CARCINOHISTOGENESIS AND EXPRESSION OF ALPHA FETOPROTEIN IN EXPERIMENTAL HEPATOCARCINOMA

ZHANG Jizeng 张继增

Department of Pathology, General Hospital, Lanzhou Military Commend, PLA, Lanzhou 730050, China

ABSTRACT

Subjective: The purpose of investigating the carcinohistogenesis of hepatocarcinoma and alpha-fetoprotein AFP expression in oncogenesis was to lay a morphological foundation for diagnosing and curing hepatocarcinoma. Methods: Experimental hepato carcinoma of Wistar rats induced by 0.04% 3`-Me-DAB and killed according to different dates. All the animals were killed during a period of 36 weeks. The morphologic changes of dynamics and expression of (AFP) with immunohistochemical method were observed. Results: Hyperplasia of oval cells was multipotento-differential stem cell. It was further differentiated into transitional cell and embryoid small liver cell, and the latter can form pattern of atypical hyperplasia, which scattered or crowded. The AFP in these cells showed was a strong positive expression. The feature was different between crowded small hepatocytes of atypical hyperplasia and proliferative nodules of hepatocytes. The former was similar to morphologic character of liver cell carcinoma. The oval cells were toward biliary duct differentiation to form fibroadenomatoid structure, and atypical hyperplasia was also seen in their epithelial cells. Of all the 58 experimental animals there were 26 with hepatocarcinoma, among which 18 cases of hepatocytic carcinoma and 8 mixed carcinoma were found. The hepatocellular carcinoma showed AFP strong .positive expression In the host hestocytes around cancer and hepatocytes of non-neoplastic animals of a later experimental period also expressed various degrees of AFP positive. Conclusion: Atypical hyperplasia of small hepatocytes and epithelial cells of bile canaliculi were the precancerous lesions of hepatocellular carcinoma and bile duct carcinoma.

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Correspondence to: Zhang Jizeng, Department of pathology, General Hospital, Lanzhou Military Command, PLA; No. 58, Xiao-Xi-Hu West Road, Qi-Li-He District, Lanzhou 730050, China; Phone: (0086-0931)-8975406.

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In 1973, When Anthony, et al. in Uganda[1] were studying human hepatocirrhosis and hepatocarcinoma they first reported that atypical hyperplasia (AH) of hepatocytes was precancerosis of hepatocellular carcinoma (HCC). Roncalli, et al.[2] divided AH into large hepatocytic and small hepatocytic AH, and they considered the latter was closely related to forming HCC. In 1988, Xu, et al.[3] pointed out that HCC occurs from proliferative nodules of basophilic and acidophilic hepatocytes. Indeed, carcinohistogenesis, in particular the change of alpha-fetoprotein (AFP) in the forming hepatocarcinoma was very complex. The AFP was established cell differentiation and a tumor marker in experimental course.[4] In addition, previously there was a lack of a detailed description concerning carcinohistogenesis of cholangio-carcinoma and AH of epithelial cells on small bile duct. In order to lay a morphological foundation for diagnosis and treatment of hepatocarcinoma. This work tried to profoundly study for carcinohistogenesis and AFP expression in experimental course of hepatocarcinoma.

MATERIALS AND METHODS

Wistar rats were fed mixed food containing 0.04% 3`-Me-DAB. The number of the rats and inducing cancer data were showed in Table 1. At corresponding time hepato-specimen with wax block slices of 2 normal animals takes control-experiment. The specimens of live were fixed in 10% neutral formalin. The AFP immunohistochemistry was stained by ABC method. 1st antiserum was AFP of rabbit anti-rat, 1: 400 dilution; 2nd anti-rabbit IgG serum of biotin labeled, 1: 200 dilution; 3rd antiserum was ABC-HRP (Horseradish Peroxidase), 1: 100 dilution. The routine

method was used insections. The slices coloration were stained by 0.04%-H_2O_2DAB solution, hematoxilin counter stained lightly. The positive brown granules of AFP were in cytoplasm and they were uniform in distribution. In stain of control slices the 1st antiserum was replaced by normal sheep serum. The positive cells counted with percentage. The counting method of positive AFP cells was counted in 10 fields under light microscope of 400 times. Mark +++ showed positive cells to be ≥41% and strong staining with wide spread in all 10 fields, the positive granules showed deep brown color. Mark + showed positive AFP cells to be ≤10%. Mark ++ was situated between +++ and +. The negative mark "-" had not positive expression. In order to differentiated diagnosis the cytokeratin and epithelial membrane antigen (EMA) were stained.

RESULTS

Of all the 58 experimental animals there were 26 hepatocarcinoma (Figure 1), among which 18 cases of hepatocytic carcinoma and 8 mixed carcinoma were found. There was no histo-structure change in the normal contrast of animal liver, the AFP expression showed negative. The main hepatic lesion of 58 experimental animals was indicated in Table 1. The AFP positive reaction was indicated in Table 2. During the 13th week of experiment owing to hepatocytic necrosis, hyperplasia and destruction of hepatic lobules, the hepatic pseudolobules were formed, at the same time, the hyperplastic foci and nodules of hepatocytes were formed. A lot of AH hepatocytes spreaded in hyperplastic nodules and pseudolobules. The dysplastic hepatocytes had different sizes. Dysplasia of embryonic small hepatocytes from transitional cell was usually crowded; they were different from hyperplastic nodules and were usually adjacent to hepato carcinoma nests. The crowds of dysplastic small hepatocytes did not press peripheral hepatic tissue. Sometimes these cells were not easily distinguished from liver cell carcinoma, but HCC had blood sinus between cancer cell cords. The AH small hepatocytes showed usually marked +++ AFP expression (Figure 2). The positive reaction of AFP marked ++ to +++ expression in the HCC (Figure 3). The hepatocytic cancer cords connected with "normal" hepatocytic cords were seen usually. After the destruction of hepatic lobules, the remanding hepatocytes transferred themselves into dysplastic small hepatocytes, which showed bizarre nucleus. The dysplastic giant hepatocytes were situated in dysplastic small hepatocytes or their surroundings; the AFP usually showed negative expression.

Table 1. Liver lesion of 58 experimental rats in different course

Lesion and number of animals	Experimental course (weeks)				
	<4	5-12	13-20	21-28	29-36
Oval cells hyperplasia	4	4	1	-	-
Hyperplasia of transitional cell	-	4	3	-	-
Hyperplasia of embryoid small					
Hepatocytes (HC)	-	-	6	6	8
Dysplastic HC	-	-	5	6	33
Focus and nodule of hyperplastic HC	-	-	5	5	20
Hepatic pseudolobule	-	-	4	6	38
Fibroadenoma toid hyperplasis of					
Hile canaliculi	-	-	4	6	38
Hepatocarcinoma	-	-	2	3	21
Number of killed animal	4	4	6	6	38

The proliferative bile canaliculi showed fibroadenomatoid formation, and in this regions the oval cells disappeared. Epithelial cells of with small nucleolus, a lot of mitosis could be seen. They were arranged in a crowd stratifies, and AFP expression marked ++ to +++. These cells distinguish from cholangiocarcinoma to be difficult and they transferred oneself into morphologic characteristics of bile canaliculi adenomatoid hyperplasia were arranged in a stratifies, crowded and deep blue staining (Figure 4). In 6 cases, epithelial cell AH of the small bile canaliculi were found. These cells had a giant light staining nucleus including remarkable acidophilic cholangiocarcinoma. The AFP showed negative expression in cholangiocarcinoma. The cholangicarcinoma showed irregular cholangiectasis

and various bile canals in size. They have not basic membrane (Figure 5), formed cancer nests and infiltrated in plentiful connective tissues. Sometimes, cholangiocarcinoma and adenoid HCC exists intersection. There was mucin in the canal cavity of the cholangiocarcinoma. The cholangiocarcinoma stain with EMA and CK were positive expression and the AFP was negative expression; the AFP of adenoid HCC was positive expression. The host liver cells around cancer and liver cells of non-neoplastic animals in later experimental period have showed positive AFP expression as to different degree.

Table 2. AFP positive expression in hepatic lesion of 58 animal during neoplastic process

Lesion	Animal number	AFP				
		+	++	+++	–	Positive %
Transitional cell	7	1	4	-	2	71.4
Embryoid small HC	20	4	6	10	-	100.0
Dysplastic small HE	44	7	10	27	-	100.0
Focus and nodule of hyperplastic HC	30	-	11	19	-	100.0
HC in pseudolobules	48	15	10	16	7	85.4
Epthelial cell of bile						
Canalliculi fibroadenomatosis	48	3	4	2	39	18.7
Carcinoma	26	-	10	16	-	100.0

AFP can only see in HCC

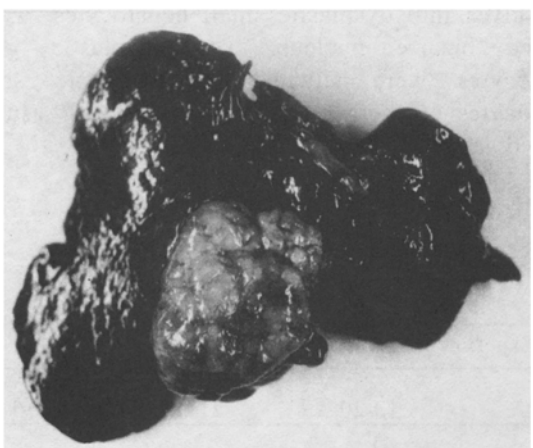

Fig. 1. Massive hepatocarcinoma of right lobe.

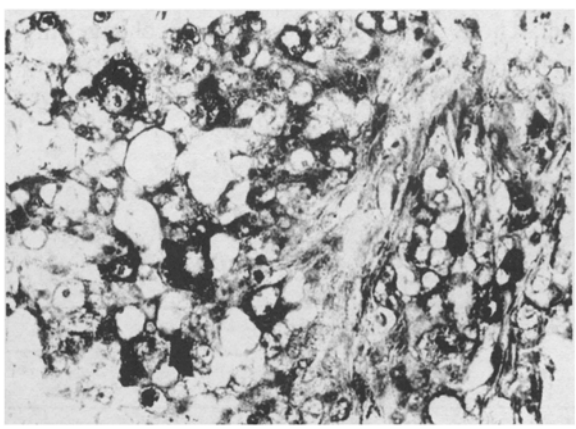

Fig. 3. AFP(+++) positive expression of HCC and the unfilled spaces of blood sinus. ABC×400

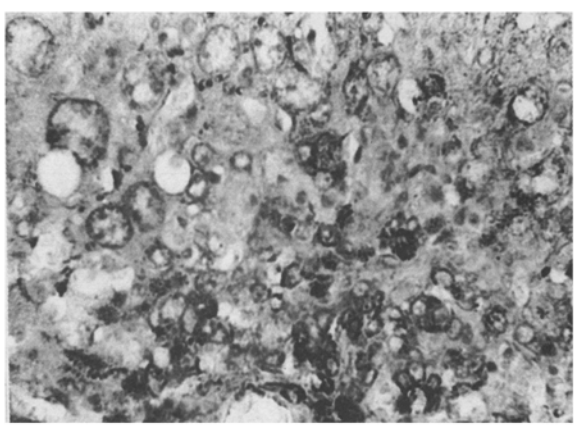

Fig. 2. AH small hepatocytes showed positive AFP(+++), AH large hepatocytes was negative. ABC ×400

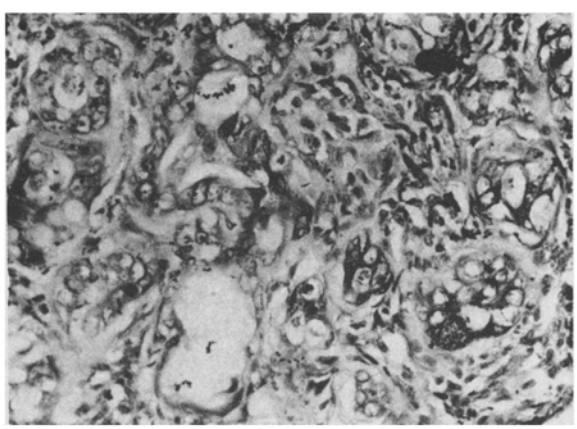

Fig. 4. Fibroadenomatoid hyperplasia of bile canaliculi and their AH of Epithelial cells (lower night). HE×200

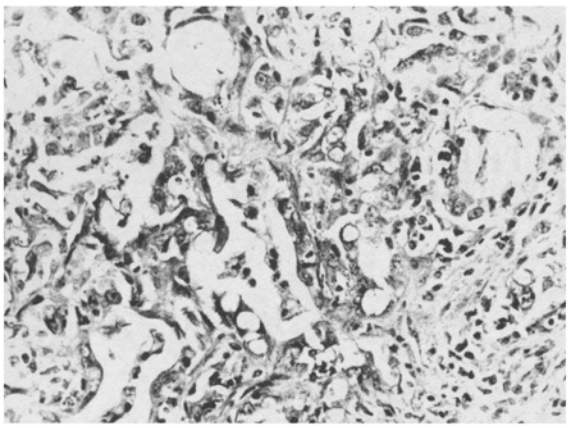

Fig. 5. Cholangiocarcinoma, irregular acini are formed by oncocytes and muco-vacuolus appear in cytoplasm of oncocytes. HE×200

DISCUSSION

AH of small hepatocytes acts as precancerous cells, these cells were situated in hepatic pseudolobules. Because they simulated embryonic hepatocytes of earlier period, thus AFP displayed positive expression. A group of small hepatocytes of AH were different from proliferative nodule, they had not obvious pressing around tissues; there were not obvious demarcation between the group of small dysplastic hepatocytes and around tissues. These cells were usually adjacent to hepatocytic carcinoma, sometimes they uneasily distinguish each other, but the latter had blood sinus between cancer cell cords. As stated above, the AH itself had morphological character and existing form in course of dynamics change. Dai, et al.[5] had studied that the host hepatocytes around human HCC could synthesize AFP. In present experiment the peripheral host hepatocytes of hepatocarcinoma and liver cells of nonneoplastic animal in later experiment period also showed positive AFP reaction but different in degree. Although the form of these hepatocytes showed normal, the hepatocytes had altered function so that atavism passing through differentiation might also be mutated into carcinoma. The hepatocytes with embryonic function appeared positive AFP. The HCC cords were connected with "normal hepatic cords", which probably have altered function and de-differentiation so that mutation of these cells occurs due to carcinogen stimulation. In the present experiment, the AFP positive expression in non-neoplastic animal hepatocytes laid morphologic basis for serum AFP high level in hepatopath of non-neoplasm.

As everyone know the embryonic bud of hepatocytes and biliary duct cells were autoploidy in embryonic period. In experimental hepatocarcinoma Dempo[6] and Hixsen, et al.[7] pointed out that oval cell was multipotento-differential stem cell, in their differentiation, it could not only form HCC and cholangiocarcinoma but also mixed hepatocarcinoma. In the regions of obvious small bile duct adenomatosis the oval cells disappeared there, it was in close relationship with differentiation of small bile canaliculi during atavism to embryonic property showed positive AFP reaction. Once they have changed cholangiocarcinoma, the AFP showed negative reaction, at the same time, the positive CK and EMA could be seen. This immunohistochemical staining method made them to differentiate from adenoid HCC.

REFERENCES

[1] Anthony PP, Vogel CL, Barker LF. Liver cell dysplasia. A premalignant condition. J Clini Pathol 1973; 26:217.

[2] Roncalli M, Borzio M, Biagi GD, et al. Liver cell dysphasia in cirrhosis serologic and immunohistochemical study. Cancer 1986; 57: 1515.

[3] Xu YD, Zhang ZC, Zhang JS, et al. Pathological study of hepatocarcinogenesis in rts induced by 3'-Me-DAB. Chin J Pathol 1988; 17:87.

[4] Khamzina L, Eraiser T, Borgeat P. Alpha fetoprotein (AFP) expression in clones of MCA-RH7777 rat hepatoma: correlation with the occurrence of homogeneously staining regions on chromosome 14. Cancer Res 1995; 55: 3615.

[5] Dai YM, Ni CR. Preliminary study of the origi n of alpha-fetoprotein (AFP) in the host hepatocytes of human liver cancer: Immunohistochemical analysis of 48 cases. Chin J Pathol 1988; 17: 247.

[6] Dempo K, Shisak N, Yoshida Y, et al. Immunofluorescent study on a –Fetoprotein producing cell in the early stage of 3-Methyl-4-dimethylaminoazobenzene. Cancer Res 1975; 35: 1282.

[7] Hixson DC, Affigne S, Faris RA, et al. Delineation of antigenic pathways of ethionine induced liver cancer in the rat. Pathology 1996; 64: 79.