Nuclear β-Catenin Expression at the Invasive Front and in the Vessels Predicts Liver Metastasis in Colorectal Carcinoma

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Abstract. *β-Catenin is a component of the Wingless/Wnt signaling pathway and can activate target genes associated with proliferation and invasion, linking with the APC gene. The purpose of this study was to investigate whether nuclear expression of β-catenin in cells at the invasive front or in the vessels was associated with liver metastasis in human colon cancer. Patients and Methods: One hundred and eighteen patients with colorectal carcinoma who underwent surgical resection (45 patients with liver metastasis and 73 patients without liver metastasis at least 5 years after surgery) were included in the study. Proliferative activity was determined in several areas (tumor center, invasive front and in the vessels) by immunohistochemistry and whether it was correlated with liver metastasis was examined. Results: In 73.1% of primary tumors, positive staining for β-catenin was detected in the membranes at the tumor center and in the nuclei at the invasive front. In 32 patients (26.9% of all cases), β-catenin was expressed exclusively in the nuclei of the carcinoma cells throughout the tumors. Significant differences in expression of nuclear β-catenin in the primary tumors were detected between the liver metastasis and non-liver metastasis groups at the tumor center (p=0.004), invasive front (p=0.021) and in the vessels (p<0.0001). Conclusion: Nuclear accumulation of β-catenin in cellular cells at the invasive front and in the vessels was the most powerful predictor of liver metastasis in colorectal cancer. This may be an important marker in the selection of patients for adjuvant therapy or other treatment modalities.*

Abbreviations: APC: adenomatous polyposis coli; NLM: no liver metastasis; LM: liver metastasis.

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Key Words: β-Catenin, invasive front, liver metastasis.

Colorectal carcinoma is one of the most commonly occurring solid tumors worldwide. Despite the considerable amount of time spent on cancer research and the introduction of new therapies, the prognosis of this type of carcinoma remains poor, with a mean 5-year survival rate of approximately 50% (1). After curative surgical treatment of colorectal cancer, the liver is the major site of recurrence. Liver metastasis is fatal if there is no chance of surgical treatment. Even after successful removal of liver metastases, the recurrence rate is still high and the prognosis is very poor, with only 20-40% of patients surviving for 5 years (2-6), whereas the long-term survival rate after medical therapy or no therapy at all is no more than 2% (6). A high incidence of dissemination is one major obstacle in curative surgery for colorectal carcinoma. However, it is not easy to identify these patients with or without a high risk of metastasis. Patients with Dukes' C disease have metastases in adjacent lymph nodes and therefore possess clones of malignant cells with the ability to spread *via* the lymphatic system into adjacent lymph nodes and grow there. However, it is a very general view that some patients in the Dukes' B stage develop recurrence and some in the Dukes' C stage do not. The possibility of obtaining more direct evidence indicating a high probability of recurrence in individual cases during the follow-up period would be very useful for selecting patients who need careful follow-up and adjuvant chemotherapy. β-catenin, a central component of the cadherin adhesion system, binds to the cytoplasmic domain of cadherin and the amino-terminal domain of α-catenin and mediates cell adhesion. Disorders of β-catenin have been reported in association with impaired intercellular adhesion of cancer cells (7-9). Furthermore, β-catenin has also attracted attention because, in addition to its role in cell adhesion, it is also a component of the Wingless/Wnt signaling pathway (10). The similarity between the β-catenin gene and the Drosophila segment polarity gene, *Armadillo* (*Arm*), suggests that β-catenin may also act as a signal transducer of the evolutionary conserved Wnt/Wingless signaling system. The oncogenic potential of β-catenin is derived from a nuclear pool associated with the T-cell factor family of transcription factors. The resulting transcription complex can activate target genes

associated with proliferation and invasion, linking *APC* gene defects to these processes. Not only target genes such as *c-myc* (11), and *cyclin D1* (12), but also genes necessary for invasive growth, such as *matryilysin* (13, 14), *fibronectin* (15), *CD44* (16), and uPAR (17), are activated by β-catenin. It is therefore thought that nuclear accumulation of β-catenin is correlated with tumor invasion and liver metastasis at the time of surgical resection. Brabletz T *et al*. (18) reported that an important driving force for the progression of colorectal carcinoma is the specific environment, initiating loss of epithelial differentiation and gain of mesenchyme-like capabilities of the tumor cells at the invasive front, two transient phenotypic transition processes that modulate intracellular β-catenin distribution in tumor cells. Nuclear accumulation of β-catenin and mesenchyme-like capabilities are thought to be correlated with tumor invasion and liver metastasis. Invasion and liver metastasis of well-differentiated carcinomas are often associated with a loss of epithelial differentiation and gain of mesenchyme-like capabilities of tumor cells at the invasive front. In the vessels, the characteristic phenotype of the tumor cells is also expected to change. The aim of this study was to establish whether the distribution of nuclear β-catenin at the invasive front or in the vessels was associated with a high risk of liver metastasis and poor prognostic outcome. If these factors prove to be predictors of a poor outcome, they may be helpful when making decisions about the need for surgical resection and the most appropriate postoperative follow-up.

Patients and Methods

Patients. One hundred and eighteen patients with colorectal carcinoma who underwent surgical resection between June 1994 and June 2000 in our hospital were investigated. Among these, 45 patients had liver metastasis (37 had synchronous liver metastasis and eight had metachronous recurrence in the liver; LM group). In contrast, 73 patients with no liver metastasis at least 5 years after surgery were selected during the same period (NLM group). The clinicopathological characteristics of these two groups are shown in Table I. The patients in the NLM group were similar to the LM group in terms of gender, age, tumor location and tumor depth. No patient had received radiation or chemotherapy preoperatively. The pathological tumor stage and disease grades were classified according to the fifth edition of the TNM classification of the International Union against Cancer (UICC) (19).

Histopathological examination and assessment of blood vessel permeation. Vessels with smooth muscle walls or those that contained red blood cells were considered to be blood vessels, and tumor nests enclosed by the lumen of the vessels were judged to be blood vessel permeation. At least two formalin-fixed and paraffin-embedded sections (2-6 sections) from each case with blood vessel permeation were examined after hematoxylin and eosin (H & E) staining, with 2 μm-thick sections cut from the deepest site. Elastica van Gieson staining (20) was performed and the sections were re-examined microscopically. For immunohistochemical staining, tissues with most vessel permeation were selected; in cases with no vessel permeation, however, sections from the deepest invasive sites were chosen.

Table I. *Clinicopathological features of the cases examined.*

Factor	Liver metastasis (–)	Liver metastasis (+)	Total	*P*-value
No. of patients	73	45	118	
Gender				
Male	49	30	79	0.842
Female	24	15	39	
Age (years)				
Mean ± SD	60.6±12.1	61.0±12.2		0.828
Tumor location				
Colon	28	29	57	0.006
Rectum	45	16	61	
Tumor size (mm)				
Mean ± SD	54.6±21.2	58.1±22.8		0.401
Differentiation				
Well	23	10	33	0.235
Moderate	46	30	76	
Poor	4	6	10	
Tumor depth				
T2 or T3	51	33	84	0.686
T4	22	12	33	
Involved lymph node				
N0	35	23	58	0.080
N1	31	12	43	
N2, N3	7	10	17	
Lymphatic invasion				
Negative	18	6	24	0.129
Positive	55	39	94	
Venous invasion				
Negative	37	8	45	0.0002
Positive	36	37	74	

Immunohistochemistry. Serial sections (2 μm thick) of selected specimens were cut, mounted on silane-coated glass slides and immunohistochemical studies performed as described elsewhere (21). In brief, sections were deparaffinized in xylene and dehydrated in a decreasing alcohol gradient. Endogenous peroxidase activity was quenched in 0.3% hydrogen peroxide in methanol for 30 min, and the slides were then heated in a microwave oven in 0.1 mol/L citrate buffer (pH 6.0) at 94˚C for 15 min before immunostaining. After rinsing in phosphate-buffered saline (PBS; pH 7.4), non-specific antibody binding was blocked with 10% normal horse serum in PBS for 30 min at room temperature. After decanting off any excess serum, each section was incubated overnight at 4˚C with mouse monoclonal anti E-cadherin antibody (clone H-ECD1; Takara, Tokyo, Japan) at 1:1000 dilution, anti α-catenin antibody or anti β-catenin antibody (clone 5 and clone 14, respectively; Transduction Laboratory, KY, USA) at 1:250 and 1:1000 dilution, respectively. After washing with PBS, the slides were incubated with 10% biotinylated anti-mouse antibodies for 30 min at room temperature, washed three times and then incubated with a 1:500 dilution of avidin-biotin peroxidase complex (Dako Corp., Carpinteria, CA, USA). After washing, bound peroxidase activity was visualized with 0.02% 3,3'-diaminobenzidine tetrahydrochloride containing 0.005% hydrogen peroxide in 50 mmol/L ammonium acetate-citric buffer (pH 6.0). After elastic fiber was stained with Maeda's resorcin-fuchsin solution (Muto Pure

Chemical Co. Ltd., Tokyo, Japan) for 20 min., nuclear counterstaining was carried out using Mayer's hematoxylin. Membrane expression in the normal adjacent colonic epithelium was used as a positive control for each antibody, and sections from each block were incubated without primary antibody as a negative control.

Evaluation of β-catenin, α-catenin and E-cadherin immunostaining. Three areas of each tissue, the tumor center, invasive front and vessels infiltrating tumors were visualized by elastic fiber staining and assessed for expression of each protein. For α-catenin and E-cadherin, areas with strong membrane staining similar to that of the normal colonic epithelium were regarded as preserved cases, and weaker staining as compared with normal colonic epithelium was classified as a reduced case. For β-catenin, membrane, cytoplasmic and nuclear staining were evaluated independently in each of the three areas, and membrane staining of β-catenin was determined in the same way as for E-cadherin and α-catenin. As cytoplasmic and nuclear staining were not seen in areas of normal colon mucosa, areas with more than 5% of cells that were positive for cytoplasmic or nuclear staining were regarded as positive for cytoplasmic and nuclear staining. Expression of β-catenin, α-catenin and E-cadherin was evaluated in the three areas and the association between protein expression and incidence of liver metastasis in colorectal cancer was determined.

Statistical analysis. The results were expressed as median±standard division. The relationships between liver metastasis and clinicopathological features, E-cadherin, α-catenin and β-catenin expression in the two groups (LM and NLM group) were determined using the χ^2 method. Fisher's exact test was used for small numbers.

Results

Clinicopathologic features of the patients. Table I shows the clinicopathologic features of the two groups of patients. The presence of lymph node metastasis was associated with developing liver metastasis. Statistically significant differences were observed in tumor location and venous invasion, whereas none of the other characteristics, including tumor depth, had a significant relationship with liver metastasis in this group.

Growth patterns of well differentiated colorectal carcinomas. Primary tumors showed an identical epithelial growth pattern, with polarized tumor cells forming clear tubular structures in the central tumor areas. At the invasive front of the primary tumor, columnar cells with a single or solitary trabecular form with indistinct polarity showed an infiltrative growth pattern. Tumor cells in the vessels, however, did not form tubular structures and were characterized by the detachment of small cell clusters and isolated cells. Despite the loss of epithelial phenotype at the invasive front, the epithelial dedifferentiation state, characterized by polarized epithelial tumor cells forming clear tubular structures, was seen once again in the liver metastases (Figure 1). Immunohistochemical staining for E-cadherin, α-catenin and β-catenin revealed that normal colorectal epithelium expressed E-cadherin and α-catenin strongly and, without exception, at the cell–cell boundaries. Membranous E-cadherin and α-catenin expression was observed at the tumor center, but in almost all primary tumors expression of E-cadherin and α-catenin was reduced at the invasive front. This reduced pattern of E-cadherin and α-catenin expression persisted in the vessel permeation (Figure 2). Conversely, membranous expression of β-catenin was detected in colonic epithelial cells in the normal mucosa and in primary tumor cells in the tumor centers. In contrast, expression of β-catenin changed towards strong nuclear staining at the invasive front, accompanied occasionally by diffuse cytoplasmic expression (Figure 3). This nuclear pattern was also observed occasionally in the vessels at the tumor permeation. It was relatively easy to detect blood vessels filled with tumor cell emboli at the same time as the Elastica van Gieson staining (Figure 4). On the other hand, in 32 patients (26.9% of all cases), β-catenin was expressed exclusively in the nuclei of the carcinoma cells throughout the tumors (Figure 5).

E-cadherin and α-catenin expression. In most of the tumor, but particularly at the center, the tumor cells were well differentiated. However, at the invasive front of the primary tumors, the tumor cells were characterized by an opening of the tubules, leading to isolated tumor cells. Without exception, all of the non-cancerous epithelium of the large bowel expressed both E-cadherin and α-catenin at the cell–cell boundaries. The expression of E-cadherin and α-catenin was preserved in 93.2% (110/118) and 97.5% (115/118) of tumors at the tumor center, respectively, but reduced expression of E-cadherin and α-catenin was observed in 92.4% (109/118) and 52.5% (62/118) of tumors at the invasive front respectively. Reduced expression of E-cadherin and α-catenin was the most frequent abnormality observed at the invasive front and in the vessels. However, reduced expression of E-cadherin was only associated with liver metastasis when the tumor was observed in the vessels. Seventeen of 37 cases with tumor in the vessels and liver metastasis (45.9%) exhibited reduced expression of E-cadherin (Table II). Because E-cadherin and α-catenin were strongly associated with each other, the pattern of coexpression of these molecules was considered to be associated with metastasis of cancer cells.

The frequency of reduced expression of α-catenin tended to be higher in tumors in the vessels than at other locations (54.2%). However, the reduced membranous expression of α-catenin in the vessels was not significantly associated with liver metastasis (Table III).

β-Catenin expression in the tumor center, invasive front and vessels. In 73.1% of primary tumors β-catenin expression was membranous in the well-differentiated tumor center. In contrast, at the invasive front, expression of β-catenin changed towards strong nuclear staining, accompanied occasionally by a diffuse cytoplasmic expression pattern. This expression pattern persisted in isolated tumor cells in the vessels. In 32

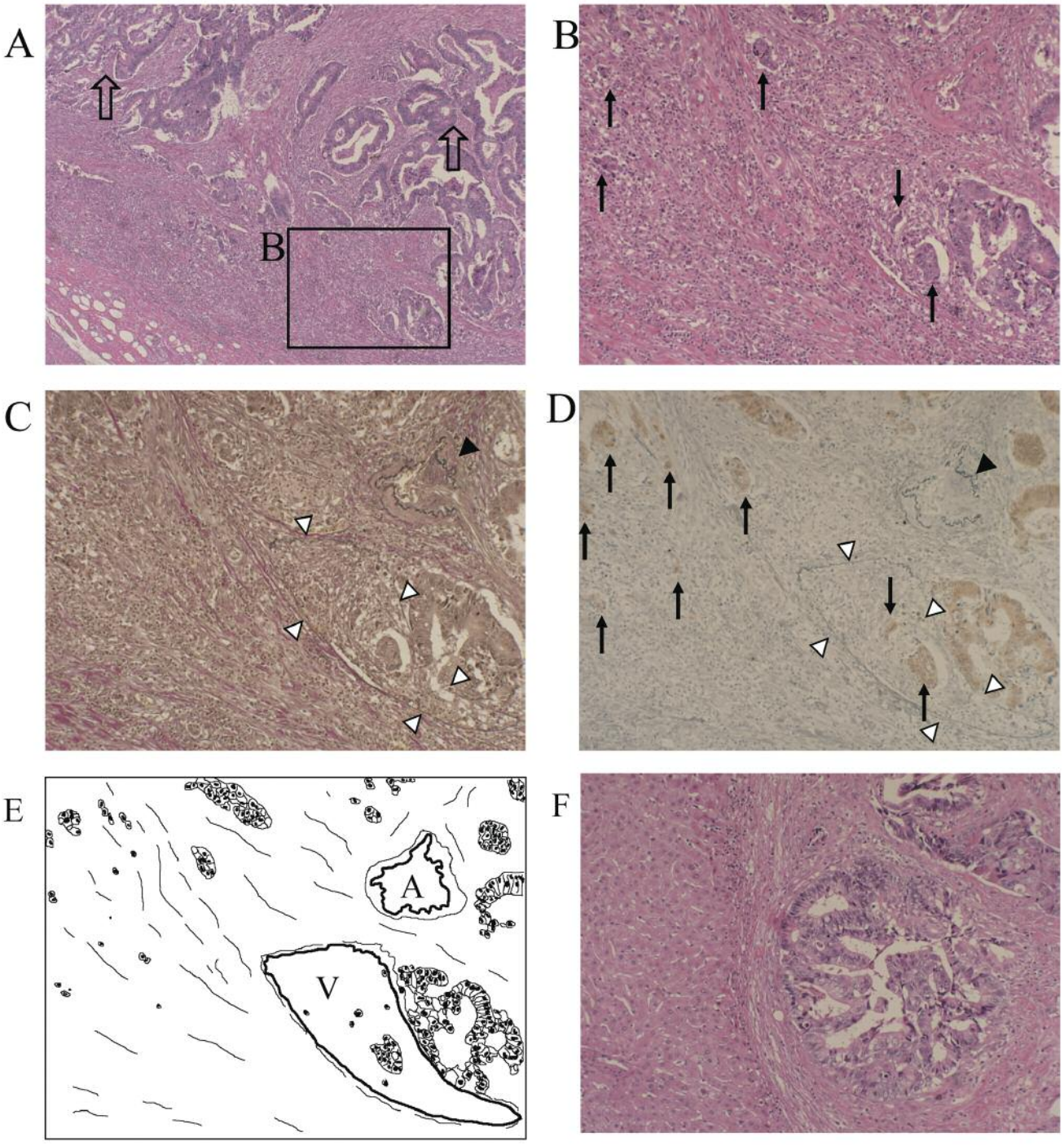

Figure 1. *A case of moderately differentiated adenocarcinoma of the colon with synchronous liver metastasis. (A) Hematoxylin and eosin (H&E) staining (×40); large cancer nests with a clear trabecular pattern can be seen in the superficial area and tumor center (open arrows), but the tumor nests gradually decrease in size. (B) Magnification of Figure 1A (H & E, ×100); carcinoma cells have formed small nests, lost their epithelial differentiation and acquired a more spindle shaped/mesenchymal morphology. (C) Vessel permeation stained by the Elastica van Gieson method. Finely stained, small tumor nests can be observed surrounded by the vessel walls. (D) β-Catenin staining with elastic fibers invading cancer cells or small cancer nests are clearly stained for β-catenin. Some of them can be defined as vessel permeation because of the surrounding elastic fiber wall stained by Maeda's resorcin-fuchsin solution (open arrowhead). The solid arrowhead shows the artery in Figure 1C. (E) Diagram of the area shown in Figure 1 B–D. (F) H & E staining of liver metastasis in the same case (×100); cancer cells form large nests with a clear trabecular pattern at the liver metastasis site.*

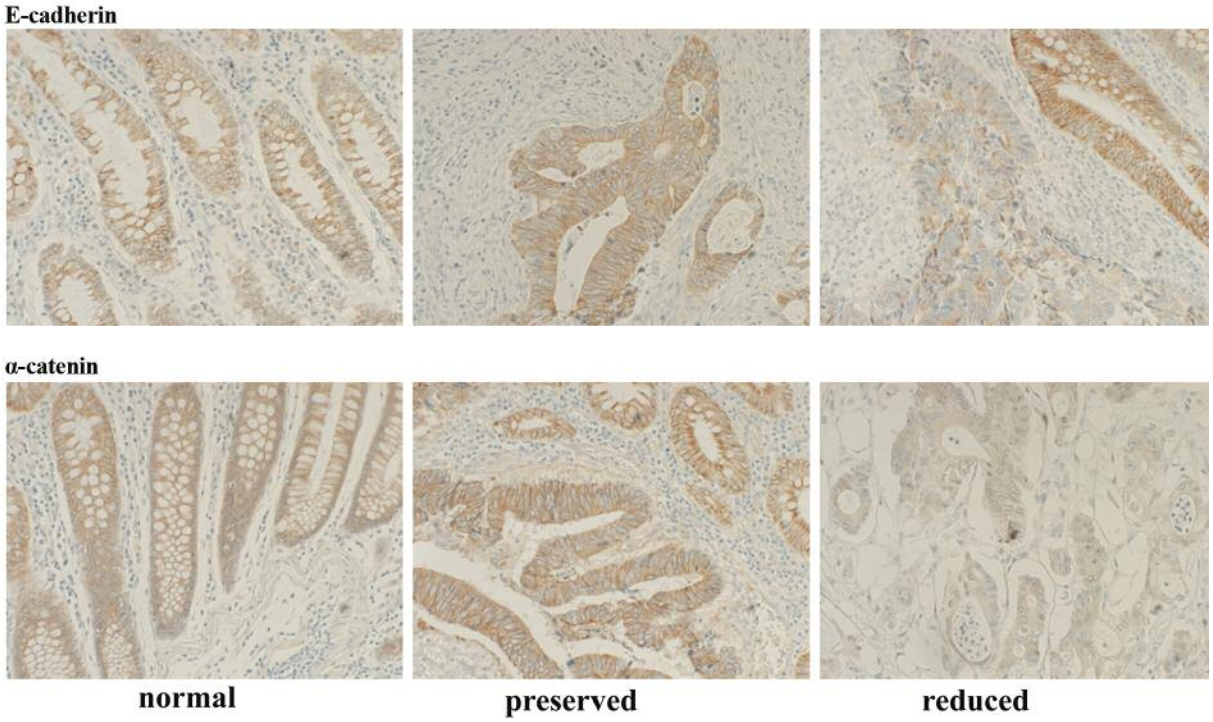

Figure 2. *Normal expression as shown by homogeneous staining of the membrane of epithelial cells at the cell–cell boundaries. The expression of E-cadherin and α-catenin was preserved at the tumor center, but was reduced at the invasive front.*

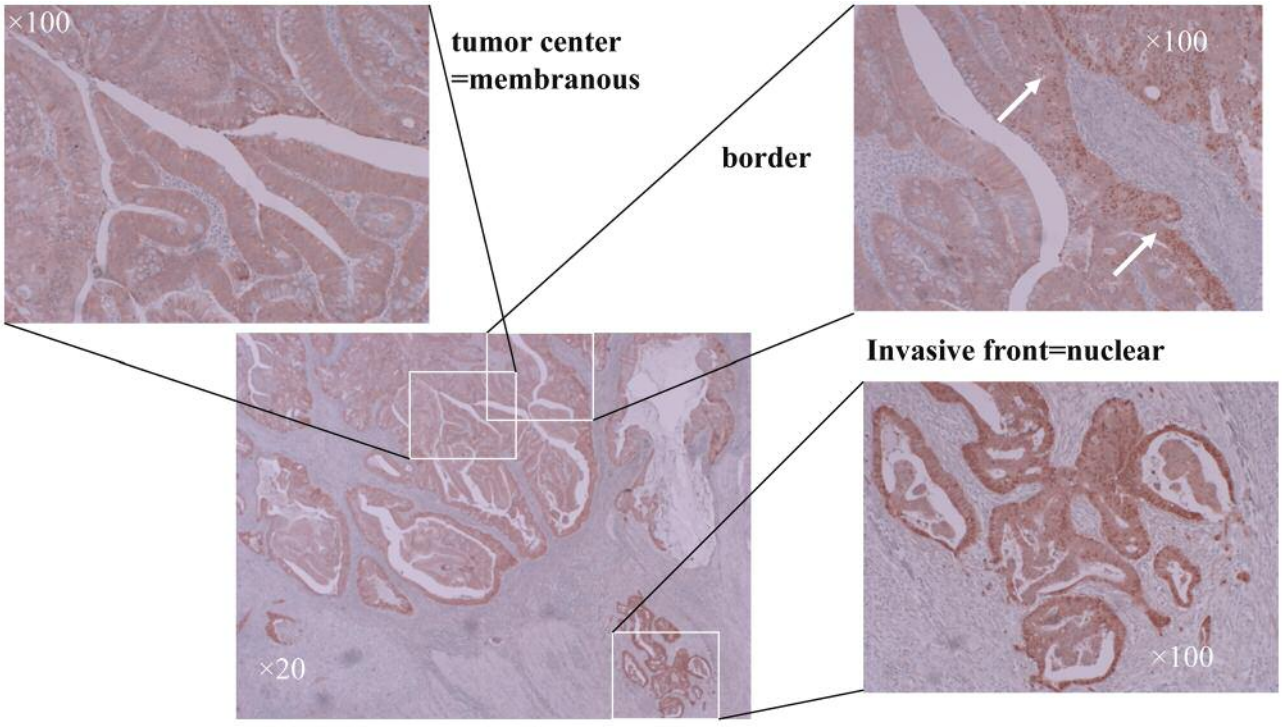

Figure 3. *More than 50% of the primary tumor cells at the tumor center show membranous expression of β-catenin. Tumor cells at the invasive front have lost their polar orientation and become dissociated. This morphological change is accompanied by nuclear accumulation of β-catenin. Dissemination of tumor cells at the invasive front has occurred permeating the vessels. These tumor cells in the vessels and the loss of epithelial phenotype were usually accompanied by strong nuclear staining for β-catenin.*

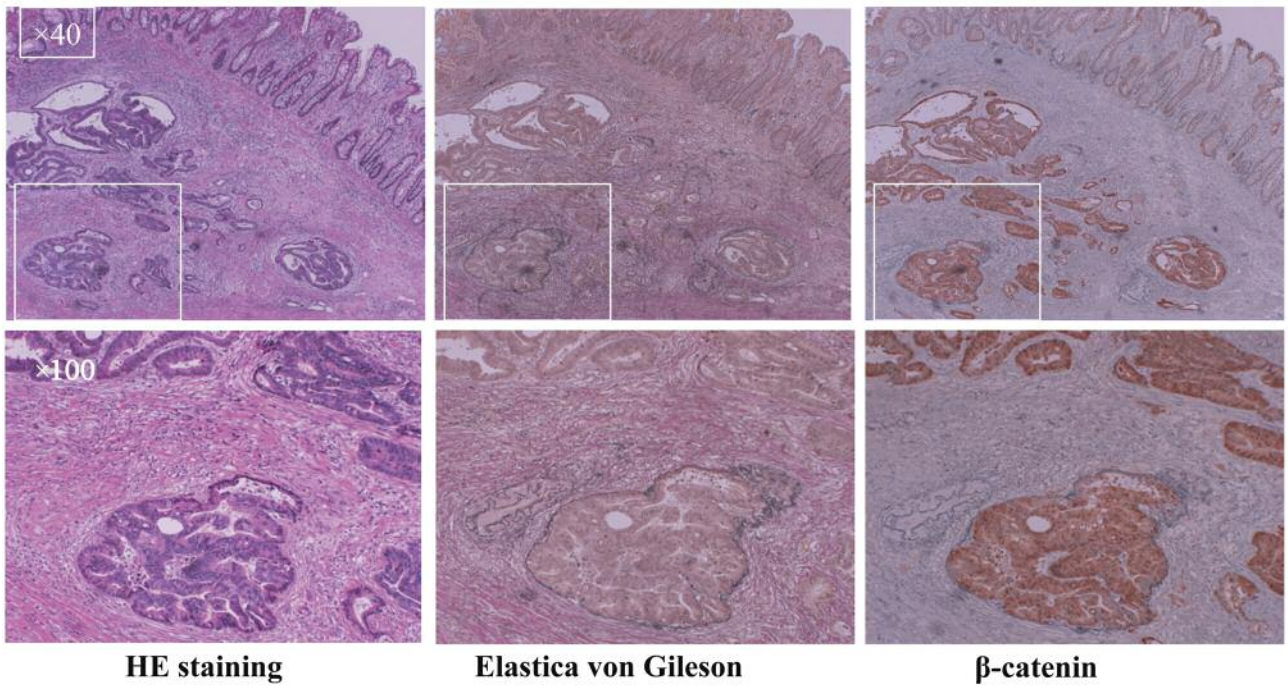

Figure 4. *It was relatively easy to detect blood vessels filled with tumor cell emboli when simultaneously using Elastica van Gieson staining.*

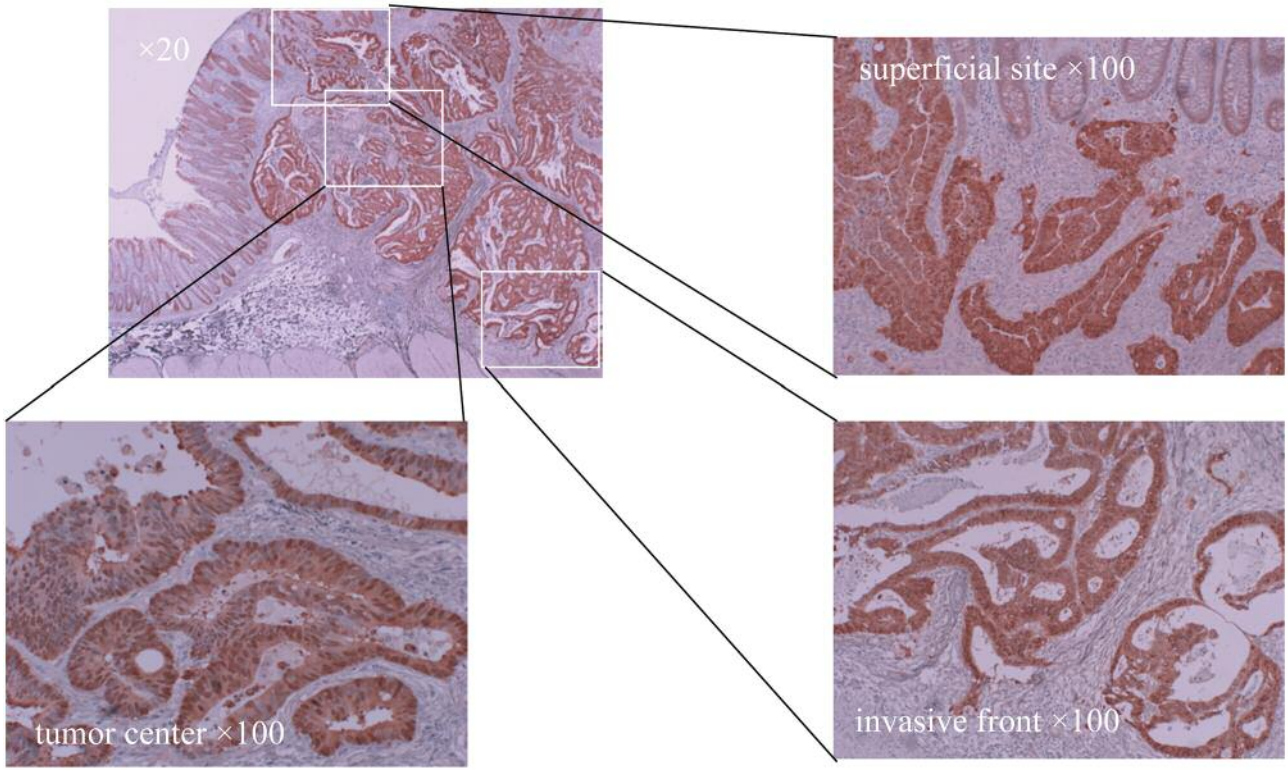

Figure 5. *In some tumors, β-catenin was expressed exclusively in the nuclei of the carcinoma cells throughout the tumors.*

Table II. *Expression pattern of E-cadherin primary tumor.*

	Number of cases			
	Non-liver metastasis	Liver metastasis	Total	*P*-value
Central tumor				
Preserved	68	42	110	0.969
Reduced	5	3	8	
Invasive front				
Preserved	6	3	9	0.755
Reduced	67	42	109	
In the vessels				
Preserved	38	20	58	0.005
Reduced	8	17	25	

Table III. *Expression pattern of α-catenin in primary tumor.*

	Number of cases			
	Non-liver metastasis	Liver metastasis	Total	*P*-value
Central tumor				
Preserved	72	43	115	0.303
Reduced	1	2	3	
Invasive front				
Preserved	30	26	56	0.780
Reduced	43	19	62	
In the vessels				
Preserved	19	19	38	0.361
Reduced	27	18	45	

Table IVa. *Membrane expression of β-catenin in primary tumor.*

	Number of cases			
	Non-liver metastasis	Liver metastasis	Total	*P*-value
Central tumor				
Preserved	72	44	116	0.731
Reduced	1	1	2	
Invasive front				
Preserved	56	32	88	0.497
Reduced	17	13	30	
In the vessels				
Preserved	38	31	69	0.887
Reduced	8	6	14	

Table IVb. *Cytoplasmic of β-catenin in primary tumor.*

	Number of cases			
	Non-liver metastasis	Liver metastasis	Total	*P*-value
Central tumor				
Preserved	54	26	80	0.069
Reduced	19	19	38	
Invasive front				
Preserved	14	12	26	0.340
Reduced	59	33	92	
In the vessels				
Preserved	30	15	45	0.058
Reduced	14	22	36	

Table IVc. *Nuclear expression of β-catenin in primary tumor.*

	Number of cases			
	Non-liver metastasis	Liver metastasis	Total	*P*-value
Central tumor				
Preserved	60	26	86	0.004
Reduced	13	19	32	
Invasive front				
Preserved	33	11	44	0.021
Reduced	40	34	74	
In the vessels				
Preserved	28	7	35	<0.0001
Reduced	18	31	49	

patients (26.9% of all cases), however, β-catenin was expressed exclusively in the nuclei of the carcinoma cells throughout the tumors. Membranous β-catenin expression was reduced or absent in 30 of 118 tumors (25.4%) at the invasive front and 14 of 83 tumors (16.9%) in the vessels. There was no significant association between membranous β-catenin expression and liver metastasis (Table IVa). Conversely, cytoplasmic β-catenin expression at the invasive front was positive in 92 of 118 patients (75.6%), but this expression was not associated with liver metastasis. Cytoplasmic expression of β-catenin in the vessels tended to be associated with a higher level of liver metastasis (Table IVb). On the other hand, significant differences were observed in the expression of β-catenin in the nuclei of primary tumors between the LM and NLM groups at the tumor center (p=0.004), invasive front (p=0.021) and in the vessels (p<0.0001) (Table IVc).

Discussion

Expression of E-cadherin, α-catenin and β-catenin was determined by immunohistochemistry in 118 cases of colorectal carcinoma. Although the expression of E-cadherin, α-catenin and β-catenin and its association with clinicopathological features has been studied in various organs, few studies of the expression of these markers at several areas (tumor center, invasive front and in the vessels) in human cancer tissue have been reported. In this study, diffuse nuclear accumulation was detected throughout the tumor in some tumors, nuclear β-catenin, however, was found at the invasive

front in most tumors. In the central areas of the primary tumor, expression was localized to the membrane and cytoplasm of polarized epithelial tumor cells. This expression pattern was accompanied by changes in E-cadherin and α-catenin expression.

Late in tumor progression, carcinoma cells lose their epithelial differentiation and acquire a more spindle-shaped/mesenchymal morphology, a phenomenon that has been suggested to correlate with metastatic potential. In this study, the tumor centers were observed to have an epithelial growth pattern characterized by adherent polarized tumor cells forming tubular structures. At the invasive front, tumor cells dissociate from the tubules and acquire the ability to invade by a process of dedifferentiation. However, metastases of colorectal carcinoma in suitable tissues, such as the liver, often completely resemble the primary tumor. Several phenotypes of cancer cells were observed in the tumor. Therefore, we investigated the expression of E-cadherin, α-catenin and β-catenin in colorectal carcinoma at the tumor center, invasive front and in the vessels. In this study, nuclear expression of β-catenin in the tumor cells in the vessels was found to be most predictive of liver metastasis. In normal colonic epithelial cells, APC, in combination with glycogen syntheses kinase 3β, regulates the free cytoplasmic β-catenin gene, and APC in combination with axin may regulates free cytoplasmic β-catenin for degradation by ubiquitination-dependent proteolysis (22-24). This regulates the availability of free β-catenin for binding with the TCF-LEF family of transcription factors (25, 26). Mutations in *APC* or β-catenin can result in failure to degrade β-catenin, and subsequently, an increase in β-catenin-TCF complex formation. This results in alternations in gene transcription (27). Several reports have shown that oncogenic activation of β-catenin is an early event in the development of colon cancer (28, 29). In the model proposed by Vogelstein *et al*. (30), APC suppressor gene mutation is found in the earliest stage, before proceeding to *K-ras* mutation and to other tumor suppressor genes, such as *p53* and *DCC*. Mutation in the APC suppressor gene leads to activation of β-catenin (31). In this study, only 32 cases (27%) showed diffuse nuclear accumulation of β-catenin throughout the tumor. Mutation in the APC suppressor gene was expected to be an early event in cancer development in these cases. On the other hand, in advanced colorectal carcinoma, most of the nuclear β-catenin pattern was found at the invasive front, and this expression pattern persisted in the vessels. Most membranous expression pattern was observed in the central areas of the primary tumor in 117 cases (98.3%). The difference between tumors with diffuse nuclear accumulation of β-catenin (early event) and nuclear accumulation at the invasive front of the tumor or in the vessels (late event) should be emphasized. It is suggested that selective activation of β-catenin at the invasive front of the tumor occurs as a late event independent of *APC* mutation accelerating tumor invasion and metastasis. The data from this study show that tumor cells at the invasive front, which exhibited nuclear accumulation of β-catenin, tended towards liver metastasis.The mechanisms leading to cytoplasmic accumulation and nuclear expression of β-catenin are unclear. Genetic mutations of *APC* or *β-catenin* are the most common disease-causing genetic events and are known to result in nuclear accumulation of β-catenin (10, 27). However, other genetic or epigenetic events may also occur, which modulate nuclear translocation. Although the tumor at the primary site expressed nuclear β-catenin in this study, membranous expression was observed in the tumors in the liver. It is also possible that a reversible mechanism such as an epigenetic event occurred. Hypermethylation of *APC* promoter CpG islands may be one of the mechanisms of inactivation of this tumor suppressor gene in primary colon cancer. Tyrosine phosphorylation of β-catenin by a biochemical molecule such intestinal trefoil factor has been reported (32). As suggested by He *et al*. (11), an inducible *APC* gene that lacks endogenous functional APC exists in a colorectal cancer cell line. The data from this study show that reduced expression of E-cadherin/catenin was associated with the degree of tumor dedifferentiation, but less obviously with infiltrative growth and liver metastasis. Tumor cells in the vessels, however, were associated with invasion and metastatic potential. Expression of E-cadherin, α-catenin and β-catenin in the vessels was also strongly associated with liver metastasis. Many studies have investigated E-cadherin and α-catenin expression in colorectal cancer tissue, but did not include the vessels. This is the first study to show a relationship between expression of E-cadherin, α-catenin and β-catenin in tumor cells in the vessels and liver metastasis. A decrease in expression of cadherin/catenin conferred tight adhesiveness to the tumor mass. Furthermore, a decrease in expression of cadherin/catenin is correlated with the degree of differentiation of tumor cells and frequently confers high metastatic capacity on tumor cells (33). Various studies have shown that tumor invasion and formation of metastases is associated with an impairment of expression or structure of the E-cadherin/catenin complex (8, 9). Furthermore, functional data demonstrate that E-cadherin controls invasiveness *in vitro* and metastasis formation *in vivo* (34). On the other hand, Dorudi *et al*. demonstrated by immunohistochemical studies of primary human colon carcinomas that E-cadherin expression correlated strongly with the dedifferentiation grade of the tumor but less obviously with invasion and metastasis potential (35). Three distinct patterns of tumor cells in the blood vessels were demonstrated by Elastica van Gieson staining. One consisted of blood vessels infiltrated directly by tumor cells; the second consisted of blood vessels with floating tumor cells; and the third, blood vessels filled with tumor cell emboli. It was relatively easy to detect blood vessels infiltrated by tumor cells or blood vessels in which tumor cells were floating. However, it was difficult to detect blood vessels filled with tumor cell emboli by H & E

staining alone. Inoue *et al*. reported that vascular invasion into the colorectal wall revealed with Elastica van Gieson staining provided a more precise prediction of the recurrence of hematogenous metastasis (20). Therefore, Elastica van Gieson staining was also used in this study to detect blood vessels with tumor cell emboli, at the same time as using the tumor stain for E-cadherin, α-catenin and β-catenin. Recently, several Japanese groups have described the dedifferentiated histology at the invasive margin of the tumor (tumor budding), in spite of well-differentiated carcinoma at the tumor center. This dedifferentiation at the invasive front is useful in predicting lymph node metastasis or hematogenous metastasis in patients with colorectal carcinoma (36). Focal dedifferentiation resembles 'tumor budding', which refers to microscopic clusters of undifferentiated cancer cells just ahead of the invasive front (37). Tumor budding has been reported to correlate with a high recurrence rate and poor prognosis of colorectal cancer. It has been reported that infiltrative colorectal cancer, characterized by deep, wide infiltration by isolated individual cells, has a worse prognosis than expanding tumors (38). However, little is known regarding the clinical relevance of oncogenic β-catenin activation in the vessels at the invasive front in colorectal cancer. The current study shows that dedifferentiated cells in the vessels at the invasive front could be more useful for predicting the occurrence of liver metastasis and prognosis. Nuclear overexpression is heterogeneous and is often found in areas in contact with the extracellular matrix (invasive front). This also implies that extracellular matrix-induced epigenetic modulations may be responsible for the nuclear translocation of β-catenin. It is suggested that the microenvironment in which cancer cells grow is one of the factors involved in the regulation of invasion and metastatic behavior. For instance, specific collagens are able to promote metastatic behavior by down-regulation of E-cadherin gene expression in a Src-kinase-dependent manner (39). Ho *et al*. present a wealth of detailed evidence for a direct influence of the extracellular matrix. These authors demonstrated that biological and synthetic matrix metalloproteinase inhibitors promoted the assembly and stabilization of both focal and cell-cell contacts, and that cadherin protein levels and localization at cell-cell contacts were increased (40). The specific microenvironment may also alter promoter methylation of relevant genes. E-cadherin expression is enhanced when tumor cells are cultured as three-dimensional spheroids and is accompanied by regional demethylation within the 5' CpG island. E-cadherin promoters are dynamic and can vary in different microenvironments (41). Even if tumors cannot be cured completely, surgeons can offer the benefit of surgery for longer survival with a high quality of life. Unfortunately, however, most tumors with nuclear accumulation of β-catenin metastasize to the liver. β-catenin expression can provide important information for the selection of patients with colorectal cancer for these treatments. Continuing discoveries in molecular biology may reveal better biological markers. Appropriate application of these markers in clinical cancer care has yet to be determined, but it may prove important in the selection of patients for adjuvant therapies or other treatment modalities. These current data are important and show that nuclear accumulation of β-catenin at the invasive front and in the vessels is indicative of hematogenous liver metastasis in patients with colorectal cancer.

In conclusion, nuclear accumulation of β-catenin at the invasive front and in the vessels was indicative of liver metastasis in colorectal cancer. Nuclear overexpression is heterogeneous and is often found in areas of contact with the extracellular matrix (invasive front). Site-dependent modulation of invasive and metastatic behavior of colorectal carcinoma appears to occur and local tissue factors may play a role in invasion and metastasis. These current data show that nuclear accumulation of β-catenin in cellular cells at the invasive front and in the vessels may be important in the selection of patients for adjuvant therapies or other treatment modalities.

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Received January 24, 2008
Revised March 21, 2008
Accepted March 26, 2008