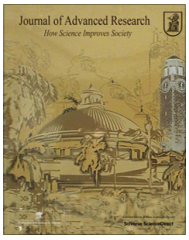
Journal of Advanced Research (2014) 5, 27–40

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Methylation of multiple genes in hepatitis C virus associated hepatocellular carcinoma

Abdel-Rahman N. Zekri [a,*], Abeer A. Bahnasy [b], Fatma elzahraa M. Shoeab [d], Waleed S. Mohamed [a], Dina H. El-Dahshan [c], Fahmey T. Ali [d], Gilane M. Sabry [d], Nairajana Dasgupta [e], Sayed S. Daoud [e]

[a] *Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Egypt*
[b] *Pathology Department, National Cancer Institute, Cairo University, Egypt*
[c] *Faculty of Medicine, Beni Suef University, Egypt*
[d] *Faculty of Science, Ain Shams, University, Egypt*
[e] *Center for Integrated Biotechnology, Washington State University, Pullman, WA, United States*

ARTICLE INFO

Article history:
Received 7 July 2012
Received in revised form 6 November 2012
Accepted 6 November 2012
Available online 26 January 2013

Keywords:
Hepatitis C virus-genotype 4
Chronic hepatitis
Hepatocellular carcinoma
Promoter methylation

ABSTRACT

We studied promoter methylation (PM) of 11 genes in Peripheral Blood Lymphocytes (PBLs) and tissues of hepatitis C virus (HCV) associated hepatocellular carcinoma (HCC) and chronic hepatitis (CH) Egyptian patients. The present study included 31 HCC with their ANT, 38 CH and 13 normal hepatic tissue (NHT) samples. In all groups, PM of *APC*, *FHIT*, *p15*, *p73*, *p14*, *p16*, *DAPK1*, *CDH1*, *RARβ*, *RASSF1A*, O^6*MGMT* was assessed by methylation-specific PCR (MSP). *APC* and *O6-MGMT* protein expression was assessed by immunohistochemistry (IHC) in the studied HCC and CH (20 samples each) as well as in a different HCC and CH set for confirmation of MSP results. PM was associated with progression from CH to HCC. Most genes showed high methylation frequency (MF) and the methylation index (MI) increased with disease progression. MF of *p14*, *p73*, *RASSF1A*, *CDH1* and O^6*MGMT* was significantly higher in HCC and their ANT. MF of *APC* was higher in CH. We reported high concordance between MF in HCC and their ANT, MF in PBL and CH tissues as well as between PM and protein expression of *APC* and O^6*MGMT*. A panel of 4 genes (*APC*, *p73*, *p14*, O^6*MGMT*) classifies the cases independently into HCC and CH with high accuracy (89.9%), sensitivity (83.9%) and specificity (94.7%). HCV infection may contribute to hepatocarcinogenesis through enhancing PM of multiple genes. PM of *APC* occurs early in the cascade while PM of *p14*, *p73*, *RASSF1A*, *RARB*, *CDH1* and O^6*MGMT* are late changes. A panel of *APC*, *p73*, *p14*, *O6-MGMT* could be used in monitoring CH patients for early detection of HCC. Also, we

* Corresponding author. Tel.: +20 101413521; fax: +20 223644720.
E-mail address: ncizekri@yahoo.com (A.-R.N. Zekri).
Peer review under responsibility of Cairo University.

http://dx.doi.org/10.1016/j.jare.2012.11.002

found that, the methylation status is not significantly affected by whether the tissue was from the liver or PBL, indicating the possibility of use PBL as indicator to genetic profile instead of liver tissue regardless the stage of disease.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common solid tumor worldwide and the fourth leading cause of cancer-related death [1]. It accounts for approximately 600,000 deaths per year [2] and it shows a wide geographical variation with low incidence areas in North America and Europe, and high incidence areas in Africa and Asia. In Egypt the incidence of HCC has doubled in the past 10 years, thus it is now the second most incident and lethal cancer in men after lung cancer [3]. The heavy burden of HCC parallels the high rates of HCV infection while hepatitis B virus (HBV) rates have declined after the introduction of the vaccine in 1992 [4,5]. Although it has been estimated that 80% of HCC occurs in cirrhotic livers, the exact molecular mechanisms underlying virus-associated hepatocarcinogenesis are still unclear.

Multiple genetic aberrations of oncogenes and tumor suppressor genes have been identified, which control hepatocytes proliferation, differentiation, maintenance of genomic integrity and death [6,7]. In addition, recent studies suggest aberrant DNA (PM) as an alternative mechanism of tumor pathogenesis because the hypermethylated promoters often lack transcriptional activity, which could result in gene inactivation [8]. DNA methylation refers to the addition of a methyl group to the cytosine residue in CpG dinucleotides. Normally, clustered CpG dinucleotides (CpG islands) are not methylated regardless of their transcriptional status, whereas in tumor cells, methylation of CpG islands in the promoter regions of many tumor suppressor genes (TSGs) and growth regulatory genes effectively silences those genes. Since different types of cancer show distinct DNA methylation profiles, it is possible to develop cancer- type specific methylation signatures [9]. The power of PM as a marker derives not only from its ability to be detected in a wide variety of samples, from fresh specimens to body fluids and archival paraffin-embedded tissues, but also from the defined localization of the lesion in promoter CpG islands of the genes. This could be an early important event in carcinogenesis and could also be of importance for treatment or prognostication [10]. DNA methylation profiles in Egypt has not been well studied, though it has the highest prevalence of HCV infection in the world with approximately 14% of the population infected, and seven million have chronic HCV induced liver disease [11].

We sought to assess DNA methylation patterns in Egyptian patients with HCV associated chronic hepatitis and HCC using a panel of genes that are commonly hypermethylated in other solid tumors (*p14*, *p15*, *p16*, *p73*, *APC*, *FHIT*, *DAPK1*, *CDH1*, *RARβ*, *RASSF1A*, and *O6MGMT*) in order to understand the role of epigenetic silencing in this patient population. The studied groups included 38 HCV/genotype-4-associated CH patients with matched PBL in 20 of them and 31 HCC cases with their ANT. Thirteen NHT obtained from healthy individuals, were used as a control group. The prognostic impact of aberrant PM was also assessed through correlations between methylation patterns and the clinic-pathological features of the studied patients.

Methodology

Study design

This prospective study encompassed three groups. The first group included 31 HCC cases, of which, 23 cases had enough adjacent normal tissue (ANT) samples to be assessed. The second group included: A) 20 cases of chronic CH patients with cirrhosis from which tissue samples and Peripheral Blood Lymphocytes (PBLs) were collected and 18 cases of asymptomatic carriers (ASC), from which tissue samples only were collected. The third group was a control group in which normal hepatic tissue (NHT) samples were obtained from 13 liver transplantation donors matched for age (±5 years) and sex.

HCC samples were obtained from patients who underwent surgical resection of their tumors at the National Cancer Institute (NCI), Cairo, Egypt. Whereas CH samples were obtained from the Endemic medicine department, Kasr Al-Aini School of Medicine, Cairo University. All cases were assessed for viral profile as a part of the routine clinical workup. All HCC and CH cases were positive for HCV/genotype-4 and negative for HBV by serological tests and/or HBV-DNA by real time PCR (qRT PCR). Histopathological diagnosis and grading of the HCC cases were done according to the World Health Organization (WHO) classification criteria [12] and staging was performed according to the American Joint Committee on Cancer [13]. Grading and staging of CH patients were performed according to the pathology activity index [14]. A written informed consent was obtained from each patient and the Institutional Review Boards of the National Cancer Institute and Kasr Al-Aini School of Medicine, Cairo University, reviewed the study protocol which was in accordance with the 2007 Declaration of Helsinki. All patients' characteristics were collected from the patients' records and illustrated in Table 1.

DNA extraction

DNA was extracted from PBL according to standard protocols (6). Briefly, equal volume of equilibrated phenol (pH 7.0–7.5) was added to samples and vortexed. The upper aqueous layer was removed and an equal volume of phenol/chloroform (1:1) was added and vortexed. The upper aqueous layer was removed again and an equal volume of chloroform/isoamyl alcohol (24:1) was added and vortexed. This was followed by the addition of 3 M Sodium acetate (pH 4.7–5.2), DNA precipitation by ice-cold ethanol and overnight incubation at −80 °C. The fluid was decanted and the DNA pellet was dissolved in sterile water. DNA was extracted from fresh tissue samples as previously described [15].