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Clinical significance of molecular diagnosis for gastric cancer lymph node micrometastasis

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Abstract

Advances in molecular diagnostic tools have allowed the identification of lymph node micrometastasis (LNM), including isolated tumor cells, in cancer patients. While immunohistochemistry and reverse transcription-polymerase chain reaction have been used to identify LNM in patients with gastric cancer, the clinical significance of this finding remains unclear. Recently, minimally invasive treatments, such as endoscopic submucosal dissection and laparoscopic surgery, are widely performed to help improve postsurgical quality of life (QOL). However, it is important to maintain the balance between QOL and curability when making treatments decision for patients with gastric cancer. If minimally invasive surgery based on accurate intraoperative LNM diagnosis was established, it could be performed safely. Therefore, we reviewed the clinical significance of LNM detected by molecular techniques as an important target for treatment decision making with gastric cancer patients.

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Key words: Gastric cancer; Lymph node micrometastasis; Molecular technique; Sentinel lymph node; Minimally invasive surgery

Core tip: Advances in molecular diagnostic tools have allowed the identification of lymph node micrometastasis in cancer patients. Minimally invasive treatments, such as endoscopic submucosal dissection and laparoscopic surgery, are widely performed to help improve postsurgical quality of life (QOL). However, it is important to maintain the balance between QOL and curability.

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INTRODUCTION

Regional lymph node metastasis is the most important prognostic factor for patients with gastric cancer. Therefore, radical gastrectomy with D2 lymph node dissection is recognized as the standard surgical treatment for early gastric cancer (EGC). Patients with EGC have good prognosis, with a 5-year survival rate for mucosal and submucosal gastric cancer of 95%-100% and 85%-95%, respectively^[1-3]. Nevertheless, some patients without lymph node metastasis who received radical gastrectomy based on conventional histological hematoxylin-eosin (HE) staining occur disease recurrence^[4-6]. Such reserches have concluded that lymph node micrometastasis (LNM) might be a key causative factor for gastric cancer recurrence. However, it is difficult to preoperatively diagnose lymph node metastasis, including micrometastasis, using imaging examinations (*e.g.*, ultrasonography, computed tomography, and positron emission tomography) in patients with gastric cancer^[7].

To increase the quality of life (QOL) of patients with EGC, minimally invasive treatments, such as endoscopic

submucosal dissection (ESD), laparoscopic surgery and a reduced form of a lymphadenectomy are preferred treatment options. Thus, it is more important to select those patients in which lymph node metastatic disease is not present. Recently, the clinical efficacy of sentinel lymph node navigation surgery (SLNNS) has been investigated in patients without lymph node metastasis preoperatively (cN0), and many researchers have reported that SLNNS is applicable in patients who are preoperatively diagnosed with cT1 and cN0 gastric cancer^[8-11]. Accordingly, the precise intraoperative assessment whether lymph node metastasis, including micrometastasis, is present or not is extremely important for treatment planning purposes when utilizing less invasive treatments. This review will focus on the clinical significance of molecular diagnosis for LNM as an important therapeutic target in gastric cancer and will discuss the recent progress in this field.

LITERATURE SEARCH

A PubMed search using the terms gastric cancer, micrometastasis was performed for article published from 1946 to 2013. The literature search was limited to English language only. From these searched articles, we selected and reviewed clinical studies about LNM of gastric cancer. Moreover, we described future perspective based on the molecular techniques for detection of the LNM.

DEFINITION OF LNM

Micrometastasis was defined as tumor cell clusters measuring from 0.2 mm to 2.0 mm in their greatest dimension, and classified as pN1 (mi) by the criteria of the tumor-node-metastasis (TNM) classification established by the International Union Against Cancer (UICC)^[12]. On the other hand, isolated tumor cells (ITC) are defined as single tumor cells or small clusters of cells measuring \leq 0.2 mm in their greatest dimension, and classified as pN0 (i+). Furthermore, patients with lymph node metastasis detected only by using reverse transcription-polymerase chain reaction (RT-PCR) are classified as pN0 (mol+).

DETECTION OF MICROMETASTASIS

Several procedures have been used for the detection of LNM in gastric cancer patients. First, Isozaki *et al.*^[13] reported that the detection rate of lymph node metastases of gastric cancer by serial sectioning was significantly higher than that of routine histological diagnosis. Immunohistochemistry (IHC) has also been used to detect LNMs. Epithelial marker, such as cytokeratin (CK), is commonly used to identify LNM in IHC. According to the previous studies, CK AE1/AE3 and CAM5.2 monoclonal antibodies (mAb) were often used to detect LNM of gastric cancer. The greatest advantage of IHC is that we can morphologically recognize cells in lymph nodes. However, there are some problems to apply IHC for the detection of LNM, such as the serial numbers of

lymph node section required. Noura *et al.*^[14] demonstrated that the diagnosis of LNM in colorectal cancer patients should be assessed by IHC for at least five sections. IHC can be time consuming, but Matsumoto *et al.*^[15] established a rapid IHC procedure that could diagnose LNM within 30 min. They have been recently applied this rapid IHC technique for the detection of LNM during surgery in the upper gastrointestinal tract cancer. However, this method has not been widely accepted.

Due to progress in molecular biological techniques, RT-PCR can now be used for the detection of LNM. CK and carcinoembryonic antigen (CEA) are usually used as target markers for the detection of LNM^[16-19]. CEA is a 200 kDa glycoprotein that is not expressed in normal gastric mucosa but expressed in most gastric cancer cells^[20]. Although the RT-PCR assay has high sensitivity for the detection of small numbers of occult cancer cells in lymph nodes, several researchers have reported that there are some problems to apply the RT-PCR assay for the detection of LNM^[21,22]. First, false-positives may be produced by RT-PCR because of the contamination or the presence of pseudogene^[21]. Second, there is a possibility of false-negatives because of the heterogeneous expression of target markers^[22]. We previously reported that the MUC2 RT-PCR assay was a sensitive and specific method to detect LNM in gastric cancer patients^[23]. Furthermore, detailed assessment by duplex^[24] or multiplex^[25] RT-PCR assay has been recommended in order to decrease the rate of false-negative results. Moreover, the use of RT-PCR assay as an intraoperative diagnostic tool for the detection of LNM requires rapid analysis during operation in a highly sensitive and specific manner. Horibe *et al.*^[26] developed a rapid method to detect LNM with the use of a reverse transcription loop mediated isothermal amplification (RT-LAMP) reaction. This technique requires less than 1 hour to obtain the final results. Recently, Sysmex Corp. (Kobe, Japan) developed a one-step nucleic acid amplification (OSNA) assay, which is an automated system that uses the RT-LAMP method for gene amplification. In this system, the cytokeratin 19 (CK19) mRNA, cancer specific molecular marker, is directly and rapidly amplified from the homogenized lymph nodes. Results are available within 30 min for one lymph node because the mRNA purification process that is usually performed in RT-PCR methods is not required in this assay. We can also analyze four lymph nodes simultaneously. Kumagai *et al.*^[27] reported that the OSNA assay could detect LNM as accurately as the histological examination of blocks sectioned at 2-mm intervals in the patients with gastric cancer. Therefore, they concluded that the OSNA assay is a useful tool for the intraoperative diagnosis of LNM in gastric cancer patients. Moreover, Yaguchi *et al.*^[28] described the use of the OSNA assay with SLNNS in patients with gastric cancer. There is some doubt as to whether the OSNA assay using a single marker is sufficient for detection of LNM in gastric cancer. However, advances in the RT-PCR assay are likely, and will likely enhance the clinical utility of this molecular system for

Table 1 Immunohistochemical studies in gastric cancer patients with histological node negatively diagnosed by hematoxylin-eosin staining *n* (%)

Ref.	Year	Marker	Depth of tumor invasion	No. of patients	No. of micrometastatic patients	5-yr survival	P value
Maehara <i>et al</i> ^[41]	1996	CAM5.2	T1	34	8 (23.5)	N/A	N/A
Cai <i>et al</i> ^[11]	2000	CAM5.2	T1b	69	17 (24.6)	82% vs 100%	< 0.010
Harrison <i>et al</i> ^[42]	2000	CAM5.2	T1-T4	25	9 (36)	35% vs 66%	0.048
Nakajo <i>et al</i> ^[41]	2001	AE1/AE3	T1-T3	67	10 (14.9)	N/A	N/A
Fukagawa <i>et al</i> ^[40]	2001	AE1/AE3	T2-T3	107	38 (35.5)	94% vs 89%	0.86
Morgagni <i>et al</i> ^[39]	2001	MNF116	T1	139	24 (17.3)	87% vs 88%	0.6564
Choi <i>et al</i> ^[38]	2002	35βH11	T1b	88	28 (31.8)	92.9% vs 95%	0.6836
Yasuda <i>et al</i> ^[29]	2002	CAM5.2	T2-T4a	64	20 (31.3)	66% vs 95%	< 0.010
Morgagni <i>et al</i> ^[37]	2003	MNF116	T1	300	30 (10)	94% vs 89%	0.7794
Miyake <i>et al</i> ^[36]	2006	AE1/AE3	T1	120	27 (22.5)	N/A	N/A
Yonemura <i>et al</i> ^[35]	2007	AE1/AE3	T1-T4	308	37 (12)	N/A	N/A
Kim <i>et al</i> ^[30]	2008	AE1/AE3	T1-T4a	184	31 (16.8)	58.5% vs 91.8%	< 0.001
Ishii <i>et al</i> ^[34]	2008	O.N.352	T1b-T2	35	4 (11)	N/A	N/A
Kim <i>et al</i> ^[33]	2009	AE1/AE3	T1	90	9 (10)	N/A	N/A
Cao <i>et al</i> ^[32]	2011	AE1/AE3	T1	160	34 (21.3)	55.9% vs 92.9%	< 0.001
Wang <i>et al</i> ^[31]	2011	AE1/AE3	T1-T3	191	54 (28.3)	27.8% vs 87.1%	< 0.001

T1: Invasion of lamina propria or submucosa; T3: Invasion of subserosa; T4: Penetration of serosa without invading adjacent structures (T4a) or invasion of adjacent structures; N/A: Not applicable.

intraoperative detection of LNM when we intend to perform minimally invasive surgery, such as SLNNS, in patients with gastric cancer.

INCIDENCE OF MICROMETASTASIS

Since 1996, there have been many studies regarding LNM detected by IHC in pN0 gastric cancer patients (Table 1). When comparing studies, there are remarkable differences in the number of patients, dissected lymph nodes, the depth of tumor invasion, and the number of node sections assessed by IHC. However, the incidence of LNM ranged from 10% to 36 % in all studies^[1,4,29-42]. It is noteworthy that the incidence of LNM in pN0 gastric cancer was 10% or more even in the patients with mucosal and submucosal cancer^[1,4,32,33,36-39].

Some studies have investigated LNM as determined by RT-PCR in pN0 gastric cancer patients^[16-19,24]. In our study^[23], we detected 49 of 286 histologically node negative lymph nodes (17.1%) by RT-PCR analysis of MUC2 mRNA. Of these 49 LNM, only six were detected by IHC using AE1/AE3 mAb. Similarly, Arigami *et al*^[18] and Kubota *et al*^[43] also reported that the incidence of LNM detected by RT-PCR was higher than that of LNM detected by IHC. These results suggest that the RT-PCR assay is now the most sensitive method for detection of LNM in gastric cancer patients.

CLINICAL SIGNIFICANCE OF MICROMETASTASIS

Many studies have reported the clinical impact of LNM in various cancers. Particularly, sentinel lymph node micrometastases were reported to be associated with adverse outcomes in patients with malignant melanoma^[44] and breast cancer^[45]. However, the clinical significance of

LNM in patients with gastric cancer remains controversial.

Some studies have investigated the clinical impact of LNM in gastric cancer using IHC. Yasuda *et al*^[29] studied 64 patients with pT2-3N0 gastric cancer and reported that the 5-year overall survival rates in patients with or without LNM were 66% and 95% ($P < 0.01$), respectively. Cao *et al*^[32] and Yonemura *et al*^[35] also reported that patients with LNM had significantly worse outcomes than those without LNM in pN0 gastric cancer. In contrast, Morgagni *et al*^[37] studied 300 patients with pT1N0 gastric cancer and reported that there were no significant differences in the 10-year overall survival rates regardless of the presence of LNM. Fukagawa *et al*^[40] studied 107 gastric cancer patients with pT2N0 or pT3N0 tumors at the Japanese National Cancer Center and also reported that there were no significant difference in the 5-year survival rates and 10-year survival rates in patients with or without LNM. On the other hand, no investigations have studied the relationship between the incidence of LNM detected by RT-PCR and patient outcomes (Table 2).

To verify these results from the viewpoint of tumor biology, Yonemura *et al*^[35] immunostained sections of lymph nodes diagnosed as pN0 by H-E staining using Ki-67 mAb (MIB-1). This IHC analysis demonstrated positive MIB-1 labeling in 12 of 25 (48.0%) with single isolated cancer cells and in 49 of 52 (94.2%) with clusters of cancer cells. Similarly, Yanagita *et al*^[46] also assessed the proliferative activity of ITC and micrometastasis using IHC analysis with Ki-67 mAb. According to this study, the Ki-67 positivity rates for macrometastasis, micrometastasis and ITC were 96%, 92% and 29%, respectively. These two studies suggest that LNM could have proliferative activity.

Although it remains clinically difficult to draw definitive conclusions regarding this issue, the clinical outcome was not affected by the presence of LNM who underwent curative gastrectomy with D2 lymph node dissec-

Table 2 Reverse transcription-polymerase chain reaction studies in gastric cancer patients with histological node negativity diagnosed by hematoxylin-eosin staining *n* (%)

Ref.	Year	Markers	Depth of tumor invasion	No. of patients	No. of total LNs	No. of micrometastatic patients	Outcomes
Okada <i>et al</i> ^[16]	2001	CEA, CK20, MAGE3	T1-T4a	24	335	10 (41.7)	N/A
Matsumoto <i>et al</i> ^[17]	2002	CEA	T1-T4	50	312	14 (28)	N/A
Arigami <i>et al</i> ^[18]	2005	CEA	T1-T3	80	1862	25 (31.3)	N/A
Sonoda <i>et al</i> ^[24]	2006	MUC2, TFF1	T1	33	310	11 (33)	N/A

T1: Invasion of lamina propria or submucosa; T3: Invasion of subserosa; T4: Penetration of serosa without invading adjacent structures (T4a) or invasion of adjacent structures; N/A: Not applicable, LN: Lymph node; RT-PCR: Reverse transcription-polymerase chain reaction; CEA: Carcinoembryonic antigen; CK: Cytokeratin.

tion in Japan.

FUTURE PERSPECTIVE

Gastrectomy with regional lymph nodes dissection has been widely accepted as the standard treatment even for the EGC. However, such extensive surgery is associated with long-term reduction of patients' QOL. The patients with EGC could have received curative endoscopic treatment by using ESD even with the submucosal invasion. A large number of studies have been published so as to the good long-term outcome of EGC treated by ESD. In the cases of the contraindication of ESD, surgery is usually required. Recently, minimally invasive surgery, such as SLNNS, has been widely performed. An accurate and rapid intraoperative diagnosis of lymph node metastasis, including LNM, is essential when performing SLNNS. Kumagai *et al*^[27] reported that the OSNA assay could detect LNM as accurately as that detected by histological examination of blocks sectioned at 2-mm intervals in the patients with gastric cancer. Moreover, such results are available within 30 min. From these points of view, the OSNA assay is considered to be the most accurate and rapid method for the intraoperative detection of LNM in patients with gastric cancer. Therefore, if intraoperative molecular examinations, such as the OSNA assay, demonstrate no metastasis in all SLN, then patients could be received laparoscopic partial or segmental gastrectomy with SLN dissection. However, Shimizu *et al*^[47] reported that seven (6.8%) of 103 patients had LNM in non-sentinel lymph nodes (all within the same lymphatic basin), according to RT-PCR performed with multiple markers (CK19, CK20 and CEA). The lymphatic basin is regarded as the most important lymphatic area in which lymph node metastasis may develops. Kinami *et al*^[48] reported that the gastric lymphatic basins were divided in the following five directions along the main arteries around the stomach: left gastric artery area (l-GA), right gastric artery area (r-GA), right gastroepiploic artery area (r-GEA), left gastroepiploic artery area (l-GEA), and posterior gastric artery (p-GA). They also reported that nodal metastasis generally occurred in the SLNs or the lymphatic basin, and rarely extended outside the lymphatic basin. Therefore, those investigators concluded that if the all SLNs are negative for cancer metastasis by intraoperative

diagnosis, patients could be received limited gastrectomy, such as partial or segmental gastrectomy with SLN basin dissection. On the other hand, in the case of positive for SLN metastasis confirmed by intraoperative diagnosis, standard gastrectomy with regional lymphadenectomy is considered necessary. Recently, Bok *et al*^[49] reported that combined ESD and sentinel lymph nodes surgery (ESN) is a feasible minimally invasive procedure for cT1 (< 3 cm) N0 EGC. We believe that, in the near future, such advanced technology allows the patients with EGC to be treated with the procedures that balance between curability and QOL. Future studies will be needed to achieve such objectives.

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