Int J Clin Exp Pathol 2016;9(8):8631-8633
www.ijcep.com /ISSN:1936-2625/IJCEP0028511

Original Article

Distribution and characterization of glypican-3 in hepatocelluar carcinoma

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Received March 17, 2016; Accepted June 27, 2016; Epub August 1, 2016; Published August 15, 2016

Abstract: Glypican-3 (GPC3), a cellular surface proteoglycan, is clinically used to diagnose hepatocellular carcinoma. However, the phenotypic characterization of GPC3 in liver cancer cells warrants to be further investigated. The objective of this study was to assess the clinical application of GPC3 as a histochemical marker when it was matched for series of diagnostic data from advanced liver cancer patients. Here, late stage of liver cancer patients was recruited for screening the disease progression on immunohistochemical analysis and clinical biochemical measurements. Consequentially, patients were diagnosed serologically with notably elevated aminotransferases levels (AST and ALT) and increased alpha-fetoprotein (AFP) content. As shown microscopically, GPC3-positive cells were widespread expressed within the liver tissue, characterized with parallel overgrowth of cancer cells that were immunostained with Ki-67. Furthermore, representative immunophenotypes, such as p53, TOPO II, CD34, CD10, and other biomarkers were abnormally altered during the disease development. Taken together, our present findings preliminarily reveal that GPC3 may serve as a potential marker using in clinical application for liver cancer diagnosis, especially in advanced stage. As well, GPC3 also may be a therapeutical target for innovative medicine design.

Keywords: Hepatoma, GPC3, phenotype, marker

Introduction

Primary hepatocelluar carcinoma represents a fatal type of cancer in which diseases from the liver tissue. Worldwide, incidence of liver cancer increases sharply, especially in China [1]. More notably, symptoms of liver cancer seem to be vague in early stage, whereas the disease is diagnosed when is at an advanced outcome. As a result, the liver cancer patients commonly are given treatments, such as chemotherapy, radiotherapy [2, 3]. However, high risk of relapse in treated patients may occur after managed regimen. Therefore, more effective screening method can aid in contributive reduction of cancer cell developing. Recently, some evidences show that glypican 3 (GPC3) is employed as a promising serum biomarker for diagnosis of hepatocellular carcinoma [4]. Further study indicates that GPC-3 exerts a crucial action in liver cancer cell proliferation and subsequent metastasis, in which is associated with regulating signal pathways in hepatocyte malignant transformation [5]. Here, clinical observations and related analysis were subject to assessment of the potential clinical utility of GPC-3 in liver cancer development, in which was based on representative clinical biochemical data and histopathological investigation in advanced liver cancer patients.

Patients and methods

The patients (n = 7) with liver cancer were hospitalized and given treatments after being identified via serologically testing and pathologically diagnosing. As a statement, all the procedures were according to the Ethical Guidelines issued in the Declaration of Helsinki.

In brief, patient liver specimen was harvested through biopsy and further preparation as paraffin-embedded block. 5 μm liver section was subject to immunohistochemical staining as

Table 1. Association between hepatocellular GPC3 expression and positive immunophenotypes in patients with hepatocelluar carcinoma

Parameters		Pooled	GPC3$^+$
Sex	Male	5	-
	Female	2	-
p53$^+$	Male	4	5
	Female	2	2
Ki-67$^+$ (≥20)	Male	3	5
	Female	2	2
CD34$^+$	Male	2	5
	Female	0	2
CD10$^+$	Male	1	5
	Female	0	2
TOPO II$^+$	Male	4	5
	Female	2	2

described previously [6, 7]. Liver section was dewaxed and rehydrated via series of xylene and ethanol concentrations. After rishing and blocking, liver sample was incubated with primary antibodies (1:100; Fuzhou Maixin Biotech. Co., Ltd., China) at 4°C overnight. Further, the sample was exposed to secondary antibodies for 1 h at room temperature prior to incubation antigen-antibody complex with 3, 3'-diaminobenzidine (DAB) for chromatic visualization, followed by counterstained with hematoxylin on cell nuclei, as well as captured pictures.

Results

Basal data from clinical examinations

As shown in clinical diagnosis results, all patients were exsanguinated for biochemical parameter testing, resulting in significant elevation of liver functional enzymes (AST and ALT), indicator for hepatitis B infection (HBsAg), and target for diagnosis of liver cancer (AFP). Further, diagnostic immunophenotype markers were abnormally changed during treatment period, and there was relationship between hepatocellular GPC3 expression and related positive immunophenotypes in patients with hepatoma, especially in male patients. In these patients, there were 5 males and 2 female, with average age of 46.1 (**Table 1**).

Immunohistochemical staining

In addition to common histopathological examination, the antibody-specific immunostaining was used to assess identification and characterization of GPC-3-markers in liver cancer cells. As analyzed outcomes, GPC-3 positive cells were detectable in all liver cancer patients, in which the higher expression of GPC-3 accounted for 60-95%/field area, even in different hepatic architectures. In addition, immunoactivity for Ki-67, a nuclear protein necessary for cellular proliferation, was labeled in cell nuclei with higher expression, while cytoplasm-stained AFP, a liver cancer marker, was expressed in the liver cancer tissue widely (**Figure 1**).

Discussion

Mounting evidences suggest that advanced liver cancer has high fatality due to liver failure and further metastasis [8, 9]. In clinical practice, diagnostic test is a routine method for identifying liver cancer development. Thereby, more effective pathological screening shows economic benefit for cancer diagnosis. A sensitive and specific biomarker needs to be represented in clinical application.

Biologically, groups of the glypican-associated membrane proteoglycan family include a vital protein anchored to cytoplasmic membrane through glycosyl phosphatidylinositol binding, in which the type of protein can exert an important action of regulating cell division and growth [10]. GPC-3 refers to a heparin sulphate proteoglycan, and functions as an oncofetal protein. Recent reports describe that glypican-3 (GPC-3) may be one of the most promising plasma biomarkers for hepatocellular carcinoma, characterized by diagnostic accuracy with AFP [11, 12]. Nevertheless, GPC-3 functioned as immunostaining marker is still limited in histopathological practice.

Therefore, our present clinicopathologic findings demonstrate that GPC-3-labeled immunopositive cells widely express around different parts of liver cancer tissue, showing in cell proliferative marker of Ki-67 and hepatoma marker of AFP *in situ*. In addition, high proportion of GPC-3-positive outcome is also consistent with conventional pathological markers with immunoactivity (**Table 1**). These preliminary observations provide potential applicable prospect that high level of GPC-3 can be traced during development of liver cancer.

As visible limitation in this report, further large sample could be analyzed and verified prior to

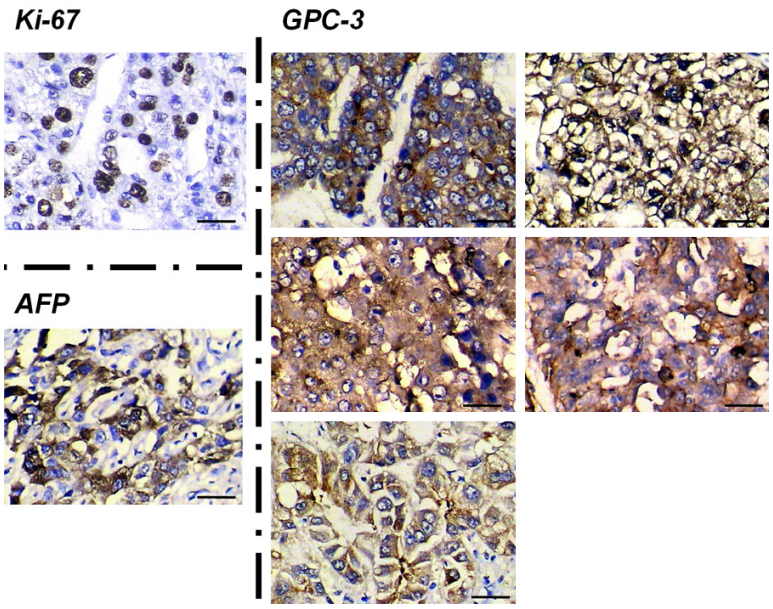

Figure 1. Representative micrographs from immunohistochemical staining showed the widespread distribution of immunoreactive GPC-3 cells in liver cancer tissue (60%-95%/view area), in which the cell proliferative marker of Ki-67 and hepatoma marker of AFP were detected positively round the livers during liver cancer development (Immunohistochemistry analysis; scale bar = 100 mm).

clinical usefulness of GPC-3. In addition, function-gain and -loss of GPC-3 experiments in vitro and in vivo need to be conducted.

Acknowledgements

This study was granted from Science and Technology Research Projects of Guangxi Universities (No. KY2014048 and KY2015LX283), and National Nature Science Foundation of China (No. 81560134).

Disclosure of conflict of interest

None.

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