

EFNA1 ligand and its receptor EphA2: potential biomarkers for hepatocellular carcinoma

Xiang-Dan Cui¹, Mi-Jin Lee¹, Goung-Ran Yu¹, In-Hee Kim¹, Hee-Chul Yu², Eun-Young Song³ and Dae-Ghon Kim¹

¹Division of Gastroenterology and Hepatology, The Institute for Medical Science, Department of Internal Medicine, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk, Republic of Korea

²Department of Surgery, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk, Republic of Korea

³Laboratory of Cellular Signaling Modulator, Korea Research Institute of Bioscience and Biotechnology, Yuseong, Daejeon, Republic of Korea

Novel biomarkers are needed for early detection and progression evaluation of hepatocellular carcinoma (HCC). The purpose of this study was to identify useful biomolecular markers for HCC. The 26 genes that encode membrane or secretory proteins were identified from cDNA microarray data. We further examined the expression of EFNA1 and its receptor EphA2 and determined their biological implications during the development and progression of HCC. The EFNA1 mRNA was overexpressed in most HCCs as compared with its expression in corresponding nontumor tissues (36 out of 40 cases, 90%), but EphA2 expression was noted in only half of the HCC tissues (20 of 40 cases, 50%). In most of the hepatoma cell lines, the EFNA1 protein expression was positively associated with alpha-fetoprotein (AFP) expression but inversely associated with EphA2 expression. Furthermore, EFNA1 levels were detectable in the supernatant of the cultured hepatoma cells and in the serum of patients with HCC. In contrast, EphA2 expression was prominent in highly invasive hepatoma cells, and its overexpression was significantly correlated with decreased differentiation ($r = 0.0248$, $p < 0.010$) and poor survival ($p = 0.0453$) for HCC patients. EFNA1 and EphA2 may be useful serum markers for the detection of HCC development and progression, respectively.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and it is known to be the third most common cause of cancer-related mortality.¹ The incidence of HCC is increasing in Western countries such as Europe and the United States.² One of the reasons for the high mortality is that HCC tumors are commonly detected at a stage when curative resection is no longer feasible because of intrahepatic and extrahepatic metastasis. The diagnosis of HCC currently relies on observation of a liver mass in radiology imaging studies such as ultrasonography, computed tomography scanning or magnetic resonance imaging. However, the diagnosis of small lesions is relatively inaccurate.³ The only approach

to screen for the presence of HCC in a high risk-population is the combination of serum alpha-fetoprotein (AFP) level determination and ultrasonography.^{4,5} However, the AFP level test has low sensitivity and specificity, particularly in patients with smaller HCCs. Several biomarkers such as Des-gamma carboxyprothrombin (DCP), lens culinaris agglutinin-reactive AFP and glypican-3 (GPC3) have yet to be validated for their abilities to detect an early HCC.⁶ Therefore, there is an urgent need to identify adequate biochemical markers for early detection and to evaluate the progression of HCC. Genome-wide microarray analysis offers a systemic approach for obtaining comprehensive information on the transcriptional profiles of HCC. Using microarray technology, we previously determined the molecular nature of multistep hepatocarcinogenesis and the specific genetic changes associated with the oncogenic differentiation of HCC.⁷ In our previous study, we used a modified analytical approach to identify several molecular markers, confined to two critical steps in development and advancement, for HCC. In this present study, we focused on the differential expression of the genes that encode membrane or secretory proteins, *i.e.*, histochemical or serum markers for HCC. We are particularly interested in the preferential expression of EFNA1 in HCC. EFNA1 is a prototypic ligand for EphA2 receptor tyrosine kinase and is bound to the cell membrane by a glycosyl-phosphatidylinositol (GPI) linkage. The binding of ligand EFNA1 to its receptor EphA2 promotes autophosphorylation of EphA2, which in turn triggers downstream signals that regulate cell growth and migration. EFNA1 also promotes rapid turnover of phosphorylated EphA2.^{8,9} The expression of EFNA1 has also

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Correspondence to: Dae-Ghon Kim, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk, Republic of Korea, Fax: +82-63-254-1609, E-mail: daeghon@chonbuk.ac.kr