Clinica Chimica Acta 433 (2014) 111–122

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Monitoring fibrogenic progression in the liver

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ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form 21 February 2014
Accepted 23 February 2014
Available online 5 March 2014

Keywords:
Biological markers
Liver cirrhosis
Cytokines
Blood chemical analysis
Serologic tests

ABSTRACT

The clinical course of chronic liver diseases is significantly dependent on the progression rate of fibrosis which is the unstructured replacement of injured parenchyma by extracellular matrix. Despite intensive studies, the clinical opportunities for patients with fibrosing liver diseases have not improved. This will be changed by increasing knowledge of new pathogenetic mechanisms, which complement the "canonical principle" of fibrogenesis. The latter is based on the activation of hepatic stellate cells and their transdifferentiation to myofibroblasts induced by hepatocellular injury and consecutive inflammatory mediators such as TGF-β. Stellate cells express a broad spectrum of matrix components. New mechanisms indicate that the heterogeneous pool of (myo-)fibroblasts can be supplemented by epithelial–mesenchymal transition (EMT) from cholangiocytes and potentially also from hepatocytes to fibroblasts, by influx of bone marrow-derived fibrocytes in the damaged liver tissue and by differentiation of a subgroup of monocytes to fibroblasts after homing in the damaged tissue. These processes are regulated by the cytokines TGF-β and BMP-7, chemokines, colony-stimulating factors, metalloproteinases and numerous trapping proteins. They offer innovative diagnostic and therapeutic options. As an example, modulation of TGF-β/BMP-7 ratio changes the rate of EMT, and so the simultaneous determination of these parameters and of the connective tissue growth factor (CTGF) in serum might provide information on fibrogenic activity. Also, proteomic and glycomic approaches of serum are under investigation to set up specific protein profiles in patients with liver fibrosis. The aim of this article is to present the current pathogenetic concepts of liver fibrosis and to discuss established and novel diagnostic approaches to reflect the process of hepatic fibrogenesis in the medical laboratory.

Contents

1. Introduction

Chronic liver diseases are the fifth most frequent cause of death in the European Union, as they entail multiple risks, such as portal

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http://dx.doi.org/10.1016/j.cca.2014.02.021
0009-8981/

hypertension, ascites, spontaneous bacterial peritonitis, hepatorenal and hepatopulmonary syndromes, hepatic encephalopathy and, of course, hepatocellular carcinoma (HCC) [1].

Liver fibrosis, and ultimately liver cirrhosis, is the common end-stage of all chronic liver diseases. Experimental and clinical studies of the past twenty years provide a detailed knowledge of structure and composition of the extracellular matrix (ECM) in normal and fibrotic liver tissue [2,3], of the cellular origin of the various matrix components [4], of the cytokine- and growth factor-regulated stimulation of ECM synthesis (fibrogenesis) and regulation of matrix degradation (fibrolysis) [1,5,6], of several genetic conditions predisposing for fibrogenesis [1,2,7], and of multiple, experimentally successful therapeutic approaches. Until now the clinical benefit derived from basic research in the context of translational medicine is scarce with regard to an effective, harmless and site-directed antifibrotic therapy and approved non-invasive diagnostic measures of the activity of fibrogenesis ("grading") and/or of the extent of the fibrotic organ transition ("staging") using serum parameters [8]. The failure of clinical success boosts current research on fibrosis and fibrogenesis not only of the liver, but also of the lung, kidney, pancreas, heart, skin, bone marrow and other organs with the result that during the last four to five years very important new insights into the pathogenesis of fibrosis and of related diagnostic and therapeutic options have been made [9]. Evolving pathogenetic concepts supplement the so-called "canonical principle" of liver fibrogenesis, which has been worked out in detail during the last twenty years and which is based, in principle, on the activation of hepatic stellate cells (HSCs).

Fibrosis is characterized by a severalfold increase of the extracellular matrix that comprises collagens, structural glycoproteins, sulfated proteoglycans and hyaluronan by a histological redistribution with a preferred initial matrix deposition in the subendothelial space of Disse leading to the formation of an incomplete subendothelial basement membrane creating additional diffusion barriers between hepatocytes and the liver sinusoid ("capillarization of sinusoids"). It is also characterized by changes in the microstructure of collagens (e.g. degree of hydroxylation of proline and lysine), glycoproteins (variations of the carbohydrate structure) and proteoglycans (changes of the degree of sulfation of the glycosaminoglycan side chains) (Fig. 1). It is known for a long time that the increase of ECM in the parenchyma is not a passive process caused by condensation of pre-existing septa of connective tissue due to necrotic and apoptotic collapses of the parenchyma. It is rather an active biosynthetic process, which is attributed to stimulated matrix production in portal or peribiliary fibroblasts and, in particular, in contractile myofibroblasts (MFBs) localized initially in the subendothelial space of Disse. The development of MFB is the result of a multi-step sequence which originates from liver cell necrosis induced by various noxious agents (toxic, immunologic) [10,11] (Fig. 2). As a consequence, HSC, formerly called vitamin A-storing cells, fat-storing cells, arachnocytes and Ito-cells [12,13], which are localized in the immediate vicinity of hepatocytes are activated (Fig. 2). HSCs are liver pericytes, which embrace the endothelial cell layer of the sinusoids with thorn-like micro-projections providing physical contact not only to sinusoidal endothelial cells, but also to the cell body of the hepatocytes [14]. HSCs constitute about one third of the non-parenchymal cell population (Kupffer cells, endothelial cells, HSC) and about 15% of the total liver resident cells including hepatocytes. The "hepatic stellate cell index", i.e. the number of HSC per 1000 hepatocytes was estimated to be 109 in the healthy rat liver [15]. The spindle-like cell body of HSC contains multiple triglyceride-rich vacuoles in which vitamin A metabolites (retinoids) are dissolved and stored [16]. About 85% of the vitamin A of the liver is found in HSC. Additional functions of these cells were recently discovered: They seem to play a role as antigen presenting cells (APCs) [17–19]. Being $CD133^+$ progenitor cells with the ability to differentiate to progenitor endothelial cells and hepatocytes suggesting important roles in liver regeneration and repair [20]. For example, they are involved in endocytosis of apoptotic parenchymal cells [21,22], or in the secretion of apolipoproteins, matrix metalloproteinases (MMPs), respective MMP-inhibitors (TIMPs) [23,24] and growth factors [4]. Furthermore, they support liver regeneration through promotion of hepatocyte proliferation involving the neurotrophin receptor p75 [25], through the regulation of angiogenesis and vascular remodeling, and

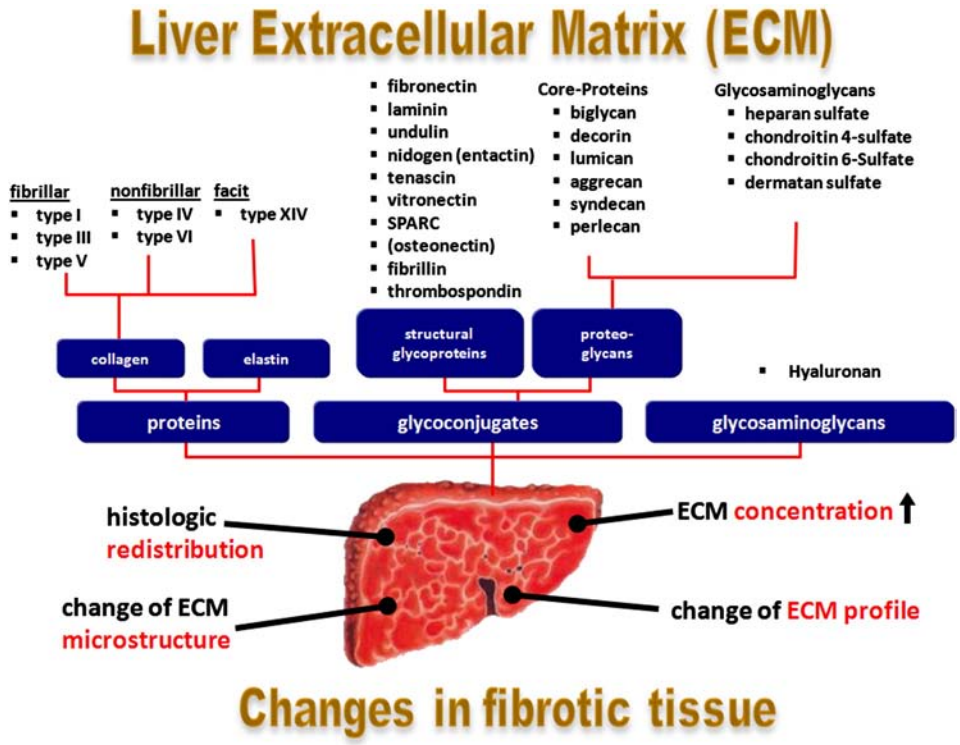

Fig. 1. Components of the extracellular matrix (connective tissue) of the fibrotic liver and their major changes. The binding of glycosaminoglycans (GAG) to the respective core proteins (CP) of proteoglycans (PG) is shown. BM: Basement membranes; FACIT: Fibril-associated collagens with interrupted triple-helices.