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Original Article

Clinical biocharacterization of immunophenotype in hepatocellular carcinoma patients

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Abstract: In current observations, we investigated the clinical immunophenotypes in liver cells to assess the metastasic predisposition in advance hepatocellular carcinoma patients. In method, we harvested the clinically diagnosed data from 8 liver cancer subjects. Definitely, all patients were received standard chemotherapeutics when being confirmed as advanced liver cancer via clinical diagnosis. In parallel, biopsy liver samples were subjected to histopathological and immunoblotting assays. Representatively, clinical laboratory results showed that blood parameters resulted in notable elevations of aminotransferases (ALT, AST), hepatitis B e antibody (HBeAb), alpha-fetoprotein (APF) and carcinoembryonic antigen (CEA). In addition, immunoassays exhibited significant hepatocellular expressions of Ki-67 (cell proliferation), CD34 (angiogenesis), as well as strong production of CK8, CK10 (metastasis) in liver cells, as revealed in both the immunostaining and Western blotting analyses. Collectively, the present clinical findings elucidate that the metastasis of hepatocellular carcinoma relates to unregulated cell proliferation and angiogenesis. In particular, current representative immunophenotypes may be served as potential biomarkers for screening metastasis of advanced liver cancer.

Keywords: Liver cancer, immunophenotype, metastasis, biomarker

Introduction

Liver cancer refers to the global sixth most lethal cancer, displaying the significant pathological features of dysregulated cell growth and widespread metastasis [1]. As a vital metabolic organ, hepatocellular oncogenesis can become lethal over time, especially in cancer metastasis. Under pathophysiological condition, overgrowth of liver cancer cell is involvement of metastasis into other tissues/organs [2]. Once occurred in neoplasm metastasis, majority of patients are advanced state of disease. Normally, clinical practice suggests that there is limited to control the development of migrated cancer cells until the emergence of death [3, 4]. To restrain cancer cell metastasis, clinicopathologic evidences that trace the condition of diseased cells prior to metastasis can aid in management of cancer, such as hepatocellular carcinoma. As expected, our current human study intends to delve the potential diagnostic biomarkers in use for advanced liver tumor, characterized with applicable immunophenotypes in cancer cells.

Patients and methods

Total number of 8 patients was recruited and diagnosed as hepatocellular carcinoma before they were administrated with routine chemotherapy. All the patients were traced in subsequent visits. The ethical guidelines stated in the Declaration of Helsinki were followed.

Patient blood sera were subjected to a series of biochemical assays in clinical laboratory.

Hepatic samples via biopsy were processed as 5 μm-paraffin slice. After the steps of dewax and dehydration in different concentrations of xylene and ethanol, the liver sections were incubated with corresponding primary antibodies (diluted as 1:100; Fuzhou Maixin, China) at 4°C overnight, followed by incubation of correspond-

Table 1. Pooled data of diagnosed liver cancer patients

Clinical parameters	*Variation*	*Cases*	*P* Value
Age (Year)	$\bar{x}$ = 58.6	8	0.045
Gender	Male	6	-
	Female	2	-
HBeAb	Negative	4	0.238
	Positive	4	0.349
AFP (μg/l)	>20	6	0.043
CEA (ng/ml)	>10	8	0.016
ALT (U/L)	>50	8	0.022
AST (U/L)	>40	8	0.009

Note: HBeAb: hepatitis B e antibody; APF: alpha-fetoprotein; CEA: carcinoembryonic antigen; ALT, AST: aminotransferases.

ing secondary antibodies (diluted as 1:1500; Fuzhou Maixin, China) for 1 h at 37°C. Chromogenic complex was produced by 3, 3'-diaminobenzidine (DAB) prior to counterstaining with haematoxylin in cell nucleus [5].

As reported previously [6], paraffin-embedded liver sample was dewaxed and blocked with 5% BSA buffer (Beyotime Biotechnology, China) for 1 hour at room temperature, followed by incubation of corresponding primary antibodies (1:200; Boster, Wuhan, China) overnight at 4°C, and reincubation of IgG H&L (Alexa Fluor® 488) (1:200; Abcam, UK) for 1 h at room temperature. Then, DAPI (Abcam, UK) was used to nuclear staining ahead of imaging and assaying.

Protein lysate from RIPA was prepared by using a commercial kit (Beyotime Biotechnology, China). Equivalent protein (30 μg/well) was separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and further blotting to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocked with 5% non-fat milk buffer for 1 h, corresponding primary antibody (Boster Biotechnology, China) was incubated in the samples overnight at 4°C, followed by incubation with horseradish peroxidase-coupled secondary antibodies (Boster Biotechnology, China). Membrane was washed and detected using an enhanced chemiluminescence (ECL) system (Boster Biotechnology, China). Quantity software (Image J, USA) was used to quantify the intensities of protein bands compared to that in Actin for equal protein loading.

Statistical analysis

Statistical data was produced through statistical product and service solutions (SPSS) 19.0 software. Differences in compared groups were assayed via Student's t test. Result was expressed as mean ± SD, in which P less than 0.05 was considered as statistically significant.

Results

Conclusion for the features of patients

As shown in hospitalized records (**Table 1**), blood plasma contents of aminotransferases (ALT, AST), hepatitis B e antibody (HBeAb), and alpha-fetoprotein (APF), carcinoembryonic antigen (CEA) were significantly increased during the oncogenesis development. The patients were labeled with the average age of 58.6, and gender ratio with 6:2 of male:female.

Representative immunophenotypes in liver cancer cells

To characterize the distribution of immunophenotypes in liver cancer cells, we performed immunohistochemical and immunofluorescence analyses on the liver sections. As featured in **Figure 1**, Ki-67 labeled cells were widespread expressed in diseased liver cells. Notably, portal area and plexus venosus inside the liver showed the strongly stained CD34-positive cells. Further, detectable CK8, CK10 cells with significant expression was found in hepatic bile duct.

To further validate these target proteins in liver cancer cells, immunobloting assay was conducted. As a result, hepatic expressions of CD34, CD8 and CD10 were significantly increased when compared to those in controls ($P<0.05$) (**Figure 2**). Interestingly, these results were consistent with the observations revealed in the immunohistochemistry stains.

Discussion

Increasing reports indicate that some of biological cascade events, such as deregulated cell proliferation, are necessary to induce further cellular oncogenesis [7, 8]. Physiologically, tumor overgrowth and pan-metastasis rely on angiogenesis via induction of cellular and cell-cell signals from cancer cells as a result of

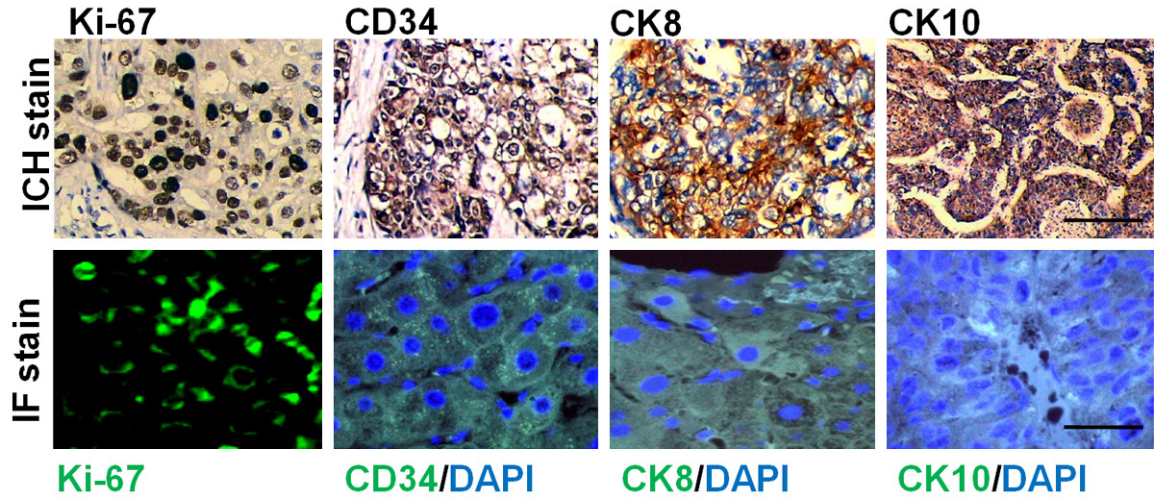

Figure 1. Representative images marked with specific antibodies displayed immuno-phenotypes of biomarkers when hepatocellular oncogenesis (Immunohistochemical assay; scale bar: 200 μm). Visualization outcomes resulted in immunoreactivity for Ki-67 (proliferation), CD34 (angiogenesis), and CK8/10 (metastasis) in liver cancer cells. Note: ICH = immunohistochemistry, IF = immunofluorescence.

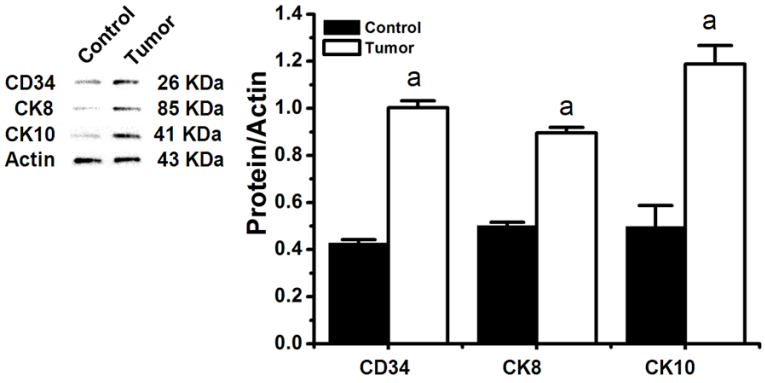

Figure 2. Validation of specific protein expressions during oncogenesis in the liver (Western blotting assay). These data showed significant upregulation of CD34 (angiogenesis), and CK8/10 (metastasis) expressions in liver tumor cells when compared to those in liver paracancerous tissue (Control). Differences in compared groups were assayed via Student's t test. Result was expressed as mean ± SD. Notes: vs. Control, $^{a}P<0.05$. Control.

rapid proliferation [9]. More specifically, cluster of differentiation 34 (CD34) functions as a promising biomarker for assessing angiogenesis in the initiation of neoplastic metastasis [10]. Some publications suggest that increased expression patterns of cytokeratins cytokeratin-8 (CK8) and CK10 are involved in epithelial differentiation, characterized with indicating metastasis in cell/tissue [11, 12]. Collectively, an intervention strategy through modulating proliferation-angiogenesis-metastasis events may contribute to inhibition of cancer development and migration, such as hepatocellular carcinoma.

In present clinical data, immunostaining images displayed up-regulated expressions of Ki-67, CD34, CK8/10 positive cells in hepatocellular carcinoma tissue in patients. Basically, these progressions from proliferation to angiogenesis/metastasis should be managed for inhibition of hepatocellular carcinoma developing. In addition, CK8/10 may serve as applicable markers for screening metastasis in advanced liver cancer patients. However, more in depth mechanisms need to be conducted in future study.

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Disclosure of conflict of interest

None.

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