ANALYSIS OF SOME CELL CYCLE REGULATORY MOLECULES IN CHRONIC HEPATITIS C VIRUS LIVER DISEASE

Thesis

Submitted for Partial Fulfilment of Master Degree (M.Sc.)
in
Science (Immunology)

By

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AA	Amino acid.
Ag	Antigen.
ALT	Alanin aminotransferase.
AP	Activating protein.
Apaf-1	Apoptosis protease activating factor-1.
ASK1	Apoptosis signal-regulating kinase 1.
AST	Aspartate aminotransferase.
ATM	Protein kinase that recognize damaged DNA leading to cell cycle arrest.
Bax	Bcl-2–associated X protein.
C	Core protein.
CAK	Cdk-activating kinases.
Cdk	Cyclin-dependent kinase.
CdkI	Cyclin-dependent kinase inhibitor.
C/EBP	CAATT enhancer binding protein.
CHC	Chronic hepatitis C.
CHK2	Check-point kinase 2.
Cip	Cyclin inhibitory protein.
c-myc	Cellular avian myleocytoma virus gene.
CSA	Circulating schistosomal antibody.
DNA	Deoxyribonucleic acid.
E1, E2	Envelop proteins.
ECM	Extracellular matrix.
E2F	Transcription factor activating adenovirus E2 gene.
ELISA	Enzyme-linked immunosorbent assay.
ER	Endoplasmic reticulum.
ERK	Extracellular signal related protein kinase.
FAK	Focal adhesion kinase.
FCS	Fetal calf serum.
G1, G2	Gap1, Gap2 phases.
GF	Growth factor.
HBc	Hepatitis B core antigen.

HBs	Hepatitis B surface antigen.
HCC	Hepatocellular carcinoma.
HCV	Hepatitis C virus.
H & E	Hematoxylin and eosin stain.
H_2O_2	Hydrogen peroxide.
H_2SO_4	Sulfuric acid.
Ig	Immunoglobulin.
IHC	Immunohistochemistry.
INK	Inhibitory protein of Cdk4.
IRES	Internal ribosome entry site.
JAB1	Jun activation domain binding protein-1.
JNK	c-Jun NH_2-terminal kinase
kDa	Kilo Dalton.
Kip	Kinase inhibitory protein.
LC	Liver cirrhosis.
M	Mitosis.
MAb	Monoclonal antibody.
MAPK	Mitogen activated protein kinase.
MCM	Multicopy maintenance.
Mdm2	Murine double minute 2.
MEK	Mitogen activated ERK activated kinase.
mRNA	Messenger ribonucleic acid.
NF	Nuclear factor.
nm	Nanometer.
NS	Non-structural protein.
ORC	Origin recognition complex.
PAT	Parental anti-schistosome therapy.
PBS/T	Phosphate buffer saline/Tween 20.
PCNA	Proliferating cell nuclear antigen.
pRb	Retinoblastoma protein.
PT	Prothrombine time.
PUMA	p53 up-regulated modulator of apoptosis.

raf	Cytoplasmic serine/thereonine protein kinase.
ras	Rat sarcoma.
RNA	Ribonucleic acid.
RT-PCR	Reverse transcriptase-polymerase chain reaction.
S	Synthesis.
SAPK	Stress-activated protein kinase.
SD	Standard deviation.
SE	Standard error.
SEA	Soluble egg antigen.
SPSS	Statistical Package for Social Sciences.
SR-BI	Scavenger receptor class B1.
src	Rous sarcoma virus.
STAT	Signal transducer and activation transcription.
TBP	TATA-box-binding protein.
TGF-β	Transforming growth factor-*beta*.
UTR	Untranslated region.
UV	Ultraviolet.

LIST OF TABLES

LIST OF FIGURES

Differential expression of cell cycle regulators in HCVinfection and related hepatocellular carcinoma

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Abstract

AIM: To investigate cell cycle proteins in chronic hepatitis C virus infection in order to analyze their role in the process of hepatocyte transformation and to characterize their prognostic properties.

METHODS: Subjects of the current study included 50 cases of chronic hepatitis C (CHC) without cirrhosis, 30 cases of CHC with liver cirrhosis (LC), and 30 cases of hepatitis C-related hepatocellular carcinoma (HCC) admitted to the Department of Hepato- Gastroenterology, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Fifteen wedge liver biopsies, taken during laparoscopic cholecystectomy, were also included as normal controls. Laboratory investigations including urine and stool analysis, liver function tests and prothrombin concentration; serologic markers for viral hepatitis and ultrasonography were done for all cases of the study together with immunohistochemical analysis using primary antibodies against cyclin D1, cyclin E, p21, p27 and Rb1/p105 proteins.

RESULTS: Normal wedge liver biopsies didn't express cyclin E or Rb1/p105 immunostaining but show positive staining for cyclin D1, p21 and p27. Cyclin D1 expressed nuclear staining that was sequentially increased from CHC to LC ($p<0.01$) to HCC ($p<0.01$) cases; meanwhile, cyclin E revealed nuclear positivity only in the case of HCC patients that was directly correlated to Rb1/p105 immuno-reactivity. The expression of p21 and p27 was significantly increased in CHC and LC cases compared to normal controls and HCC with no significant difference between well- and poorly-differentiated tumors. p21 showed only a nuclear pattern of staining, while, p27 presented with either cytoplasmic and/or nuclear reactivity in all studied cases. Correlation analysis revealed a direct relation between cyclin D1 and p21 in CHC cases ($p<0.01$), between cyclin D1 and cyclin E in HCC ($p<0.01$); however, an inverse relationship was detected between cyclin D1 and p21 or p27 ($p<0.01$) and between p21 and Rb1/p105 ($p<0.05$) in HCC.

CONCLUSION: These data suggest that upregulation of cyclin D1 in CHC plays a vital role in the development and differentiation of HCC; while, cyclin E may be a useful marker for monitoring tumor behavior. p21 and p27 can be used as predictive markers for HCC. Furthermore, expression of Rb1/p105 in CHC and LC may help to protect those patients from malignant transformation. Its higher presentation as well as inverse relation with p21 and histologic grades suggests its important role in hepatic carcinogenesis.

Key words: Chronic hepatitis C; Liver cirrhosis; Hepatocellular carcinoma; Cell cycle; Cyclin D1; Cyclin E; p21; p27; Rb1/p105

INTRODUCTION

The cell cycle is divided into four sequential phases. G1 is the first gap phase in which cells prepare for deoxyribonucleic acid (DNA) replication; S (synthesis) phase is the period of DNA synthesis for the reproduction of the whole genome; G2 is the second gap phase in which cells prepare mitosis; and M (mitosis) phase in which cell division occurs for the generation of two genetically identical daughter cells (Owa *et al.,* 2001). Quiescent cells that have not entered the cell cycle are referred to as being in G0 (Morgan, 2007_a).

Cyclins are the prime cell cycle regulators that play a central role in the control of cell proliferation by forming complexes with different cyclin-dependent kinases (Cdks) (Bornfeldt, 2003). Members of cyclin family are often quite distinct from each other in amino acid sequence (Morgan, 2007_a). At least, 15 different cyclins and 10 Cdks have been identified (Rosania and Chang, 2000). In response to mitogenic signals, G1 cyclins (cyclin D1 and cyclin E) participate in the initiation and progression of the cell cycle; where cyclin D1 is activated during the mid G1, while cyclin E is required for G1/S transition (Jung *et al.,* 2001). They can accelerate and shorten the G1 phase and reinforce the ability of cells to loose growth control suggesting an oncogenic role for G1 cyclins (Mann *et al.,* 2007).

Cyclin-dependent kinase inhibitors (CdkIs), on the other hand, are potent negative regulators of the cell cycle that inhibit the G1/S transition (Mann *et al.,* 2007) and include two families on the basis of sequence homology: The INK4 family including $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p19^{INK4d}$ that specifically binds to Cdk4 and Cdk6 and inhibits cyclin D binding (Nan *et al.,* 2004) and the Cip/Kip family including $p21^{Cip1/Waf1}$, $p27^{Kip1}$

and $p57^{Kip2}$ that bind to and inhibit cyclin bound Cdks (Polak *et al.,* 2003). Moreover, the two main regulatory proteins of the cell cycle are the retinoblastoma proteins (pRb) and p53. The Rb gene family is composed of three members that share many structural and functional features and play a fundamental role in growth control (Santopietro *et al.,* 2006). It includes the Rb susceptibility gene which encodes a nuclear phosphoprotein (pRb1/p105) and two related genes pRb/p107 and pRb2/p130 (Baldi *et al.,* 1996). The Rb1/p105 gene maps to the 13q14 chromosome, where deletions and heterozygous mutations are frequent in many human malignancies (Nevins, 1998; Sanseverino *et al.,* 2003). The balance between cell cycle regulators and cell proliferation is an important determinant of tumor development and/or behavior (Lü *et al.,* 2005).

Hepatitis C virus (HCV) is one of the most common etiologic agents of chronic liver disease (Li *et al.,* 2004). Infection with HCV becomes persistent in most infected individuals and can lead to the development of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (Bian *et al*., 2009).

Chronic hepatitis C is one of the most serious liver diseases in Egypt (El-Zayadi *et al.,* 2005). Hepatitis C patients tend to retain the causative virus for long periods and attain a carrier state with high risk for developing complications (Okumura *et al*., 2005).

It has been suggested that hepatocyte turnover is increased in chronic HCV infection as markers of cell proliferation are elevated (Lake-Bakaar *et al.,* 2002) and telomere shortening is reported (Miura *et al.,* 1997). However, mitotic activity is usually sparse or absent as hepatocytes expressing “proliferation markers” could enter the cell cycle but have been arrested and unable to complete cell division or progress to S phase (Marshall *et al.,* 2005).

Viral replication is enhanced by induction of both cell cycle entry and cell cycle arrest by viral factors (Flemington, 2001). Accordingly, a relationship between viral replication and the host cell cycle state exists in HCV infection (Marshall *et al.*, 2005). There are several potential consequences of cell cycle arrest and senescence for the liver. Cellular senescence is a risk factor for cancer development and senescent hepatocytes may act synergistically with oncogenic mutations in neighboring hepatocytes leading to the development of HCC (Krtolica *et al.*, 2001; Ozturk *et al.*, 2009).