Human Pathology (2014) **45**, 160–165

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Different collagen types show distinct rates of increase from early to late stages of hepatitis C–related liver fibrosis☆

Wei Chen MD, PhD[a], Jonathan B. Rock MD[a], Martha M. Yearsley MD[a], Linda D. Ferrell MD[b], Wendy L. Frankel MD[a,*]

[a]*Department of Pathology, Ohio State University Wexner Medical Center, Columbus, OH 43210, USA*
[b]*Department of Pathology and Laboratory Medicine, University of California, San Francisco, San Francisco, CA 94143, USA*

Received 21 June 2013; revised 16 August 2013; accepted 21 August 2013

Keywords:
Liver fibrosis;
Extracellular matrix;
Collagen;
Vitronectin;
Immunohistochemistry;
Hepatitis C

Summary During progression from normal liver to cirrhosis, total collagen increases nearly 10-fold with an abnormal increase in fibril-forming collagen and other extracellular matrix molecules. However, little is known regarding the changes each collagen type undergoes during fibrogenesis. We assessed the different collagen types by immunohistochemistry at various stages of hepatitis C–related liver fibrosis in core biopsies and compared changes in each with trichrome stain to better understand fibrogenesis. The possible utility in staging fibrosis was investigated. We found collagens III, IV, V, VI, vitronectin, and trichrome all showed statistically significant increases from early to late stages of fibrosis, but with temporal and quantitative differences. During the transition from early to late fibrosis, trichrome (stains primarily collagen I) and collagen IV showed the steepest increase and appear to be the most useful discriminators between early and late stages of fibrosis. Collagens V and VI have strong reactivity even in stage 1, which may be helpful in identifying early fibrosis when trichrome is weak or negative. Collagen III and vitronectin showed the most gradual increase. Interestingly, collagen V also showed increased staining in areas around inflammation/edema, which may overestimate established fibrosis as compared with trichrome.

1. Introduction

It is well known that during progression from normal liver to cirrhosis, significant changes occur in extracellular matrix. Total collagen increases nearly 10-fold with an abnormal increase in fibril-forming collagen and other extracellular matrix molecules [1,2]. However, little is known regarding the changes each collagen type undergoes during fibrogenesis. To recognize quantitative and qualitative changes of extracellular matrix during fibrogenesis, it is important to understand the normal constituents in liver extracellular matrix.

Previous studies [3-6] of normal rat and human liver have shown that collagen types I and III are the most abundant collagens present in the liver capsule, portal stroma, Disse space, and fibrous septa. Type IV collagen codistributes with laminin in all basement membranes of vessels and bile ducts, and there is also "free" type IV collagen that is not associated with laminin and distributed in the sinusoids as small, discrete, discontinuous deposits. Type V collagen is

☆ Conflict of interest and funding disclosures. All authors: None.
* Corresponding author. Department of Pathology, The Ohio State University Wexner Medical Center, Columbus, OH 43210.
E-mail address: wendy.frankel@osumc.edu (W. L. Frankel).

0046-8177/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.08.015

fine collagen fibrils that constitute the core of larger fibrils of collagens I and III. Type VI collagen is a nonfibril, network-forming collagen that dominates the subendothelial space. It functions as an important bridging molecule that anchors structures such as nerves, blood vessels, and other cells to the basement membrane collagen IV.

Numerous noncollagen glycoproteins are also present in the liver. Vitronectin is a glycoprotein found in normal serum and pathologic extracellular matrix, promoting cell adhesion, spreading, and migration [7]. Laminin is present in the basement membrane and endoplasmic reticulum of mesothelial cells. Fibronectin, a structural glycoprotein, is found in the liver capsule, portal stroma, and Disse space, but not in basement membranes. It mediates adhesion of collagen, fibrin, and heparin to cells. Elastin is present in the wall of blood vessels, in the capsule, and in long-standing fibrosis.

As the liver becomes fibrotic, all collagen types increase [1]. Of note, there is abnormal increase in fibril-forming collagens as well as other extracellular matrix molecules in the subendothelial space, leading to the pathologic transformation of the normal low-density base membrane–like matrix to the interstitial type [8]. We studied hepatic fibrosis using liver core biopsies to assess different collagen types and vitronectin by immunohistochemistry at various stages of hepatitis C–related liver fibrosis. Our goal is to gain a better understanding of hepatic fibrogenesis by comparing the changes of each collagen type and vitronectin at different stages of liver fibrosis.

2. Materials and methods

The research was approved by Ohio State University Institutional Review Board. Seventy-three liver core biopsies from hepatitis C patients during a 5-year period (2007-2012) were identified. Only liver cores with a total length of equal to or more than 2 cm were selected because previous studies showed optimal liver biopsy is > 2 to 2.5 cm long for accurate grading of the degree of fibrosis [9]. Staging was performed using the hematoxylin and eosin– and trichrome-stained slides and the Batts and Ludwig scoring system [10]. Transplanted livers and cases with other known liver diseases, except steatosis, were excluded to help limit variability due to different etiologies of liver disease. Because steatosis was common with hepatitis C, we included these cases (present in 15 cases).

Peroxidase immunohistochemical staining was performed to examine the expression of collagen types III, IV, V, and VI as well as vitronectin. Briefly, paraffin-embedded tissue was cut at 4 μm and placed on positively charged slides. The slides were deparaffinized, rehydrated, and then were placed in a 3% hydrogen peroxide solution in water for 5 minutes to block for endogenous peroxidase. Antigen retrieval was performed by heat-induced epitope retrieval, in which the slides were placed in a 1× solution of Target Retrieval Solution (S1699; Dako, Carpinteria, CA) for 25 minutes at 96°C using a vegetable steamer (Black & Decker, New Britain, CT, USA) and cooled for 15 minutes in solution. Slides were then incubated on a Dako Autostainer Immunostaining System at room temperature.

The primary antibodies were diluted with an antibody diluent (S0809; Dako) at the following dilutions: collagen III (1:1000; Abcam, Cambridge, MA), collagen IV (1:25; Dako), collagen V (1:1000; Abcam), collagen VI (1:6000; Abcam), and vitronectin (1:400; Abcam). All antibodies were incubated for 60 minutes. Mach 3 Probe and Mach 3 Polymer HRP (M3M530 or M3M531; Biocare, Concord, CA, USA) were applied sequentially for 20 minutes each (collagen III, collagen V, collagen VI, and vitronectin). For collagen IV, the slides were first blocked for endogenous biotin using the Biotin Blocking System (X0590; Dako) before the application of the LSAB+ detection system (K0690; Dako), a labeled streptavidin-biotin-horseradish peroxidase (HRP) complex. Staining was visualized with the DAB + chromogen (K3468; Dako) using a 5-minute development. Slides were then counterstained in Richard Allen hematoxylin, dehydrated through graded ethanol solutions, cleared in xylene, and coverslipped. Trichrome stain (Dako, Cambridge, MA) was performed on Dako Artisan special stainer. Positive and negative controls (cirrhotic livers) stained appropriately. All immunohistochemical stains were performed in the same laboratory, under same quality controls, and by the same technologist. Trichrome stains were performed at different times, but in the same laboratory and under the same quality controls. The controls appear to be consistent by microscopic examination.

Staining intensity was graded by 2 pathologists as negative (0), weak (1), moderate (2), and strong (3), and distribution, as patchy if (1) ≤ 50% of the portal areas stained or diffuse (2) if > 50% of the portal areas stained. The final score was calculated as the product of intensity and distribution. The statistic analysis was performed using an unpaired *t* test by GraphPad Software online *t* test calculator (http://graphpad.com/quickcalcs/ttest1.cfm).

3. Results

The 73 viral hepatitis C patients ranged from 48 to 55 years old, with male predominance in all stages (total 56 men and 17 women). As expected, immunohistochemical stains for collagens and vitronectin highlighted the periportal and intralobular fibrotic areas in the liver cores (Figs. 1 and 2). Trichrome stain as well as collagens III, IV, V, VI, and vitronectin all showed increased interstitial staining from early to late stages of fibrosis. Interestingly, collagens IV and VI also showed a perisinusoidal staining pattern in all stages. Collagen V showed increased staining in areas around inflammation or edema (Fig. 3). In these areas, the trichrome stain is pale blue rather than the darker blue typically considered to represent true fibrosis rather than edema [11]. Because of this pattern, the degree of staining with collagen