"DHCR24" RELATION TO HEPATOCELLULAR CARCINOMA

Thesis:

Submitted for Fulfillment of the Master Degree
In
Medical Oncology
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2010

ACKNOWLEDGEMENT

I would like to thank Professor Heba Elzawahry for her help and support all through my work.

I would like to thank Professor Koyoko Kohara for all the help and supplement of the kits used in this work.

I would like to thank Professor Ahmed El-Gohary for his valuable work, effort and guidance.

I would also like to thank my Professors and colleagues in the Medical Oncology Department.

Finally I would like to thank my husband and family for being always there for me and their support in every step of my life.

Nahla El-Gohary

Abstract

Hepatocellular carcinoma is one of the most lethal forms of cancer in the world. So early detection and treatment with the use of tumor markers is very important to improve the patient's outcome.

The presence of DHCR24 in hepatocytes indicated tumorigenicity. A monoclonal antibody on a genome-wide scale against an HCV-expressing human hepatoblastoma-derived cell line was generated (DHCR24 monoclonal antibody).

HCV induced the over-expression of (DHCR24) and showed that its expression reflected tumorigenicity in human hepatocytes.

The aim of this study was to examine the possibility of using DHCR24 monoclonal antibody in the detection of HCC. 50 control, 50 HCV patients and 50 HCC patients presented to National Cancer Institute, Cairo University were recruited.

Serum levels of DHCR24 were assayed by ELISA in these three groups.

(KEY WORDS): hepatocellular carcinoma, DHCR24, early diagnosis of hepatocellular carcinoma, liver fibrosis, HCV, HBV

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List of Abbreviations

- **A549 cells:** Adenocarcinomic human alveolar basal epithelial cells
- **AF**: Aflatoxin
- **AFP**: α-fetoprotein
- **AFP-L3**: Lens culinaris agglutinin-reactive AFP
- **AFU**: α-L-fucosidase
- **AJCC**: American Joint Committee on Cancer
- **AU**: Arbitrary unit
- **BMI**: Body mass index
- **CECT**: Contrast-enhanced helical CT
- **CEUS**: Contrast-enhanced Ultrasonography
- **CGH**: Comparative genomic hybridization
- **CT**: Computed tomography
- **CTP** (CT): Child Turcotte Pugh
- **DCP**: Des-g-carboxy prothrombin
- **DEVD**: Capsase inhibitor
- **DEVD**: DNA repair enzyme. The sequence DEVD is cleaved by caspase-3 during cell death by apoptosis.
- **DHCR24**: Seladin-1, diminuto/dwarf1 homolog
- **ECOG**: Eastern Cooperative oncology group
- **G418**: Aminoglycoside antibiotic similar in structure to gentamycine
- **GFP**: The green fluorescent protein is a protein composed of 238 amino acids (26.9kDa), which exhibits bright green fluorescence when exposed to blue light
- **GPC3**: Glypican-3

- **GPDA**: Glycylproline dipeptidyl aminopeptidase
- **HBsAg**: Hepatitis B surface antigen
- **HBV**: Hepatitis B virus
- **HCC**: Hepatocellular carcinoma
- **HCCR** protein: Human cervical cancer oncogene
- **HCV**: Hepatitis C virus
- **HepG2**: Human liver carcinoma cell line
- **HGF**: Hepatocytes growth factor
- **HuH**-7: human hepatoma cell line 7
- **IGF-II**: Insulin-like growth factor-II
- **ISOGEN Reagent** (**Nippon Gene**): reagent complete and ready to use for isolation of total RNA or for simultaneous isolation of RNA, DNA and protein
- **LOH**: Loss of heterozygosity
- **MHC**: Major histocompatibility complex
- **MPCT**: Multiphase helical CT
- **MDM2**: Negative regulator of p53
- **MSI**: Microsatellite instability
- **NAFLD**: Nonalcoholic fatty liver disease
- **NANB-H** : non-A, non-B hepatitis
- **NASH**: Nonalcoholic steatohepatitis
- **Opti-MEM**: reduced serum media is a modification of Eagle's min essential media
- **P21WAF1/CLIP1**: Family that inhibit all kinase involved in the G1/S transition
- **PBC**: Primary biliary cirrhosis
- **PEI**: Percutaneous local ablation therapy

- **PET**: Positron emission tomography
- **PIVKAII**: protein induced by vitamin K absence or antagonist-II
- **PMCT**: percutaneous microwave coagulation therapy
- **PS**: Performance Status
- **pWWP-**Luc: mutagenesis template
- **Rb**: Retinoblastoma
- **TACE**: Transarterial chemoembolization
- **TUNEL assay**: Terminal deoxynucleotidyl transferase DUTP nick end labelling
- **US**: Ultrasonography
- **VEGF**: Vascular endothelial growth factor
- **WI-38**: Human diploid cell line from normal embryonic (3-month gestation) lung tissue of a female
- **WRL 68**: Human liver embryonic cells
- **Xhol**: Restriction enzyme
- **18F-FDG**: 18F-fluorodeoxyglucose
- **293FT**: Fast growing, highly transfectable cells derived from human embryonal kidney cells

Child-Pugh Classification of Severity of Liver Disease

Modified Child-Pugh classification of severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy.

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin, mg/dL	</= 2	2-3	>3
Albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time * Seconds over control * INR	1-3 <1.8	4-6 1.8-2.3	>6 >2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (descompensated disease). These grades correlate with one- and two-year patient survival.

Grade	Points	One-year patient survival (%)	Two-year patient survival (%)
A: well-compensated disease	5-6	100	85
B: significant functional compromise	7-9	80	60
C: descompensated disease	10-15	45	35

INTRODUCTION

Hepatocellular carcinoma (HCC) is the major primary liver cancer. It is the fifth most common cancer and the third cause of cancer-related death worldwide ***(Bosch et al, 2005)***. According to the National Cancer Registry ***(NCR)***, HCC is the fourth cancer in Egypt (8.1%) and the first cause (14.8%) of deaths due to all malignancies ***(Department of Biostatistics & Epidemiology NCI Egypt: http://www.nci.edu.eg).***

The main risk factor for HCC is cirrhosis of any etiology, but mainly due to hepatitis B virus (HBV), hepatitis C virus (HCV) and chronic alcohol consumption. In Eastern Asia, Middle and Western Africa where HBV infection is hyperendemic, HCC is a public health problem and the incidence is more than 20 per 100,000 per year. It is less than 5 per 100,000 in Europe, but the yearly incidence of HCC in cirrhotics is higher and varies between 2% and 6%, with a 5-year cumulative incidence ranging between 15% and 20% ***(Bruix et al, 2001)***.

The incidence of HCC depends on several factors, including age over 50 years, male sex, etiology and severity stage of the cirrhosis. Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in African and Asian countries where hepatitis viral infection is endemic. Despite recent progress in imaging techniques and treatment modalities, the prognosis of a patient with HCC is poor. The 5-year survival rate is 5% in the USA, and 40% in Japan ***(Morris, 2005)***.

A computer cohort simulation analysis predicted approximately 165,900 and 27,200 deaths due to chronic liver diseases and HCC

respectively in the USA from the year 2010 to 2019, suggesting that hepatitis virus-related HCC will become a worldwide challenge in the near future ***(Montalto et al, 2002)***.

As the majority of HCCs develop from cirrhotic livers, particularly the post hepatitis or macronodular variety, patients with cirrhosis are recommended to undergo regular examinations for early detection of possible HCC. Measurements of protein induced by vitamin K absence or antagonist-II (PIVKAII) and α-fetoprotein (AFP) concentration, along with several imaging modalities, have been used widely in Japan and North America (***Hattori et al, 1988).***

Ideally, serological markers for HCC should possess the following characteristics (1) high sensitivity and specificity for the diagnosis of HCC; (2) assays that are easy to perform and (3) assays that are comparatively cheap.

Hepatocellular carcinoma is asymptomatic during its course of neoplastic development, and patients present in an advanced stage when first diagnosed. With the aim to allow early diagnosis of HCC, researchers in many biological areas are now routinely identifying and characterizing molecular biomarkers for HCC development and progression ***(Man-Fung, Ching-Lung, 2005)***.

RATIONALE OF THE STUDY

HCC is a primary liver tumor with a poor prognosis for which treatment efficiency is closely related to the timing of the diagnosis.
AFP, still the only marker routinely used at present, remains a controversial and unsatisfactory marker. The discovery of new biomarkers is urgently needed. The current research approaches are directed toward the discovery of more sensitive and early diagnostic markers and the identification of new predictive tools for recurrence.

Up till now, none of known markers are sufficiently validated and their development is limited by routine adaptation of techniques and cost. However, in the future, the development of global genomic or proteomic technologies will likely amplify the discovery of new markers that will hopefully be measurable by simple and inexpensive methods. The development of restricted expression profile analysis could also represent a significant aid for prognosis evaluation ***(Man-Fung et al, 2005)***.

DHCR24 is an enzyme that is elevated in the follow up of HCV patients. Its identification may help in the early diagnosis HCC with other sensitive biomarker ***(Nishimura et al, 2009).***

AIM OF THE WORK

The aim of this study is to evaluate the level of DHCR 24 protein in HCC patients as well as HCV patients in comparison to the control group.

OBJECTIVES

1. Correlate the level of DHCR 24 protein to clinico-pathologic features of 50 HCC patients.
2. Possibility of using DHCR 24 protein in serum (ELISA technique) as biomarker in HCC.

HEPATOCELLULAR CARCINOMA

I. INCIDENCE

Liver cancer is among the most common neoplasms and causes of cancer death in the world, occurring most commonly in Africa and Asia. Up to 1 million deaths due to hepatocellular carcinoma (HCC) occur each year worldwide. In the United States, 17,000 new cases of cancer of the liver and biliary passages develop annually. Incidence throughout the world varies dramatically with 115 cases per 100,000 people noted in China and Thailand, compared with 1 to 2 cases per 100,000 in Britain ***(Morris, 2005)***.

II. ETIOLOGY OF HEPATOCELLULAR CARCINOMA

The etiology of HCC is multifactorial. The most important causes are cirrhosis, HCV, HBV and alcohol.

1. Cirrhosis

Cirrhosis is defined as the histological development of regenerative nodules, surrounded by fibrous bands, in response to chronic liver injury. Complications of cirrhosis include HCC and portal hypertension. The majority of patients with HCC have underlying cirrhosis. In an autopsy series from Italy and Japan, the prevalence of cirrhosis in patients with HCC was between 80 and 90%. With the exception of hepatitis B virus (HBV) and aflatoxin (AF), all other etiological risks for HCC are associated with cirrhosis. Furthermore, in the USA and parts of Europe, the mortality rate of HCC incidence is increasing, but the rate of mortality due to non-HCC complications of cirrhosis is decreasing or static ***(Fattovich et al, 2004)***. This would suggest that the improved