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Relationships among Tenascin Expression, DNA Ploidy Patterns, and Multidrug Resistance Gene Product (P-Glycoprotein) in Human Colon Carcinoma

Isamu Sugawara,[1,3] Jun Hirakoshi,[1] Moriaki Kusakabe,[2] Atsuko Masunaga,[1] Shinji Itoyama[1] and Teruyo Sakakura[2]

[1]Department of Pathology, Saitama Medical Center, Saitama Medical School, 1981 Kamoda, Kawagoe, Saitama 350 and [2]Laboratory of Cell Biology, Tsukuba Life Science Center, Riken Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305

Relationships among tenascin expression, DNA ploidy, and P-glycoprotein were examined in 81 primary human colon cancers and 61 metastatic lymph nodes. First, the DNA ploidy patterns of colon cancerous tissue surrounded (TN^+) and not surrounded (TN^-) by tenascin immunoreactivity were investigated. Then the expression of P-glycoprotein, one of two multidrug resistance gene products, was examined in TN^+ and TN^- colon cancer tissues by immunohistochemistry. Aneuploid DNA patterns were observed at high frequency in TN^- colon cancer tissues (37/61) and metastatic lymph nodes (44/52). In contrast, diploid DNA patterns were observed predominantly in TN^+ colon cancer tissues (50/56). Although P-glycoprotein expression was observed in primary TN^+ and TN^- colon cancer (9/81), the level of P-glycoprotein expression was not correlated with DNA aneuploidy in TN^- colon cancer tissues. Overall, reduced tenascin expression was correlated well with DNA aneuploidy, but no significant correlation was found between DNA aneuploidy and P-glycoprotein appearing when cancer cells become resistant to several anti-cancer drugs. Thus, tenascin may play an important role in preventing colon cancer cells from invading surrounding tissues.

Key words: Tenascin — DNA ploidy — P-glycoprotein — Colon carcinoma

It is important to examine the degree of malignancy in primary cancers and metastatic lymph nodes because it gives an insight into the prognosis of cancer patients and the feasibility of preventing cancer cell invasion into surrounding tissues. It is well known that patients showing aneuploid DNA patterns in gastrointestinal cancer have a poor prognosis.[1-4] Therefore, analysis of DNA ploidy patterns is useful for determining cancer malignancy.[5]

Tenascin (TN) known formerly as myotendinous antigen, is an extracellular matrix glycoprotein[6,7] which is thought to play a role in limiting or preventing local tumor invasion. TN derived from chickens has been demonstrated to have molecular masses of 230, 200 and 190 kDa,[6] and the molecular masses of human TN are 250 and 190 kDa.[8] Recently, we have found that TN was reduced in colonic carcinoma with lymphogenous metastasis, and that the affected patients had a poor prognosis.[9]

P-Glycoprotein (P-GP) encoded by *mdr*1 mRNA appears when cancer cells become resistant to certain kinds of anti-cancer drug.[10] It is reported that a high proportion of colonic carcinomas possess P-GP.[11] When cancer cells resistant to anti-cancer drugs invade the surrounding tissues and eventually metastasize to organs via small blood vessels and lymphatic vessels, patients die of cachexia. Thus, it is expected that anti-cancer drug-resistant cancer cells would have a more malignant nature than cancer cells sensitive to these drugs.

The present experiments were designed to investigate relationships among TN as a marker of ability to prevent local invasion by cancer cells, DNA ploidy as a marker of malignancy, and P-GP as a multidrug resistance marker.

The materials were obtained from the Department of Pathology, Saitama Medical Center, Saitama Medical School, Saitama. We analyzed 10% formalin-fixed, paraffin-embedded sections prepared from surgical pathology specimens from 81 patients with primary colonic carcinoma with and without lymphogenous metastasis (61 and 20 cases, respectively). The tumors were staged according to Dukes' classification.[12] As we did not encounter many cases of stage C colonic cancer, all the cases in this study were at stage B or greater.

An anti-tenascin monoclonal antibody (MAb), MAb RCB1, was used. This is a well characterized MAb described by Oike *et al.*[13] In addition, an anti-P-GP MAb, JSB-1, prepared and well characterized by Scheper *et al.*,[14] was used. Another anti-P-GP MAb, UIC2, was kindly provided by Dr. Igor B. Roninson, Department of Genetics, University of Illinois at Chicago.[15]

[3] To whom requests for reprints should be addressed.

Table I. Expression of TN and P-GP in Primary Colonic Carcinoma and Metastatic Lymph Nodes

Case	Extent of TN[a]			Extent of P-GP[a]	
	−	+	++	−	+
Non-metastatic colonic carcinoma	0	0	20[b]	18	2
Primary colonic carcinoma with lymphogenous metastasis	9	52	0	54	7
Metastatic lymph nodes	37	24	0	58	3

a) The scoring of intensity of TN and P-GP immunoreactivities using MAbs JSB-1 and UIC2 is described in "Materials and Methods."
b) Differs significantly ($P<0.01$) from primary colonic carcinoma with lymphogenous metastasis according to Student's t test.

Immunohistochemical staining was carried out by the avidin-biotin-peroxidase complex (ABC-PO) technique as described elsewhere.[16-18] For TN and P-GP detection, MAbs RCB1 (1 mg/ml), JSB-1 (1 mg/ml) and UIC2 were diluted 1:200, 1:100 and 1:100, respectively. A negative control tumor sample from each patient was prepared according to this protocol, except that the primary antibody was substituted by an irrelevant (anti-keratin Ab, Dakopatts), isotype-matched MAb. For each case, the anti-tenascin MAb reactivity scoring was based on evaluation of the paraffin tissue sections immunostained with MAb RCB1. The immunoreactivity and localization patterns of the MAb were identical. The anti-TN reactivity was scored as negative when the stroma lacked immunostaining, + when 50% or less of the stroma was immunostained and ++ when more than 50% of the stroma was immunostained. Anti-P-GP reactivity was scored as negative when the cancerous tissue lacked immunostaining, and + when the cancerous tissue was positively immunostained with MAbs JSB-1 and UIC2. The immunoreactivities of each of the tissue sections were reviewed by three experienced immunopathologists (I.S., A.M., S.I.), and similar results were obtained by each of them.

After deparaffinization, the tissue sections were stained by the Feulgen method. The DNA content of the sections was analyzed using a computerized microscopic image processor (OSP-1, Olympus Optical Co., Tokyo, and SAMBA 200, Thomson-TITN, USA).[19-21] Both cell-image processors yielded similar results. Nuclear DNA content was measured in 100 full-size cancer cells, each surrounded or not surrounded by tenascin, and 100 full-size cancer cells in metastatic lymph nodes.

Very strong TN expression was observed in every case of non-metastatic colonic carcinoma. Strong TN expression was noted in primary colonic carcinoma with lymphogenous metastasis (52/61) and in metastatic lymph nodes (24/61) (Table I and Fig. 1).

On the other hand, P-GP expression was recognized in 9 of 81 primary colonic carcinomas (7 of 61 colonic carcinomas with lymphogenous metastasis and 2 of 20 non-metastatic colonic carcinomas) and 3 cases of 61 metastatic lymph nodes. P-GP-expressing colonic cancer cells were recognized in both cancerous tissues surrounded (TN^+) and not surrounded by TN (TN^-).

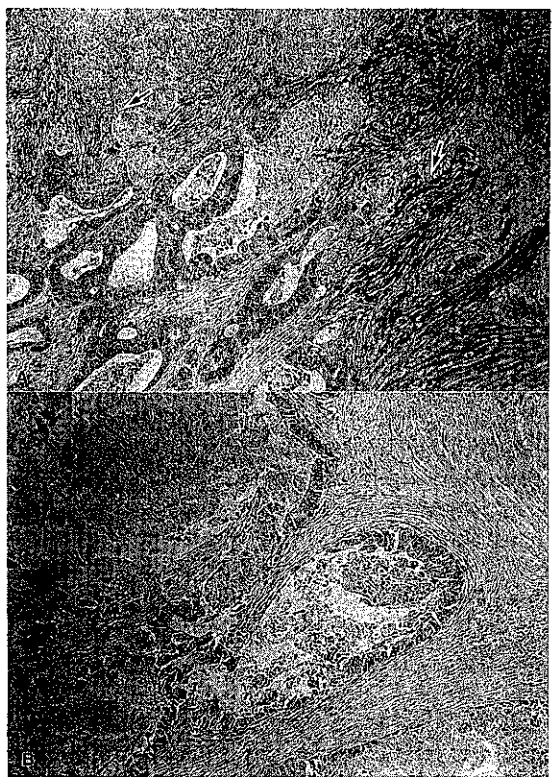

Fig. 1. Colonic cancer cells surrounded (A) and not surrounded (B) by tenascin (TN). Colonic cancer cells are surrounded by dense deposits of TN (→). ABC-PO method. Counterstained with hematoxylin. ×100.

Although it is reported that C219 reacts with P-GP in paraffin blocks of colonic cancer tissues,[22-24] we could not obtain positive immunoreactivity with this MAb.

Instead, MAbs JSB-1 and UIC2 were used to react with P-GP-expressing colonic carcinoma. The difference in immunoreactivity between the reported experiments and ours may be due to the fact that they used 10% neutral buffered formalin as a fixative and we used 10% unbuffered formalin as a routine procedure; 10% unbuffered formalin might affect P-GP reactivity.

Next, we examined whether or not colon cancer tissue surrounded by TN expressed P-GP. Seven cases showed P-GP expression: five of 52 TN-positive colonic carcinomas with lymphogenous metastasis (Fig. 2A and 2B) and two of 20 TN-positive non-metastatic carcinomas. Only two P-GP-expressing colonic cancers were not surrounded by TN. There was no correlation between P-GP expression by colonic carcinoma and TN-immunopositivity ($P<0.01$). It has been reported that there is a relationship between the expression of P-GP in human colon carcinoma, and local tumor aggressiveness and lymph node metastasis.[25] As stated above, in our study there was no relationship between P-GP expression and cancer cell invasion and metastasis, although different parameters were utilized for evaluating the degree of malignancy.

Nuclear DNA content was measured in 100 full-size cancer cells surrounded or not surrounded by TN. As shown in Table II, in colonic carcinoma with lymphogenous metastasis, 50 colon cancer specimens surrounded by TN showed a diploid DNA pattern and 24 colonic cancer specimens not surrounded by TN showed a diploid DNA pattern. DNA aneuploidy was noted in 6

Fig. 2. P-glycoprotein (P-GP)-expressing colonic cancer cells (55-year-old, male patient with lymphogenous metastasis). ABC-PO method. Counterstained with hematoxylin. ×200. A. Colonic cancer cells immunostained with MAb JSB-1. B. Colonic cancer cells immunostained with MAb UIC2. C. Adrenal cortex immunostained with MAb UIC2 (→) (positive control).

Table II. Correlation of DNA Ploidy with Presence or Absence of TN[a]

Case		DNA diploid	DNA aneuploid
Colonic carcinoma without lymphogenous metastasis	surrounded by TN (TN^+)	18[b]	2
	not surrounded by TN (TN^-)	6	14
Colonic carcinoma with lymphogenous metastasis	surrounded by TN (TN^+)	50[c]	6
	not surrounded by TN (TN^-)	24	37
Metastatic lymph node		8	44

a) DNA ploidy patterns were evaluated by an OSP-1 or SAMBA cell image processor by counting 100 full-size cancer cells after staining by the Feulgen method.
b) Differs significantly ($P<0.01$) from colonic carcinoma without lymphogenous metastasis (TN^-) according to Student's *t* test.
c) Differs significantly ($P<0.01$) from colonic carcinoma with lymphogenous metastasis (TN^-) according to Student's *t* test.

colon cancer specimens surrounded by TN and 37 not surrounded by TN. There was a significant correlation in the frequency of DNA aneuploidy between colon cancer tissues surrounded and not surrounded by TN ($P<0.01$). In colonic carcinoma without lymphogenous metastasis, cancerous tissues surrounded by TN showed an aneuploid DNA pattern in 2/20 cases, whereas cancerous tissues not surrounded by TN showed DNA aneuploidy in 14/20 cases. There was also a significant correlation in the frequency of DNA aneuploidy between colon cancer tissues surrounded and not surrounded by TN ($P<0.01$). In metastatic lymph nodes, DNA aneuploidy was recognized regardless of the presence of TN. Once colonic cancer cells had become more aggressive, no TN accumulation was found. Cancer cells that had metastasized to lymph nodes were more aggressive even though TN was deposited there.

In summary, colonic cancer cells were not as invasive when they were surrounded by TN. Then the colonic cancer cells became more invasive in nature, TN was barely seen surrounding them. Thus, TN may play an important role in preventing cancer cells from infiltrating into surrounding tissues. On the other hand, P-GP was observed in both colon cancer cells showing DNA diploid and aneuploid patterns. Thus, multidrug resistance seems not to be directly related to the degree of malignancy and cancer cell invasion.

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