



Histopathology and immunohistochemistry of large hepatocellular carcinoma with undetectable or low serum levels of alpha-fetoprotein

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Abstract

AIM: To explore the pathomorphological characteristics of large hepatocellular carcinoma (LHCC) with low serum alpha-fetoprotein (AFP) level.

METHODS: Specimens obtained from surgically resected LHCC with undetectable or low levels of serum AFP were fixed in formalin, embedded in paraffin, and prepared as serial sections. Routine hematoxylin and eosin as well as immunohistochemical stains (LSAB method) were used to test for expression of AFP, alpha-1-antitrypsin, epithelial membrane antigen, and vimentin. Some characteristics of the histopathological changes and immunohistochemical reactions of the cancerous tissues were observed under the light microscope.

RESULTS: The majority of the cases (19/30) of LHCC with undetectable or low levels of serum AFP were of the clear-cell-type HCC, with 2 being positive for AFP expression at the periphery of the cytoplasm.

CONCLUSION: The clear cell is the morphological manifestation of disturbance in glycogen and/or lipid metabolism of hepatoma cells. Such changes might be one of the factors hindering the synthesis of AFP and resulting in negative or low level serum AFP of the patient.

Key words: Liver neoplasms/pathology; Alpha fetoproteins/analysis; Immunohistochemistry

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Wu QM, Hu MH, Tan YS. Histopathology and immunohistochemistry of large hepatocellular carcinoma with undetectable or low serum levels of alpha-fetoprotein. *World J Gastroenterol* 1997; 3(2): 64-66 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/64.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.64>

INTRODUCTION

Serum alpha-fetoprotein (AFP) is generally detected and often elevated in patients with hepatocellular carcinoma (HCC); however, some patients with small HCC (SHCC, diameter ≤ 3 cm) in early stage or large HCC (LHCC; Diameter, ≥ 3 cm) in the mid and late stages show absence or low levels of serum AFP. In the present study, 30 cases of LHCC with undetectable or low levels serum AFP were assessed histopathologically and immunohistochemically, and the relationship between some pathomorphological characteristics and the level of serum AFP was evaluated.

MATERIALS AND METHODS

Specimens were obtained from the surgical resection of 30 LHCC with diameter of > 3 cm and undetectable or low levels of serum AFP (counter immunoelectrophoresis, negative; Rocket electrophoresis, ≤ 399 $\mu\text{g/L}$). Eight specimens from cases positive for serum AFP with high serum AFP levels (rocket electrophoresis ≥ 400 $\mu\text{g/L}$) were used as controls.

All specimens were fixed in 10% formalin normal saline, embedded in paraffin, prepared as serial sections, stained by routine hematoxylin-eosin and immunohistochemical technique with labeled streptavidin biotin (LSAB), and observed under the light microscope.

Immunohistochemistry was performed to detect the following: AFP, alpha-1-antitrypsin (AAT), epithelial membrane antigen (EMA), and vimentin. All antibodies and agents were products from DAKO Company.

RESULTS

Degree of cell differentiation of HCC tissue

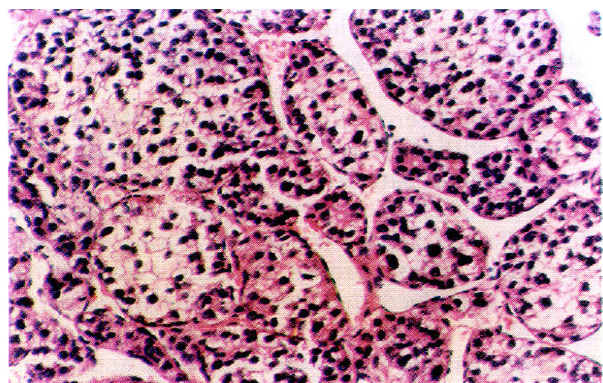
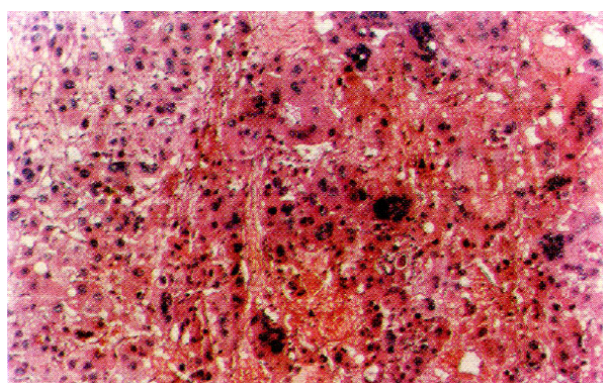
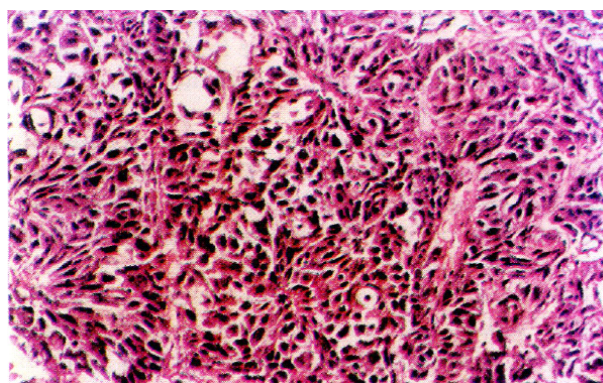
Classification of the 30 cases of LHCC with undetectable or low levels of serum AFP according to the Edmondson 4-grade method^[1] revealed that cell differentiation was of grade I in 1 case, grade II in 1 case, grade III in 18 cases, and grade IV in 10 cases (Table 1).

Pathomorphological types of cells in hepatocarcinomatous tissue

According to the WHO classification system^[1], cells of carcinomatous tissues in the 30 cases of LHCC with undetectable or low levels of

Table 1 Degree of cell differentiation of hepatocarcinomatous tissues

Serum alpha-fetoprotein level (μg/L)	Cases	Degree of differentiation			
		I	II	III	IV
≤ 20	18	1	9	7	1
21-299	12	0	9	3	0
Total	30	1	18	10	1

**Figure 1** Clear cell hepatocellular carcinoma. HE × 400.**Figure 2** Giant cell hepatocellular carcinoma. HE × 400.**Figure 3** Spindle cell hepatocellular carcinoma. HE × 400.

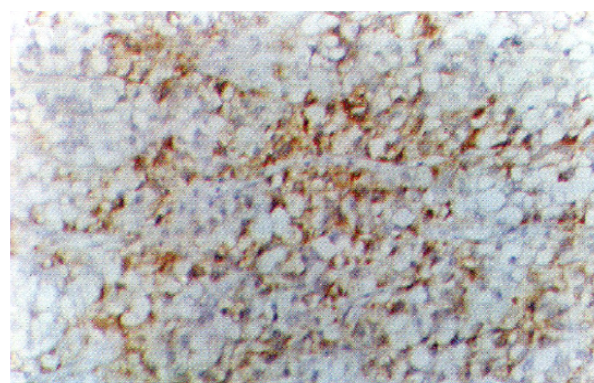
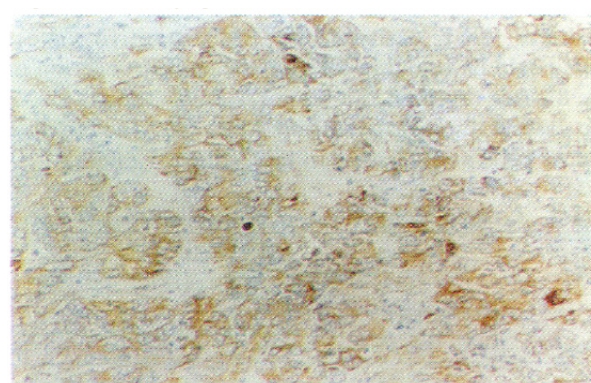
serum AFP showed peculiar morphological types. Among them, 19 were of the clear-cell type (clear cells occupied 30%-90% of total cancer cells), (Figure 1), 1 was of the giant-cell type (Figure 2), and 1 was of the spindle-cell type (Figure 3, Table 2).

Immunohistochemical reactions of cells in hepatocarcinomatous tissues

In histological sections of the 30 cases of LHCC with undetectable or low levels of serum AFP, only 3 were found to be positive for serum AFP in cells, 2 were of the clear-cell type, and 1 was of the non-clear-cell type. AFP-positive cancer cells were focally distributed and the AFP-positive portion in the clear cells was expressed in the periphery of the cytoplasm (Figure 4). AAT-positive cancer cells were found in 8 cases (Figure 5) and EMA-positive cancer cells in 28 cases (Figure 6). Tests for vimentin expression in cancer cells of all the 30 cases were negative, while those in the interstitial tissues (including

Table 2 Cytomorphological types of hepatocarcinomatous tissue

Serum alpha-fetoprotein level (μg/L)	Number of cases	Morphological types				
		Trabecular compact	Clear cell	Giant cell	Spindle cell	
≤ 20	18	6	10	1	1	
21-299	12	3	9	0	0	
Total	30	9	19	1	1	

**Figure 4** Clear cell hepatocellular carcinoma. The AFP-positive portion in clear cells was expressed in the periphery of the cytoplasm. Immunohistochemical stains × 400. AFP: Alpha-fetoprotein.**Figure 5** Clear cell hepatocellular carcinoma. The AAT-positive portion was expressed in the cytoplasm. Immunohistochemical stains × 100. AAT: Alpha-1-antitrypsin.**Figure 6** Clear cell hepatocellular carcinoma. The EMA-positive portion was expressed in the cancer cell membrane. Immunohistochemical stains × 100. EMA: Epithelial membrane antigen.

endothelial cells of blood sinuses and blood vessels, smooth muscle cells of the vascular wall, and fibroblasts) were positive (Figure 7).

In paraffin sections of 8 cases of LHCC with positive and high expression levels of serum AFP observed immunohistochemically, various concentrations of AFP-positive, AAT-positive, and EMA-positive cancer cells were found in all cases; tests for vimentin expression in the 8 cases yielded the same results as those obtained in the cases with undetectable serum AFP levels, *i.e.* negative results for cancer cells and positive results for interstitial tissues (Table 3).

DISCUSSION

Different degrees of cell differentiation were observed in the 30 cases investigated in this study, with Grade III ranking the first and

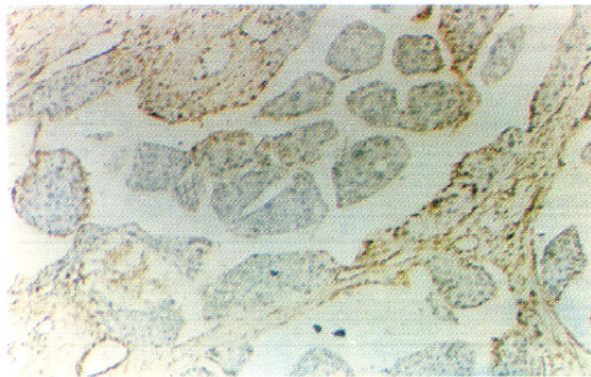


Figure 7 Clear cell hepatocellular carcinoma. Tests for vimentin expression in cancer cells were negative, but those in the interstitial tissues appear to be positive. Immunohistochemical stains × 100

Table 3 Immunohistochemical reactions of hepatocarcinoma						
Serum alpha-fetoprotein level (μg/L)	Number of cases	Morphological types				
		Trabecular	compact	Clear cell	Giant cell	Spindle cell
≤ 20	18	6	10	1	1	
21-299	12	3	9	0	0	
Total	30	9	19	1	1	

Grade IV the next. Grade III and IV constituted the great majority of the cases, but this grade distribution was not only peculiar for those cases with undetectable or low levels of serum AFP. Two massive case reports from our country^[2,3] indicated that Grades III and IV occupied the great majority of HCC, including both cases positive and negative for serum AFP expression. The distribution of Grades III and IV was also predominant in this study of 30 cases of LHCC that had undetectable or low levels of serum AFP, without any significant difference from that observed in HCC that were positive or negative for serum AFP expression. There was no significant correlation between the degree of differentiation of cancer cells and serum AFP level.

Cells of cancerous tissues in most of the 30 cases of LHCC with undetectable or low levels of serum AFP showed peculiar pathological morphology, most of which were of the clear-cell type, with a few belonging to the giant- and spindle-cell types. Wu *et al*^[4] used periodic acid-Schiff and Sudan black stains and proved that the cytoplasm of clear cells were rich in glycogen and lipid, partly as

ordinary cytoplasmic constituents (the glycogen and lipid dissolved and disappeared during the preparation of histological slides, which were left clear). On electron microscopy, cell organelles decreased, the rough surfaced endoplasmic reticulum (RER), free ribosomes and polyribosomes seemed to have been pushed aside by glycogen and lipid. Zhang *et al*^[5] observed by immune-electronmicroscopy that AFP was mainly located in the membrane of RER and ribosomes. Our immunohistochemical study on 19 cases of clear-cell carcinoma showed positive results for AFP expression in only 2 cases, wherein AFP was focally distributed with positive staining of the peripheral cytoplasm. The latter findings coincided with the above mentioned phenomenon, *i.e.*, the pushing of RER and ribosomes aside. It is suggested that clear cells are morphological manifestations of glycometabolic and lipometabolic disturbances in hepatocarcinomatous cells. It is a type of degeneration of the carcinoma cells that possibly hinders the synthesis of AFP. This might be one of the factors responsible for the undetectable or low levels of serum AFP in patients with HCC.

Thus, samples testing negative and positive for serum AFP expression did not show any significant differences in the results of the immunohistochemical studies such as those for EMA and vimentin. The positive results for EMA expression and negative results for vimentin expression in the cancer cells are indicative of their epithelial origin. Further, AAT-positive cases among those testing negative for serum AFP expression were apparently fewer than those testing positive for serum AFP for control. The reasons for these findings remain obscure and further studies are required to elucidate them.

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