ROLE OF HEPATIC STELLATE CELLS IN THE PATHOGENESIS OF HEPATIC FIBROSIS IN CHRONIC HEPATITIS C: AN IMMUNOHISTOCHEMICAL STUDY

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By

OMAIMA MOSTAFA MAHMOUD
M.B.B.Ch., M.Sc., of Tropical Medicine

Supervised by

SOHEIR ZAKARIA M. EISSA, M.D.
Professor of Tropical Medicine
Faculty of Medicine, Cairo University

ALI A. FOUAD EL-HINDAWI, M.D.
Professor of Pathology
Faculty of Medicine, Cairo University

MAGDY MAHMOUD YOUSEF, M.D.
Professor of Tropical Medicine
Theodor Bilharz Research Institute

MAISSA EL SAID EL RAZIKY, M.D.
Professor of Tropical Medicine
Faculty of Medicine, Cairo University

Faculty of Medicine
Cairo University
(2008)

ABSTRACT

Chronic hepatitis C is one of the most important health problems. End-stage liver disease due to hepatitis C is the leading indication for liver transplantation. Hepatic fibrosis, the common final manifestation of several chronic liver diseases, is the result of a prominent accumulation of extracellular matrix (ECM) materials and ultimately can lead to cirrhosis. HSCs have a crucial role in determining the pathogenesis and clinical course of liver fibrosis and cirrhosis. The alpha isotype of actin (α-SMA) expressed by hepatic stellate cells reflects their activation to myofibroblast-like cell and has been directly related to experimental liver fibrogenesis, and indirectly to human fibrosis in chronic liver disease. The role of GFAP expression in HSCs is currently unknown. Previous studies in rodents showed that, when rodent HSCs were activated, the expression of GFAP decreased. The decreased GFAP expression in an advanced stage of fibrosis suggested that GFAP could be a marker for quiescent cells in rodents.

This study was conducted on 69 chronic HCV patients, presented to the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute, Egypt, in the period between 2004-2007. Amongst whom, there were 11 patients having mixed chronic HCV and schistosomal infections (served as pathological controls). In addition to 10 liver disease-free individuals serving as normal controls.

Clinical characteristics of the studied patients revealed the presence of fatigue as a common symptom among them, occurring in 87%. Rt hypochondrial dull aching pain was reported in 65.2%. None of the studied patients had clinically palpable spleen or ascites. Manifestations of liver cell failure in the form of Jaundice, spider nevi, palmer erythema, encephalopathy and bleeding tendency were totally absent in the study 69 patients. Quantitative PCR-HCV RNA performed to all patients revealed that, 30.4% had high viremia, 65.2% had low viremia. Only 3/69 (4.4%) had negative PCR test. Alpha-SMA-positive HSCs were detected in all stages of hepatic fibrosis. The α-SMA-positive cells were encountered mostly in the perisinusoidal region. Patients with low grade of necro-inflammatory activity showed a significantly higher α-SMA immunoexpression on *perisinusoidal* HSCs when compared with those having a high grade of activity. The control group had a significantly lower α-SMA expression when compared with the other groups. Patients with no evident fibrosis (F0 group) showed higher α-SMA immunoreactivity than both the cirrhotic and bilharzial groups. CHC group had the highest α-SMA immunoreactivity when compared with the bilharzial group and the cirrhotic groups. Alpha-SMA expression was significantly higher in the cirrhotic group when compared to the bilharzial group. Patients with low grade of necro-inflammatory activity showed a significantly higher expression of GFAP-positive perisinusoidal HSCs when compared to those with high grade of activity. The control group had a significantly lower GFAP expression when compared with the other groups. Also, F0 group showed the highest GFAP immunoreactivity than both the CHC and cirrhotic groups. Finally, cirrhotic group had lower GFAP expression than bilharzial group. Among the 69 studied patients, there was no statistically significant correlation between the immunoexpression of both markers and clinical and laboratory variables of those patients. Also, no statistical significant correlation was recorded between the immunoexpression of both markers and both stages of fibrosis and necro-inflammatory grading of those patients. On the other hand, the α-SMA expression correlated significantly with GFAP expression in the same studied patients.

Alpha-SMA could be a useful marker to identify the earliest grades of necro-inflammatory activity and also earliest stages of hepatic fibrosis. GFAP could represent a useful marker of early activation of HSCs in the HCV chronic hepatitis setting. In addition, GFAP-dependent activation of HSCs precedes fibrotic tissue deposition.

Key words

Immunopathogenesis of hepatitis C, Hepatic stellate cells, Liver fibrosis, Alph-smooth muscle actin, Glial fibrillary acidic protein.

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LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
A-II	Angiotensin II
ALT	Alanine aminotransferase
ANA	Anti-nuclear antibody
Ang-1	Angiopoietin 1
ANP	Atrial natriuretic peptide
APRI	(AST)-platelet ratio index
ARFP	Alternate reading frame protein
A-SMA	Alpha-smooth muscle actin
AST	Aspartate aminotransferase
ATPase	Adenosine triphosphatase
AUC	Areas under the curve
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CBS	Cystathionine-β-synthase
CCR	Chemokine receptor
CD	Crohn's disease
CD	Cluster of differentiation
cGMP	Cyclic guanosine monophosphate
CINC	Cytokine-induced neutrophil chemoattractant
CLDs	Chronic liver diseases
CMV	Cytomegalovirus
CNS	Central nervous system
CO	Carbon monoxide
COX	Cyclooxygenase
CRP	C-reactive protein
CT	Cysteinyl leukotrienes
CTGF	Connective tissue growth factor
CTL	Cytotoxic T-lymphocyte
DAG	Diacylglycerol
DCs	Denderitic Cells
DPIV	Dipeptidyl peptidase IV
ECM	Extracellular matrix
ECM	Extracellular matrix
EDCFs	Endothelial derived contracting factors
EGF	Epidermal growth factor
ELFG	European Liver Fibrosis Group
ELISA	Enzyme-linked immunosorbent assays

ENS	Enteric nervous system
ER	Endoplasmic reticulum
ET-1	Endothelin-1
ET-A	Endothelin A
ET-B	Endothelin B
FAK	Focal adhesion kinase
FAP	Fibroblast activation protein
FT	FibroTest™
FXR	Farnesoid X receptor
GFAP	Glial fibrillary acidic protein
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCV-C	Hepatitis C virus cirrhosis
HCV-CH	Hepatitis C virus chronic hepatitis
HCV-PTR	HCV-posttransplant recurrent hepatitis
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HO-1	Heme oxygenase-1
HRT	Hormone reposition therapy
HSCs	Hepatic stellate cells
ICAM-1	Intracellular adhesion molecule 1
IF	Intermediate filament
IFN- γ	Interferon gamma
Ig	Immunoglobulin
IGF	Insulin-like growth factor
IHVR	Intrahepatic vascular resistance
IL	Interleukin
IP3	Inositol 1,4,5-trisphosphate
IRF	Interferon regulatory factor
ISGF	IFN- stimulated gene factor
ISGs	IFN-stimulated genes
ISRE	IFN-stimulated response elements
JAK	Janus kinase
LDL	Low-density lipoproteins
LMCs	Liver mesenchymal cells
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
MBL	Mannose-binding lectin
MCP-1	Monocyte chemoattractant protein 1

M-CSF	Macrophage colony-stimulating factor
MFBs	Myofibroblasts
MFs	Myofibroblasts
MHC	Major histocompatibility complex
MICA/B	MHC class-I related chain A/B
MLC	Myosin light chain
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP-2	Matrix metalloproteinase 2
MW	Membranous web
NASH	Non-alcoholic steatohepatitis
NCAM	Neural cell adhesion molecule
NEC	Necrotising enterocolitis
NF-L	Neurofilament protein
NF-κ B	Nuclear factor-kappa B
NGF	Nerve growth factor
NIH	The National Institutes of Health
NK	Natural killer
NO	Nitric oxide
NOs	Nitric oxide synthase
NS	Nonstructural
OLT	Orthotopic liver transplantation
PA	Phosphatidic acid
PAF	Platelet activating factor
PAT	Parentral anti-schistosomal therapy
PBC	Primary biliary cirrhosis
PBMCs	Peripheral blood mononuclear cells
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PDL-1	Programmed death ligand-1
PG	Prostaglandin
PGP9.5	Beta-tubulin, protein gene product 9.5
PGs	Prostaglandins
PI 3-kinase	Phosphoinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PKC	Protein kinase C
PKC	Protein kinase C
PLC	Phospholipase C
PLD	Phospholipase D

PNS	Post neuronal synapse
PPARs	Peroxisome proliferators activated receptors
RANTES	Regulated upon activation normal T-cell expressed and secreted.
RAR	Retinoic acid receptors
RAS	Renin- angiotensin system
RBP	Retinol-binding protein
rMLC	Regulatory myosin light chain
ROS	Reactive oxygen species
RT-PCR	Reverse transcription-PCR
RT-PCR	Real-time PCR
RXR	Retinoid X receptors
SCF	Stem cell factor
SHP	Small heterodimer partner
siRNA	Small interfering RNA
SR-BI	Scavenger receptor class B1
STAT	Signal transducer and activation of transcription
SVR	Sustained virologic response
TCR	T-cell receptor
TGF-α	Transforming growth factor alpha
TGF-β	Transforming growth factor beta
Th	T helper
TLRs	Toll-like receptors
TNF	Tumor necrosis factor
Tollip	Toll-interacting protein
TRAIL	TNF-related apoptosis inducing ligand
TXA2	Thromboxane A2
TXs	Thromboxanes
UTR	Untranslated regions
VCAM-1	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VEGFR-1&2	VEGF receptor-1 and -2
WBC	White blood cells

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION AND AIM OF THE WORK

Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease (CLD) in Egypt, where prevalence of antibodies to HCV (anti-HCV) is approximately 10-fold greater than in the United States and Europe (Strickland *et al*., 2002).

Chronic hepatitis C is responsible for significant morbidity and mortality rates (Bataller and Brenner, 2005). Currently, the cirrhosis resulting from chronic virus C infection is the main cause of hepatic transplantation worldwide (Codes *et al*., 2007).

The main injury caused by hepatitis C virus is the hepatic fibrosis, as a result of a chronic inflammatory process in the liver. The development of the chronic hepatitis C is better estimated by the fibrosis stage rather than by the necro-inflammatory activity level (Poynard *et al*., 1997).

Fibrosis develops as a multicellular process involving paracrine signaling between the resident liver cells and inflammatory cells (Friedman, 2000).

Central events include the activation of hepatic stellate cells (HSCs) in association with tissue necrosis and inflammation (Friedman, 2004). In response to liver injury, human HSCs express alpha-smooth muscle actin (α-SMA), becoming "activated" and myofibroblast-like. Immunohistochemical staining for α-SMA correlates with HSC activation (Guido *et al*., 1997).

So, the expression of (α-SMA) is a reliable and widely used marker of activation of HSCs to myofibroblast-like cells in patients with chronic hepatitis C

(Friedman, 2008). Although correlation between HSC activation and necro-inflammatory activity and/or fibrosis stage is a point of large depate (Carpino *et al*., 2005).

GFAP is an intermediate filament (IF) protein that is found in glial cells (Fuchs and Weber, 1994).

GFAP is expressed in the central nervous system in astrocyte cells. It is involved in many cellular functioning processes, such as cell structure and movement, cell communication, and the functioning of the blood brain barrier (Tardy et al., 1990).

GFAP expression was reported in quiescent stellate cells in vivo, with an increased expression in the acute response to injury in the rat, and a down regulation in the chronic one (Morini et al., 2005).

Reports concerning GFAP expression in human liver are conflicting. Few studies have been performed in order to quantify the hepatic expression of GFAP at different stages of human chronic hepatitis (Martinelli et al., 2004).

GFAP becomes down-regulated in chronically activated HSCs and therefore is used as a unique marker for the activation of quiescent HSCs to proliferative myofibroblast-like HSCs, a critical step during inflammation and injury in the liver (Neubauer et al., 1996; Niki et al., 1996).

However, despite an increasing appreciation of the manner in which HSCs are regulated, the factors responsible for initiation of fibrogenesis and progression of fibrosis in chronic hepatitis C remain poorly understood (Lau et al., 2005). Hence,