Increased Claudin-1 Protein Expression in Hepatic Metastatic Lesions of Colorectal Cancer

TETSUSHI KINUGASA[1], YOSHITO AKAGI[1], TAKAFUMI OCHI[1], NATSUKI TANAKA[1], AKIHIKO KAWAHARA[2], YOSHINOBU ISHIBASHI[1], YUKITO GOTANDA[1], KEIZO YAMAGUCHI[1], ICHITAROU SHIRATUCHI[1], YOUSUKE OKA[1], MASAYOSHI KAGE[2] and KAZUO SHIROUZU[1]

Departments of [1]Surgery and [2]Pathology, Kurume University School of Medicine, Fukuoka, Japan

Abstract. *Background: The molecular and morphological alterations of the tight junctions in hepatic metastatic lesions are poorly understood. The possible involvement of claudin-1 (CL-1), which is one of the major tight junctional proteins, was investigated in the tumorigenesis of hepatic metastasis in patients with colorectal cancer (CRC). Patients and Methods: A total of 14 patients with hepatic metastasis of CRC who underwent surgical treatment from January 2007 until December 2010 at the Kurume University Hospital in Fukuoka, were examined. CRC tissue specimens were analyzed to determine whether the levels of CL-1 correlated with clinicopathological factors and to determine the roles of CL-1, β-catenin, and E-cadherin in the alterations of the tight junctions during tumorigenesis. Results: In seven cases, the tumors were located in the colon, while the other seven tumors were found in the rectum. There were eight cases of synchronous liver metastasis, while there were six cases of metachronous liver metastasis. The levels of the CL-1 protein were up-regulated in CRC and in hepatic metastatic lesions. The levels of β-catenin were positive or up-regulated in the primary CRC lesions and in hepatic metastatic lesions. Despite the finding that the levels of E-cadherin were decreased in CRC, they were clearly up-regulated in hepatic metastatic lesions in this study. Conclusion: This study demonstrated that CL-1 levels were up-regulated in liver metastatic lesions from primary CRC lesions. Moreover, the levels of E-cadherin were increased in liver metastatic lesions, which may point to the existence of interactions between CL-1 and E-cadherin in hepatic metastatic lesions. These observations suggest that CL-1 plays a pivotal role in the regulation of cellular morphology and in the behavior of metastatic processes in CRC.*

Correspondence to: Dr. Tetsushi Kinugasa, Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka, Japan. Tel: +81 942353311, Fax: +81 942340709, e-mail: kinugasa_tetsushi@med.kurume-u.ac.jp

Key Words: Hepatic metastasis, colorectal cancer, claudin-1.

Colorectal cancer (CRC) is one of the most frequently diagnosed types of cancer worldwide. There were more than one million diagnosed new cases (9.8% of worldwide cancer diagnoses) and 600,000 deaths (8.1% of all worldwide cancer deaths) caused by the specific disease in 2008 (1). CRC has been ranked as a leading type of cancer in many developed countries including Japan. In Japan, the incidence of CRC has increased significantly in recent years, due to changing in lifestyles, and CRC is one of the most prominent causes of death from neoplastic diseases (2). Clinically, the major cause of death from CRC is due to distant metastases to organs such as the liver and the lung (3), while an improvement of CRC patients' survival rates can be expected if metastasis can be controlled. Hepatic metastasis, which is the most common form of distal spread of primary CRC, occurs in over 50% of patients with metastasis (4). Some studies have recently shown that these patients have variable 5-year survival rates that range from 36% to 58% (5-9). Characterization of the key molecules involved in these processes is particularly promising for the development of novel approaches to the treatment of CRC.

Tight junctions (TJs) are the most apical structures of cells, and tight junction proteins (TJPs) are very important in the maintenance of normal epithelial physiology. Recent studies have shown that considerable changes in the expression of TJPs are associated in various types of cancers. Neoplastic cells frequently exhibit both structural and functional TJ disorganization. Claudins (CLs), which are key components of TJs, form the backbone of TJ strands (10). CLs which belong to a family consisting of at least 24 members, and contain four transmembrane domains, a C-terminal domain that serves as a binding site for interactions with a complex set of proteins, and a site for PDZ-domain proteins that are involved in intracellular signaling (11-13).

The altered expression of CL-1 has been shown to have prognostic value in colon cancer (12, 14, 15). Differential expressions of genes encoding TJPs have been detected in CRC (12, 16). In addition, the up-regulated expression of CL-1 in CRC has been reported (14, 15, 17-19). These studies suggest that the CL family, especially CL-1, plays a causal role in the process of

cellular transformation and invasion in CRC. However, there have been few studies that have focused on the expression of CL-1 in the distant metastases. Therefore, it is important to investigate the expression of CL-1 in CRC tissues of patients with hepatic metastases, in order to determine the potential clinical efficacy of CL-1 in the therapy and diagnosis of hepatic metastasis of CRC.

The aim of this study was to analyze the correlation between the levels of expression of CL-1 in hepatic metastases from CRC and in primary lesions in order to investigate its possible functions in tumorigenesis and metastasis in patients with CRC.

Patients and Methods

Patient and tissue samples. This study included 14 patients with hepatic metastases of CRC who underwent liver resection (LR) with surgical treatment from January 2007 until December 2010 at the Kurume University Hospital in Fukuoka. Informed consent was obtained from all patients before surgical resections were performed. Approval from the Institutional Review Committee for Research on Human Subjects at the Kurume University Hospital was also obtained. Tumor differentiation and the degree of invasion were examined by pathologists, and histopathological classification was performed according to the General Rules for Colorectal Cancer Study (20). In addition, clinicopathological factors were assessed according to the tumor node metastasis (TMN) classification system of the International Union Against Cancer (UICC). Hepatic metastasis of CRC was confirmed in all patients by histological examinations of specimens derived from the LRs.

LR for hepatic metastasis of CRC. Before surgery, all patients were thoroughly evaluated with appropriate imaging studies, including computed tomography (CT) of the abdominal and pelvic areas and, chest CT, in order to determine the clinical status of the CRC and the hepatic metastasis status. Positron-emission tomography (PET), and PET/CT was not routinely performed. Resectability with curative intent required complete resection of all hepatic metastatic lesions and preservation of a sufficient volume of remnant liver.

Histopathology. After surgery, the tumor specimens, the resection margin, and the lymph nodes were fixed in formalin and were embedded in paraffin. Hematoxylin and eosin (H.E.)-stained sections were reviewed in order to establish the pathological diagnosis.

Immunohistochemistry. Immunohistochemistry was performed as described previously (14, 21). The tissue sections, CRC lesions and hepatic metastatic lesions, were stained with mono-/polyclonal antibodies (diluted 1:100) against CL-1 (Zymed Laboratories Inc., San Francisco, CA, USA), E-cadherin (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA), and β-catenin (Dako Denmark A/S, Glostrup, Denmark). Assessments of immunostaining were rated as being positive (>10%) or negative (<10%).

Results

Clinicopathological features. The clinical information of the patients is summarized in Table I. The mean age of the patients on the date when surgery was performed was 66.9±10.1 (minimum: 43; maximum: 81) years. Fourteen patients were included in the study, eight were men and six women. In seven cases, the tumors were located in the colon, while in the other seven, the tumors were found in the rectum. All cases were of advanced cancer that had invaded the subserosa, the serosa (T3), and the surrounding organs (T4). Four cases had well-differentiated tumors; eight had moderately differentiated tumors, and only one had poorly differentiated tumor. Another case was of a carcinoid tumor. In our data, there was no relationship between preoperative carcinoembryonic antigen (CEA) or carbohydrate antigen (CA19-9) values in serum and liver metastases. Eight cases had synchronous liver metastases, while six cases had metachronous liver metastases. The histopathological findings that were seen in almost all of the cases were definite venous invasion and/or lymphatic invasion.

Levels of expression of CL-1, β-catenin, and E-cadherin protein in primary CRC and hepatic metastatic tissue specimens. Immunostaining was used to confirm the changes in the distribution pattern of CL-1, β-catenin and E-cadherin (Figure 1). In the normal colonic mucosa, all of the epithelial cells expressed CL-1 along the cell membrane, but not in the cytoplasm. Figure 1 demonstrates that the expression of CL-1 protein was up-regulated in CRC and in hepatic metastatic lesions. The immunostaining pattern of the CRC and hepatic metastatic lesions for CL-1 showed much stronger and more diffuse staining not only of the membrane, but also of the cytoplasm in both types of lesions, excluding one case of hepatic metastatic lesions.

The immunostaining pattern for β-catenin was much stronger and more diffuse in the primary CRC lesions and in hepatic metastatic lesions than in normal colonic mucosa (Figure 1). The expression of β-catenin was also positive or up-regulated in the primary CRC lesions and in the hepatic metastatic lesions. The β-catenin expression pattern was the same as the CL-1 and was found along the cell membrane and cytoplasm in both types of lesions. In addition, we demonstrated that β-catenin was expressed in the nucleus, a finding that is noteworthy.

The expression of E-cadherin was negative or down-regulated in all of the CRC primary tissue specimens. Interestingly, the immunostaining clearly demonstrated that the levels of E-cadherin expression were up-regulated in hepatic metastases, especially in the membranes of the lesions (Figure 1). An increase in the expression of the E-cadherin protein was observed in 13 out of 14 cases, as was found for the CL-1 expression.

Discussion

This study demonstrated that CL-1 was up-regulated in hepatic metastatic lesions from primary CRC compared to normal colonic mucosa. Moreover, the expression of E-cadherin was also increased in the hepatic metastatic lesions, and this may

Table I. *Clinical information on patients and their colorectal cancer specimens (n=14).*

Case	Age (years)	Gender location	Primary grade	Histological	Depth type	Metastatic (ng/mL)	CEA (Unit/mL)	CA19-9	ly	v	CL-1	
											P	H
1	53	M	R	Well	T3	Meta	708.3	635.1	+	+	+	+
2	43	F	R	Carcinoid	T4	Synch	8.1	33.1	+	+	+	–
3	76	F	R	Mod	T3	Meta	230.7	534.7	+	+	+	+
4	60	M	R	Well	T3	Synch	8.7	82.5	+	+	+	+
5	72	F	C	Mod	T3	Meta	1	0	+	+	+	+
6	73	F	C	Well	T3	Synch	19.8	36.8	+	+	+	+
7	65	M	C	Well	T4	Synch	2.5	18.6	+	+	+	+
8	68	M	R	Mod	T3	Meta	5.9	10.7	+	+	+	+
9	59	M	R	Mod	T4	Synch	42	99.5	+	+	+	+
10	70	M	C	Mod	T3	Meta	151.8	4270	+	+	+	+
11	73	M	R	Mod	T4	Synch	26.3	1	–	+	+	+
12	81	F	C	Mod	T3	Meta	69.3	287.2	+	+	+	+
13	69	F	C	Mod	T3	Synch	16.9	1.3	+	+	+	+
14	74	M	C	Poor	T3	Synch	14.6	2.7	+	–	+	+

CEA: Carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; CL-1: claudin-1. P: Primary lesion; H: hepatic metastasis; M: male; F: female; C: colon; R: rectum; Well: well-differentiated adenocarcinoma; Mod: moderately-differentiated adenocarcinoma; Poor: poorly-differentiated adnocarcinoma; Synch: synchronous; Meta: metachronous; ly: lymphatic invasion; v: venous invasion; n: negative; +: positively stained; –: negatively stained.

have contributed to the changes in the expression of CL-1. These observations suggest that CL-1 plays a pivotal role in the regulation of cellular morphology and the behavior of metastatic processes in CRC. We speculate that increased CL-1 expression may be involved in the early stages of transformation in CRC-associated metastasis. CL-1 protein may therefore be a good candidate for the targeted therapy of CRC patients with hepatic metastasis.

Disruption of the cell-cell junction and detachment of tumor cells from the primary lesion is the first step in cancer cell invasion and metastasis. There have been some reports that CL-1 expression enhances invasive abilities and metastatic properties, possibly by increasing matrix metalloproteinase-2 (MMP-2) activity. This has been shown, for example, in colon cancer (13), melanoma (22), and oral squamous cell carcinoma (23). CL-1 has been shown to directly interact with membrane-type-1 (MT1)-MMP and pro-MMP2 and, recruit them to the cell surface, where it consequently activates pro-MMP-2 (24). Dhawan *et al*. reported that E-cadherin transcription increased and the expression of mesenchymal markers and characteristics decreased after the inhibition of CL-1 expression. This was consistent with data showing that the loss of E-cadherin consistently occurred at sites of epithelial- mesenchymal transition (EMT), during development and in cancer athymic mice (13).

Taken together, these data strongly suggest that CL-1 induces cancer invasion/metastasis. The overexpression of CL-1 in cancer cells not only promotes MMP-2 activation, but also influences the expression of several important tumor invasiveness- and metastasis-related genes (25). Additional mechanisms and downstream signaling pathways that are relevant to CL-1 expression in colon adenocarcinoma need further investigation. However, the present observation raises the possibility that CL-1 could be exploited as a biomarker for progression of colonic adenocarcinoma, and might provide new opportunities for therapeutic intervention.

This study was undertaken in order to gain insight into the biological significance of altered CL-1 expression in CRC. Our data demonstrated increased CL-1 expression in human CRC, particularly in metastatic lesions, where it was frequently mislocalized from the cell membrane to the cell cytoplasm and nucleus.

Mutations of the adenomatous polyposis coli (*APC*) gene (thus, β-catenin activation and nuclear translocation) are present in the majority of human CRCs (26). CL-1 was recently identified as a probable target of β-catenin/T-cell factor (Tcf) transcriptional activation in SW480 cells (27). It is interesting that colon cancer cells that expressed CL-1 (HT29, SW480, and SW620), all harbor mutations in *APC* and have activated β-catenin/Tcf signaling. SW480 cells which are transfected with the CL-1 gene formed tumors at a significantly faster rate and caused multiple liver metastases compared to control SW480 cells, which did not form any liver metastasis (13).

Several molecular alterations that contribute to sporadic CRC have been found, including the loss of *APC* and *p53* tumor suppressor gene function. However, the timing and sequence in which these genetic mutations occur differ from those of sporadic CRC, especially for the metastatic processes.

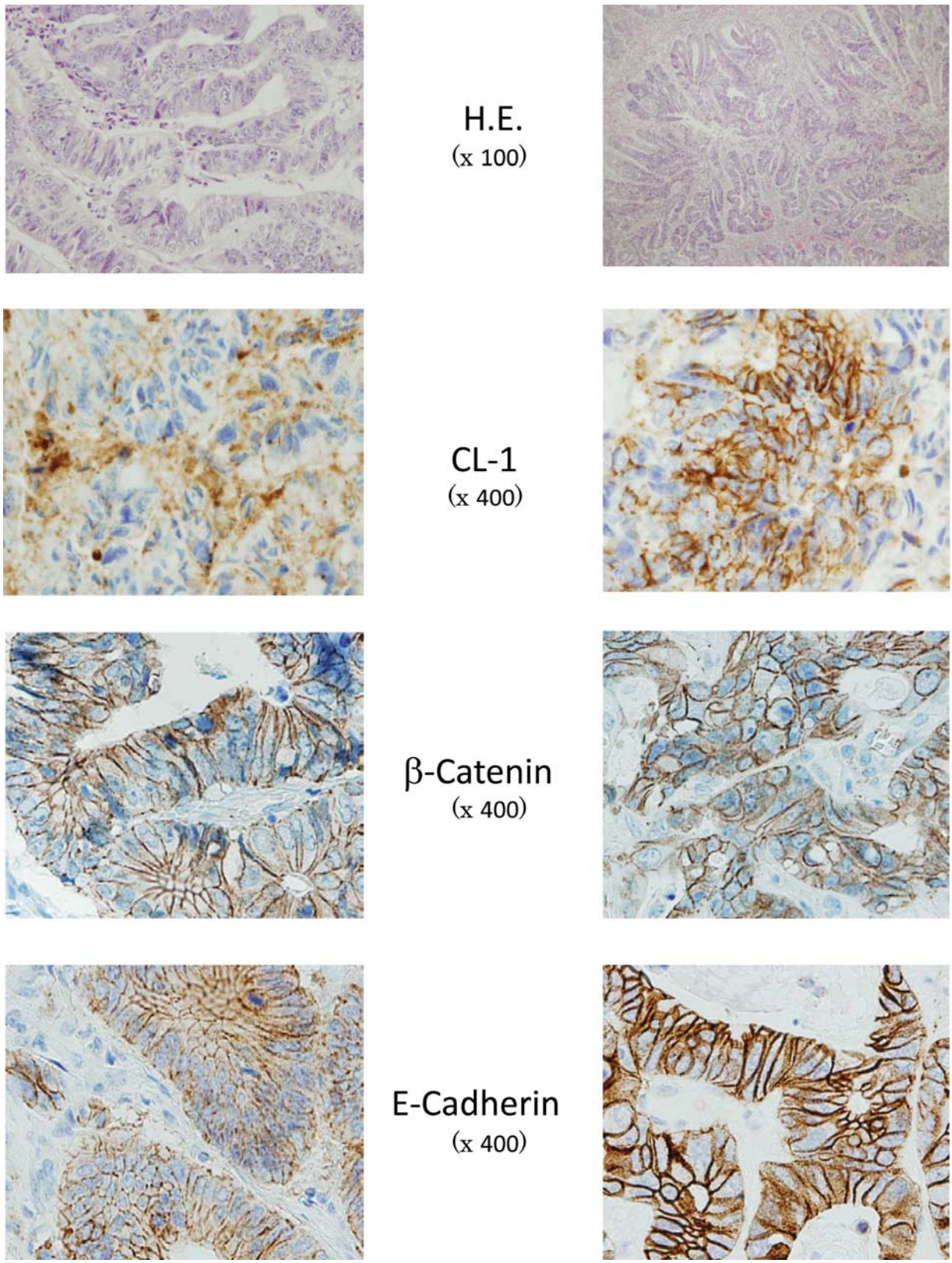

Figure 1. *Hematoxylin-eosin (H.E; top: magnification: x 100) and immunostaining (magnification: ×400) of claudin-1 (CL-1), β-catenin, and of E-cadherin in primary colorectal cancer (CRC) (left), and the corresponding liver metastases from CRC (right).*

Based on previous reports that suggested that the levels of CL-1 expression are altered in invasive colon cancer (13, 28-33), we hypothesized that modified CL-1 expression might be associated with the increased risk of hepatic metastasis of CRC. In order to assess the association between altered levels of CL-1 expression in CRC and liver metastatic risk, we performed an expression analysis in primary CRC lesions and in metastatic lesions of CRC. Increased expressions of CL-1 were found in both types of lesions. Given the emerging association of metastatic risk in CRC with pathological findings, these data suggest that increased expression of CL-1 in this setting may contribute to metastatic progression. Although the change in CL-1 expression is expected to impact the barrier function, it is not clear that barrier modulation is the mechanism by which the CL protein expression influences the metastatic process. It is possible that CL proteins interact with signaling pathways, including transforming growth factor-beta (TGFβ)/SMAD) and β-catenin (13, 34), in a manner that is separate from their effects on barrier function. The observation that CL-1 is regulated by β-catenin/TCF/lymphocyte-enhancer factor (LEF) signaling (13, 27, 35) suggested that the increased CL-1 expression, that is observed, may be due to β-catenin activation. We, therefore, assessed β-catenin that was expressed in liver metastatic lesions and CRC tissues. As expected, the increased translocation of β-catenin from the lateral membranes to the nucleus was strikingly present in both types of tissues. We previously generated a CL-1-overexpressing CRC cell line, and the effects on the tight junctional barrier, on cell morphology, proliferation, and migration were analyzed in order to determine the relationship between this protein and CRC (15). CL-1-overexpressing cells grew as aggregates in contrast to the monolayer formation of the parental cells, and the expression of CL-1 at the mRNA and protein levels was found to increase in the CRC tissue in comparison of that in the normal tissue specimens.

An aberrant expression of E-cadherin, which is one of the key cell-cell adhesion molecules in all epithelia, has been implicated in tumor invasion and metastasis (36). As shown in Figure 1, E-cadherin expression was markedly reduced in primary CRC tissues, indicating that the expressions of CL-1 and E-cadherin are differently mediated in the CRC tissue specimens (37). These results suggest that distinct signaling pathways regulate TJ and adherence junctions differently under the same conditions. Despite the fact that the expression of E-cadherin was reduced in CRC, it was found to be clearly up-regulated in the hepatic metastatic lesions in this study. These are very impressive and surprising findings.

In conclusion, CL-1 plays a pivotal role in the regulation of cellular morphology and behavior in the colonic epithelium. This study also suggested that liver metastatic lesions from CRC exhibit an increase in β-catenin transcriptional activity, which may contribute to increased CL-1 expression. Thus, increased CL-1 expression may contribute to carcinogenesis in CRC with liver metastasis. We speculate that increased CL-1 expression may be involved in the early stages of transformation in the hepatic metastatic process. The present observations raise the possibility of exploiting CL-1 as a potential biomarker for CRC progression and might provide new opportunities for therapeutic intervention.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (no. 21591740), Japan.

References

1 Ferlay J, Parkin DM and Steliarova-Foucher E: Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer *46*: 765-781, 2010.

2 Kohno SI, Luo C, Nawa A, Fujimoto Y, Watanabe D, Goshima F, Tsurumi T and Nishiyama Y: Oncolytic virotherapy with an HSV amplicon vector expressing granulocyte-macrophage colony-stimulating factor using the replication-competent HSV type 1 mutant HF10 as a helper virus. Cancer Gene Ther *14*: 918-926, 2007.

3 Yamasaki M, Takemasa I, Komori T, Watanabe S, Sekimoto M, Doki Y, Matsubara K and Monden M: The gene expression profile represents the molecular nature of liver metastasis in colorectal cancer. Int J Oncol *30*: 129-138, 2007.

4 Chan KM, Chiang JM, Lee CF, Yu MC, Lee WC, Chen JS and Wang JY: Outcomes of resection for colorectal cancer hepatic metastases stratified by evolving eras of treatment. World J Surg Oncol *9*: 174, 2011.

5 Rees M, Tekkis PP, Welsh FK, O'Rourke T and John TG: Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. Ann Surg *247*: 125-135, 2008.

6 Kato T, Yasui K, Hirai T, Kanemitsu Y, Mori T, Sugihara K, Mochizuki H and Yamamoto J: Therapeutic results for hepatic metastasis of colorectal cancer with special reference to effectiveness of hepatectomy: analysis of prognostic factors for 763 cases recorded at 18 institutions. Dis Colon Rectum 46: S22-31, 2003.

7 Wei AC, Greig PD, Grant D, Taylor B, Langer B and Gallinger S: Survival after hepatic resection for colorectal metastases: a 10-year experience. Ann Surg Oncol *13*: 668-676, 2006.

8 Fernandez FG, Drebin JA, Linehan DC, Dehdashti F, Siegel BA and Strasberg SM: Five-year survival after resection of hepatic metastases from colorectal cancer in patients screened by positron-emission tomography with F-18 fluorodeoxyglucose (FDG-PET). Ann Surg *240*: 438-447; discussion 447-450, 2004.

9 Choti MA, Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Schulick RD, Lillemoe KD, Yeo CJ and Cameron JL: Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg *235*: 759-766, 2002.

10 Tsukita S, Furuse M and Itoh M: Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol *2*: 285-293, 2001.

11 Itoh M, Furuse M, Morita K, Kubota K, Saitou M and Tsukita S: Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. J Cell Biol *147*: 1351-1363, 1999.
12 Hamazaki Y, Itoh M, Sasaki H, Furuse M and Tsukita S: Multi-PDZ domain protein 1 (MUPP1) is concentrated at tight junctions through its possible interaction with claudin-1 and junctional adhesion molecule. J Biol Chem *277*: 455-461, 2002.
13 Dhawan P, Singh AB, Deane NG, No Y, Shiou SR, Schmidt C, Neff J, Washington MK and Beauchamp RD: Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer. J Clin Invest *115*: 1765-1776, 2005.
14 Kinugasa T, Huo Q, Higashi D, Shibaguchi H, Kuroki M, Tanaka T, Futami K, Yamashita Y, Hachimine K, Maekawa S, Nabeshima K and Iwasaki H: Selective up-regulation of claudin-1 and claudin-2 in colorectal cancer. Anticancer Res *27*: 3729-3734, 2007.
15 Huo Q, Kinugasa T, Wang L, Huang J, Zhao J, Shibaguchi H, Kuroki M, Tanaka T, Yamashita Y, Nabeshima K and Iwasaki H: Claudin-1 protein is a major factor involved in the tumorigenesis of colorectal cancer. Anticancer Res *29*: 851-857, 2009.
16 Cheung ST, Leung KL, Ip YC, Chen X, Fong DY, Ng IO, Fan ST and So S: Claudin-10 expression level is associated with recurrence of primary hepatocellular carcinoma. Clin Cancer Res *11*: 551-556, 2005.
17 Howe HL, Wu X, Ries LA, Cokkinides V, Ahmed F, Jemal A, Miller B, Williams M, Ward E, Wingo PA, Ramirez A and Edwards BK: Annual report to the nation on the status of cancer, 1975-2003, featuring cancer among U.S. Hispanic/Latino populations. Cancer *107*: 1711-1742, 2006.
18 Peto J: Cancer epidemiology in the last century and the next decade. Nature *411*: 390-395, 2001.
19 Tobioka H, Isomura H, Kokai Y and Sawada N: Polarized distribution of carcinoembryonic antigen is associated with a tight junction molecule in human colorectal adenocarcinoma. J Pathol *198*: 207-212, 2002.
20 General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus 7th Edition Tokyo, Japan: Kanehara Shuppan 2006.
21 Kinugasa T, Sakaguchi T, Gu X and Reinecker HC: Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology *118*: 1001-1011, 2000.
22 Leotlela PD, Wade MS, Duray PH, Rhode MJ, Brown HF, Rosenthal DT, Dissanayake SK, Earley R, Indig FE, Nickoloff BJ, Taub DD, Kallioniemi OP, Meltzer P, Morin PJ and Weeraratna AT: Claudin-1 overexpression in melanoma is regulated by PKC and contributes to melanoma cell motility. Oncogene *26*: 3846-3856, 2007.
23 Oku N, Sasabe E, Ueta E, Yamamoto T and Osaki T: Tight junction protein claudin-1 enhances the invasive activity of oral squamous cell carcinoma cells by promoting cleavage of laminin-5 gamma2 chain *via* matrix metalloproteinase (MMP)-2 and membrane-type MMP-1. Cancer Res *66*: 5251-5257, 2006.
24 Miyamori H, Takino T, Kobayashi Y, Tokai H, Itoh Y, Seiki M and Sato H: Claudin promotes activation of pro-matrix metalloproteinase-2 mediated by membrane-type matrix metalloproteinases. J Biol Chem *276*: 28204-28211, 2001.
25 Chao YC, Pan SH, Yang SC, Yu SL, Che TF, Lin CW, Tsai MS, Chang GC, Wu CH, Wu YY, Lee YC, Hong TM and Yang PC: Claudin-1 is a metastasis suppressor and correlates with clinical outcome in lung adenocarcinoma. Am J Respir Crit Care Med *179*: 123-133, 2009.
26 Kinzler KW and Vogelstein B: Lessons from hereditary colorectal cancer. Cell *87*: 159-170, 1996.
27 Miwa N, Furuse M, Tsukita S, Niikawa N, Nakamura Y and Furukawa Y: Involvement of claudin-1 in the beta-catenin/Tcf signaling pathway and its frequent upregulation in human colorectal cancers. Oncol Res *12*: 469-476, 2001.
28 Hewitt KJ, Agarwal R and Morin PJ: The claudin gene family: expression in normal and neoplastic tissues. BMC Cancer *6*: 186, 2006.
29 Soini Y: Expression of claudins 1, 2, 3, 4, 5 and 7 in various types of tumours. Histopathology *46*: 551-560, 2005.
30 Swisshelm K, Macek R and Kubbies M: Role of claudins in tumorigenesis. Adv Drug Deliv Rev *57*: 919-928, 2005.
31 Morin PJ: Claudin proteins in human cancer: promising new targets for diagnosis and therapy. Cancer Res *65*: 9603-9606, 2005.
32 Michl P, Barth C, Buchholz M, Lerch MM, Rolke M, Holzmann KH, Menke A, Fensterer H, Giehl K, Lohr M, Leder G, Iwamura T, Adler G and Gress TM: Claudin-4 expression decreases invasiveness and metastatic potential of pancreatic cancer. Cancer Res *63*: 6265-6271, 2003.
33 Resnick MB, Konkin T, Routhier J, Sabo E and Pricolo VE: Claudin-1 is a strong prognostic indicator in stage II colonic cancer: a tissue microarray study. Mod Pathol *18*: 511-518, 2005.
34 Shiou SR, Singh AB, Moorthy K, Datta PK, Washington MK, Beauchamp RD and Dhawan P: Smad4 regulates claudin-1 expression in a transforming growth factor-beta-independent manner in colon cancer cells. Cancer Res *67*: 1571-1579, 2007.
35 Mankertz J, Hillenbrand B, Tavalali S, Huber O, Fromm M and Schulzke JD: Functional crosstalk between Wnt signaling and Cdx-related transcriptional activation in the regulation of the claudin-2 promoter activity. Biochem Biophys Res Commun *314*: 1001-1007, 2004.
36 Dorudi S, Sheffield JP, Poulsom R, Northover JM and Hart IR: E-cadherin expression in colorectal cancer. An immunocytochemical and *in situ* hybridization study. Am J Pathol *142*: 981-986, 1993.
37 Hollande F, Lee DJ, Choquet A, Roche S and Baldwin GS: Adherens junctions and tight junctions are regulated *via* different pathways by progastrin in epithelial cells. J Cell Sci *116*: 1187-1197, 2003.

Received April 4, 2012
Revised May 14, 2012
Accepted May 15, 2012