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Original article

Micrometastasis in lymph nodes of mucosal gastric cancer

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Abstract

Background. **Endoscopic mucosal resection is frequently used in the treatment of mucosal gastric cancer. Micrometastasis in the lymph nodes of mucosal gastric cancer remains unclear.**

Methods. **We examined 2526 lymph nodes from 84 patients with mucosal gastric cancer. Two consecutive sections were prepared, for simultaneous staining with hematoxylin and eosin and immunostaining with CAM 5.2 monoclonal antibody against cytokeratin (CK), respectively. A clinicopathological comparison was made between patients with and without lymph node involvement.**

Results. **Lymph node involvement was detected in 45 of 2526 (1.8%) lymph nodes. The incidence of nodal involvement was significantly increased, from 1.2% (1/84 patients) with hematoxylin and eosin staining, to 19% (16/84 patients) with CK immunostaining. Although no significant difference was found, micrometastasis to lymph nodes was more frequently detected in tumors larger than 1.0 cm (15/72 patients, 21%) than in those less than or equal to 1.0 cm (1/12 patients; 8%, $P = 0.307$). However, discrete CK-positive cancer cells or clusters of CK-positive cancer cells were detected only in tumors larger than 2 cm.**

Conclusion. **Because mucosal gastric cancer of more than 1.0 cm in superficial diameter may indicate a risk of micrometastasis to lymph nodes, endoscopic mucosal resection is not recommended for these patients.**

Key words Mucosal gastric cancer · Micrometastasis · Cytokeratin · Immunohistochemistry

Introduction

In patients with gastric cancer, metastasis to lymph nodes has been recognized as the most important factor in prognosis, and lymphadenectomy has long been considered to improve postoperative survival [1–3]. A recent trend in the treatment of early gastric cancer, however, involves less invasive or limited surgery to improve the patient's quality of life [4–6]. Recent investigations have reported that micrometastases to lymph nodes that were overlooked by ordinary hematoxylin and eosin (H&E) staining could be detected by cytokeratin (CK) immunostaining, and these so-called micrometastases were found to have prognostic significance [7–10]. Mucosal gastric cancer (m-cancer) has been the prime candidate for less invasive surgery or endoscopic mucosal resection (EMR) [11,12]. However, the frequency or outcome of occult involvement in lymph nodes from m-cancer remains unclear. In this study, in order to assess the critical indications for EMR or less invasive surgery for m-cancer, we investigated the incidence of micrometastasis in lymph nodes in m-cancer, using CK immunostaining.

Patients and methods

A total of 84 patients (46 men and 38 women) with m-cancer, who underwent curative gastrectomy combined with lymphadenectomy at our hospital between 1986 and 1991, were investigated. The patients ranged in age from 37 to 82 years, with a mean age of 63 years. Total gastrectomy was performed in 6 patients (7%), and distal and proximal subtotal gastrectomies in 71 patients (85%) and 7 patients (8%), respectively. Fifty-eight patients underwent standard D2 lymphadenectomy. D2 plus part of group 3 lymph node dissections (lymph nodes in the hepatoduodenal ligament, around the common hepatic artery, behind the head of the pancreas, or at the root of the mesentery) were performed in 26 patients. All of the patients were followed-up for more than 5 years after surgery.

Clinicopathologic data were evaluated according to the *General rules for gastric cancer study in surgery and*

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pathology of the Japanese Research Society for Gastric Cancer [13]. For the purpose of describing tumor location, we divided the gastric area into three equal regions: the upper-third, the middle-third, and the lower-third. Tumor size was determined based on the maximum superficial diameter of the lesion. Macroscopic types were described as 0-I, protruded; 0-IIa, superficial elevated; 0-IIb, flat; 0-IIc, superficial depressed; and 0-III, excavated.

A total of 2526 lymph nodes dissected from the 84 patients were investigated in the present study. The mean number of dissected lymph nodes per patient was 30 (range, 10–61 nodes). All samples were fixed in formalin and embedded in paraffin. Two consecutive sections, of 4-μm-thickness, were prepared from each sample. One section was stained with H&E and the other was subjected to CK specific immunostaining. Eight lymph nodes with metastatic cancer cells detected by H&E staining were used as positive controls, and 40 perigastric nodes obtained from 15 patients with benign gastric ulcer were used as negative controls.

Immunohistochemical staining was performed by the streptavidin-biotin (SAB) immunoperoxidase method with murine monoclonal antibody CAM 5.2 (Becton Dickinson, San Jose, CA, USA), which specifically recognizes intracellular CK components numbers 8 and 18 [14]. Briefly, dewaxed and dehydrated sections were heated in a microwave oven (700 W) for 10 min for the retrieval of antigens in the specimens. Endogenous peroxidase was blocked by incubation of the samples with 3% hydrogen peroxide in methanol. The tissue sections were incubated with the primary monoclonal antibody, CAM 5.2, at 25 μg per ml overnight at 4 °C. The second antibodies, biotinylated antibodies against mouse immunoglobulin, were applied, followed by the application of peroxidase-labeled streptavidin. The reaction products were visualized with diaminobenzidine as the chromogen, and sections were counterstained with methyl green.

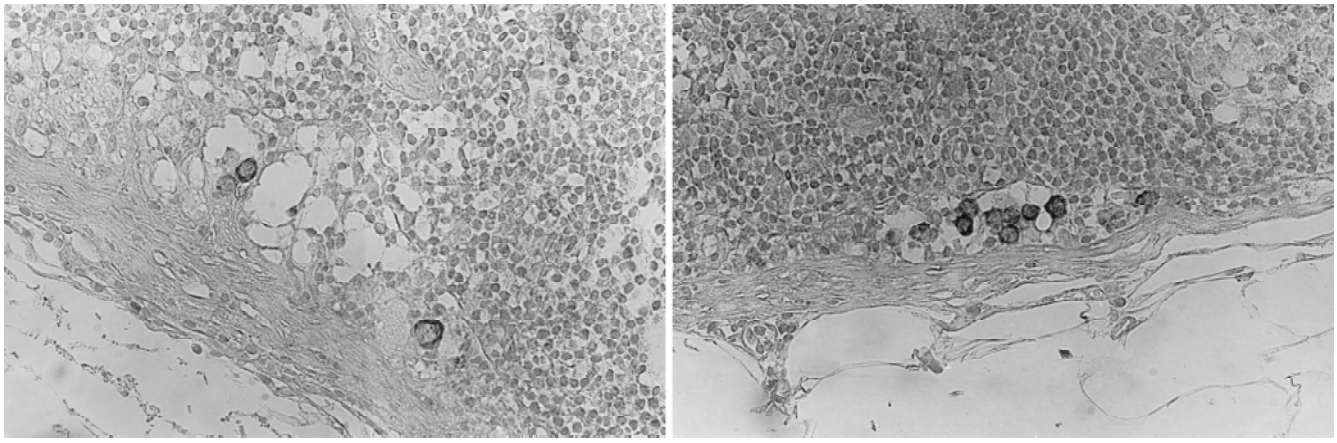
The H&E-stained slides were first assessed for the presence of metastases, at a magnification of 200×, by an experienced pathologist without the knowledge of sample groupings and previous diagnosis. Then the immunostained slides were examined, and the results were compared with those obtained from the H&E-stained slides. Micrometastasis in lymph nodes was recognized when tumor cells were detected only by CK-specific immunostaining, having been overlooked after ordinary H&E staining.

Statistical analysis was performed by the χ^2 test to examine the differences between CK immunostaining and the clinicopathological characteristics of the primary tumors. A P value of less than 0.05 was considered an indication of statistical significance.

Results

Features of nodal involvement

Metastases in lymph nodes were confirmed by ordinary H&E staining in 8 lymph nodes from only one patient (1.2%). All 8 nodes were CK-positive; in addition, another 9 lymph nodes of this patient were shown to be cancer-positive by CK immunostaining. Micrometastases were also detected in 28 lymph nodes from 15 other patients (18%), none of whom were observed to have metastases by conventional H&E staining. Thus, the frequency of lymph node involvement in relation to the total number of dissected lymph nodes was increased, from 0.3% (8/2526 nodes) with H&E staining,



a b

Fig. 1a,b. Cytokeratin immunostaining in lymph nodes. **a** Micrometastasis in the form of discrete cancer cells. **b** Micrometastasis in the form of clustered cancer cells

to 1.8% (45/2526 nodes) with CK immunostaining ($P < 0.0001$). The incidence of nodal involvement in all 84 patients with m-cancer was increased, from 1.2% (1/84 patients) with H&E staining, to 19% (16/84 patients) with CK immunostaining ($P < 0.05$). The clinicopathological characteristics of these 16 patients with lymph node involvement are shown in Table 1. In these 16 patients with metastasis or micrometastasis, a single cancer cell was detected in the lymph node in 9 patients (56%), two or more discrete cancer cells were detected in the lymph node in 2 patients (13%; Fig. 1a), and clustered cancer cells were detected in the lymph node in 5 patients (31%; Fig. 1b). In the patient with lymph node metastasis evidenced by H&E staining, large clustered cancer cells were located in the medullar and marginal sinus of the lymph nodes. By contrast, micrometastasized cancer cells detected by CK immunostaining were found to be located in the marginal sinus in the form of a single cell, or discrete cells, or small cell clusters.

Cancer-positive lymph nodes were detected only in group 1 nodes in 11 patients, and 8 of these patients were found to have only a single cancer cell in their lymph nodes. Cancer-positive lymph nodes were detected in group 2 or 3 nodes in 5 patients, and 4 of these 5 patients had discrete or clustered cancer cells in their lymph nodes. Of these 5 patients with extra-perigastric lymph node involvement, 2 patients had micrometastasis only in group 3 lymph nodes, but did not show any involvement in any of the group 1 or 2 lymph nodes. One of these two rare tumors with only group 3 lymph node micrometastasis was located in the lesser curvature of the middle-third of the stomach, with only a single CK-positive cell in the station 8p lymph node. The other of these rare tumors was located in the greater curvature of the middle-third of the stomach, with one cluster of CK-positive cells in each of the station 2 and 4sa lymph nodes. An additional 10 consecutive sections of station 3, 1, 7, and 8a lymph nodes in the former patient, and station 4sb, 4d, and 3 lymph nodes in the latter patient, were cut again from the remaining half of the lymph node blocks and immunostained, but no CK-positive cells were found in any of these lymph nodes. No CK-positive lymph node was found in any of the 20 lymph nodes from benign gastric ulcer patients (negative controls).

Association with clinicopathological characteristics

We compared the clinicopathological factors in the 16 patients with and the 68 patients without nodal involvement. More nodal involvement occurred in tumors larger than 1.0 cm in superficial diameter (21%), then in those with depressed macroscopic type (IIc or III; 23%) and in those with ulcer formation (36%), but no

Table 1. Clinicopathological and immunohistochemical data of 16 patients with CK-positive cancer cells in lymph nodes

Patient number	Age (years)	Tumor location	Tumor size (cm)	Mac. type[a]	Hist. type[b]	Ulceration	H&E staining	Survival (months)	No. of nodes examined	No. of nodes with CK (+)	Stations of nodal involvement	Features of nodal involvement
1	77	L	2	IIc	Por.	–	–	Alive (120)	27	1	4d	Single cell
2	59	M	2.5	IIc	Por.	+	–	Alive (117)	21	1	8p	Single cell
3	58	L	1.7	IIc	Tub 1.	–	–	Alive (70)	33	1	6	Single cell
4	39	U	2.8	IIc	Tub 2.	–	–	Alive (141)	22	3	2, 3	Single cell
5	46	L	4.7	IIc	Por.	+	+	Alive (60)	61	17	1, 3, 4d, 6, 7	Cluster
6	51	M	2.6	IIc	Por.	–	–	Alive (130)	25	1	3	Discrete
7	77	M	2.7	IIc	Pap.	–	–	Alive (96)	25	1	9	Discrete
8	59	M	5.2	IIc	Por.	+	–	Alive (120)	44	5	2, 4sa	Cluster
9	76	L	0.6	IIc	Tub 2.	–	–	Alive (118)	12	1	5	Single cell
10	70	U	1.4	IIa	Pap.	–	–	Alive (120)	46	2	3	Single cell
11	57	L	1.1	IIc	Tub 2.	–	–	Alive (124)	51	2	3, 4sb	Single cell
12	42	M	1.1	IIc	Sig.	–	–	Alive (60)	36	1	4d	Single cell
13	57	L	8.0	IIc	Tub 2.	+	–	Alive (124)	39	3	3, 6	Single cell
14	37	M	5.4	IIc+III	Por.	+	–	Alive (72)	34	1	3	Cluster
15	58	M	2.5	IIb+IIc	Por.	–	–	Alive (105)	38	1	4d	Cluster
16	82	L	5.5	IIc	Tub 2.	–	–	Died, peritoneal recurrence (48)	18	4	3, 7	Cluster

CK, Cytokeratin; L, lower-third of stomach; M, middle-third; U, upper-third

[a] Macroscopic type, according to reference [13]

[b] Histological type, according to reference [13]

Table 2. Relationship between CK immunostaining and clinicohistopathological characteristics[a] of 84 patients with mucosal gastric cancer

Variables	CK-negative	CK-positive (%)	*P*
Location			
Upper-third	6	2 (25)	
Middle-third	32	7 (18)	
Lower-third	30	7 (19)	0.8982
Superficial diameter (cm)			
≦1.0	11	1 (8)	
1.1–2.0	17	5 (23)	
>2.0	40	10 (20)	0.5724
Macroscopic type			
I, or IIa	10	1 (9)	
IIa + IIc, or IIc + IIa	9	0	
IIc, or III	49	15 (23)	0.1633
Ulceration			
Negative	59	11 (16)	
Positive	9	5 (36)	0.0819
Histopathology			
Well differentiated	26	3 (10)	
Moderately differentiated	22	5 (18)	
Poorly differentiated	20	8 (29)	0.2148

[a] According to reference [13]

Table 3. Relationship between features of lymph node involvement and tumor size in 16 patients with CK-positive staining in lymph nodes

Features of nodal involvement	No. of patients	Superficial diameter (%)			
		≦1.0 cm	1.1–2.0 cm	>2.0 cm	*P*
Single cell only	9	1	5	3	
Discrete or clusters	7	0	0	7	0.0239

significant difference was found (Table 2). Six of the 9 patients with only a single cancer cell in the lymph nodes had tumors smaller than or equal to 2 cm, while all 7 (100%) patients with discrete or clustered cancer cells in the lymph nodes had tumors larger than 2 cm in diameter ($P = 0.0239$; Table 3). All 5 patients with group 2 or 3 lymph node involvement had tumors larger than 2 cm ($P = 0.0367$). In addition, the number of involved lymph nodes was much higher in patients with tumors larger than 2 cm than in those with tumors smaller than or equal to 2 cm ($P = 0.0367$). One of our 84 patients with m-cancer who died of peritoneal dissemination was postoperatively found to have micrometastasis in group 2 lymph nodes, in the form of clustered cancer cells. There was no postoperative recurrence in the patients without lymph node involvement.

Discussion

Traditional D2 lymphadenectomy is widely used for patients with early gastric cancer in Japan. Histologically, the incidence of lymph node metastasis in mucosal gastric cancer has been reported as 1.3% to 5.0% [11,12,15–17]. Recently, it has been reported that m-cancer with a tumor smaller than 2 cm in diameter, of the well differentiated type or the elevated macroscopic type, has no lymph node metastasis. Thus, for patients with this type of m-cancer, EMR or limited lymphadenectomy has generally been performed [4,5,11]. Micrometastasis of lymph nodes in such patients with m-cancer has not been well discussed. Lymph node metastases are known to be one of the most important prognostic factors [15–18]. Maehara et al. [7] and Ishida et al. [8] reported that patients with micrometastasis in the lymph nodes showed poor prognosis. Our previous investigations in advanced [9] and in submucosal gastric cancers [10] also estimated an unfavorable outcome in patients with lymph node micrometastasis. In the present study, we investigated a sufficiently large number of lymph nodes (2526) from 84 patients with m-cancer by cytokeratin (CK) immunostaining, and the incidence of micrometastasis in lymph nodes was found to be 18% in patients with cancer-negative lymph nodes

shown by ordinary H&E staining. This incidence is significantly higher than that reported by ordinary H&E staining.

Whether a single cancer cell in the regional lymph node can have clinicopathological significance remains unclear. Discrete or large clusters of cancer cells were frequently found in H&E-positive lymph nodes. By comparison, 9 of 15 patients were shown to have micrometastasis by CK immunostaining on the basis of only a single cancer cell in their lymph nodes, and in all but 1 of these patients, only the group 1 lymph node was involved. These cases of micrometastases seem to be at a very early stage of tumor invasion to the lymph nodes. Histological research has shown a very low incidence of postoperative recurrence in patients with m-cancer [19]. Whether these micrometastasized single cells in lymph nodes are subsequently removed by the immune response of the host is still unclear [8].

Tumor size has been reported to be one of the risk factors for lymph node metastasis [10,20]. In the present study, we found that tumors larger than 2 cm frequently showed micrometastasis in the form of discrete or clustered cancer cells in lymph nodes. Moreover, we found that tumors larger than 2 cm were much more likely to invade the distant lymph nodes such as those in group 2 or 3. These findings indicate that m-cancers with tumors larger than 2 cm are at risk for micrometastasis in lymph nodes far from the primary tumors. The present study demonstrated that a considerable number of small m-cancers with tumors of 1–2 cm, in fact, had micrometastasis in the lymph nodes. Therefore, patients with m-cancer with a tumor larger than 1.0 cm may have lymph node involvement, which possibility should be given due consideration.

Lymph node metastasis has been observed mainly in perigastric nodes in m- and submucosal (sm)-cancers, although jumping metastases to extra-perigastric lymph nodes have been reported histologically in early gastric cancer [17,21]. In our study, two patients showed micrometastasis in the group 3 lymph nodes, but not in any of the group 1 or 2 lymph nodes. An additional ten consecutive sections from the perigastric lymph nodes of each of these two patients were checked in detail, but no additional cancer cells were found. Therefore, two patients with jumping micrometastases in distant lymph nodes were shown in the present study. Both of these patients had undifferentiated tumors larger than 4.5 cm in diameter. Jumping micrometastasis of lymph nodes shown by CK immunostaining appears to occur more frequently than has been previously reported histologically in m-cancers [21–23].

Recently, EMR or limited lymphadenectomy has been performed for patients with m-cancer. Because m-cancer with a tumor larger than 1.0 cm in superficial diameter has a risk of micrometastasis to the lymph nodes, EMR is not recommended for these patients.

References

1. Maekawa S, Takeo S, Ikejiri K, Anai H, Saku M. Clinicopathological features of lymph node metastasis in early gastric cancer. Int Surg 1995;80:200–3.
2. Yamao Y, Shirao K, Ono H, Kondo H, Saito D, Yamaguchi H, et al. Risk factors for lymph node metastasis from intramucosal gastric carcinoma. Cancer 1996;77:602–6.
3. Shen KH, Wu CW, Lo SS, Hsieh MC, Hsia CY, Chiang SC, et al. Factors correlated with the number of metastatic lymph nodes in gastric cancer. Am J Gastroenterol 1999;94:104–8.
4. Sano T, Kobori O, Muto T. Lymph node metastasis from early gastric cancer: endoscopic resection of tumor. Br J Surg 1992;79: 241–4.
5. Kitamura K, Yamaguchi T, Taniguchi H, Hagiwara A, Sawai K, Takahashi T. Analysis of lymph node metastasis in early gastric cancer: rationale of limited surgery. J Surg Oncol 1997;64:42–7.
6. Yokota T, Saito T, Teshima S, Kikuchi S, Kunii Y, Yamauchi H. Lymph node metastasis in early gastric cancer: how can surgeons perform limited surgery? Int Surg 1998;83:287–90.
7. Maehara Y, Oshiro T, Endo K, Baba H, Oda S, Ichiyoshi Y, et al. Clinical significance of occult micrometastasis in lymph nodes from patients with early gastric cancer who died of recurrence. Surgery 1996;119:397–402.
8. Ishida K, Katsuyama T, Sugiyama A, Kawasaki S. Immunohistochemical evaluation of lymph node micrometastases from gastric carcinomas. Cancer 1997;79:1069–76.
9. Cai J, Ikeguchi M, Maeta M, Kaibara N, Sakatani T. Clinicopathological value of immunohistochemical detection of occult involvement in pT3N0 gastric cancer. Gastric Cancer 1999;2:95–100.
10. Cai J, Ikeguchi M, Maeta M, Kaibara N. Micrometastasis in lymph nodes and microinvasion of the muscularis propria in primary lesions of submucosal gastric cancer. Surgery 2000; 127:32–9.
11. Koufuji K, Takeda J, Toyonaga A, Yoshihara S, Tanaka Y, Ohta J, et al. Early gastric cancer and lymph node metastasis. Kurume Med J 1997;44:157–64.
12. Suzuki H, Miho O, Watanabe Y, Kohyama M, Nagao F. Endoscopic laser therapy in the curative and palliative treatment of upper gastrointestinal cancer. World J Surg 1989;13:158–64.
13. Japanese Research Society for Gastric Cancer. The general rules for gastric cancer study in surgery and pathology. Jpn J Surg 1981;11:127–45.
14. Sasaki M, Watanabe H, Jass JR, Ajioka Y, Kobayashi M, Matsuda K, et al. Occult lymph node metastases detected by cytokeratin immuno-histochemistry predict recurrence in "node negative" colorectal cancer. J Gastroenterol 1997;32:758–64.
15. Okamura T, Tsujitani S, Korenaga D, Haraguchi M, Baba H, Hiramoto Y, et al. Lymphadenectomy for cure in patients with early gastric cancer and lymph node metastasis. Am J Surg 1988;155:476–80.
16. Baba H, Maehara Y, Okuyama H, Orita H, Anai H, Akazawa K, et al. Lymph node metastasis and macroscopic features in early gastric cancer. Hepatogastroenterology 1994;41:380–3.
17. Iwashita T, Nishihara K, Abe Y, Ihara T, Nakahara S, Katsumoto F, et al. Risk factors of lymph node metastases and recurrence from intramucosal gastric cancer. J Jpn Surg Soc 1996;57:2119–23.
18. Natsugoe S, Aikou T, Shimada M, Yoshinaka H, Takao S, Shimazu H, et al. Occult lymph node metastasis in gastric cancer with submucosal invasion. Surg Today 1994;24:870–5.

19. Sano T, Sasako M, Kinoshita T, Maruyama K. Recurrence of early gastric cancer: follow up of 1475 patients and review of the Japanese literature. Cancer 1993;72:3174–8.
20. Maehara Y, Orita H, Okuyama T, Moriguchi S, Tsujitani S, Korenaga D, et al. Predictors of lymph node metastasis in early gastric cancer. Br J Surg 1992;79:245–7.
21. Koufuji K, Takeda J, Tanaka T, Hashimoto K, Kakegawa T. Early mucosal gastric cancer with extra-perigastric (n2) lymph node metastasis. Kurume Med J 1991;38:191–3.
22. Tsuji Y, Takeda J, Koufuji K, Kodama I, Maruiwa M, Kawabata S, et al. Early gastric cancer with juxta-lymph node (n3) metastasis. Kurume Med J 1993;40:45–8.
23. Kosaka T, Ueshige N, Sugaya J, Nakano Y, Akiyama T, Tomita F, et al. Lymphatic routes of the stomach demonstrated by gastric carcinomas with solitary lymph node metastasis. Surg Today 1999;29:695–700.