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Relationship between collagen IV expression and biological behavior of gastric cancer

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INTRODUCTION

Conceivably the presence of basement membrane (BM) in a neoplasm might be a result of interaction of tumor cells with the extracellular matrix. Collagen IV is one of the major intrinsic components of BM. Recent study[1] has shown that collagen IV has cell adhesion function and is involved in the process of tumor invasion and metastasis, including colorectal cancer[2] and breast cancer. But there are few systematic studies on gastric cancer and the results are equivocal. In this study we evaluated the expression of collagen IV immunohistochemically in 148 advanced gastric cancer cases in an attempt to clarify the relationship between the patterns of expression and the biological behavior of gastric cancer.

MATERIALS AND METHODS

Patients

Surgical specimens (148) of gastric carcinomas resected at the Oncology Department of China Medical University from 1988 to 1992 were studied. Routinely formalin-fixed and paraffin-embedded tissue blocks were sectioned at 5 μm thickness. HE-stained slides were also collected and available.

Immunohistochemistry

The avidin-biotin-peroxidase complex (ABC) method by Hsu *et al*[3] was applied. Briefly, paraffin sections were deparaffinized, dehydrated and pretreated with pepsin (0.1% in 0.1M- HCl for one hour at 37℃) to restore the immunoreactivity to type IV collagen. After blocking of endogenous peroxidase with 0.3% H_2O_2 in methanol and washing in phosphate-buffered saline (PBS) for 3 × 5 min, the sections were incubated with anti-collagen IV monoclonal antibody (Dako Putts Co., diluted 1:75) overnight at 4℃ in a moist chamber. After washing in PBS the sections were incubated with secondary antibody for 30 min and ABC for 60 min at 37℃, then sections were stained with 0.05% DAB freshly prepared to visualize the immunoreactivity. Tumors were classified as "positive" with regard to the immunoreactivity for collagen IV when there was unequivocal immunostaining of the matrix components at least in one representative area of the tumor.

Statistical analysis

Statistical analysis was performed by χ^2 test.

RESULTS

Collagen IV stained the basement membrane of normal gastric glands and vessels and also the basement membrane of the smooth muscle cells in the muscular layers of the gastric wall.

Relationship between collagen IV *expression and histological type, growth pattern of gastric cancer*

Seventy patients (47%) has continuous or, more frequently, disrupted linear structures around cancerous gland ducts or solid nests of tumor cells. The positive rates in undifferentiated or signet ring cell cancers were much lower than those in other cancers (P<0.01). Fifty-five percent of carcinomas with the nest-fashioned growth pattern showed collagen IV positive staining (Table 1), which was slightly lower than the percentage of collagen-IV positive carcinomas with mass-fashioned growth pattern, but significantly higher than that of diffuse-gastric carcinomas (P<0.01).

Relationship between the distribution of collagen IV *and liver metastasis of gastric cancer*

The distribution patterns of collagen IV in cancer tissue in gastric wall are quite different. Collagen IV was seen less often in the deep layer than in the mucosa. Collagen IV positive BM was found in 70 (47%) of 148 patients in the mucosa and only 36 (24%) in the deep layer of stomach. Based on the difference of collagen IV distribution, we divided the cases into three groups as follow: Group A, tumors positive for collagen IV in both mucosa and deep layer; Group B, tumors positive for collagen IV in mucosa but negative in deep layer; Group C,

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tumors negative for collagen IV. From Table 2, we can find that liver metastasis rate in Group A is much higher than that in Group B or C ($P<0.01$).

Table 1 Relationship between collagen IV expression and histological type, growth pattern of gastric cancer

Type	Number	Collagen IV *n*(%)	
		(+)	(-)
Growth pattern			
Mass-like	56	43(77)	13(23)
Nest-like	38	21(55)	17(45)
Diffuse	54	7(13)	47(87)
Histological type			
Well differentiated	37	30(81)	7(19)
Moderately differentiated	22	13(59)	9(41)
Poorly differentiated	23	10(43)	13(57)
Undifferentiated	27	3(11)	24(89)
Signet-net cell	25	2(8)	23(92)
Mucoid	14	12(86)	2(14)

Table 2 Relationship between the distribution of collagen IV and liver metastasis of gastric cancer

Liver-metastasis	Group A *n* = 36(%)	Group B *n* = 34(%)	Group C *n* = 78(%)
(+)	10(28)	2(6)	1(1)
(-)	26(72)	32(94)	77(99)

The blood vessels BM in gastric wall are also positively stained

In differentiated carcinoma, a large number of blood vessels were observed in the vicinity of cancer glands and collagen IV was localized around the blood vessels and cancer glands. But in poorly differentiated carcinoma, only a small number of blood vessels were distributed sporadically in stroma. In addition, vasoinvasion of tumor cells were highlighted by collagen IV immunostaining of the blood vessels BM.

DISCUSSION

Normal basement membrane is one of the biological barriers to tumor invasion and metastasis. In carcinomas, a dynamic interaction occurs at the interface between tumor cells and the surrounding extracellular matrix components. Tumor cells not only destroyed the basement membrane by producing collagenase, including the specific type IV collagenase, but also synthesize the components of basement membrane[4]. And also as a host reaction to the invading tumor, extracellular matrix components may be deposited around the tumor cells. The appearance of these components may symbolize the characteristic of the tumor and reflect its biological behavior. Collagen IV is one of the major components of the basement membrane, Burtin *et al*[5] showed that expression of collagen IV was related to the differentiation of the colorectal cancer.

Histological growth pattern could be considered as the objective indicator of the biological behavior of gastric cancer. In this study, we found that the presence of collagen IV containing basement membrane was closely related to the growth pattern. In mass or nest-fashioned growth pattern of gastric carcinomas, continuous or disrupted linear structures stained positively for collagen IV can be seen around cancerous glands or solid nests of tumor cells. But only a few scattered spots or patches were positively stained in diffuse gastric carcinomas, most of which were undifferentiated or signet ring cell carcinomas. Our study demonstrated that the attenuation or absence of collagen IV expression was frequently seen in poorly differentiated and diffusely infiltrating gastric cancers. That is, the loss and irregular distribution of collagen IV expression could be considered as a biological marker of cancer cells which had strong invading ability. This finding may be concerned with these tumor cells which could produce collagenase with higher activity or had poorer potential of collagen IV synthesis.

The prognosis of patients with advanced gastric cancer is poor because the likelihood of recurrence is high. The most common patterns of recurrence are peritoneal implantation and liver metastasis. According to our study, the distribution patterns of collagen IV are related to liver metastasis in gastric cancer. Positive expressions of collagen IV in mucosa and deep layer of gastric cancer are accompanied by much higher incidence of liver metastasis. Vascular spreading of gastric cancer, which is different from direct invasion, is a multi-step process in which cells must migrate from the primary tumor and invade blood vessels. David L *et al*[6] found that the expression of collagen IV was related to invading potential of tumor cells to blood vessels. All these findings support the hypothesis that collagen IV may play an important role in the metastatic process after migration from the primary tumor. The principal mechanism is an interesting subject for further studies. In conclusion, collagen IV-positive basement membrane in the deep layer of gastric wall in cancer tissue might be a risk factor for liver metastasis. In addition, collagen IV immunostaining facilitates recognition of vasoinvasion by highlighting the basement membrane of vessels.

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