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The Progression Potential of Peritoneal Dissemination Nodules From Gastrointestinal Tumors

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It is necessary to examine the characteristics of the dissemination nodules to establish a therapeutic strategy for peritoneal dissemination from digestive malignancy. Ki-67 expression as a proliferation marker in peritoneal dissemination nodules was investigated. The subjects were 15 patients with gastrointestinal cancers who underwent resection of the primary tumor and disseminated nodules. The expression of Ki-67 in both primary tumor and peritoneal dissemination nodule from each patient was evaluated by immunohistochemistry. Ki-67 labeling index in the original tumor was higher than that in the disseminated nodule in 13 of 15 patients ($P < 0.0001$). The mean value of Ki-67 labeling index was 42.2% in the 15 original tumors and 18.7% in the 15 disseminated nodules. Proliferative activity in the disseminated nodules was lower than that in the primary tumors. Further examination about characteristics of cancer dissemination is needed to treat patients with peritoneal metastasis.

Key words: Peritoneal dissemination – Ki-67 – Gastrointestinal cancer

The presence of peritoneal dissemination is a poor prognostic factor for patients with digestive malignancies,[1–4] and a therapeutic strategy is urgently required to be established for peritoneal dissemination. In the past few years, the introduction of cytoreductive surgery combined with hyperthermic intraperitoneal chemoperfusion has shown promising results in selected patients with peritoneal carcinomatosis of colorectal cancer. However, further studies are needed to standardize the indications and techniques of this therapy.[5]

Oncology researchers have previously examined the role of certain biological features of the tumor and expression of molecular markers, which may

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Table 1 Patient characteristics and Ki-67 labeling index in the primary tumor and disseminated nodule resected simultaneously or allochronically

Case	Sex	Age	Primary organ	Histologic type	Section site	Dissemination type	Ki-67 LI (%)
1	M	64	Stomach	Differentiated	Origin	—	51.3
				Differentiated	Dissemination	Simultaneously	24.0
2	F	38	Stomach	Undifferentiated	Origin	—	33.0
				Undifferentiated	Dissemination	Allochronically	20.4
3	F	75	Stomach	Differentiated	Origin	—	20.4
				Differentiated	Dissemination	Allochronically	21.5
4	M	62	Stomach	Undifferentiated	Origin	—	35.2
				Undifferentiated	Dissemination	Simultaneously	0.0
5	M	59	Stomach	Nen	Origin	—	39.7
				Nen	Dissemination	Allochronically	17.2
6	M	64	Stomach	Undifferentiated	Origin	—	48.7
				Undifferentiated	Dissemination	Simultaneously	17.6
7	M	66	Stomach	Differentiated	Origin	—	62.6
				Differentiated	Dissemination	Allochronically	15.1
8	F	47	Colon	Differentiated	Origin	—	40.2
				Differentiated	Dissemination	Simultaneously	24.0
9	F	70	Colon	Undifferentiated	Origin	—	33.2
				Undifferentiated	Dissemination	Allochronically	20.0
10	M	71	Colon	Differentiated	Origin	—	39.1
				Differentiated	Dissemination	Simultaneously	16.8
11	M	74	Colon	Differentiated	Origin	—	16.8
				Differentiated	Dissemination	Allochronically	19.0
12	F	68	Colon	Differentiated	Origin	—	36.5
				Differentiated	Dissemination	Simultaneously	15.3
13	M	71	Colon	Undifferentiated	Origin	—	62.2
				Undifferentiated	Dissemination	Allochronically	29.5
14	M	59	Rectum	Differentiated	Origin	—	44.8
				Differentiated	Dissemination	Allochronically	1.8
15	F	57	Pancreas	Differentiated	Origin	—	69.0
				Differentiated	Dissemination	Simultaneously	38.3

F, female; LI, labeling index; M, male; Nen, neuroendocrine cell carcinoma.

enable selection of the tumors most likely to be sensitive to chemotherapy. Many cytotoxic drugs are effective against proliferating cells, and quiescent cells show a degree of drug resistance relative to cycling cells.[6] Markers of cell proliferation such as Ki-67 (MIB-1), which is a nuclear antigen and is expressed in all stages of the cell cycle, except in resting cells in the G_0 phase, may be useful in identifying tumors that will be sensitive to drugs.[7–11] Biological characteristics, especially the proportion of proliferating cells in the dissemination nodules from the original tumor, should be elucidated to discover the most effective treatment against the peritoneal disseminating type of cancer spread. On the other hand, the histologic marker of Ki-67 labeling index (LI) has been widely validated as a prognostic marker in various tumors.[12,13] The tumors that have a high level of proliferation are very aggressive and bring poor prognosis.

Relatively little has been reported regarding the clinical and histologic features of peritoneal carcinomatosis from digestive malignancies. The aim of the present study was to evaluate Ki-67 LI in cancer dissemination nodules in the peritoneum to define whether the disseminating type of cancer has a lower Ki-67 LI than the original tumor, which would suggest that the disseminating type of cancer is apt to survive under multidisciplinary therapy.

Methods

Patients and samples

Thirty samples were obtained from 15 patients diagnosed with gastric cancer, colorectal cancer, and pancreatic cancer who underwent surgery for the removal of tumors and disseminated nodules either simultaneously or allochronically at the Department of General Surgical Science, Gunma University Hospital, from January 2001 to December 2009. Written informed consent was obtained from each subject in accordance with institutional guidelines. All surgical specimens were evaluated by two pathologists (H. Yamauchi and T. Asao).

Table 2 The results of immunohistochemical examination using Ki-67 LI are summarized for each diagnosis

	Origin	Dissemination	*P* value
Gastric cancer (n = 7)	41.6 ± 13.9	16.5 ± 7.9	0.0013
Colon cancer (n = 7)	39.0 ± 13.6	18.1 ± 8.6	0.0049
Pancreatic cancer (n = 1)	69.0	38.3	—
All the cases (n = 15)	42.2 ± 14.8	18.7 ± 9.4	< 0.0001

Immunohistochemistry

The tissue samples were fixed in 10% neutral-buffered formalin, and the prepared paraffin-embedded tissues were sectioned at 4–5 μm in thickness. Paraffin was removed and the samples were autoclaved in a 10 mMol/L citrate buffer (pH 6.0) at 120°C for 3 minutes. The samples were then blocked and incubated at 4°C overnight with an anti-Ki-67 antibody (clones MIB-1 from DAKO Japan, Kyoto, Japan) at a dilution of 1:50. The sections were incubated with biotinylated anti-mouse IgG and finally incubated in a streptavidin-biotin peroxidase complex solution (Nichirei Co, Tokyo, Japan). All the sections were counterstained with hematoxylin. The percentage of Ki-67-positive nuclei among 1000 tumor cells was evaluated as the Ki-67 LI. Sections from each block were incubated without a primary antibody as a negative control.

Statistical analyses

Statistical differences were analyzed using Student *t* test. Statistical analyses were performed using Stat View software (ver. 5.0, SAS Institute Inc, North Carolina). The criterion for significance was $P < 0.05$ for all comparisons. Data are presented as means ± SD.

Results

Expression of Ki-67 in clinical samples

Immunohistochemistry using the Ki-67 antibody for progression activity was performed in the 30 samples, which included gastric cancer, colorectal cancer, and pancreatic cancer in 15 patients who underwent surgery for the removal of tumors and disseminated nodules (Table 1). The results of the immunohistochemical examination using Ki-67 LI are summarized for each diagnosis in Table 2. Ki-67 LI in the original tumor was higher than that in the disseminated nodule in 13 of 15 cases ($P < 0.0001$; Fig. 1). The other 2 cases included 1 gastric cancer and 1 colon cancer, which pathologically expressed moderately differentiated adenocarcinoma. The

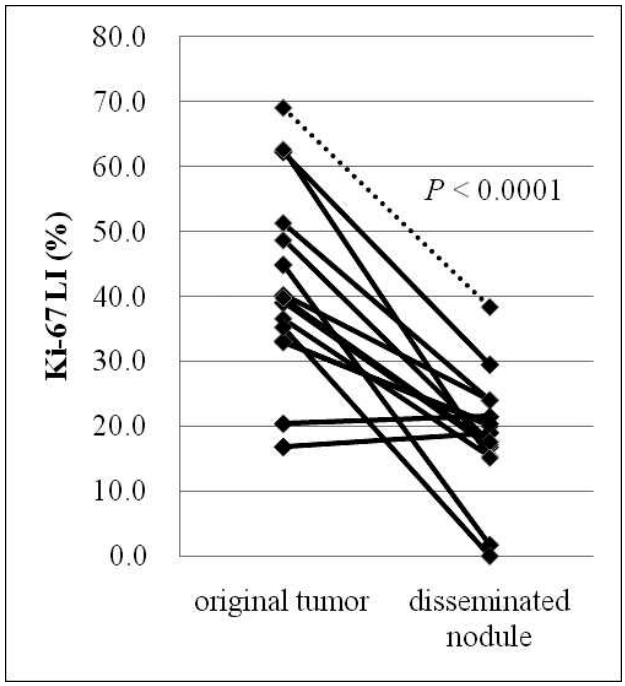

Fig. 1 Ki-67 LI in clinical samples including 7 gastric cancers, 7 colorectal cancers, and 1 pancreatic cancer in 15 patients. Ki-67 LI was measured by immunohistochemistry from the paired original tumor and disseminated nodule. The mean value of Ki-67 LI in the 15 original tumors was 42.2% (range, 16.8%–69.0%). In all the disseminated tumors, the mean value of Ki-67 LI was 18.7% (range, 0–38.3%). The dotted line represents a pancreatic cancer case. The 2 lines that exhibit an uptrend to the right include 1 female patient with gastric cancer and 1 male patient with colon cancer.

mean values of Ki-67 LI in the original tumors and disseminated tumors of 7 gastric cancers were 41.6% (range, 20.4%–62.6%) and 16.5% (range, 0–24.0%), respectively. The mean value of Ki-67 LI in the original tumors of 7 colorectal cancers was 39.0% (range, 16.8%–62.2%). In disseminated tumors of those cases, the mean value of Ki-67 LI was 18.1% (range, 1.8%–29.5%). There was no significant difference in Ki-67 LI between the original tumors of 7 gastric cancers and those of 7 colorectal cancers ($P > 0.05$). The mean value of Ki-67 LI in the 15 original tumors, which included 7 gastric cancers, 7 colorectal cancers, and 1 pancreatic cancer, was 42.2% (range, 16.8%–69.0%). In all the disseminated tumors, the mean value of Ki-67 LI was 18.7% (range, 0–38.3%). Histopathologic images of selected cases with gastric cancer (Fig. 2a and 2b) and colon cancer (Fig. 2c and 2d) are demonstrated individually.

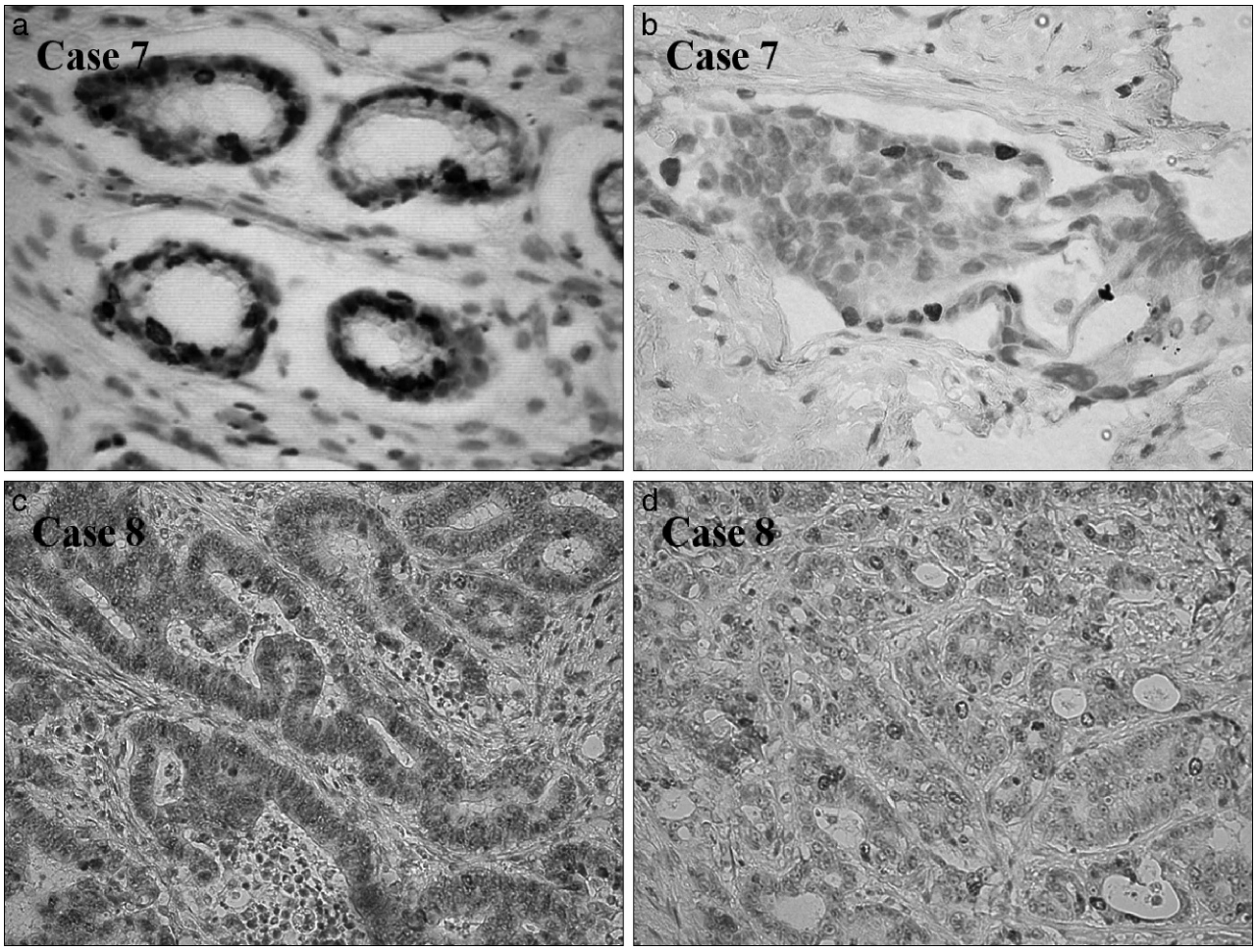

Fig. 2 Representative photomicrographs of tissue sections immunostained for Ki-67 (×400). Proliferation abilities of the original tumors and the dissemination nodules were assessed by immunohistochemical examination using Ki-67 LI. Images show representative examples of patient samples 7 and 8 in the original tumor (a and c) and the dissemination nodule (b and d). Ki-67 LI were 62.6% (a), 15.1% (b), 40.2% (c), and 24.0% (d).

Discussion

Peritoneal dissemination was first described as the regional spread of ovarian carcinoma in 1931.[14] The mechanisms responsible for the development of peritoneal dissemination are multifactorial: spreading of free cancer cells due to serosal involvement of the primary tumor,[15] implantation of free cancer cells due to the presence of adherence molecules, invasion of the underlying connective tissue, and induction of angiogenesis to sustain tumor proliferation.[16]

The presence of the peritoneal seeding type of cancer spread is a poor prognostic factor for patients with advanced gastric cancer[1,4] and colon cancer.[2,3] In recent years, systemic therapy regimens have increased response rates and have contributed to overall survival improvement. However, selection criteria for therapeutic modalities at the time of diagnosis of peritoneal dissemination from digestive malignancy have yet to be defined. Therefore, the biological features of dissemination tumors must be determined to select those patients who are likely to receive benefit from aggressive therapy. In this report, Ki-67 LI as a proliferation marker in the cancer dissemination nodules in the abdominal cavity was evaluated in 15 patients who underwent surgery for the removal of tumors and disseminated nodules.

Peritoneal dissemination is the most frequent pattern of metastasis and recurrence in patients with gastric cancer.[17–19] Of patients scheduled for curative resection of gastric cancer 10%–20% will have

peritoneal seeding at the time of abdominal examination, and some patients will have peritoneal carcinomatosis.[20–22] About 8% of patients at the time of primary resection and up to 25% of patients with recurrent colorectal cancer will have disseminated disease detected in the peritoneal cavity.[2,3] For the majority of patients with pancreatic cancer, even for patients who have undergone curative resection, a poor survival rate due to cancer recurrence was revealed.[23] The majority of postoperative recurrences are due to hepatic metastasis, local recurrence, and peritoneal dissemination.[24–27] The present analysis included 7 patients with gastric cancer, 7 patients with colorectal cancer, and 1 patient with pancreatic cancer who underwent surgery for the removal of tumors and disseminated nodules either synchronously or metachronously from January 2001 to December 2009. The number of patients analyzed in the present study was restricted because of the exclusion of cases in which either the original tumor or the dissemination nodule was unresectable.

In this report, Ki-67 LI in the original tumor was higher than in the disseminated nodule in 13 of 15 cases (Fig. 1). The other 2 cases included 1 female patient with gastric cancer and 1 male patient with colon cancer, pathologically expressing moderately differentiated adenocarcinoma. Both of these patients had poor proliferation potential in the primary tumor, which contributed to lower Ki-67 LI in the original tumor than in the disseminated nodule. The primary tumor had a greater proportion of proliferating cells than the disseminated nodule even when the value of Ki-67 LI was assessed for each kind of cancer (Table 2). Ki-67 LI of the dissemination nodule might be likely to be lower than that of primary tumor in other malignancies, but further studies including those on other types of cancer are needed.

Many current therapies are directed against cancer cells in the proliferating phase. Radiation sensitivity also varies with different phases of the cell cycle and it is known that proliferating cells are radiosensitive. Radiation-induced DNA damage and its manifestations on genomic instability are well reported.[28–31] On the other hand, the present study explained that the disseminated type of cancer cells generally divide rarely. A small population of dividing cells in the dissemination nodules will cause uncertainty of the results of current therapies. Therefore, no single drug therapy, but rather a multidisciplinary approach, such as multidrug chemotherapy combined with radiation and hyperthermia, may be a reasonable strategy for patients with malignant tumors that have a large population of nonproliferating cells.

In the present 15 patients, the progression potential in the peritoneal disseminated type of digestive cancer was poorer than that in primary tumor. Further investigation about characteristics of cancer dissemination is needed to find a suitable treatment for patients with peritoneal metastasis.

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