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Preoperative Plasma Transcript AA454543 Level Is an Independent Prognostic Factor for Hepatocellular Carcinoma after Partial Hepatectomy[1]*

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Abstract

BACKGROUND*: We have previously reported that tissue expression levels of transcript AA454543 in hepatocellular carcinoma (HCC) are significantly higher than those of normal livers, livers with cirrhosis, and livers with hepatitis. In addition, a higher level of transcript AA454543 in tumor tissues is associated with poor prognosis. We aim to examine whether quantitative measurement of preoperative plasma transcript AA454543 can provide similar prognostic information.** ***PATIENTS AND METHODS: **Blood samples were obtained from 84 HCC patients before surgery. Real-time quantitative reverse transcription–polymerase chain reaction, using *TaqMan* system, was employed to measure plasma transcript AA454543 and α-fetoprotein (AFP) RNA levels. We assessed their prediction power in prognosis using univariate and multivariate analyses.** ***RESULTS:*** **High plasma transcript AA454543 RNA levels were associated with poor overall survival (log-rank test, $P < .01$). Patients with different plasma AFP RNA levels revealed no difference in overall survival (log-rank test, $P = .88$). By multivariate Cox regression analysis, plasma transcript AA454543 RNA level (hazard ratio = 4.8, $P < .01$) and tumor stage (hazard ratio = 1.7, $P < .01$) were determined to be independent risk factors for the prediction of overall survival.** ***CONCLUSION:*** **Preoperative plasma transcript AA454543 RNA level can provide prognostic information for HCC patients receiving curative partial hepatectomy.**

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Keywords: Plasma RNA, circulating nucleic acid, liver cancer, prognosis, biomarker.

Introduction

Studies on the detection of cell-free nucleic acids in plasma and serum have revealed potential in the initial diagnosis and follow-up monitoring of a variety of cancers [1–3]. Different RNA targets have been used in diverse cancer studies, and cytokeratin-19 frequently serves as a universal marker for solid tumors of epithelial cell origin [4]. In addition, studies on RNA targets of viral and cellular origins have been reported, including studies on Epstein-Barr virus RNA in nasopharyngeal carcinoma, tyrosinase RNA in melanoma, catenin RNA in adenoma and colorectal cancer, hnRNP B1 RNA in lung cancer, and telomerase RNA in breast cancer, melanoma, and thyroid cancer [5–10].

We have previously reported that the tissue expression of transcript AA454543 (GenBank accession no. BC043195) was specific for hepatocellular carcinoma (HCC), with a significantly higher level in tumor tissues than in liver tissues adjacent to tumors (comprising hepatitis and cirrhotic livers) and in normal livers [11,12]. Furthermore, a higher level of transcript AA454543 in tumor tissues was associated with aggressive tumor features, including venous infiltration, microsatellite nodules, late tumor stage, and poor overall survival [12]. Such association could be explained by the biologic role of AA454543 in cell invasion. An increased level of transcript AA454543 might enhance the invasive ability of tumor cells, subsequently resulting in venous infiltration and formation of microsatellite nodules. Importantly, transcript AA45453 levels in tumor tissues and tumor stage were independent prognostic factors for overall survival [12]. The prognostic significance of transcript AA454543 was first identified in our earlier cDNA microarray dataset [11], in which data were validated by quantitative reverse transcription–polymerase chain reaction (RT-PCR) and then confirmed in a separate HCC sample set [12]. Thus, quantitative assay of the molecular marker transcript AA454543 in tumor tissues can provide general prognostic information (two independent cohorts of HCC patients, and prediction is independent of the assay method used).

Abbreviations: HCC, hepatocellular carcinoma; AFP, α-fetoprotein

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In the current study, we aim to investigate whether preoperative plasma RNA levels of transcript AA454543 can provide prognostic information. Plasma RNA levels of transcript AA454543 and α-fetoprotein (AFP) were examined. Plasma RNA data were compared with serum AFP protein data and tumor stage to determine their prediction power on overall survival. We report here that preoperative plasma transcript AA454543 level is one of the independent prognostic factors for HCC following partial hepatectomy.

Patients and Methods

Patients

The study protocol was approved by the Ethics Committee of The University of Hong Kong, and informed consent was obtained from the patients. Between October 1999 and May 2004, 84 patients undergoing partial hepatectomy for HCC at the Department of Surgery, The University of Hong Kong, Queen Mary Hospital, were recruited for the current study. The same team of surgeons performed all operations throughout this period. All patients had been diagnosed with primary HCC and have not received other treatments before surgery. Clinicopathological data were prospectively collected (Table 1). Overall survival was measured from the date of surgery to the date of death due to liver cancer as endpoint by the end of follow-up. Disease-free survival was calculated from the date of surgery to the date of recurrence and/or liver cancer death as endpoint by the end of follow-up. Diagnosis of recurrence was based on typical imaging findings in a contrast-enhanced computed tomography scan and an increased serum AFP level. In case of uncertainty, hepatic arteriography and post-Lipiodol computed tomography scan were performed; when necessary, fine-needle aspiration cytology was used for confirmation. Up to the date of analysis, the median follow-up time was 25.4 months.

Table 1. Clinicopathological Features of HCC.

HCC Features	Total (n = 84)
Age in years [median (range)]	52 (24–79)
Gender	
Male	72
Female	12
Tumor stage*	
I and II	46
III and IV	38
Tumor size (cm) [median (range)]	11 (3–22)
Serum AFP level (ng/ml) [median (range)]	249.5 (1–1,043,700)
HBsAg	
Positive	74
Negative	10
Underlying liver disease	
Noncirrhosis	59
Cirrhosis	25
Disease recurrence	
With	50
Without	34
Disease mortality	
Death	31
Living	53

*Tumor stage according to the *American Joint Committee on Cancer Staging Manual* [15].

Isolation of Plasma RNA

Blood samples (10 ml each) were collected from each patient before the operation. The blood samples collected were stored at 4°C, and the plasma separated within 24 hours. The blood samples were centrifuged at 3000 rpm for 20 minutes to separate the plasma. Parallel plasma samples that had been centrifuged once and twice were compared, and they did not reveal a significant difference in plasma RNA levels, including 18*S*, AFP, and AA454543 in our pilot study. Thereafter, including all plasma samples used in the current study, the blood samples were centrifuged once. Plasma specimens were stored at −70°C until use. Total RNA was extracted using TRIZOL LS reagent (Invitrogen, Carlsbad, CA). Briefly, 200 μl of plasma was added to 600 μl of TRIZOL LS, mixed, incubated at room temperature for 5 minutes, and added to 160 μl of chloroform. After thorough mixing, the mixture was centrifuged at 13,000 rpm for 30 minutes at 4°C. The aqueous phase (360 μl) on top was transferred to a clean tube, then added to 10 μg of glycogen and 400 μl of isopropanol. RNA was precipitated by centrifugation, and RNA pellet was washed once with 75% ethanol. The RNA pellet was then dissolved with 50 μl of diethylpyrocarbonate (DEPC)–water for subsequent quantitative assays.

Real-Time Quantitative RT-PCR

Real-time quantitative RT-PCR was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA), as described [12]. First-strand cDNA was synthesized with 4.5 μl of total RNA in 50 μl of reaction mixture using High-Capacity cDNA Achive kit (Applied Biosystems), following the manufacturer's instructions. The 5-μl first-strand cDNA was then used in each 25-μl quantitative assay with 1× PCR buffer II; 5.5 mM $MgCl_2$; 0.2 mM dATP, dCTP, and dGTP; 0.4 mM dUTP; and 0.625 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). The primers and probe for transcript AA454543 were AA454543-F (5′-CAA TCA GAC AGG CTG CTT TTC TC-3′), AA454543-R (5′-CTT CCT AAT TAA TGT TTG CAC CAT ATG-3′), and AA454543-P (5′-6FAM AAC TCA TAG GTA ACA AAC ACA AA-MGBNFQ-3′). The primers and probe for AFP RNA were AFP-F (5′-ACT CCA GTA AAC CCT GGT GTT G-3′), AFP-R (5′-ACA TAT GTT TCA TCC ACC ACC AA-3′), and AFP-P (5′-6FAM CAC TTC TTC ATA TGC C-MGBNFQ-3′). The primers and probe reagents for control ribosomal 18*S* were ready-made reagents (Pre-Developed *TaqMan* Assay Reagents; Applied Biosystems) and served as quality control for all samples in all assays. Transcript quantification was performed at least in duplicate for every sample. The PCR profile for the quantitative assay included incubation at 95°C for 10 minutes and then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The amplification plots of PCR were used to determine the threshold cycle (C_T), and the threshold line was usually set at R_n = 0.1. The C_T value represented the PCR cycle at which an increase in reporter fluorescence above a baseline signal could first be detected. Calibrator