murphy G. Biochemical characterization of human collagenase-3. J Biol Chem 1996; 271: 1544-1550.

[49] O'Brien KD, Lewis K, Fischer JW, Johnson P, Hwang JY, Knopp EA, Kinsella MG, Barrett PH, Chait A, and Wight TN. Smooth muscle cell biglycan overexpression results in increased lipoprotein retention on extracellular matrix: implications for the retention of lipoproteins in atherosclerosis. Atherosclerosis 2004; 177: 29-35.

[50] Van Wart HE and Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci U S A 1990; 87: 5578-5582.

[51] Van Wart HE and Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci U S A 1990; 87: 5578-5582.

[52] Murphy G. Matrix metalloproteinases and their inhibitors. Acta Orthop Scand Suppl 1995; 266: 55-60.

[53] Herron GS, Banda MJ, Clark EJ, Gavrilovic J, and Werb Z. Secretion of metalloproteinases by stimulated capillary endothelial cells. II. Expression of collagenase and stromelysin activities is regulated by endogenous inhibitors. J Biol Chem 1986; 261: 2814-2818.

[54] Herron GS, Werb Z, Dwyer K, and Banda MJ. Secretion of metalloproteinases by stimulated capillary endothelial cells. I. Production of procollagenase and prostromelysin exceeds expression of proteolytic activity. J Biol Chem 1986; 261: 2810-2813.

[55] Hanemaaijer R, Koolwijk P, le CL, de Vree WJ, and van H, V. Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. Biochem J 1993; 296: 803-809.

[56] Hanemaaijer R, Koolwijk P, le CL, de Vree WJ, and van H, V. Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. Biochem J 1993; 296: 803-809.

[57] Herron GS, Werb Z, Dwyer K, and Banda MJ. Secretion of metalloproteinases by stimulated capillary endothelial cells. I. Production of procollagenase and prostromelysin exceeds expression of proteolytic activity. J Biol Chem 1986; 261: 2810-2813.

[58] Rajavashisth TB, Liao JK, Galis ZS, Tripathi S, Laufs U, Tripathi J, Chai NN, Xu XP, Jovinge S, Shah PK, and Libby P. Inflammatory cytokines and oxidized low density lipoproteins increase endothelial cell expression of membrane type 1-matrix metalloproteinase. J Biol Chem 1999; 274: 11924-11929.

[59] Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, Lark MW, Amento E, and Libby P. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. Circ Res 1994; 75: 181-189.

[60] Evans CH, Georgescu HI, Lin CW, Mendelow D, Steed DL, and Webster MW. Inducible synthesis of collagenase and other neutral metalloproteinases by cells of aortic origin. J Surg Res 1991; 51: 399-404.

[61] Yanagi H, Sasaguri Y, Sugama K, Morimatsu M, and Nagase H. Production of tissue collagenase (matrix metalloproteinase 1) by human aortic smooth muscle cells in response to platelet-derived growth factor. Atherosclerosis 1991; 91: 207-216.

[62] Au YP, Montgomery KF, and Clowes AW. Heparin inhibits collagenase gene expression mediated by phorbol ester-responsive element in primate arterial smooth muscle cells. Circ Res 1992; 70: 1062-1069.

[63] Pauly RR, Passaniti A, Bilato C, Monticone R, Cheng L, Papadopoulos N, Gluzband YA, Smith

L, Weinstein C, Lakatta EG, et al. Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity and is inhibited by cellular differentiation. Circ Res 1994; 75: 41-54.

[64] Southgate KM, Davies M, Booth RF, and Newby AC. Involvement of extracellular-matrix-degrading metalloproteinases in rabbit aortic smooth-muscle cell proliferation. Biochem J 1992; 288: 93-99.

[65] Libby P and Hansson GK. Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions. Lab Invest 1991; 64: 5-15.

[66] Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, and Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996; 98: 2572-2579.

[67] Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, and Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996; 98: 2572-2579.

[68] Goetzl EJ, Banda MJ, and Leppert D. Matrix metalloproteinases in immunity. J Immunol 1996; 156: 1-4.

[69] Wight TN and Potter-Perigo S. The Extracellular Matrix: An Active or Passive Player in Fibrosis. Am J Physiol Gastrointest Liver Physiol 2011; 301: G950-5.

[70] Schuppan D, Ruehl M, Somasundaram R, and Hahn EG. Matrix as a modulator of hepatic fibrogenesis. Semin Liver Dis 2001; 21: 351-372.

[71] Vassiliadis E, Veidal SS, Simonsen H, Larsen DV, Vainer B, Chen X, Zheng Q, Karsdal MA, and Leeming DJ. Immunological detection of the type V collagen propeptide fragment, PVCP-1230, in connective tissue remodeling associated with liver fibrosis. Biomarkers 2011; 16: 426-433.

[72] Vassiliadis E, Larsen DV, Clausen RE, Veidal SS, Barascuk N, Larsen L, Simonsen H, Silvestre TS, Hansen C, Overgaard T, Leeming DJ, and Karsdal MA. 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.

[73] Gorrell MD. Liver fibrosis: the hepatocyte revisited. Hepatology 2007; 46: 1659-1661.

[74] Streuli C. Extracellular matrix remodelling and cellular differentiation. Curr Opin Cell Biol 1999; 11: 634-640.

[75] Moriya K, Bae E, Honda K, Sakai K, Sakaguchi T, Tsujimoto I, Kamisoyama H, Keene DR, Sasaki T, and Sakai T. A fibronectin-independent mechanism of collagen fibrillogenesis in adult liver remodeling. Gastroenterology 2011; 140: 1653-1663.

[76] Kershenobich SD and Weissbrod AB. Liver fibrosis and inflammation. A review. Ann Hepatol 2003; 2: 159-163.

[77] Karsdal MA, Henriksen K, Leeming DJ, Woodworth T, Vassiliadis E, and Bay-Jensen AC. 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.

[78] Kagan HM. Lysyl oxidase: mechanism, regulation and relationship to liver fibrosis. Pathol Res Pract 1994; 190: 910-919.

[79] Piemonte F, Petrini S, Gaeta LM, Tozzi G, Bertini E, Devito R, Boldrini R, Marcellini M, Ciacco E, and Nobili V. Protein glutathionylation increases in the liver of patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2008; 23: e457-e464.

[80] Kivirikko KI. Collagens and their abnormalities in a wide spectrum of diseases. Ann Med 1993; 25: 113-126.

[81] Kisseleva T and Brenner DA. Fibrogenesis of parenchymal organs. Proc Am Thorac Soc 2008; 5: 338-342.

[82] Strieter RM, Keeley EC, Hughes MA, Burdick MD, and Mehrad B. The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. J Leukoc Biol 2009; 86: 1111-1118.

[83] Lee Y and Friedman SL. Fibrosis in the liver: acute protection and chronic disease. Prog Mol Biol Transl Sci 2010; 97: 151-200.

[84] Watanabe A, Sohail MA, Gomes DA, Hashmi A, Nagata J, Sutterwala FS, Mahmood S, Jhandier MN, Shi Y, Flavell RA, and Mehal WZ. Inflammasome-mediated regulation of hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2009; 296: G1248-G1257.

[85] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134: 1655-1669.

[86] Marra F, Aleffi S, Galastri S, and Provenzano A. Mononuclear cells in liver fibrosis. Semin Immunopathol 2009; 31: 345-358.

[87] Bilzer M, Roggel F, and Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver Int 2006; 26: 1175-1186.

[88] Seki E, De MS, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, and Schwabe RF. TLR4 en-

hances TGF-beta signaling and hepatic fibrosis. Nat Med 2007; 13: 1324-1332.

[89] Kolios G, Valatas V, and Kouroumalis E. Role of Kupffer cells in the pathogenesis of liver disease. World J Gastroenterol 2006; 12: 7413-7420.

[90] Canbay A, Feldstein AE, Higuchi H, Werneburg N, Grambihler A, Bronk SF, and Gores GJ. Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. Hepatology 2003; 38: 1188-1198.

[91] Fischer R, Cariers A, Reinehr R, and Haussinger D. Caspase 9-dependent killing of hepatic stellate cells by activated Kupffer cells. Gastroenterology 2002; 123: 845-861.

[92] Shi J, Hao JH, Ren WH, Zhu JR, Wang SY, and Xie YB. Pathogenesis of liver fibrosis in patients with chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi 2009; 17: 443-445.

[93] Bhunchet E and Fujieda K. Capillarization and venularization of hepatic sinusoids in porcine serum-induced rat liver fibrosis: a mechanism to maintain liver blood flow. Hepatology 1993; 18: 1450-1458.

[94] Thabut D and Shah V. Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? J Hepatol 2010; 53: 976-980.

[95] DeLeve LD, Wang X, and Guo Y. Sinusoidal endothelial cells prevent rat stellate cell activation and promote reversion to quiescence. Hepatology 2008; 48: 920-930.

[96] DeLeve LD. Hepatic microvasculature in liver injury. Semin Liver Dis 2007; 27: 390-400.

[97] Hemmann S, Graf J, Roderfeld M, and 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.

[98] Benyon RC and Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liver Dis 2001; 21: 373-384.

[99] Amalinei C, Caruntu ID, Giusca SE, and Balan RA. Matrix metalloproteinases involvement in pathologic conditions. Rom J Morphol Embryol 2010; 51: 215-228.

[100] Skjot-Arkil H, Clausen RE, Nguyen QH, Wang Y, Zheng Q, Martinez FJ, Hogaboam CM, Han M, Klickstein LB, Larsen MR, Nawrocki A, Leeming DJ, and Karsdal MA. Measurement of MMP-9 and -12 degraded elastin (ELM) provides unique information on lung tissue degradation. BMC Pulm Med 2012; 12: 34

[101] Skjot-Arkil H, Schett G, Zhang C, Larsen DV, Wang Y, Zheng Q, Larsen MR, Nawrocki A, Bay-Jensen AC, Henriksen K, Christiansen C, Alexandersen P, Leeming DJ, and Karsdal MA. 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.

[102] Veidal SS, Karsdal MA, Vassiliadis E, Nawrocki A, Larsen MR, Nguyen QH, Hagglund P, Luo Y, Zheng Q, Vainer B, and Leeming DJ. 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

[103] Segovia-Silvestre T, Reichenbach V, Fernandez-Varo G, Vassiliadis E, Barascuk N, Morales-Ruiz M, Karsdal MA, and Jimenez W. 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

[104] Barascuk N, Vassiliadis E, Larsen L, Wang J, Zheng Q, Xing R, Cao Y, Crespo C, Lapret I, Sabatini M, Villeneuve N, Vilaine JP, Rasmussen LM, Register TC, and Karsdal MA. 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.

[105] Vassiliadis E, Veidal SS, Barascuk N, Mullick JB, Clausen RE, Larsen L, Simonsen H, Larsen DV, Bay-Jensen AC, Segovia-Silvestre T, Leeming DJ, and Karsdal MA. Measurement of matrix metalloproteinase 9-mediated collagen type III degradation fragment as a marker of skin fibrosis. BMC Dermatol 2011; 11: 6

[106] Veidal SS, Vassiliadis E, Barascuk N, Zhang C, Segovia-Silvestre T, Klickstein L, Larsen MR, Qvist P, Christiansen C, Vainer B, and Karsdal MA. Matrix metalloproteinase-9-mediated type III collagen degradation as a novel serological biochemical marker for liver fibrogenesis. Liver Int 2010; 30: 1293-1304.

[107] Veidal SS, Vassiliadis E, Bay-Jensen AC, Tougas G, Vainer B, and Karsdal MA. 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

[108] Skjot-Arkil H, Barascuk N, Register T, and 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.

[109] Barascuk N, Vassiliadis E, Zheng Q, Wang Y, Wang W, Larsen L, Rasmussen LM, and Karsdal MA. 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.

[110] Barascuk N, Veidal SS, Larsen L, Larsen DV, Larsen MR, Wang J, Zheng Q, Xing R, Cao Y, Rasmussen LM, and Karsdal MA. 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.

[111] Vassiliadis E, Melholt RL, Byrjalsen I, Larsen DV, Chaturvedi R, Hosbond S, Saabye L, Diederichsen AC, Genovese F, Duffin KL, Zheng Q, Chen X, Leeming DJ, Christiansen C, and Kars-dal MA. Clinical evaluation of a matrix metallo-proteinase-12 cleaved fragment of titin as a cardiovascular-specific serological biomarker. J Transl Med 2012; 10: 140

[112] Vassiliadis E, Melholt RL, Byrjalsen I, Larsen DV, Chaturvedi R, Hosbond S, Saabye L, Diederichsen AC, Genovese F, Duffin KL, Zheng Q, Chen X, Leeming DJ, Christiansen C, and Kars-dal MA. Clinical evaluation of a matrix metallo-proteinase-12 cleaved fragment of titin as a cardiovascular-specific serological biomarker. J Transl Med 2012; 10: 140.