JOURNAL OF SURGICAL RESEARCH 195 (2015) 444–453

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Protein tyrosine kinase 7: a hepatocellular carcinoma-related gene detected by triple-combination array

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ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form
20 November 2014
Accepted 23 December 2014
Available online 31 December 2014

Keywords:
Hepatocellular carcinoma
Protein tyrosine kinase 7
Cancer-related genes
Array analysis
Methylation

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the top five causes of cancer-related deaths worldwide. We developed a novel technique to identify cancer-related genes of HCC as follows: triple-combination array analysis, which combines gene expression profiles, single nucleotide polymorphism arrays, and methylation arrays.

Materials and methods: Triple-combination array analysis was performed on one HCC sample from a 68-y-old female patient, and one candidate cancer-related gene was selected. Subsequently, we analyzed the identified gene by quantitative real-time reverse-transcriptase polymerase chain reaction (PCR) and methylation-specific PCR in nine HCC cell lines and in samples from 48 HCC patients. Additionally, we evaluated gene expression by immunohistochemistry and Western blotting.

Results: Using this method, protein tyrosine kinase 7 (*PTK7*) was detected as a candidate cancer-related gene. *PTK7* was revealed to be hypermethylated (methylation value 0.826, range 0–1.0) in cancer tissue, compared with that of adjacent noncancerous tissues (0.047) by methylation array. Of the 48 clinical samples, 30 HCC samples (62.5%) showed *PTK7* promoter hypermethylation. Downregulation of *PTK7* (expressions in tumor tissues decreased by ≥50% compared with the noncancerous tissues) was significantly associated with age >60 y ($P = 0.030$) and elevation in serum protein induced by vitamin K absence or antagonists-II ($P = 0.033$). Moreover, patients with downregulation were significantly inferior in overall survival ($P < 0.001$) than the others.

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2014.12.045

Conclusions: Our data imply that *PTK7* acts as a cancer-related gene and may be a potent prognostic marker for HCC. Triple-combination array analysis was once again found to be useful in identifying cancer-related genes.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the top five causes of cancer-related deaths worldwide [1]. Although recent developments in imaging and multidisciplinary treatment of HCC have improved the survival rate, HCC remains a disease with poor prognosis. Therefore, it is important to elucidate the mechanism of hepatocarcinogenesis and find the genetic markers of HCC.

The development of high-throughput technology such as gene expression microarrays and single nucleotide polymorphism (SNP) arrays that can simultaneously screen thousands of genes has enabled comprehensive identification of alterations in gene expression caused by oncogenesis [2–4]. We have previously reported a method named double-combination array analysis that combines SNP with gene expression arrays [5–10]. Additionally, we have hypothesized that the decrease in gene expression is due to hypermethylation of the CpG islands. Aberrant DNA methylation of the promoter and other genomic regions can lead to changes in gene expression. Therefore, we additionally performed methylation array analysis by using the Ilumina Infinium HumanMethylation27 BeadChip platform (Ilumina, San Diego, CA) to evaluate the methylation status comprehensively. Data from all three analyses can be combined to detect aberrant gene expression in tumor tissue. Through this approach named triple-combination array analysis, we have identified various molecules that could be associated with carcinogenesis and prognosis in various types of cancer [11–15].

In the assessment of data from the triple-combination array analysis, protein tyrosine kinase 7 (*PTK7*), also known as colon cancer kinase 4 [16], was identified as a promising candidate for a novel cancer-related gene in HCC. *PTK7* is one of the protein tyrosine kinases belonging to a large multigene family with particular relevance to many human diseases, including cancer [16]. *PTK7* has functions in cell adhesion, migration, polarity, proliferation, actin cytoskeleton reorganization, and apoptosis and plays an important role in embryogenesis and epithelial tissue organization [16–18]. A mutation of *PTK7* in mice disrupts neural tube closure and stereociliary bundle orientation [16,17]. Several studies have discussed the relationship between the *PTK7* gene and common human malignancies [18–25], but not for HCC. Thus, in this study, we explored the expression and clinical relevance of *PTK7* in surgically resected specimens of HCC.

Table 1 – Patient characteristics.

Characteristics	Value
Age (y), mean ± SD (range)	62.4 ± 7.8 (39–77)
Sex (*n*, %), male: female	43 (89.6): 5 (10.4)
Viral infection (*n*, %) HBV: HCV: non-HBV/HCV	7 (14.6): 38 (79.2): 3 (6.2)
Tumor size (cm), mean ± SD (range)	3.86 ± 2.62 (1.2–14)
Tumor number (%), solitary: multiple	31 (64.6): 17 (35.4)
Japanese stage (*n*, %), I: II: III: IV	6 (12.5): 26 (54.2): 11 (22.9): 5 (10.4)

HBV = hepatitis B virus; HCV = hepatitis C virus; SD = standard deviation.

2. Materials and methods

2.1. Triple-combination array analysis

Total RNA and DNA were extracted from HCC and noncancerous liver tissue derived from a 68-y-old woman with chronic hepatitis C. Total RNA was sent to the manufacturer of Affymetrix (Affymetrix, Santa Clara, CA) for expression array analysis; genomic DNA was used for SNP array analysis; and bisulfite-converted DNA was subjected to the Ilumina Infinium HumanMethylation27 BeadChip methylation array analysis. Each array analysis was performed as described previously [5–10,26,27]. The data were extensively analyzed, and *PTK7* was eventually identified as an interesting cancer-related gene. The gene was identified from a specimen from a single patient; therefore, the result could have been applicable only to that particular patient. We therefore conducted the following experiments to verify the clinical relevance of *PTK7*.

2.2. Sample collection

Nine HCC cell lines (HuH1, HuH2, HuH7, HepG2, Hep3B, HLE, HLF, SK-Hep1, and PLC/PRF/5) were obtained from the American Type Culture Collection (Manassas, VA). HCC and corresponding noncancerous tissues were obtained from 48 patients who had undergone liver resection at Nagoya University Hospital, Japan, between 1994 and 2001. The median

Table 2 – Expression array analysis of PTK7 gene.

Gene symbol	Log_2 ratio	Noncancer signal	Detection	Tumor signal	Detection	Probe ID	Chromosomal location
PTK7	−1.5	92.3	P	18.6	P	HU133p2_16,458	6q21.1