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ORIGINAL ARTICLE

Circulating microRNAs panel as a diagnostic tool for discrimination of HCV-associated hepatocellular carcinoma

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Summary Early diagnosis of hepatocellular carcinoma (HCC) can significantly improve the overall survival of HCC patients. However, current diagnostic markers are compromised and limited by their low sensitivity and specificity. In this work, circulating microRNAs (miRs) were utilized as a diagnostic tool to test their efficiency to segregate HCC and hepatitis C virus (HCV)-infected patients from healthy subjects. Nine HCC-related miRs (miR-21, miR-30c, miR-93, miR-122, miR-125b, miR-126, miR-130a, miR-193b and miR-222) were analyzed by Real-Time PCR in 86 serum samples; 34 HCC and 52 HCV patients in addition to 25 healthy subjects. The sensitivity and specificity of these miRs were assessed. Our results demonstrated that the median serum level of seven miRs was significantly reduced (P ranges from < 0.01 to < 0.001) in HCC patients whereas nine miRs were reduced ($P < 0.001$) in HCV compared to healthy controls. Receiver operating characteristic (ROC) curve analyses had shown high diagnostic accuracy (AUC = 1.0) when seven and nine combined miRs were considered in HCC and HCV groups, respectively compared to their counterparts. However, a combination of differentially expressed miRs did not improve the discriminatory power (AUC = 0.742) when HCC compared to non-HCC groups.

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miR-122 showed the highest sensitivity and specificity to stratify HCC and HCV versus normal individuals and HCC versus HCV patients. We conclude that differentially expressed miRs in the serum of HCV and HCC patients can be utilized as surrogate and non-invasive biomarker for segregation of HCV and HCC patients from healthy subjects.

Introduction

Liver cancer is the fifth most common cancer in men and the seventh in women living in developing countries [1]. The most common liver cancer is hepatocellular carcinoma (HCC) which constitutes 13% of all other cancers in Egypt [2]. HCC is associated with viral etiologic factors such as chronic viral hepatitis HBV or HCV [3,4]. It has been reported that Egypt has the highest prevalence of HCV worldwide and most of HCC cases (~94%) developed in the context of chronic HCV infection [5]. Liver cirrhosis is the most common route of hepatocarcinogenesis after HCV infection [6].

Early diagnosis can significantly improve the overall survival of HCC patients. However, currently available diagnostic markers are still inadequate and limited by their low sensitivity and specificity. For instance, the gold standard marker, alpha-fetoprotein (AFP), has a false negative rate up to 40% for early stage of HCC. It is worthy to mention that the level of AFP was reported in a normal range of 25% of patients with advanced HCC [7]. As such, AFP has been excluded from being a marker for the diagnosis of HCC by Practice Guidelines of the American Association for the Study of Liver Diseases (AASLD) and confirmed by other studies [8,9]. Moreover, des-γ-carboxy prothrombin and Lens culinaris agglutinin-reactive fraction of AFP are unsatisfactory for early diagnosis of HCC [10]. These discrepancies suggest the need of discovering new reliable diagnostic markers for patients with HCC.

Shortly after discovered in nematodes, microRNAs (miRs) were introduced as potent regulators of most of important cellular processes in eukaryotes including humans. As post-transcriptional regulators, altered expression of miRs has shown to be associated with carcinogenesis and was suggested as potential candidates for diagnosis of cancer in its early stage. Indeed, miRs are able to act as tumour suppressors, oncogenes, or even possess a dual nature playing both roles depending on the cellular needs [11].

A growing body of evidence demonstrates that miRs profiling in tumour versus normal tissues has revealed a number of significantly dysregulated miRs in human cancers. miRs expression profile analysis has allowed the characterization of 'identity' associated with each type of human cancer and this 'identity' is correlated with carcinogenesis, tumor progression, and response to tumor treatment [12,13]. Of note, miRs are very stable in body fluids such as plasma, serum, saliva, and urine even after being subjected to severe conditions such as extreme temperature and low pH [14]. Their expression level can be easily estimated [15], and are tissue specific [11,15]. These characteristics of miRs make them attractive candidates for early diagnosis of cancer. Several studies have reported the use of miR-200 [16], miR-500 [17], miR-122a [18], miR-126 [19], miR-21 [20], miR-125b [21] and miR-30c [22] as diagnostic markers in HCC.

Developing a platform of these combined miRs could improve their sensitivity and specificity for HCC detection. Therefore, our study was designed to determine the diagnostic ability of these miRs in combination to segregate HCC and HCV-infected patients from healthy groups. We assessed a panel of nine liver-associated miRs (miR-21, miR-30c, miR-93, miR-122, miR-125b, miR-126, miR-130a, miR-193b and miR-222) and our findings demonstrated that this panel could discriminate healthy individuals from patients with HCC and HCV.

Materials and methods

Patients and samples

Blood samples were collected from 34 newly diagnosed patients with primary HCC and 52 chronic HCV patients attending the Radio-Diagnosis clinic, National Cancer Institute, Cairo University, Egypt in the period from May 2011 to April 2014. Blood samples were collected from another 25 healthy individuals confirmed not to have HCC or HCV, and with no history of any other diseases. All patients and healthy individuals have participated in this study after giving a written consent approved by the Institutional Review Board (IRB) of National Cancer Institute, Cairo University. Patients were considered as HCV-positive if their serum tested positive by the third generation HCV- Enzyme-linked immunosorbent assay (ELISA, Adaltis, Italy) for HCV antibodies following the manufacturer's protocol. Infection with HBV was assessed by hepatitis B surface antigen (HBsAg) and anti-core B serological analysis. Liver function tests were conducted to determine patients' levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and α-fetoprotein (AFP) according to the manufacturer's instructions. In 20 out of the 34 patients (58.8%), HCC was developed in non-cirrhotic background. Fourteen of those patients (70%) had HCV infection and their average level of AST and ALT activities were 51.1 and 85.3 U/L, respectively. Their average level of AFP was 188.85 ng/mL. In those 14 patients, HCC was associated with cirrhosis with HCV infection in eleven of them (78.6%). The average levels of AST and ALT activities were 70.1 and 100.8 U/L, respectively. Their average AFP level was 156.1 ng/mL. Out of 5 non-fibrotic patients, only one patient was cirrhotic (20%) and the second one was mild. Regarding the association between cirrhosis and fibrosis in HCC patients, 12 of 29 (41.4%) of fibrotic patients had liver cirrhosis. The rest of clinicopathological features of the patients were depicted in Table 1.

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