

DISTRIBUTION PATTERNS OF EXTRACELLULAR MATRICES IN HEPATOCELLULAR CARCINOMA AND THEIR CLINICAL SIGNIFICANCE

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ABSTRACT

Objective: In order to find out the distribution patterns of extracellular matrix (ECM) in hepatocellular carcinoma (HCC) and explore the relationshi between distribution patterns and hepatocellular caricnoma malignancy. **Methods:** Forty cases of HCC were studied by immunohistochemistry with 5 antibodies of anti-ECM. **Results;** Four types of distribution patterns were found: 1. continuous peritrabecular or periacinar type; 2. discontinuous peritrabecular or periacinar type; 3. vascular stroma type; 4. membrane and cytoplasmic type. The former 3 types were correlated closely with the growth pattern, cell differntiation and proliferation of tumor. **Conclusions:** ECM were useful marker for valuation of malignant degree in HCC.

Key words: Hepatocellular carcinoma, Extracellular matrix, Immunohistochemistry

Extracellular matrix (ECM) including collagen, matrix glycoprotein and proteoglycans is now believed to be not only supportive structure, but also metabolic components of tissues. Moreover, it has also been proved that ECM is closely related to tumor growth, invasion and metastasis.^[1-5] There have been some studies linking ECM and hepatocellular caricnoma (HCC) in the literature,^[6,7] but in China still few.^[8] In order to study the distribution patterns of ECM in HCC, 40 cases of HCC were studied by immunohistochemistry with 5 kinds of anti-ECM antibodies.

MATERIALS AND METHODS

Materials

Forty HCC cases were taken from the biopsy files of Department of pathology, shantou University Medical College during the last 5 years. According to Kojiro and nakashima's classifying standard on gross HCC,^[9] there were 18 expansive hepatocellular carcinomas (EHCC) and 22 infiltrative hepatocellular carcinomas (IHCC) among 40 cases. According to modified Edmondson's histological grading standards,^[10] there were 2 cases with grade I; 9 with grade II; 27 with grade III and 2 with grade IV. All samples included tumor tissue and nontumor liver tissue.

ImmunohistoChemistry Staining

Sections of 4 μ were cut from the formalin fixed, paraffin-embeded tissues of the 40 samples, and stained immunohistochemically with antibodies anti-FN, LN, Col III, Col IV, Col V. FN, Col III, Col V were tested by PAP method, LN and Col IV by ABC method. DAB visualization. Col III and Col V were afforded by Sina-Ame Co, Shanghai; the other agents were supplied by Dako Co.

In order to evaluate the proliferation degree of HCC, 40 cases were stained immunohistochemically by antibody PC10 of anti-PCNA with ABC method and DAB.

Statistical Assay

The relationship between the distribution patterns of ECM and the growth types, histological grades and proliferation degree of HCC was assayed. The significance was tested by X^2 test.

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RESULTS

The Distribution of ECM in Adjacent Non-Neoplastic Liver Tissue of HCC

There were 35 cases of liver cirrhosis and 5 cases of non-cirrhosis among the 40 adjacent nonneoplastic liver tissue of HCC. The distribution of FN, Col III, Col IV, Col V were similar in cirrhosis and non-cirrhosis tissues. The positive stain appeared as dark brown line, around central vein and along blood sinusoids. However, LN was negative in perisinusoidal lining. This was significant in contrast to the positive reaction of cancer tissue (Figure 1). In portal area, FN, LN, Col III, Col IV, Col V encircled the blood vessels and bile ductules.

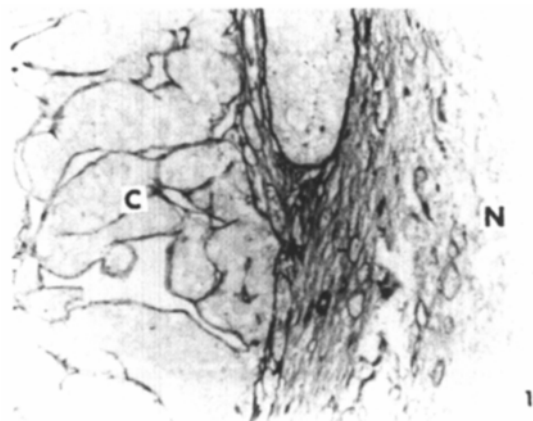


Fig 1. EHCC (grade II) with adjacent non-tumor liver tissue. Immunostaining for LN. Positive substance distributed in the cancer tissue ©, but not in non-tumor liver (N). ABC×120

The Distribution of ECM Positive Staining in HCC Cancer Tissue

The distribution of EcM component FN, LN, Col III, Col IV, Col V was similar among the 40 HCC, which could be classified into 4 types of distribution patterns: (1) continuous peritrabecular or periacinar type, (2) discontinuous peritrabecular or periacinar type, (3) vascular stroma type, (4) membrane and cytoplasmic type. Continuous peritrabecular or periacinar type was characterized by continuous immunostaining around tumor trabeculae or acini (Figure 2). Sometimes sinusoid structure still remain around cancer nest in this type and the positive staining distributed along the blood sinusoids. While in the discontinuous peritrabecular or periacinar type, the positive staining encircled trabecular or acinar cancer nest discontinuously, and the degree of

positive was weaker than that of the former type (Figure 3). The vascular stroma type was characterized by strands of ECM around or adjacent to the vessels within the tumor nests and there was no positive staining around the cancer nest. (Figure 4). There was only positive reaction to FN, LN and Col IV in membrane and cytoplasmic type, and the positive staining distributed on the membrane of cancer cells making the cancer tissue honeycomb-like (Figure 5); the positivity also distributed inside the cytoplasm of the cancer cells with dark brown granulas. Because the positivity of membrane coexisted with that of the cytoplasm, this type was refer to membrane and cytoplasmic type.

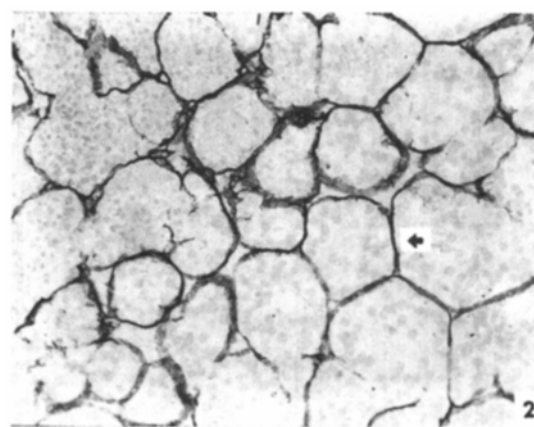


Fig. 2. EHCC (grade II). Immunostaining for LN. Continuous peritrabecular type. Positive substance encapsulate around the cancer nest entirely (↑). ABC×120

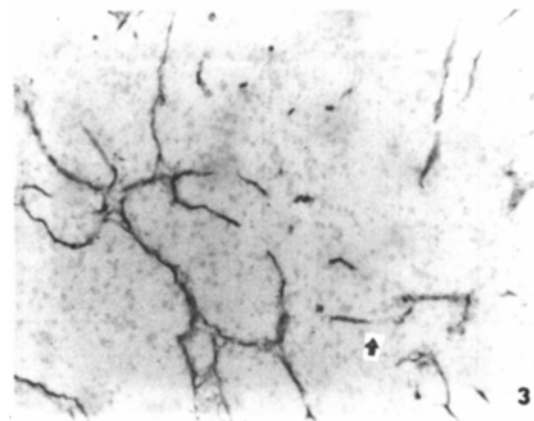


Fig. 3. IHCC (grade III). Immunostaining for LN. Discontinuous peritrabecular type. Positive substance encapsulate around the cancer nest discontinuously (↑). ABC×120

The Relationship between Distribution Patterns of ECM and Growth Patterns, Differentiation and Proliferation of HCC

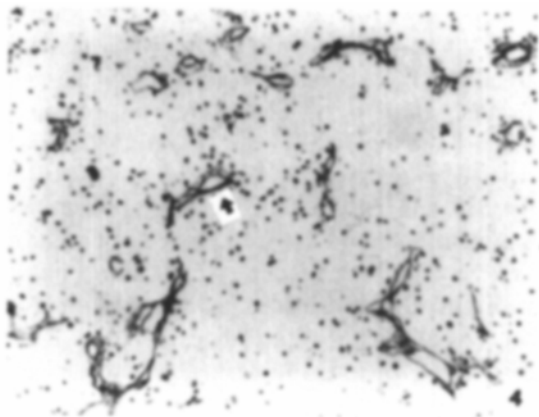


Fig. 4. IHCC(grade III). Immunostaining for FN. Vascular stromal type. Positive substance distributed around the vessels within tumor nest (↑). PAP×120.

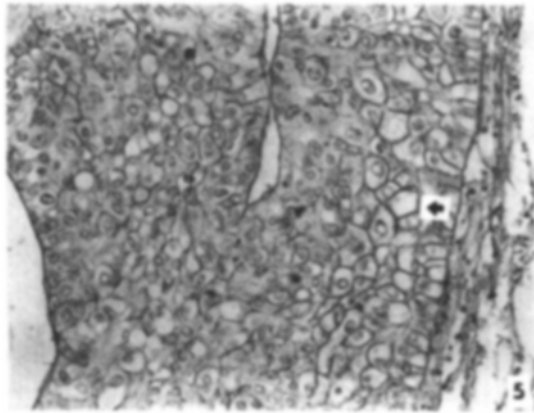


Fig. 5. IHCC (grade III). Immunostaining for FN. Membrane and cytoplasm type. Positive substance distributed on membrane of tumor cells (↑). PAP×240

Only 7 out of 40 HCC cases were membrane and cytoplasmic type, and only FN, LN and Col IV were positive. They existed in both well differentiated and undifferentiated HCCs; so have not been assayed

statistically.

The relationship between distribution pattern of ECM and the tumor growth type in the 40 cases was show on Table 1. continuous peritrabecular or periacinar type mainly existed in EHCC; discontinuous peritrabecular or periacinar type mainly existed in IHCC, and vascular stroma type also mainly existed in IHCC ($P<0.05$).

The relationship between the distribution patterns of ECM and the tumor histological grading was show on Table 2. Most of continuous peritrabecular or periacinar type belong to well-differentiated type (grade I, II), discontinuous peritrabecular or periacinar type belong to poorly-differentiated type (grade III, IV) and Vascular stroma type also belonged to poorly-differentiated type ($P<0.001$).

There were only 34 cases positive among the 40 HCCs with PC10 immunohistochemistry staining positive, so the relationship between the distribution patterns of ECM and the proliferating degree of PCNA positive cells $>20\%$ was considered as high proliferating index, while $<20\%$ as low proliferating index. Through by statistical assay it was revealed that the continuous peritrabecular or periacinar type was of low proliferating index type, the discontinuous peritrabecular or periacinar type and the vascular stroma type were completely high proliferating index type ($P<0.001$).

DISCUSSION

ECM is the indispensable structure of formation and orientation of organs and tissues, however, they are not stable structure biologically and they change continuously during the live activities. In their changing processes, parenchymal cells perform an important role, cancer cells can affect the process of

Table 1. The distribution pattern of ECM in EHCC and IHCC

	Continuous peritrabecular type	Discontinuous peritrabecular type	Vascular stromal type
EHCC	10	4	4
IHCC	2	13	7
Total	12	17	11

$X=8.058, P<0.05.$

Table 2. the relationship between ECM distribution pattern and HCC histological grade

	Continuous peritrabecular type	Discontinuous peritrabecular type	Vascular stromal
Grade I or II	8	1	2
Grade III or IV	4	16	9
Total	12	17	11

$X=13.71, P<0.01$

Table 3. The relationship between ECM distribution pattern and PCNA

	Continuous peritrabecular type	Discontinuous peritrabecular type	Vascular stromal
PCNA<20%	8	0	0
PCNA>20%	2	15	9
Total	10	15	9

$\chi^2=25.10, P<0.001$

basement membrane formation.^[6] 40 cases HCC with different growth patterns, differentiation and proliferation were immunohistochemically studied by 5 antibodies against ECM. The results supported the above view point. The distribution patterns of ECM in 40 HCC have been found to be including 4 categories: (1) continuous peritrabecular or periacinar type, (2) discontinuous peritrabecular or periacinar type, (3) vascular stroma type, (4) membrane and cytoplasmic type. Our results were similar to the reports of Donato and Grigioni.^[6,7] There was positive expression in all the 5 antibodies and the expression patterns were similar basically in the former three distribution patterns. Intact capsule-like basement membrane formed around the cancer nests in the continuous peritrabecular or periacinar type. The basement membrane encircling the cancer nests was developed badly, interrupted or broken in the discontinuous peritrabecular or periacinar type. There was no developed basement membrane around the cancer nests in vascular stroma type. The positive ECM only distributed along the blood vessels and in the interstitial spaces of the cancer nests. According to the correlation analysis, the distribution patterns of former 3 types were correlated with the growth patterns, differentiation and proliferation of HCC. The continuous peritrabecular or periacinar type appeared in the expansive, highly-differentiated and low-proliferated index type of HCC. While the discontinuous peritrabecular or periacinar type and vascular stroma type mostly appeared in the infiltrative, low-differentiated, high-proliferated index type. This suggested that the distribution patterns of ECM reflected the malignant degree of HCC. So we believe that the distribution patterns of ECM may be a useful factor in the assessment of the malignant degree as well as a prediction of prognosis in HCC. As to the distribution patterns of the membrane and cytoplasmic type, Donato has reported that it only existed in the highly differentiated HCC.^[6] But there were only 7 cases of this distribution pattern in the present study, and it appeared not only in the highly differentiated cases but also in the low-differentiated cases, thus it difficult to determine its clinical correlation.

The origin of basement membrane component of

HCC is controversial now. Donato has found that FN can encircle a single cancer cell and there are FN positive in the acina cavity. So, it is considered that FN can be produced by hepatic tumor cells in highly differentiated HCC.^[6] In the membrane and cytoplasmic type of our group, the positive ECM components (FN, LN and Col IV) can be found in the membrane and cytoplasm of cancer cells. Although positive cases and positive cells are not many, we suggest anyway that the hepatic tumor cells can produce ECM for the existence of membrane and cytoplasmic type.

Our immunohistochemistry studies also find that LN is negative in normal hepatic lobules and liver cirrhotic pseudolobules, but LN is highly positive in HCC cancer tissues. So it can be distinguished between HCC cancer and non-cancer nodes. This might be a useful tool for different diagnosis.

REFERENCES

- [1] Dumas V, Kanitakis J, Charvat S, et al. Expression of basement membrane antigens and matrix metalloproteinases 2 and 9 in cutaneous basal and squamous cell carcinomas. *Anticancer Res* 1999; 19:2929.
- [2] Vitolo D, Ciocci L, Ferrauti P, et al. Alpha 5 integrin distribution and TGF beta1 gene expression in supraglottic carcinoma: their role in neoplastic local invasion and metastasis. *Head neck* 2000; 22: 48.
- [3] Perumpanani AJ, Byrne HM. Extracellular matrix concentration exerts selection pressure on invasive cells. *Eur J Cancer* 1999; 35: 1274.
- [4] Kosmehl H, Berndt A, Strassburger S, et al. Distribution of laminin and fibronectin isoforms in oral mucosa and oral squamous cell carcinoma. *Br J Cancer* 1999; 81: 1071.
- [5] Barsky SH, Siegal GP, Jannolta F, et al. Loss of basement membrane components by invasive tumors but not by their benign counterparts. *Lab Invest* 1983; 49: 140.
- [6] Donato MF, Colombo M, Matarazzo M, et al. Distribution of basement membrane components in human hepatocellular carcinoma. *Cancer* 1989; 63: 272.

- [7] Grigioni WF, Garbisa S, Derrico A, et al. Evaluation of hepatocellular carcinoma aggressiveness by a panel of extracellular matrix antigens. *Am J Pathol* 1991; 138: 647.
- [8] Dai YM, Wang YJ, Ni CR, et al. Intersitail collagen distribuion and its mRNA expressiion in human primary liver cancer. *Chin J Pathol* 1994; 23: 151.
- [9] Kojiro M, Nakashima T. Pathology of hepatocellular carcinoma. In: Okuda K, Ishak KG eds. *Neoplasms of the liver*. 1st ed. Tokyo: Spring-Verlay, 1987; 81.
- [10] Grigioni WF, Derrico A, Bacci F, et al. Primary liver neoplasms: evaluation of proliferative index using MoAb Ki-67. *J Pathol* 1989; 158: 23.
- [11] Ng IOL, Path MRC, Lai ECS, et al. Prognostic significance of proliferating cell nuclear antigen expression in hepatocellular carcinoma. *Cancer* 1994; 73: 2268.