Redifferentiation and ZO-1 reexpression in liver-metastasized colorectal cancer: Possible association with epidermal growth factor receptor-induced tyrosine phosphorylation of ZO-1

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Tubular gland structures of colorectal cancer (CRC) have been demonstrated to undergo dedifferentiation at the primary site, and then the gland structures are re-formed in the liver metastases. In this study, we examined the degree of differentiation of the gland structure of 48 cases of CRCs (24 cases with synchronous liver metastasis, 24 cases without metastasis) by the modified Gleason grading system. We also investigated the role of ZO-1, one of the tight junction proteins, in the morphological changes, i.e., dedifferentiation and redifferentiation, of CRCs at the primary site and liver metastases. Liver-metastasized CRCs (2.47±0.37) showed a lower score in the modified Gleason grading system than the corresponding primary tumors (3.28±0.36) did, i.e., the tumor cells had undergone redifferentiation at liver metastases. ZO-1 was expressed at the apical cell borders of normal colorectal epithelium, the luminal side of which has tubular gland structures. In comparison with this normal epithelium, the ZO-1 expression level was frequently reduced in primary CRC with liver metastasis (20.8%) and ZO-1 was reexpressed in liver metastasized cancers (79.2%). Furthermore, it was demonstrated by an immunoprecipitation-western blotting analysis on 5 cases of CRC with liver metastasis that ZO-1 bound to epidermal growth factor receptor (EGFR) irrespective of the phosphorylation status of EGFR, and that EGFR associated ZO-1 was highly tyrosine-phosphorylated only in the primary CRC, but was dephosphorylated in the liver-metastasized cancers. Our observations suggest that tyrosine phosphorylation of ZO-1 leads to down-regulation of the function of ZO-1 and dedifferentiation of the glands in CRCs, and these phenomena contribute to liver metastases, and redifferentiation of the glands occurs in the liver metastases. (Cancer Sci 2003; 94: 166–172)

Tubular gland structures of colorectal cancer (CRC) have been demonstrated to undergo dedifferentiation at the primary site, i.e., the gland structures are broken and the cancer cells, either single or a few cells at a time, invade diffusely into the deepest portion. Subsequently, when the cancer cells reach the liver via the portal vein, the gland structures are re-formed in the liver metastases.[1–3] Brablez *et al.* called this process "redifferentiation" of the CRC cells in the liver metastases, and they also noted that adhesion molecules in the CRC lost their functions at the primary site and then recovered them in the metastases.[3] The loss and then recovery of the adhesion molecule function can not be explained by genetic alterations, but appears to be due to transcriptional and/or posttranslational modification. For example, phosphorylation of β-catenin is reported to contribute to such adhesion molecule function.[4,5]

Several investigators have reported that dysfunction of the adherens junction was associated with invasion and metastases of various tumors.[6–8] However, little information is available concerning the tight junction function in association with tumor metastasis. The tight junction is composed of several proteins such as occludin, claudin, ZO-1, ZO-2, and ZO-3. ZO-1 binds to occludin and claudin, and plays a crucial role in the function of the epithelial tight junction. Reduced expression of ZO-1 has been reported to be associated with invasion of breast and gastrointestinal cancers.[9,10] However, the precise molecular mechanism of the reduction of ZO-1 expression in the cancer cells is not understood.

Italie *et al.* noted that epidermal growth factor receptor (EGFR) induced tyrosine phosphorylation of ZO-1 and reorganized ZO-1 at the tight junctions in A431 cells.[11] We recently showed that reduced expression of ZO-1 in primary CRCs was closely related to liver metastases, and that CRCs with metastases showed more dedifferentiated phenotype than those without metastases did.[12]

In this study, we investigated the degree of differentiation of the gland structure of CRCs at the primary site and liver metastases by applying the modified Gleason grading system. We also examined the expression of ZO-1 in CRCs, and the possible mechanisms of the alteration of ZO-1 expression at the primary site and liver metastases.

Materials and Methods

Tissue specimens. Forty-eight patients who underwent surgery for CRCs and metastases at several hospitals, which routinely sent surgical specimens to our laboratory for histological diagnosis between 1986 and 1995, were examined retrospectively. Twenty-four of these patients had synchronous liver-metastasized cancers, whereas the other 24 had undergone curative resection with no signs of late occurrence of the liver metastases by February 2001. Only well-differentiated colorectal adenocarcinomas were included in this study, irrespective of other criteria. Poorly differentiated or anaplastic carcinomas showing no clear epithelial phenotype were excluded. None of the patients investigated had received chemotherapy or radiotherapy prior to resection of either the primary or metastasized tumors. This work was done with the approval of the Dokkyo University Surgical and Molecular Pathology Committee, and informed consent was obtained from all the patients.

Cell culture. A431 cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% (v/v) fetal calf serum (Bio-Whittaker, Walkersville, MD), 100 μg/ml streptomycin, 100 U/ml penicillin (Life Technologies, Inc.), and 0.25 μg/ml amphotericin B

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(Life Technologies, Inc.) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Modified Gleason grading system. The Gleason grading system was originally applied to prostatic carcinoma.[13] We slightly modified it for evaluating differentiation of colorectal cancers, as follows: pattern 1, well-differentiated uniform single glands, closely packed in masses with relatively circumscribed boundaries (Fig. 1A); pattern 2, well-differentiated but more variable single glands, spaced slightly apart, boundaries of tumor less well circumscribed (Fig. 1B); pattern 3, moderately differentiated glands, possibly ranging from small to large, growing in spaced-out infiltrative patterns, being either papillary or cribriform (Fig. 1C); pattern 4, raggedly infiltrating, fused-glandular tumor, frequently with pale cells, possibly resembling hypernephroma of the kidney (Fig. 1D); pattern 5, anaplastic carcinoma with minimal glandular differentiation, and diffusely infiltrating the stroma (Fig. 1E). The modified Gleason score was calculated by taking the average of the number of Gleason grading patterns in at least seven fields in the entire area of the tumor (at a power field of ×100).

Immunohistochemistry. A rabbit polyclonal antibody against rabbit human-ZO-1 (Zymed, San Francisco, CA) was used for immunohistochemical staining, which was performed using a labeled streptavidin-biotin kit according to the manufacturer's protocol (DAKO, Carpinteria, CA). All the tissue specimens were fixed with 10% (v/v) formalin, then embedded in paraffin, and sections (4-μm-thick) were cut, placed on glass slides and deparaffinized. The slides were immersed in 0.3% (v/v) hydrogen peroxide in methanol for 30 min to deplete the endogenous peroxidase, treated with microwave radiation (400 W, 10 min) for antigen retrieval, and then incubated with the primary anti-ZO-1 antibody (diluted 1:100) in a humidified chamber at 37°C for 1 h. Biotinylated anti-rabbit immunoglobulin G was used as the secondary antibody. Following treatment with peroxidase-conjugated streptavidin, the sections were washed with cold 10 m*M* phosphate buffer (pH 7.2) containing 140 m*M* NaCl, allowed to react with a mixture of 0.05% (v/v) 3,3′-diaminobenzidine tetrahydrochloride solution and 0.01% (v/v) hydrogen peroxidase in 10 m*M* phosphate buffer (pH 7.2) containing 140 m*M* NaCl, and then counterstained with hematoxy-

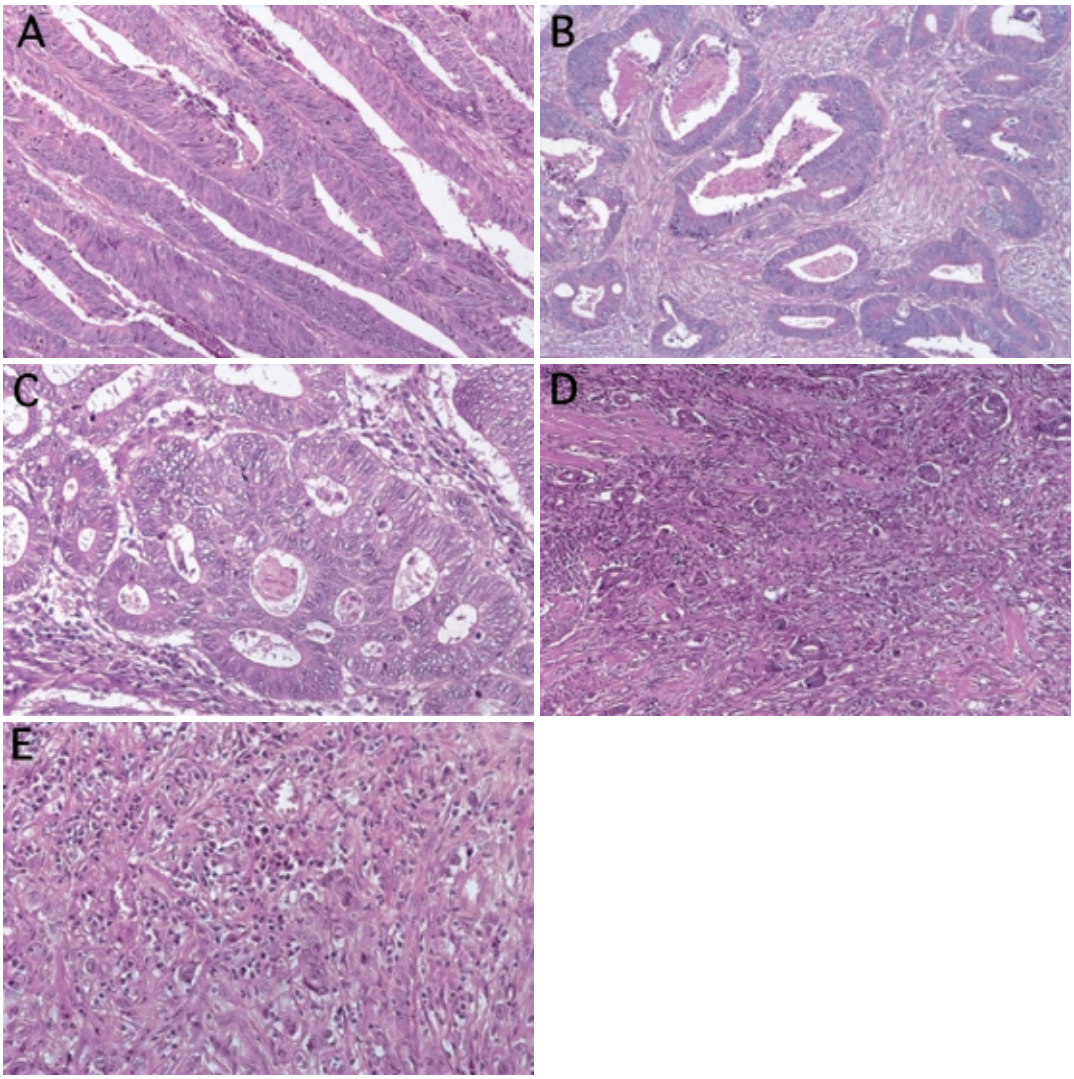

Fig. 1. Hematoxylin and eosin staining of colorectal cancers with various Gleason gradings. (A) Pattern 1, well-differentiated uniform single glands, closely packed in masses with relatively circumscribed boundaries; (B) pattern 2, well-differentiated but more variable single glands, spaced slightly apart, boundaries of the tumor less well circumscribed; (C) pattern 3, moderately differentiated glands, possibly ranging from small to large, growing in spaced-out infiltrative patterns, possibly papillary or cribriform; (D) pattern 4, raggedly infiltrating, fused-glandular tumor, frequently with pale cells, possibly resembling hypernephroma of kidney; (E) pattern 5, anaplastic carcinoma with minimal glandular differentiation, and diffusely infiltrating the stroma.

lin. Normal colonic epithelium was used as a positive control. For the majority of the primary tumors, normal epithelium adjacent to the tumor served as an internal positive control. Negative controls consisted of duplicate sections stained simultaneously without the primary antibody.

Evaluation of immunostaining. The intensity of ZO-1 staining of the cancer cells was compared with that of normal colonic epithelium. The pattern of ZO-1 expression of the cancer cells was judged from the dominant pattern of ZO-1-positive cells. ZO-1 staining was intense at the apicolateral boundaries of epithelial cells in normal colorectal glands. Cancer cells that were stained at the apical cell borders in the same manner as normal epithelial cells were classified as having a preserved pattern (Fig. 2A). Cancer cells showing less intense (relative to that of normal colonic epithelium) staining at the apical cell borders and staining of the cytoplasm (Fig. 2B) were classified as having a cytoplasm pattern, and cancer cells with no immunolabeling were classified as negative (Fig. 2C). Both the cytoplasm and negative patterns were classified as reduced patterns. Before conducting the immunohistochemical staining, we confirmed the specificity of the anti-ZO-1 antibody by western blotting on A431 cells, which were reported to express ZO-1 protein[11] and also on colon cancer tissues (Fig. 2D). The antibody recognized both α+ form and α− form of ZO-1 as the provider described.

Immunoprecipitation and western blotting. A431 cells were treated with or without epidermal growth factor (EGF; 100 ng/ml) for 15 min at 37°C, and the cells were harvested with a rubber policeman on ice. The cells were lysed in 10 m*M* 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (pH 7.4), 150 m*M* NaCl, 0.2% sodium dodecyl sulfate, 0.5% Na deoxycholate, 1% Triton X-100, 1 m*M* phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 10 U/ml aprotinin, 2 m*M* ethylenediaminetetraacetic acid, 400 μ*M* Na_3VO_4 and 10 m*M* NaF. The cell lysates were incubated with a sheep anti-EGFR (UBI, Lake Placid, NY) or the rabbit anti-ZO-1 antibody for 6 h at 4°C, and then the complexes of the antibodies and the target protein were precipitated with protein A-agarose (Sigma, St. Louis, MO). Immunoprecipitated materials and whole tissue lysates were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the proteins on the gels were transferred electrophoretically to a nitrocellulose membrane (Bio-Rad, Hercules, CA) at 50 V for 4 h. The nitrocellulose membrane was blocked with 5% skim milk in 100 m*M* Tris-HCl (pH 7.5) containing 150 m*M* NaCl, 0.1% Tween 20, and incubated with the sheep anti-EGFR diluted 1:500, the rabbit anti-human ZO-1 antibody diluted 1:500 or an anti-phosphotyrosine mouse monoclonal antibody (UBI) diluted 1:500. An Amersham ECL kit (Amersham, Arlington Heights, IL) was used for detection.

Statistical analysis. χ^2 analysis was performed to determine the correlations among the various clinicopathological parameters and Fisher's exact test or paired Student's *t* test was performed as necessary. A correlation with a *P* value of <0.05 was accepted as statistically significant.

Results

Patients. Table 1 shows the details of the 24 CRC patients with synchronous liver metastasis and the 24 CRC patients without liver metastases. The age and sex distributions of the two groups were matched.

Modified Gleason scores in primary CRCs without metastasis, primary CRCs with metastasis and liver-metastasized cancers. The modified Gleason score of primary CRCs without metastases was lower than that of the primary CRCs with metastases (Table 1). However, interestingly, the modified Gleason score of the liver metastases was significantly lower than that of the corresponding primary tumors (21 cases), except for three tumors

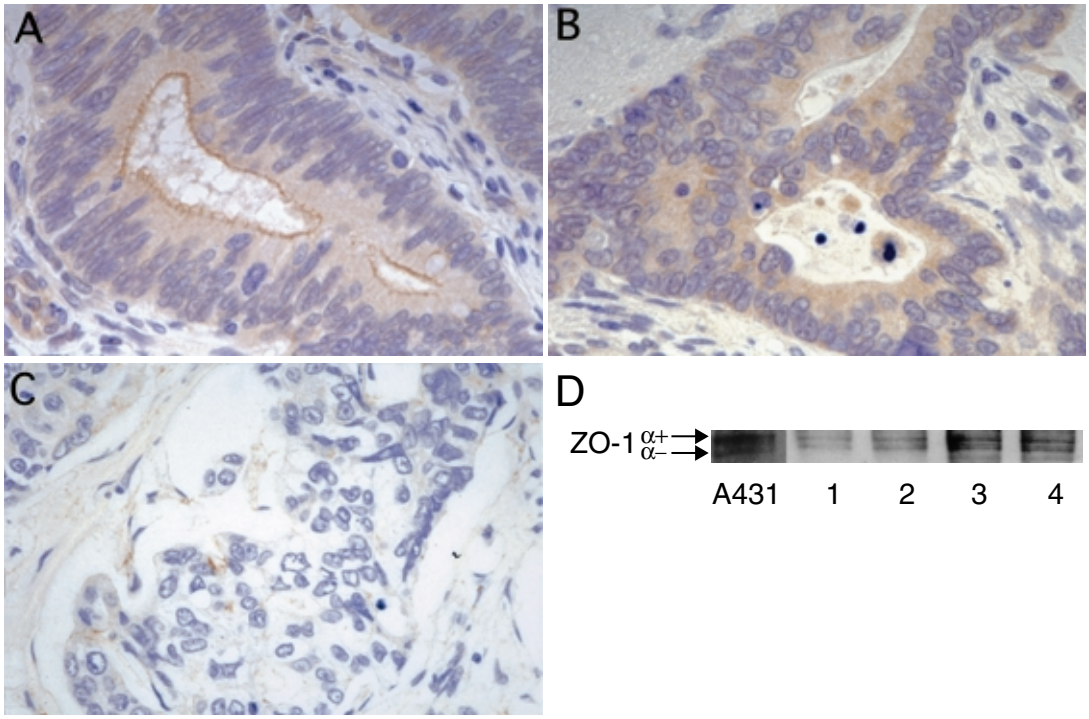

Fig. 2. Immunohistochemical staining of colorectal cancer for ZO-1. (A) Expression of ZO-1 is observed at the apical cell border. These tumors were classified as "preserved" type. (B) Expression of ZO-1 is observed at the cell membrane and in the cytoplasm. These tumors were classified as "cytoplasm" type. (C) No expression of ZO-1 in colorectal cancer. These tumors were classified as "negative" type. Western blot analysis of A431 cells and colon cancer tissues by the anti-ZO-1 antibody which was used for immunohistochemistry. A431 cells and colon cancer tissues (lanes 1–4) expressed 225 kDa (ZO-1 α+ form) and 200 kDa (ZO-1 α− form) proteins, which reacted with the anti-ZO-1 antibody.

Table 1. Modified Gleason score and expression of ZO-1 in colorectal cancer

Patient	Age (yrs)	Sex	TNM classification	Modified Gleason grading		Expression of ZO-1	
				Primary	Metastases	Primary	Metastases
Primary CRC[1] with metastases							
1	65	M[2]	T3N1M1	3.0	1.8	Rd[4]	Pre[5]
2	79	M	T3N1M1	3.3	2.0	Rd	Pre
3	47	M	T3N1M1	3.5	2.0	Rd	Pre
4	65	F[3]	T3N0M1	3.3	2.0	Rd	Pre
5	72	M	T3N1M1	3.7	2.0	Rd	Pre
6	79	M	T3N1M1	3.0	2.2	Pre	Pre
7	77	M	T3N1M1	3.3	2.2	Rd	Pre
8	47	M	T3N0M1	2.4	2.3	Pre	Pre
9	63	F	T3N1M1	3.2	2.5	Rd	Pre
10	68	M	T3N1M1	4.0	2.6	Rd	Pre
11	57	M	T2N1M1	2.9	2.6	Rd	Pre
12	59	F	T3N0M1	3.3	2.6	Rd	Pre
13	43	F	T3N1M1	4.0	2.7	Rd	Rd
14	63	F	T3N1M1	3.4	2.7	Rd	Pre
15	63	M	T3N1M1	3.2	2.7	Pre	Pre
16	33	F	T3N1M1	3.5	2.7	Rd	Pre
17	55	F	T3N1M1	3.1	2.9	Rd	Pre
18	73	M	T3N1M1	3.2	3.0	Rd	Rd
19	58	F	T3N1M1	3.0	2.9	Rd	Rd
20	68	F	T3N1M1	3.4	3.0	Rd	Pre
21	53	M	T3N2M1	3.2	2.5	Pre	Pre
22	51	M	T3N0M1			Rd	Pre
23	53	M	T3N1M1			Rd	Rd
24	56	M	T3N0M1			Rd	Rd
Primary CRC without metastasis							
1	74	F	T2N1M0	1.4		Pre	
2	48	M	T3N1M0	1.8		Pre	
3	60	M	T3N0M0	1.9		Pre	
4	65	M	T3N0M0	2.0		Pre	
5	50	F	T3N0M0	2.0		Rd	
6	73	M	T3N0M0	2.1		Pre	
7	56	F	T3N1M0	2.1		Rd	
8	65	M	T3N1M0	2.2		Pre	
9	60	M	T3N1M0	2.3		Pre	
10	66	F	T3N0M0	2.3		Pre	
11	57	M	T3N0M0	2.5		Pre	
12	58	M	T3N1M0	2.6		Pre	
13	82	M	T3N0M0	2.6		Pre	
14	33	M	T3N0M0	2.6		Pre	
15	67	M	T3N2M0	3.1		Pre	
16	61	F	T3N0M0	2.8		Rd	
17	67	M	T3NOM0	2.8		Pre	
18	72	F	T3N2M0	2.8		Pre	
19	85	M	T3N0M0	3.1		Pre	
20	51	F	T3N0M0	3.1		Pre	
21	65	M	T3N0M0	3.2		Pre	
22	57	F	T3N0M0	3.2		Rd	
23	51	F	T3N1M0	3.3		Rd	
24	58	M	T3N2M0	3.3		Rd	

1) CRC, colorectal cancer.
2) M, male; *3*) F, female.
4) Rd, reduced; *5*) Pre, preserved.

with mucinous components (case numbers 22, 23 and 24 in CRC with liver metastasis) ($P<0.001$, Table 1, Fig. 3). Thus, decrease of the modified Gleason score, in other words, redifferentiation, was demonstrated in 21/24 liver metastases (87.5%). Mucinous adenocarcinomas of the colon and rectum appear to show a more aggressive clinical course and have a less favorable prognosis than lesions classified as adenocarcino-mas.[14–16] Therefore, we excluded three tumors with mucinous components for evaluating the modified Gleason score (Table 1, Fig. 3).

Immunohistochemical expression of ZO-1 in primary CRCs without metastasis, primary CRCs with metastasis and liver-metastasized cancers. The preserved staining ratio of ZO-1 in primary CRCs with liver metastases (5/24: 20.8%, Table 2) was lower than that in primary CRCs without liver metastases (18/24: 75.0%, $P=0.0002$, Table 2, Fig. 4). The preserved staining ratio of ZO-1 in the liver-metastasized cancers (19/24: 79.2%, Table 2) was higher than that in the corresponding primary CRCs ($P<0.0001$, Table 2). Fifteen primary CRCs with liver metas-

tases which had reduced ZO-1 expression showed reexpression of ZO-1 in the liver-metastasized cancer.

Relationship between the modified Gleason score and ZO-1 expression. We classified the modified Gleason scores into 2 categories, low (score <3) and high (score ≥3), for all cancers. The cancers with high Gleason scores were less differentiated or dedifferentiated than those with low scores (Table 3). Twenty-one (75.0%) of the 28 patients with high scores had preserved expression of ZO-1 (Table 3). The high modified Gleason scores correlated highly with reduced expression of ZO-1 (P<0.0001, Table 3).

Association of ZO-1 with EGFR irrespective of the phosphorylation status of EGFR in colorectal cancers. We investigated the tyrosine-phosphorylation of EGFR in normal colorectal mucosa and CRCs. Samples were immunoprecipitated with the anti-EGFR antibody and the immunoprecipitates were immunoblotted with a specific anti-phosphotyrosine antibody. EGFR in CRC tissue was tyrosine-phosphorylated, whereas EGFR in normal colonic mucosa was not (Fig. 5). In this experiment, we used EGF-stimulated A431 cells as a positive control, and detected a clear tyrosine-phosphorylated EGFR band (Fig. 5A). Moreover, we confirmed that the anti-EGFR antibody precipitated a similar amount of EGFR (data not shown). When we immunoblotted the same immunoprecipitates with anti-ZO-1 antibody, we detected the EGFR-associated ZO-1 in all lanes (Fig. 5B). These observations suggested that ZO-1 bound to EGFR irrespective of the phosphorylation status of EGFR.

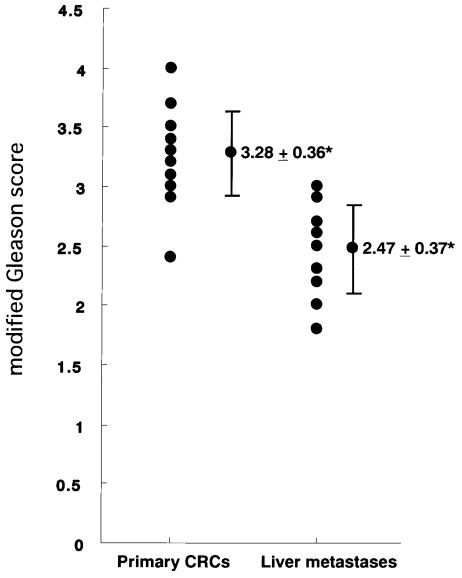

Fig. 3. Modified Gleason scores of CRCs at the primary site and liver metastases. Average score for liver-metastasized cancers was significantly lower than that for primary CRCs (P<0.001, paired Student's t test). * mean±standard deviation.

Table 2. Expression of ZO-1 in colorectal cancer

	Expression of ZO-1		P value[1]
	Preserved (%)	Reduced (%)	
Primary CRC[2] without metastasis (n=24)	18 (75.0)	6 (25.0)	0.0002
Primary CRC with metastases (n=24)	5 (20.8)	19 (79.2)	<0.0001
Liver-metastasized cancer (n=24)	19 (79.2)	5 (20.8)	

1) Evaluation by the χ^2 test.
2) Colorectal cancer.

Tyrosine phosphorylation of ZO-1 in CRCs. We analyzed the tyrosine phosphorylation of ZO-1 in 5 cases of primary CRCs with metastases, normal colorectal mucosa, liver-metastasized cancers and normal liver tissue. An example of the results for CRC with liver metastases is shown in Fig. 6. We detected the EGFR-associated ZO-1 in all samples tested, normal colonic mucosa, primary CRC, normal liver tissue, and liver-metasta-

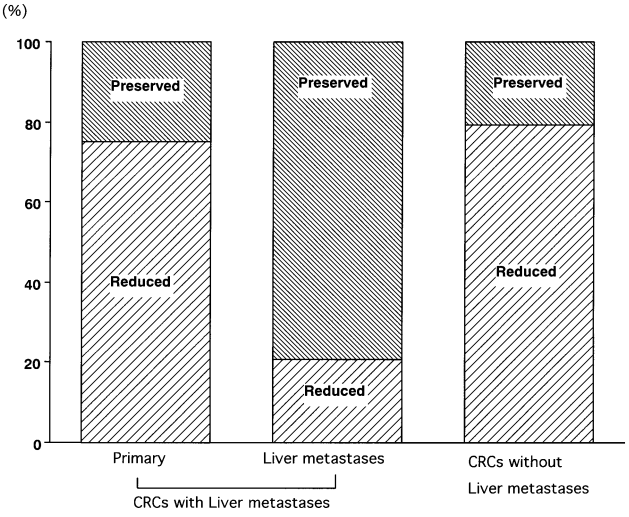

Fig. 4. Immunohistochemical expression of ZO-1 in primary CRCs with liver metastases, liver-metastasized cancer and primary CRCs without liver metastases. The percentage of the preserved membrane staining of ZO-1 in primary CRC with liver metastases is lower than that in primary CRC without metastasis. The percentage of the preserved membrane staining of ZO-1 in liver-metastasized cancer is higher than that in the corresponding primary CRCs.

Table 3. Correlation between modified Gleason grading and ZO-1 expression

	Expression pattern of ZO-1		P value[1]
	Preserved (%)	Reduced (%)	
Modified Gleason grading			
High score (3≤)	7 (25.0)	21 (75.0)	<0.0001
Low score (<3)	32 (84.2)	6 (15.8)	

1) Evaluation by the χ^2 test.

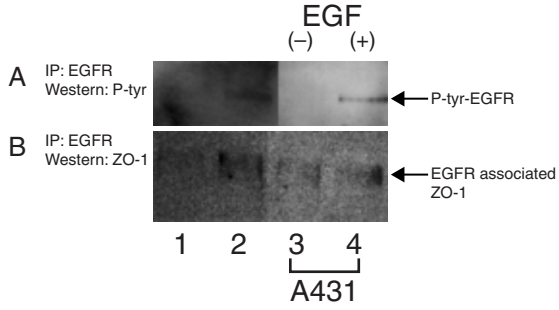

Fig. 5. Association of EGFR with ZO-1. Lane 1, normal colorectal mucosa; lane 2, CRC tissue; lane 3, non-stimulated A431 cells; lane 4, EGF-stimulated A431 cells. (A) EGFR in CRC tissue (lane 2) and EGF-stimulated A431 cells (lane 4) are tyrosine-phosphorylated, whereas EGFR in normal colorectal mucosa (lane 1) and non-stimulated A431 cells (lane 3) are not tyrosine-phosphorylated. (B) ZO-1 binds to EGFR in normal colorectal mucosa (lane 1), CRC tissue (lane 2), non-stimulated A431 cells (lane 3), and EGF stimulated A431 cells (lane 4).

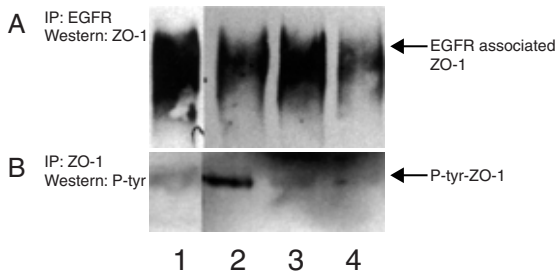

Fig. 6. Immunoprecipitation and western blotting of CRC tissue with liver metastases. Lane 1, adjacent normal colorectal mucosa; lane 2, primary CRC tissue; lane 3, normal liver tissue; lane 4, liver-metastasized cancer. (A) EGFR binds to ZO-1 in all of the tissues. (B) ZO-1 is highly tyrosine-phosphorylated only in the primary CRC, but was dephosphorylated in the liver-metastasized cancers.

sized cancer. Most interestingly, ZO-1 was highly tyrosine-phosphorylated only in the primary CRC, but was dephosphorylated in the liver-metastasized cancers. Although the results are not shown, we observed the same pattern of ZO-1 tyrosine phosphorylation in other cases, i.e., tyrosine phosphorylation at the primary site, and dephosphorylation at liver-metastasized sites.

Discussion

In this study, we demonstrated that liver-metastasized CRCs showed lower score in the modified Gleason grading system than the corresponding primary tumors did, i.e., the tumor cells had undergone redifferentiation at liver metastases. In order to clarify the mechanism responsible for this phenotypical alteration, i.e., redifferentiation, we investigated ZO-1 expression in primary CRCs and liver-metastasized cancers. ZO-1 was expressed at the apical cell borders of normal colorectal epithelium, the luminal side of which has tubular gland structures. In contrast with this normal epithelium, the ZO-1 expression level was frequently reduced in primary CRC and ZO-1 was reexpressed in liver-metastasized cancers. Furthermore, we clearly showed that ZO-1 bound to EGFR irrespective of the phosphorylation status of EGFR, and that EGFR-associated ZO-1 was highly tyrosine-phosphorylated only in the primary CRC, but was dephosphorylated in the liver-metastasized cancers.

Tumor dedifferentiation and redifferentiation have been reported to be related to transient loss of function of the adherens junction proteins E-cadherin and β-catenin.[1–3] However, to our knowledge, it has rarely been reported that tumor differentiation is related to the function of tight junction proteins. We hypothesize that adherens junctions are primarily important for the adhesion of cell-cell junctions, but the function of the tight junctions may be more important than that of the adherens junctions for the construction of tubular gland structures. If the cellular polarity is maintained in the cancer cells, cells adhere to each other at the adherens junction, and form a basement membrane, then the tight junctions at the apical cell borders are closed by the tight junction proteins and subsequently tubular gland structures are formed.

Italie *et al.* reported that ZO-1 bound to EGFR and EGFR induced tyrosine phosphorylation of ZO-1 in cultured cells.[11] However, as far as we are aware, these phenomena have not been observed in tissue specimens. In this study, we clearly demonstrated that ZO-1 bound to EGFR irrespective of the phosphorylation status of EGFR, and that EGFR-associated ZO-1 was highly tyrosine-phosphorylated only in the primary CRC, but was dephosphorylated in the liver-metastasized cancers. We preliminarily investigated the tyrosine phosphorylation levels of normal colorectal mucosa, CRCs and normal liver tissue. The tyrosine-phosphorylated protein levels of CRCs and normal colorectal mucosa were much higher than that of normal liver tissue (data not shown). In the static normal liver, most of the tyrosine kinase, such as EGFR may not be activated, and growth-stimulatory signals may be shut off.

Recently, EGFR has been shown to be associated with F-actin,[17, 18] and to phosphorylate actin-binding proteins, such as ezurin and spectrin, as well as modifying the function of actin.[19, 20] On the other hand, ZO-1 has been shown to bind directly to F-actin *in vitro*.[21, 22] Then, EGFR binds to ZO-1 via F-actin, and modifies the function of ZO-1 by tyrosine phosphorylation. However, it is also possible that EGFR directly binds to ZO-1 via the PDZ domain.

Taking the previous reports and our current study together, activation of EGFR and tyrosine phosphorylation of ZO-1 lead to down-regulation of the function of ZO-1 and dedifferentiation of the glands in CRCs, and these phenomena contribute to the development of liver metastases. Subsequently, decrease of the tyrosine-phosphorylated ZO-1 level may lead to recovery of the function of ZO-1 and redifferentiation of the glands in liver metastases.

We are now conducting studies to determine whether tyrosine phosphorylation of ZO-1 changes the binding affinity of ZO-1 to occludin and claudin, thereby changing the function of the tight junction and consequently influencing the metastatic potential of CRCs.

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