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KIAA0101 mRNA expression in the peripheral blood of hepatocellular carcinoma patients: Association with some clinicopathological features

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ABSTRACT

Objectives: The development of hepatocellular carcinoma (HCC) is multi-factorial, multi-step and involving many genes. Recent studies have revealed the involvement of KIAA0101 in HCC development and progression. KIAA0101 is involved in the regulation of DNA repair, cell cycle progression, and cell proliferation.
This study aims to elucidate the clinicopathological significance of KIAA0101 mRNA expression in the whole blood of HCC patients.

Design and methods: This study was conducted on 77 patients with proven HCC who presented to the outpatient clinic at the National Cancer Institute — Cairo University over a period of 8 consecutive months. Thirty patients with cirrhosis and forty apparently healthy volunteers were included as control groups.
Detection of KIAA0101 mRNA was done on whole blood collected on EDTA for all patients and control subjects using real-time PCR.

Results: KIAA0101 mRNA was over-expressed in the HCC group compared to the control groups. Overexpression of KIAA0101 mRNA was significantly associated with distant metastasis, advanced stage, high serum alkaline phosphatase and low serum albumin levels. Both sensitivity and specificity of KIAA0101 mRNA were higher than those of AFP and CEA.

Conclusion: Being associated with some of the prognostic factors of HCC which reflect tumor progression; as advanced stage, distant metastasis, hypoalbuminemia and elevated serum alkaline phosphatase, together with its relatively high diagnostic performance; KIAA0101 mRNA might be nominated to play a probable role in the diagnosis and prognosis prediction of HCC. Further studies on a wider scale are recommended to confirm these results.

1. Introduction

Hepatocellular carcinoma (HCC) is the third most deadly malignancy worldwide characterized by phenotypic and molecular heterogeneity [1].

The development of HCC is multi-factorial, multi-step and involving many genes during the processes of carcinogenesis and progression. Recent studies have revealed a number of genes with novel sequences and unknown functions, including KIAA0101 [2] that are involved in the development and progression of HCC [3].

P15^{PAF} (PCNA-associated factor) [4], OEATC-1 (overexpressed in anaplastic thyroid carcinoma-1) [3], and L5 [5] are alternate names for KIAA0101, which is a 15-kDa protein containing a conserved proliferating cell nuclear antigen (PCNA)-binding motif [6,7]. PCNA is an essential factor for DNA polymerase hence required for DNA replication or repair [8,9]. The PCNA-binding motif is not only present in KIAA0101, but also in many PCNA-binding proteins, such as p21^{WAF}, p57^{Kip2}, and p33ING1b [10,11]. The p21^{WAF}, p57^{Kip2}, and p33ING1b can prevent PCNA from binding to DNA polymerase by competing with one another [4,11] as well as other PCNA-binding proteins in forming complexes with PCNA, which in turn leads to inhibition of DNA synthesis and cell cycle progression [12,13,14] and eventually G_1 cell cycle arrest [15], hence, KIAA0101 is involved in the regulation of DNA repair [16], cell cycle progression [16], apoptosis and cell proliferation [4].

KIAA0101 shows aberrant expression in various cancers [5,3]. During cancer development, the lack of KIAA0101 activity alters chromosome instability [17] while, after tumors have developed, expression of KIAA0101 may contribute to chemo-resistance in human cancers.

Abbreviations: HCC, Hepatocellular carcinoma; DNA, Deoxyribonucleic acid; mRNA, Messenger ribonucleic acid; AFP, Alpha feto protein; CEA, Carcinoembryonic antigen; MEIA, Microparticle enzyme immunoassay; PCR, Polymerase chain reaction; PCNA, Proliferating cell nuclear antigen; OEATC-1, Overexpressed in anaplastic thyroid carcinoma-1; CT, Computed tomography; MRI, Magnetic resonance imaging.

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This study aims to elucidate the clinicopathological significance of KIAA0101 mRNA expression in the whole blood of HCC patients.

2. Patients and methods

This study was conducted on 77 newly diagnosed patients with HCC who presented to the outpatient clinic at the National Cancer Institute — Cairo University over a period of 8 consecutive months from January to August 2014. All patients who were eligible to the study and were proven to have HCC by computed tomography (CT), magnetic resonance imaging (MRI), or liver biopsy were included. They were 50 males and 27 females, and their age ranged from 47 to 77 years. Thirty patients with cirrhosis were included as a benign control group; they were 19 males and 11 females. Their age ranged from 50 to 71 years; 10 of whom were proven to have hepatitis C virus infection, and 3 had hepatitis B virus infection. Forty apparently healthy volunteers were also included as a normal control group; they were 24 males and 16 females with their age ranged from 45 to 69 years.

A written consent from all patients according to the international ethics committee guidelines, and IRB approval were obtained.

Inclusion criteria:

- Patients proven to have HCC.
- Either sex was eligible.
- Patients were recruited in the study before receiving any line of treatment.

Exclusion criteria:

- Patients having any concomitant malignancies.
- Patients received any line of treatment.

Blood was withdrawn from all patients prior to receiving any line of treatment.

Serum samples from all patients and control subjects were subjected to the following:

(1) Liver function tests using Beckman CX9 auto-analyzer.
(2) Tumor markers: AFP and CEA were done using Axsym based on the microparticle enzyme immunoassay (MEIA) technology.

Detection of KIAA0101 mRNA was done on whole blood collected on EDTA for all patients and control subjects using real-time PCR.

2.1. cDNA synthesis and real-time quantitative PCR

Isolation of mononuclear cells was performed using Ficoll–Hypaque. RNA extraction was done from mononuclear cells using InviTrap Spin Blood RNA Mini Kit according to manufacturer's instructions. One μg RNA was reversely transcribed using high capacity cDNA reverse transcription kit (Applied Biosystems). Reverse transcription was performed in 20 μl reaction containing 1× RT buffer, 0.2 mM dNTP mixture, 1× RT random primer, 50 U multiscribe TM reverse transcriptase and nuclease free water. The reaction was performed at 25 °C for 10 min, followed by 37 °C for 120 min and 85 °C for 5 min then kept at 4 °C.

Primers designed for KIAA0101 detection were according to a previous report [18]. The primer sequences were 5′-AGCTTTGTTGAACAGGCA TTT-3′ and 5′-GGCAGCAGTACAACAATCTAAGC-3′ for KIAA0101 gene and 5′-TGGCACCCAGCACAATGAA-3′ and 5′-CTAAGTCATAGTCCG CCTA GAAGCA-3′ for β-actin mRNA. Real-time quantitative PCR amplifications were performed in a total volume of 20 μl; each reaction contained 2 μl 10× buffer, 10 μl 2× QuantiTect SyberGreen PCR master mix (Qiagen), 1 μl (10 μmol/l) of each primer, 2 μl cDNA, and 5 μl ddH_2O. PCR regime involved a 95 °C 5-min initial denaturation step followed by 23 cycles (for β-actin) and 28 cycles (for KIAA0101) at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s.

2.2. Analysis of KIAA0101 mRNA expression using the $2-\Delta\Delta CT$ method

The mean target KIAA0101 mRNA expression level for the 2 mRNA measurements was calculated. The $2-\Delta\Delta CT$ method was used to calculate relative changes in KIAA0101 mRNA expression determined from real-time quantitative PCR experiments. In the present study, the data are presented as the fold change in KIAA0101 mRNA expression in the samples of HCC patients normalized to the internal control gene (β actin) and relative to the normal sample control (matched normal as calibrator). Results of the real-time PCR data were represented as CT values, where CT was defined as the threshold cycle number of PCRs at which amplified product was first detected. There is an inverse correlation between CT and amount of target: higher amounts of target have lower CT values and lower amounts of target correspond to a higher CT value. The average CT was calculated for both KIAA0101 mRNA and β actin and the ΔCT was determined as (the mean of the duplicate CT values for KIAA0101 mRNA) minus (the mean of the duplicate CT values for β actin). The ΔΔCT represented the difference between the paired blood samples, as calculated by the formula $\Delta\Delta CT = (\Delta CT \text{ of HCC sample} - \Delta CT \text{ of normal sample})$. The N-fold differential expression in KIAA0101 mRNA of an HCC sample compared to the normal sample counterpart was expressed as $2-\Delta\Delta CT$. In the present study, increased mRNA expression was defined as N-fold >1.0, "normal" expression was an N-fold = 1.0, and decreased mRNA expression was N-fold < 1.0.

Cutoff level of KIAA0101 mRNA expression was determined as the mean value of KIAA0101 mRNA levels among subjects of the normal control group.

2.3. Statistical analysis

Data analysis was done by using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). The descriptive measures were presented in frequencies and percentages. For quantitative data, comparison between two groups was done using Mann–Whitney (non-parametric t-test). Chi square test was used to test prevalence of

Table 1
Patients' characteristics.

			HCC		Cirrhosis
Age (median and interquartile range)			61 (55–67)		58.5(55–64)
Characteristic		N (77)	Percentage	N(30)	Percentage
Sex	Males	50	65	19	63
	Females	27	35	11	37
KIAA0101 mRNA	Over-expressed	52	67.5	2	7
	Under-expressed	25	32.5	28	93
Stage	I	6	8		
	II	23	30		
	III	25	32		
	IV	23	30		
Distant metastasis	Present	31	40		
	Absent	46	60		
Lymph nodes involvement	Present	17	22		
	Absent	60	78		
Cirrhosis	Present	27	35		
	Absent	50	65		
Portal vein thrombosis	Present	11	14		
	Absent	66	86		
Ascites	Present	33	43		
	Absent	44	57		
Hepatitis virus infection	HCV	17	22	10	33
	HBV	7	9	3	10
	None	53	69	17	57

HCV: Hepatitis C virus.
HBV: Hepatitis B virus.