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Fibrodynamics-elucidation of the mechanisms and sites of liver fibrogenesis

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ORIGINAL ARTICLE

Dynamic changes of type I, III and IV collagen synthesis and distribution of col lagen-producing cells in carbon tetrachloride- induced rat liver fibrosis.

MAJOR POINTS OF THE COMMENTED ARTICLE

In their article that appears in this issue, Du and colleagues used a combination of immunohistochemistry and *in situ* hybridization to demonstrate increased levels of collagen types I, III and IV in CCl_4/choline deficient rat model of hepatic fibrosis. Over the course of 20 weeks of treatment mRNA levels for all three types of collagen were increased, but there was a preferential increase of type III collagen mRNA over the other two types. This is consistent with the results of previous investigators[1,2] where increases in protein levels of collagen types I, III and IV were found in CCl_4 induced liver fibrosis. The authors clearly demonstrated that collagen type I, III and IV mRNAs were localized on sinusoidal cells using *in situ* hybridization. This result is also consistent with previous work of Maher and co-workers[3,4] who used the same technique to demonstrate the localization of both interstitial and basement collagen mRNAs in hepatic stellate cells in normal rat and human livers. The aut hors have utilized state-of-the-art technology to examine an important questi on in liver fibrosis-the cells responsible for overproduction of liver biomatrix components. Their results are consistent with the results of other investigato rs in the field. However, caution should be taken not to overinterpret results. Additional controls in which the *in situ* localization of collagen mRNAs in untreated normal liver as compared to that seen in fibrotic liver could have given a clearer picture of changes in the fibrotic liver. Nevertheless, the results add strong confirmation to the temporal course of fibrogenesis, and localization of the products in an important model.

COMMENTARY

Liver fibrosis is the common result of chronic hepatic injury of diverse origins such as chronic viral infections (HBV, HCV), metabolic/storage diseases (hemochromatosis), helminthic infections (schistosomiasis), chronic toxin exposure (alcohol and environmental poisons) and biliary obstruction (biliary cirrhosis). In end stage liver fibrosis or cirrhosis, the liver biomatrix may contain up to six to ten times more collagen and proteoglycans than in the normal state[5,6]. Because the connective tissue support of the liver parenchyma is particularly critical to its function, research that emphasizes the nature of liver biomatrix, the molecular regulation of the tumover of components of the biomatrix, and identification of liver cells responsible for the synthesis of biomatrix proteins are especially crucial for the ultimate design of effective therapies for liver fibrosis.

In the late 50's, Hans Popper, the eminent hepatologist, observed a correlation between the histomorphology and biochemistry of liver collagens in chronic liver diseases[7]. In the four decades following Dr. Popper's original obser vation, a great deal of research on liver biomatrix has resulted in our current knowledge of the pathogenesis of liver fibrosis. Progress has been made in three areas of liver fibrosis: characterization and quantitation of matrix component s in normal and fibrotic liver; identification of hepatic cells responsible for the increased synthesis of matrix proteins; and the role of cellular mediators of fibrogenesis.

Quantitation of matrix proteins in normal and fibrotic livers

The use of animal models of liver fibrosis such as the administration of liver toxins CCl_4[8], dimethylnitrosamine[9], alcohol[10], helminthic

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infections[11,12] have greatly helped in the characterization of the temporal expression of various components of the biomatrix during fibrogenesis. There is increase in the amounts of collagens types I, III, IV, V and VI[1]. In early fibrosis, the amounts of types III and IV collagens increase relative to other collagens. In late fibrosis, type I collagen predominates[13]. Other components of liver biomatrix such as laminin, fibronectin and proteoglycans are also increased in fibrosis[14,15]. Although most investigations have shown changes at the protein and mRNA levels of various biomatrix components, the significance of the changes in the fibrogenic process remain hotly debated[16]. The biomatrix in the normal liver changes from bein g rich in basement membrane collagens to interstitial collagens during fibrogenesis.

Hepatic stellate cells is the major effector cell type in hepatic fibrosis

The search for effector cells in the liver responsible for collagen synthesis became feasible with the development of molecular probes[17] and antibodies[14,18] to components of liver biomatrix. Stellate cells are responsible for the increased synthesis in liver biomatrix proteins such as basement membrane collagens, interstitial collagens, fibronectin, laminin and proteoglycans[3,4]. Activation of hepatic stellate cells is the earliest response to liver injury. Upon activation, stellate cells lose stored lipids and retinoids[19] and rapidly undergo morphological changes to myofibroblast-like phenot ype[20]. Other phenotypic changes of activated stellate cells include st imulation of α-actin gene expression[21,22] and increased synthesis of hepatic biomatrix components[3]. Activation also results in loss of an important feedback regulation of collagen synthesis by its terminal propeptides. In the normal liver, stellate cells are capable of controlling the amount of co llagen needed for normal biomatrix formation by a feedback inhibition of collage n synthesis by its terminal propeptides[23,24]. Following activation, stellate cells lose their normal feedback regulation of collagen synthesis leading to increased accumulation of collagen[25]. In particular, there is an increased synthesis of types I and III collagen resulting in a biomatrix rich in interstitial collagens. There is increasing evidence that accumulation of fibers in the sinusoids is not only due to increased synthesis of collagens, but also is a result of decreased synthesis of tissue collagenases and increased synthesis of inhibitors of collagenase (TIMP-1: tissue inhibitors of metalloproteinase)[26]. Thus fibrogenesis is a net result of increased synthesis and decreased degradation of interstitial collagens of activated stellate cells.

Cellular mediators of hepatic fibrosis

Understanding the underlying molecular mechanisms responsible for hepatic fibrosis became feasible with the availability of molecular probes to cytokines. It is now accepted that the initial liver injury results in a host of cytokine responses from liver cells. Specifically, TGFβ[27], TNFα[28], PDGF[29] and Kupffer cell soluble factors[30] have been implicated in stellate cell activation and proliferation. TGFβ mRNA and protein levels are increased in activated stellate cells[27]. Over-expression of TGFβ gene in cultured fibroblasts[27] and in stellate cells[31] results in increased synthesis of collagens. Inhibitors of TGFβ decrease collagen synthesis *in vivo*[32,33] while transgenic mice over expressing TGFβ have kidney and liver fibrosis[34]. Both PDGF[29] and TNFα[35] are stellate cell mitogens. PDGF-induced stellate cell proliferation and matrix protein synthesis is mediated by factors secreted by Kupffer cells[30]. TNFα acts via transcription regulation of tissue collagenase and TIMP-1 genes in activated stellate cells[28].

Current research

The elucidation of the molecular mechanisms of cytokine regulation of liver biomatrix protein synthesis continue to be a focus of current research efforts. There is increasing evidence that cytokines may act via interactions with DNA binding proteins to affect matrix proteins synthesis. Both TGFβ and TNFá interact with known transcription factors such as C/EBP[36] and NFkappaB[37,38]. Transcription factors are DNA binding proteins which act as regulators of gene transcriptions[39,40]. There is continued interest in the search for regulatory elements within genes of matrix proteins[41]. Research on interactions of DNA binding proteins to regulatory elements on matrix protein genes are underway and may provide a link between cytokines and regulation of liver biomatrix.

Future directions

Effective therapy for chronic hepatic fibrosis can be designed only with complete understanding of the molecular mechanisms that regulate matrix protein gene expression. Future research may be centered on the application of gene therapy to control hepatic fibrosis[42]. Over-expression of tissue collagenase gene, inhibition of TGFβ gene expression are potential approach in controlling and regulating hepatic fibrosis[43].

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