The Egyptian Journal of Medical Human Genetics (2016) xxx, xxx–xxx

HOSTED BY

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The Egyptian Journal of Medical Human Genetics

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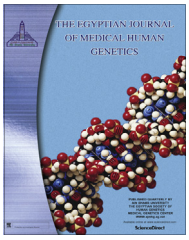

ORIGINAL ARTICLE

Circulating MiRNA-21 and programed cell death (PDCD) 4 gene expression in hepatocellular carcinoma (HCC) in Egyptian patients

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Received 9 March 2016; accepted 10 April 2016

KEYWORDS

miRNA-21;
PDCD4 gene;
Hepatocellular carcinoma;
CLD;
Apoptosis;
Real time-PCR

Abstract *Background:* Circulating microRNAs (miRNAs) are endogenous, small (17–25 nucleotides) non-coding RNAs that are overexpressed in many human cancers including hepatocellular carcinoma (HCC). Moreover, circulating miRNAs can reflect the level of tissue miRNAs, so could be potential tumor markers. miRNA-21 regulates post-transcriptional expression of tumor suppressor gene; programed cell death 4 (PDCD4) gene which implies that miRNA-21 might be a novel diagnostic and/or prognostic marker for cancer.

Objective: To evaluate the diagnostic and prognostic potential of circulating miRNA-21 and study the expression of PDCD4 gene as a target of miRNA-21 in HCC in Egyptian patients.

Subjects and methods: This study was conducted on 30 HCC patients, 20 chronic liver disease (CLD) patients due to HCV infection and 20 healthy subjects. Serum alpha fetoprotein (AFP) was measured for all participants. The relative plasma expression of each of miRNA-21 and PDCD4 gene was determined in whole blood samples using real-time polymerase chain reaction.

Results: The results revealed over expression of miRNA-21 and under expression of PDCD4 gene in HCC group ($p < 0.05$) compared to both CLD and healthy subjects, while no significant change was detected between CLD and healthy subjects. miRNA-21 expression was negatively correlated with PDCD4 gene expression. miRNA-21 expression increased significantly with presence of cirrhosis, increased number of focal lesions, larger size of tumor, advanced tumor stage and presence of vascular invasion. Receiver Operator of Characteristics (ROC) curve analysis of plasma miRNA-21 revealed that, at a cut-off value of 3.93 (fold expression), the sensitivity and specificity for differentiation of HCC cases were 93% and 90%, respectively.

Conclusion: Circulating miRNA-21 could be a novel early diagnostic and prognostic biomarker for detection of HCC. Approaches interfering with the miRNA-21/PDCD4-axis, or releasing PDCD4 expression, may have a strong basis for therapeutic uses in cancer in the future.

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2016.04.007

Please cite this article in press as: El Gedawy G et al., Circulating MiRNA-21 and programed cell death (PDCD) 4 gene expression in hepatocellular carcinoma (HCC) in Egyptian patients, Egypt J Med Hum Genet (2016), http://dx.doi.org/10.1016/j.ejmhg.2016.04.007

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and is associated with liver cirrhosis (LC) in 80% of cases [1]. In Egypt, the incidence rate of HCC has increased sharply in the last decade [2]. The development and progression of HCC is a complex process, which involves the dysregulation of oncogenes and tumor suppressor genes. It has previously been reported that microRNAs (miRNAs) are essential in oncogenesis by the regulation of oncogenes and tumor suppressor genes [3].miRNAs are approximately 22-nucleotide, noncoding, endogenous RNA molecules with an important role in various cellular biological processes, including embryonic development, cell differentiation, and tumorigenesis [4]. miRNAs regulate post-transcriptional gene expression, by binding to the 3′-untranslated region (3′-UTR) of specific target messenger RNAs (mRNAs), which in turn causes mRNA degradation or translational repression [5,6]. In humans, more than 50% of miRNA genes are located at fragile sites or in cancer-associated genomic regions that are frequently involved in chromosomal abnormalities, such as loss of heterozygosity, amplification and breakpoints [7]. miRNA-21 is one of the first oncogenic miRNAs with upregulation detected in many types of human cancer [8]. miRNA-21 has also been implicated in multiple malignancy-related processes, including cell proliferation, apoptosis, invasion and metastasis, by down regulating the expression of specific target genes, such as phosphatase and tensin homolog (PTEN), tropomyosin 1 (TPM1), programed cell death 4 (PDCD4) and B-cell lymphoma 2 (Bcl-2) [9].

PDCD4 is a tumor suppressor gene that plays an important role in regulating apoptosis, invasion and metastasis [10]. Several reports described the regulation of PDCD4 by miRNA-21. In pancreatic ductal adenocarcinoma; inhibition of miRNA-21 reduces proliferation and increases cell death by increasing PDCD4 [11]. Asangani et al. [12] found a conserved potential site for miRNA-21 within the 3′UTR (3-untranslated region) of PDCD4 mRNA. Refs. [13,14] demonstrated the functionality of this site as well as the regulation of PDCD4 levels by miRNA-21 and induction of invasion, intravasation and metastasis by elevated miRNA-21. Thus, miRNAs modulate various cellular signaling pathways involved in cell growth, proliferation, motility and survival [15].

Better understanding of the molecular mechanisms involved in hepatocellular carcinogenesis contributes to the identification of novel prognostic and diagnostic biomarkers and therapeutic targets for HCC. So, this study aimed to evaluate the diagnostic potential of circulating miRNA-21 and study the expression of programed cell death 4 (PDCD4) gene as a target of miRNA-21 in Egyptian patients with HCC and correlating them to the clinical and path logical parameters of the patients.

2. Subjects and methods

2.1. Study population

The work has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. The study was approved by ethics committee of Faculty of Medicine and National Liver Institute, Menoufia University. Enrollment of individuals in the study was conditioned by an obtained written informed consent. Seventy subjects were enrolled in the study and were divided into 3 groups. Fifty patients were randomly selected from the inpatient ward and outpatient clinic, National Liver Institute, Menoufia University from January 2014 to December 2014. Patients were subdivided to newly diagnosed naïve HCC patients and HCV positive chronic liver disease (CLD) cases. The HCC group comprised 30 patients, 26 males and 4 females, their ages ranged from 30 to 52 years. The diagnosis of HCC was based on clinical examination, laboratory tests, ultrasonography and spiral CT. The CLD group consisted of 20 cases, 15 males and 5 females, and their ages ranged from 35 to 51 years. CLD patients were diagnosed by ultrasonographical findings (shrunken liver, coarse echo pattern, attenuated hepatic vein and fine nodular surface) and biochemical evidence of parenchymal damage as well as liver biopsy. Patients with bacterial or other viral infection, chronic renal damage, Insulin-dependent diabetes mellitus (IDDM), other malignant diseases, or undergoing interferon administration or immune-suppressive or chemotherapy were excluded from this study. In addition 20 apparently healthy subjects, 18 males and 2 females, and their ages ranging from 32 to 53 years, age and gender matching, served as the control group.

All patients and control groups were subjected to full history taking, complete clinical examination, abdominal ultrasonography and/or CT.

2.2. Laboratory investigations

Ten ml venous blood samples were collected from patients and controls and divided into three parts. The first part was collected in plain tube and used for routine laboratory investigations, including liver function tests [using fully automated auto 111 analyzer SYNCHRON CX9ALX (Beckman Coulter Inc., CA, USA)] and immunoassay; serum HBs-Ag and HCV-Ab [using (Abbott Laboratories, Abbott Park, IL, USA)]. Also, serum AFP concentration was measured using the Automated Chemiluminescence System (ACS: 180 provided by Siemens Medical Solutions Diagnostics Corporation, USA). The second part was collected in an ethylene diamine tetra acetic acid (EDTA) containing tube and used for CBC assessment using Sysmex K-21, (Sysmex Corporation, Kobe, Japan). The third part was collected in an EDTA containing tube and used immediately for RNA – miRNA extraction and molecular testing.

2.3. Molecular testing

Real time PCR technology (using 7500 fast real time PCR – TaqMan® microRNA and RNA Control Assay) was used for assessments of miRNA-21 and its control gene (RNU43) and the tumor suppressor gene PDCD4 and its control gene [Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)].

2.4. Extraction and cDNA synthesis

2.4.1. RNA extraction and cDNA synthesis

RNA was extracted from fresh EDTA treated blood sample using PureLink® RNA Mini Kit (Ambion, Life Technology)

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