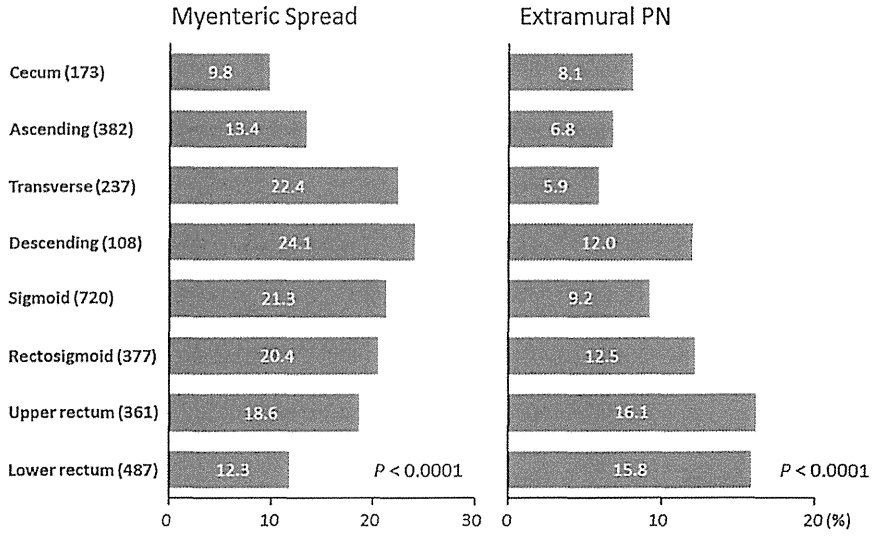

Fig. 2 Incidence of myenteric spread and extramural perineural invasion (PN) according to location of the primary tumor

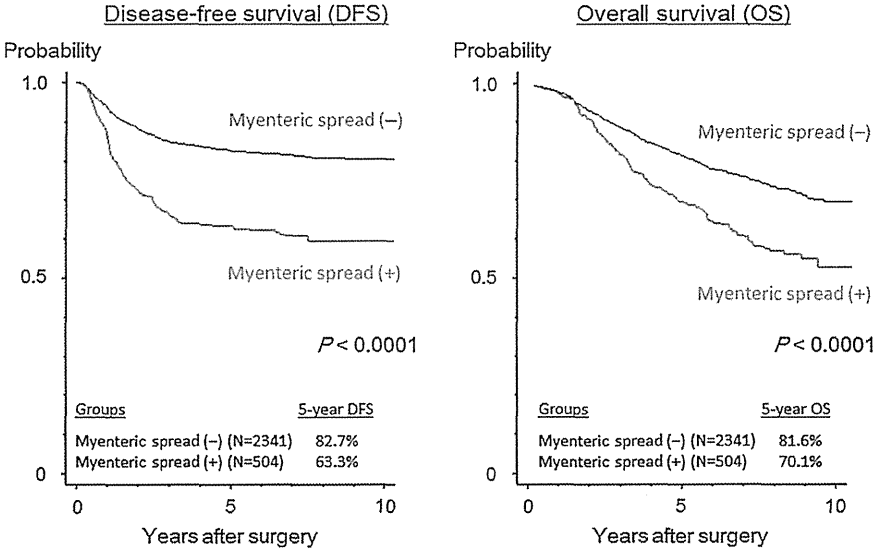

Fig. 3 Disease-free and overall survival curves with or without myenteric spread in colorectal cancer

location was also found to be unrelated to the type of myenteric spread.

With regard to prognostic relevance, there was no significant difference in the recurrence-free and overall survival rate of patients with myenteric spread in relation to the intralesion presence or absence of PN.

Immunohistochemical staining for myenteric spread

In 48 out of 50 tumors, immunohistochemical staining using neural markers revealed the existence of remnants of neural tissue within or around cancer nests (Fig. 4) located at the leading edge of myenteric spread. For 2 tumors showing negative result, additional sectioning followed by immunohistochemical staining was performed in the same manner as that performed previously, and in both the cases, a remnant of neural tissue was observed in a cancer nest located at the leading edge of the myenteric spread. Consequently, in all the 50 tumors diagnosed by H&E staining as showing myenteric spread but unaccompanied by PN, a positive result was obtained with regard to the finding of nerve involvement by the tumor.

On the other hand, on the basis of D2-40 immunostaining, cancer foci within lymphatic channels in the Auerbach's plexus area were observed only in 2 cases. Both these tumor foci were located at the center of the lesion.

Discussion

With regard to the underlying histogenesis of myenteric spread in CRC, histological evidence of PN was identified

Table 2 Univariate and multivariate analyses of disease-free survival by the Cox proportional hazards regression model

Variables	Category	Univariate		Multivariate	
		HR (95 % CI)	*P* value	HR (95 % CI)	*P* value
Tumor size (mm)		1.0 (1.0–1.0)	<0.0001	–	
Tumor differentiation	Well	1		1	
	Moderate	1.8 (1.5–2.2)	<0.0001	1.3 (1.1–1.6)	0.0041
	Others	2.9 (2.2–3.9)	<0.0001	2.0 (1.5–2.7)	<0.0001
T stage	T2	1		1	
	T3	2.6 (1.9–3.5)	<0.0001	1.7 (1.2–2.3)	0.0008
	T4	4.2 (3.0–5.7)	<0.0001	2.2 (1.6–3.0)	<0.0001
N stage	N0	1		1	
	N1	2.3 (1.9–2.8)	<0.0001	2.1 (1.7–2.5)	<0.0001
	N2	5.6 (4.6–6.9)	<0.0001	3.4 (2.7–4.3)	<0.0001
Lymphatic invasion	Negative	1		–	
	Positive	2.0 (1.6–2.4)	<0.0001		
Venous invasion	Negative	1		1	
	Positive	1.8 (1.5–2.1)	<0.0001	1.4 (1.2–1.7)	0.0001
Myenteric spread	Negative	1		1	
	Positive	2.4 (2.0–2.9)	<0.0001	1.4 (1.1–1.7)	0.0016
Extramural PN	Negative	1		1	
	Positive	4.2 (3.5–5.0)	<0.0001	2.2 (1.8–2.7)	<0.0001

HR hazard ratio, *CI* confidence interval

without exception at the advancing edge of this distinctive horizontal spread on the basis of immunohistochemical staining with neural markers. On the other hand, the results of D2-40 immunohistochemical staining confounded the hypothesis that cancer development through the lymphatic network is the underlying cause of myenteric spread. In addition, with regard to differences in the clinicopathological background of tumors with myenteric spread according to the presence or absence of intralesion PN, there were no significant differences in conventional factors associated with tumor aggression, such as tumor grade, vascular invasion, extramural PN, or lymph node metastasis. We also observed that there were no differences in prognostic outcome between the 2 tumor groups. All the findings of our study indicate that myenteric spread in CRC is the result of a single histogenesis, PN, i.e., cancer development with the replacement of the nerves of Auerbach's plexus, and this type of cancer spread is intrinsically the same, irrespective of whether PN is identified on H&E slides.

Some authors regard the nerve sheath as important in the assessment criteria of PN [1, 6, 7]. Fujita et al. [4] defined PN in Auerbach's plexus as the presence of cancer nests inside the perineurium. However, it is difficult to identify the perineurium in Auerbach's plexus on H&E slides. We believe that myenteric spread should be recognized as intramural PN, irrespective of whether cancer nests are located within the perineurium. The standard of this assessment criterion allows pathologists to diagnose intramural PN without special additional staining to identify the perineurium, and improvement in interobserver agreement in determining intramural PN may thus be expected.

There is much literature concerning extramural PN in CRC [6, 8–13], which is repeatedly shown as an independent prognostic indicator [10, 11, 14–17] and the National Comprehensive Cancer Network (NCCN) guidelines regard PN as a high-risk factor for recurrence in stage II patients [18]. However, the actual status and clinical value of intramural PN have not been fully investigated. It is noteworthy that intramural PN (i.e., myenteric spread) was demonstrated to impact survival outcome independent of conventional parameters including stage-factors and extramural PN in the present study.

We found fundamental differences between intramural PN and extramural PN. First, distribution within the large bowel varies greatly. The incidence of PN is reportedly higher in rectal cancer than in colon cancer [12]. This appeared to be the case for extramural PN in our study, although the incidence of intramural PN was relatively low in the lower rectum and cecum. Second, the survival impact differed between intramural PN and extramural PN, i.e., extramural PN exerted a greater adverse survival impact than intramural PN. Although both the AJCC staging manual and NCCN guidelines treat PN in all layers of the bowel equally as an unfavorable prognostic marker, the clinical value of this site-specific prognostic marker

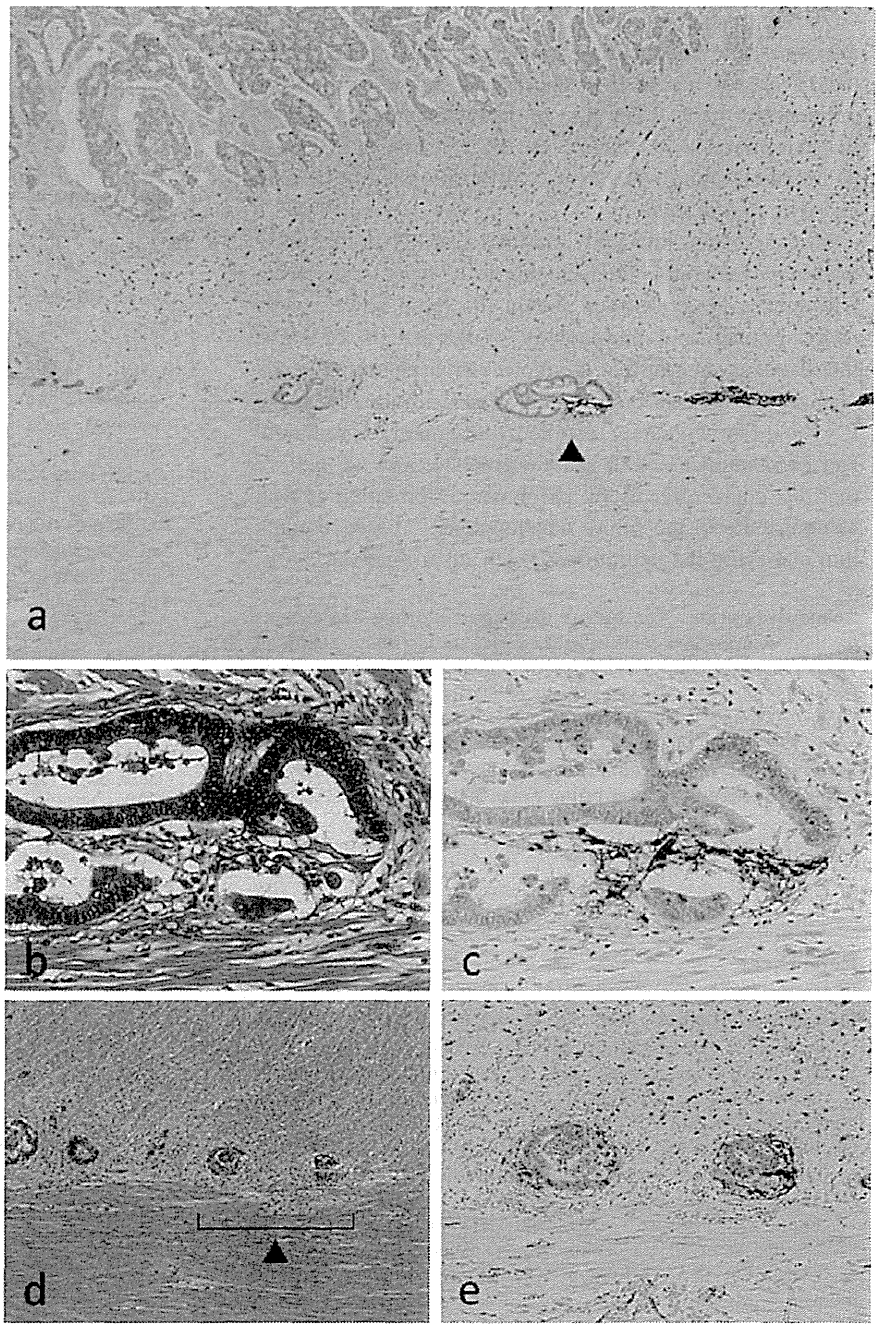

Fig. 4 Serial sectioning of a cancer nest located at the leading edge of myenteric spread. Note that remnant neural tissue exists within or around a cancer nest located at the leading edge of horizontal spread along Auerbach's plexus. **a** S100 immunostaining (×4 objective lens); **b** magnification of area indicated by arrowhead in **a**, H&E staining (×40 objective lens); **c** magnification of area indicated by arrowhead in **a**, S100 immunostaining (×40 objective lens); **d** H&E staining (×10 objective lens); **e** magnification of area indicated by arrowhead in **d**, S100 immunostaining (×20 objective lens)

would be enhanced by discriminating between intramural PN and extramural PN.

Third, there was a difference in the morphological pattern of tumor nerve involvement between intramural PN and extramural PN. In extramural PN foci, cancer nests were located along the nerve fascicles. Generally, we can easily identify nerve fascicles by cancer nests. On the other hand, in myenterically spread lesions (mostly at the advancing edge of horizontal spread), we often observe a "moth-eaten" appearance of the nerves involved, in which tiny cancer nests invade nerve fascicles. This morphological pattern leads us to a presumption that cancer develops by replacing nerve tissue, resulting in its disappearance, and this is why the typical finding of PN is rarely observed in myenterically spread lesions, except at the leading edge. Two types of nerve sheath are present in the nervous system distributed within the large bowel: the endoneurium and perineurium. The abovementioned differences in the morphological pattern of nerve involvement by cancer cells between intramural and extramural layers leads us to the

view that myenterically spread tumors may have a greater affinity to the endoneurium or neural fibers in the Auerbach's area, whereas the adhesion of tumor cells to the perineurium plays a key role in the development of PN in the extramural area. Otherwise, the function of the perineurium as a tumor barrier may differ between intramural and extramural areas.

In conclusion, this study clarified that PN in the Auerbach's plexus area is the histogenesis of myenteric spread. Differences in distribution within the large bowel, prognostic impact, and morphology of the pattern of nerve involvement suggest that the molecular background of PN may differ between the intramural and extramural layers. PN should be separately recorded with regard to intramural and extramural areas in routine practice, and we believe that this could offer useful prognostic information as well as contribute to the future development of basic research into clarifying the neurotropic mechanism involved.

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Conflict of interest The authors declare no conflict of interest.

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Original Article

Tumor-size-based morphological features of metastatic lymph node tumors from primary lung adenocarcinoma

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Most primary lung adenocarcinomas show histological diversity, however, histological diversity in the metastatic lymph node tumors (LNT) is not well defined. The aim of this study was to explore the histological characteristics of the metastatic LNT based on their sizes. We analyzed 163 primary tumors and 509 metastatic LNTs. When the primary tumor showed papillary-predominant subtype, the most frequent histological subtype in the metastatic LNT that were ≤2 mm in diameter was solid subtype (49%), followed by papillary subtype (35%); on the other hand, in the metastatic LNT measuring >2 mm in size, the frequency of tumors showing papillary-predominant subtype increased significantly to 52% ($P = 0.04$). When the primary tumor showed acinar-predominant subtype, the most predominant subtype in the ≤2 mm metastatic LN tumors was acinar subtype (55%), followed by solid subtype (40%), with the frequency of acinar subtype increasing significantly to 76% in the metastatic LNT that were >2 mm in diameter ($P = 0.04$). These results indicate that solid subtype is the characteristic histological subtype in the early phase of the LN metastatic process, and that as the metastatic LNT grow larger, they develop morphological features resembling those in the primary tumor.

Key words: diversity, lung adenocarcinoma, lymph node metastasis, micrometastasis

Adenocarcinoma is the most common histologic type of primary lung cancer and is a major focus of research to improve the patients' survival. In the new adenocarcinoma classification, International Multidisciplinary Lung Adenocarcinoma Classification, published in 2011 by the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS), invasive adenocarcinoma is divided into five predominant subtypes: the lepidic-, acinar-, papillary-, micropapillary- and solid-predominant subtypes.[1] Most adenocarcinomas are histologically heterogeneous, consisting of two or more histological subtypes. Matching the new adenocarcinoma classification, many studies have reported the prognosis and characteristics of the gene mutations according to this histological typing.[2–6] However, most previous studies focused on the primary tumors, and few studies have focused on the metastatic lesions.

Metastasis is considered as a complex and multistep process that ultimately results in the formation of a secondary mature tumor.[7–9] At the metastatic site, neoplastic cancer cells first go through an avascular growth phase to reach a size not much more than a few millimeters in diameter. During this phase, a small number of tumor cells survive and interact with the surrounding stromal cells, to then develop into macroscopically detectable metastatic lesions. During this metastatic tumor development, effective and dynamic molecular changes, including the epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) have been reported.[10,11]We previously reported that as intrapulmonary metastatic lesions grow larger, the constituent cancer cells exhibit diverse growth patterns, which results in histological diversity in the secondary tumors, just as in the case of primary tumors.[12] However, the morphological changes in early and small metastatic lymph node tumors have not yet been clarified, and it is not yet known whether larger metastatic lymph node tumors exhibit as much histological diversity as the primary tumors. In the present report, we attempt to elucidate the morphological characteristics of the metastatic lymph nodes with special reference to their size and histological diversity. In the TNM Classification of Malignant Tumors, 7th Edition, published in 2009, for breast cancer, if the size of the cancer spread is larger than 0.2 mm and/or more than 200 cells, but not larger than 2 mm, it is

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defined as *micrometastasis*.[13] Based on this model, we evaluated the morphological features of metastatic lymph node tumors in relation to their sizes: > 2 mm vs. ≤ 2 mm.

MATERIALS AND METHODS

Patient selection

Lymph node metastasis from primary lung adenocarcinoma was detected in 184 consecutive patients who underwent surgical resection without preoperative therapy at the National Cancer Center Hospital East, Chiba, Japan, between January 2008 and December 2012. After excluding 21 cases for which only incomplete clinical information was available, the remaining 163 cases with 509 metastatic lymph nodes were histologically evaluated. The data collection and analyses were performed with the approval of the institutional review board.

Pathological studies

All surgical specimens were fixed with 10% formalin and embedded in paraffin. The tumors were cut at approximately 5-mm intervals, and serial 4-μm sections were stained with hematoxylin-eosin. The median number of tissue blocks per resected specimen was 25 (range: 10–90). The materials were subsequently reviewed by two pathologists (E.Y and G.I.) to confirm the presence of lymph node metastasis and to assess the histopathological features of both the primary tumors and the metastatic lymph nodes.

Histological subtyping of the primary tumors

Histological subtyping of the primary tumors was based on the IASLC/ATS/ERS International Multidisciplinary Lung Adenocarcinoma Classification published in 2011. The lepidic subtype is defined as growth of neoplastic cells along preexisting alveolar structures. The papillary subtype is defined as growth of glandular cells along central fibrovascular cores. If a tumor shows lepidic growth, but the alveolar spaces are filled with papillary structures, the tumor is classified as the papillary subtype. The acinar subtype is defined by the formation of round to oval-shaped glandular structures with a central luminal space surrounded by tumor cells. A cribriform arrangement is regarded as representing the acinar subtype of adenocarcinoma. The micropapillary subtype is defined as tumor cells growing in papillary tufts lacking fibrovascular cores, which may appear detached and/or connected to the alveolar walls. Ring-like glandular structures floating within the alveolar spaces are also regarded as representing micropapillary components. The solid-predominant subtype is characterized by the appearance of polygonal tumor cells arranged in sheets, lacking the features of any of the other recognizable histologic subtypes of adenocarcinoma.

Comprehensive histologic subtyping was performed through a process in which the percent area occupied by each histopathological subtype present in a tumor was estimated in 10% increments, followed by identification and classification of that tumor according to the histologic subtype. The predominant subtype was defined as the subtype accounting for the largest percent area in a tumor. In this cohort, none of the primary tumors were identified as showing the micropapillary-predominant subtype. The typical appearances of the histological subtypes of the primary tumors are shown in Fig. 1(a,h,o).

Histological subtyping of the metastatic lymph node tumors

Histological subtyping of the metastatic lymph node tumors was performed according to the same classification that was used for the primary tumor, and the predominant subtype was also identified. In the case of small metastatic tumors, isolated and small clusters of tumor cells which lacked clear differentiation into the papillary or acinar pattern were divided into the solid-predominant subtype. Also, as the micropapillary-predominant subtype, characterized by tumor cells growing in papillary tufts lacking a fibrovascular core, frequently coexisted with the papillary component, it was included in the papillary-predominant subtype.

The sizes of the metastatic lymph node tumors were evaluated based on the maximum diameter of the metastatic lesion in the lymph nodes. According to the TNM classification of Malignant Tumors, 7th Edition, published in 2009, we evaluated the morphological features of the metastatic lymph node tumors in relation to their sizes, > 2 mm vs. ≤ 2 mm. The typical appearances of the histological subtypes of the metastatic lymph node tumors are shown in Fig. 1(b–g,i–n,p–s).

Histological diversity according to the sizes of the metastatic tumors

The number of histological subtypes in the metastatic lymph node tumors of the size two groups (≤2 mm vs. >2 mm) was compared statistically according to the number of subtypes in the primary tumor. As the micropapillary subtype was observed in only a very small proportion of cases, and frequently coexisted with the papillary component, it was included in the papillary subtype.

Clinical information

All available clinical information was obtained from the clinical records and reports of the referring physicians. The records were reviewed for the patient age, sex, smoking history, pathological T and N classification, stage, and number of

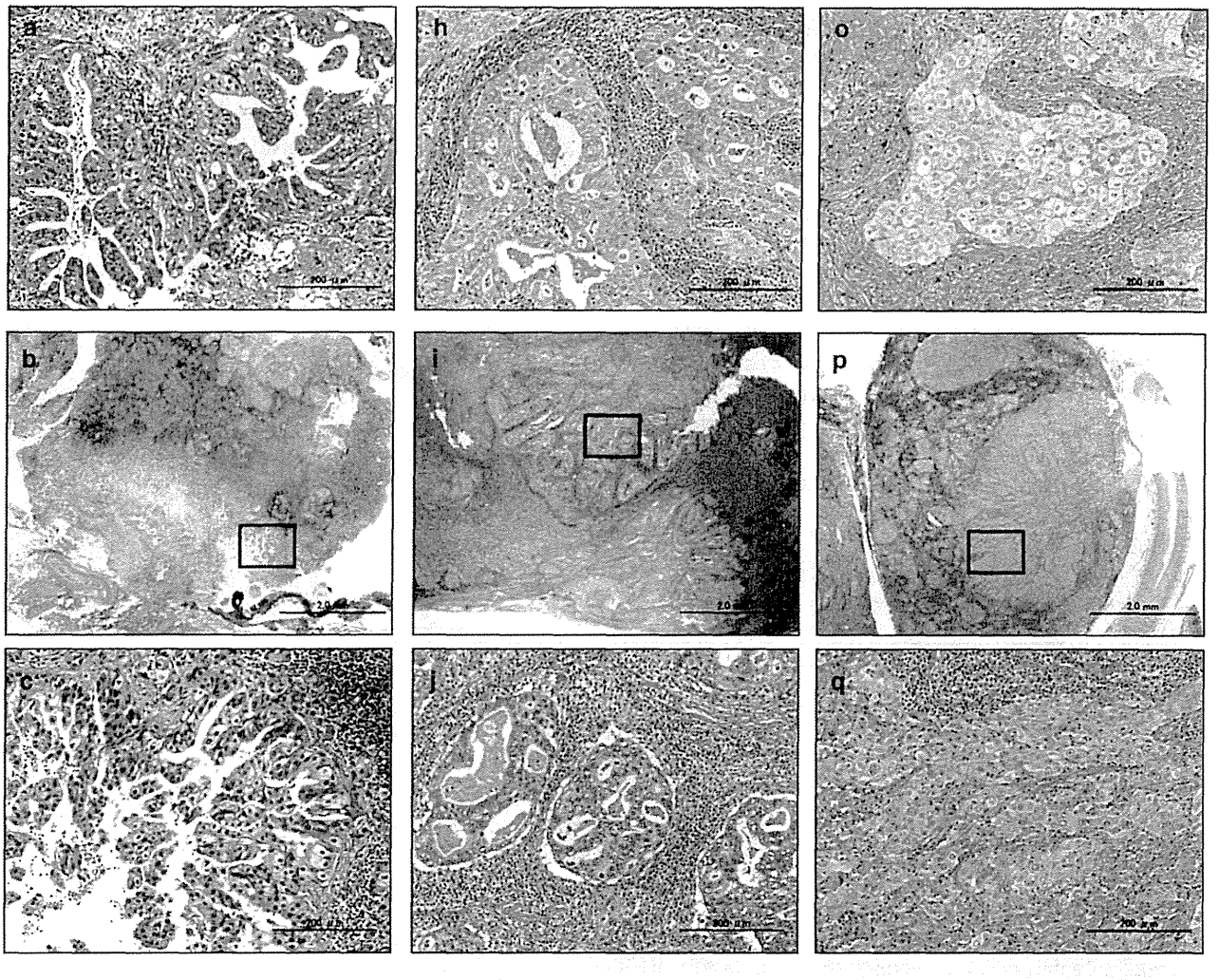

Figure 1 Histopathological features of the primary tumors and metastatic lymph node tumors(H & E). (**a**) Primary tumor showing the papillary-predominant subtype. (**b**) Metastatic lymph node tumor that was >2 mm in diameter. (**c**) Higher magnification view of the tumor shown in **b**. The tumor was mainly composed of the papillary component. (**d,f**) Metastatic lymph node tumors that werre ≤2 mm in diameter. The tumor was mainly composed of the (**d**) solid or the (**f**) papillary component. (**e,g**) Higher magnification views of the tumors shown in **d** and **f**, respectively. (**h**) Primary tumor showing the acinar-predominant subtype. (**i**) Metastatic lymph node tumor that was >2 mm in diameter. (**j**) Higher magnification view of the tumor shown in (**i**). The tumor was mainly composed of the acinar component. (**k**) and (**m**) Metastatic lymph node tumors that were ≤2 mm in diameter. The tumor was mainly composed of the (**k**) solid or the (**m**) acinar component. (**l**) and (**n**) Higher magnification views of the tumors shown in **k** and **m**, respectively. (**o**) Primary tumor showing the solid-predominant subtype. (**p**) and (**r**) Metastatic lymph node tumors that were (**p**) > 2 mm and (**r**) ≤ 2 mm in diameter. (**q**) and (**s**) Higher magnification views of the tumors shown in **p** and **r**, respectively. The tumor was mainly composed of the solid component, irrespective of the tumor size.

metastatic lymph nodes. Pathological staging was based on the TNM classification of the International Union Against Cancer (UICC).[13]

Statistical analysis

Differences in the patient characteristics between the two groups were compared by the Pearson's chi-square test. The unpaired t-test was performed to calculate the statistical significance of the differences. All *P* values are two-sided, and the significance level was set at <0.05. The analyses were performed with the SPSS 11.0 statistical software program (Dr. SPSS II for Windows, standard version 11.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Clinicopathological characteristics of the patients with lymph node metastasis

The clinicopathological characteristics of the 163 adenocarcinoma patients diagnosed as having lymph node metastasis

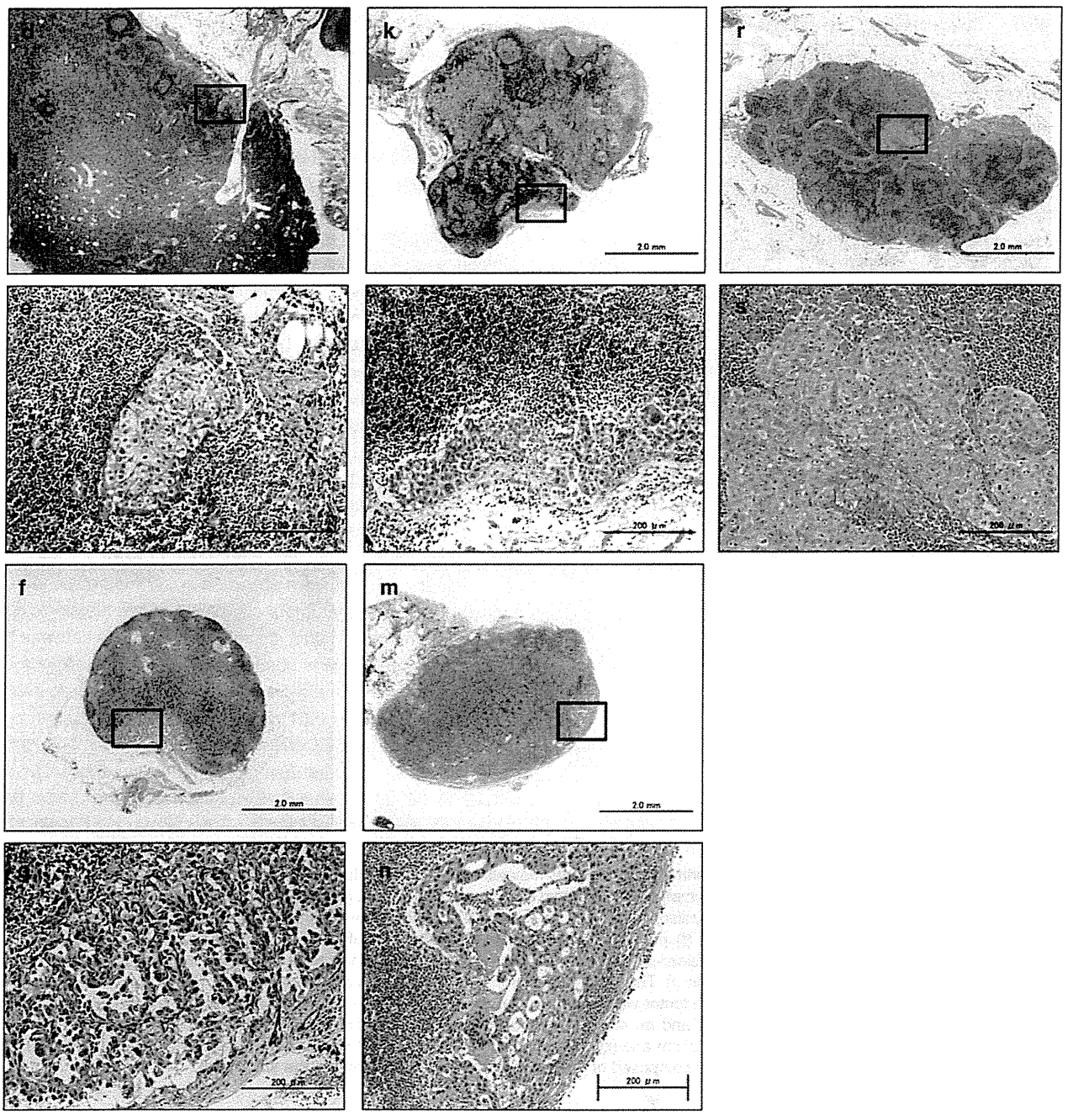

Figure 1 Continued

are summarized in Table 1. In all, 54 patients (33.1%) and 109 patients (66.9%) were pathologically diagnosed as belonging to the N1 and N2 categories, respectively.

The histologic subtypes of the primary tumors were distributed as follows: lepidic-predominant subtype, 6 tumors (3.7%); papillary-predominant subtype, 59 tumors (36.2%); acinar-predominant subtype, 35 tumors (21.5%); solid-predominant subtype, 63 tumors (38.6%).

Pathological characteristics of the metastatic lymph node tumors

Clinicopathological information pertaining to the 509 metastatic lymph node tumors is summarized in Table 2. In all, 286 (56.2%) and 223 (43.8%) of the metastatic lymph node tumors were pathologically diagnosed as belonging to the N1 region and N2 region, respectively. The histologic subtypes

Table 1 Characteristics of the patients with lymph node metastasis

Factors	*n* = 163 (%)
Age (years)	
<65	71 (43.6)
≥65	92 (56.4)
Sex	
Male	100 (61.3)
Female	63 (38.7)
Smoking history	
Never smoker	59 (36.2)
Current/Previous smoker	104 (63.8)
Pathological T classification	
pT1	40 (24.5)
pT2	81 (49.7)
pT3	33 (20.3)
pT4	9 (5.5)
Pathological N classification	
pN1	54 (33.1)
pN2	109 (66.9)
Pathological Stage (UICC7)	
Stage IIA	34 (20.8)
Stage IIB	6 (3.7)
Stage IIIA	111 (68.1)
Stage IIIB	7 (4.3)
Stage IV	5 (3.1)
Predominant subtype in primary tumor	
Lepidic	6 (3.7)
Papillary	59 (36.2)
Acinar	35 (21.5)
Solid	63 (38.6)
Number of metastatic lymph nodes	
1	47 (28.8)
2–5	82 (50.3)
≥6	34 (20.9)

Table 2 Characteristics of metastatic lymph node tumors

Factors	*n* = 509 (%)
Region	
Hilar, lobar and segmental (N1)	286 (56.2)
Mediastinal (N2)	223 (43.8)
Predominant subtype	
Papillary	107 (21.0)
Acinar	119 (23.4)
Solid	283 (55.6)
Metastatic lesion size	
≤2 mm	122 (24.0)
>2 mm, ≤5 mm	165 (32.4)
>5 mm	222 (43.6)

of the metastatic lymph node tumors were distributed as follows: papillary-predominant subtype, 107 lymph nodes (21.0%); acinar-predominant subtype, 119 lymph nodes (23.4%); solid-predominant subtype, 283 lymph nodes (55.6%).

Size distribution of the metastatic lymph node tumors

The median diameter of the lesions was 5 mm. The numbers of metastatic lymph node tumors belonging to the three size categories of ≤2 mm, >2 mm but ≤5 mm (median size) and >5 mm were 122 (24.0%), 165 (32.4%) and 222 (43.6%)(Table 2).

The number of histologic subtypes in the metastatic lymph node tumors divided according to the size

We compared the number of component histological subtypes in the primary tumors and metastatic lymph node tumors by dichotomizing the metastases according to the tumor diameter into ≤2 mm or >2 mm. The number of subtypes in the metastatic lymph node tumors in the group in which the primary tumor was composed of one subtype was 1.00 ± 0, indicating that all metastatic tumors arising from primary lesions composed of a single histologic subtype also showed a single histologic subtype (Fig. 2a). Fig. 2(b–d) shows the comparisons between the two metastatic tumor groups (divided according to the tumor size) in each case of the primary tumor being composed of two, three, or four subtypes, respectively. No significant differences between the two size groups were observed in the metastatic tumors arising from primary tumors composed of two histologic subtypes. On the other hand, significant differences between the two size groups were observed in the metastatic tumors arising from primary tumors composed of three or four subtypes (Fig. 2c,d; $P < 0.01$ and $P < 0.01$). The mean numbers ± standard error (SE) of subtypes in the metastatic lymph node tumors that were ≤2 mm and >2 mm in diameter were 1.18 ± 0.06 and 1.60 ± 0.05, respectively, and 1.32 ± 0.11 and 1.75 ± 0.06, respectively.

Comparison between the predominant histologic subtypes in the primary tumors and metastatic lymph node tumors

We compared the predominant histologic subtypes of the primary tumors and 509 metastatic lymph node tumors (Table 3). The predominant subtypes of the metastatic lymph node tumors in the cases in which the primary tumors showed the lepidic-predominant subtype were the papillary subtype (50.0%) and acinar (50.0%) subtype. The predominant subtype of the metastatic tumors in the cases in which the primary tumors showed the papillary-predominant subtype was the original papillary subtype (47.4%), followed by the solid (39.7%) and acinar (12.9%) subtypes. The predominant subtype of the metastatic tumors in the cases in which the primary tumors showed the acinar-predominant subtype was the original acinar subtype (72.0%), followed by the solid (23.4%) and papillary (4.6%) subtypes. The predominant subtype of the metastatic lymph node tumors in the cases in which the primary tumors showed the solid-predominant

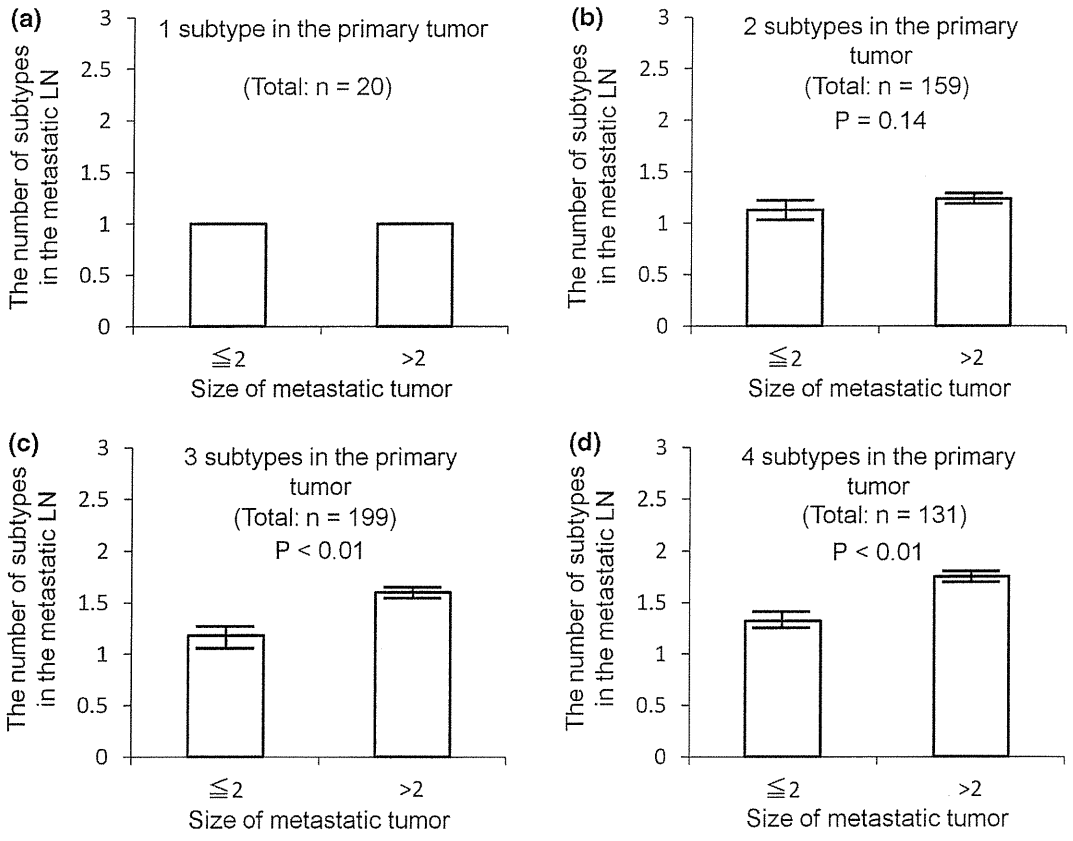

Figure 2 The number of component histologic subtypes in the metastatic lymph node tumors. (**a**) Comparison of the histologic subtypes in the group in which the primary tumor was composed of a single subtype. The mean number of histologic subtypes in both metastatic tumor size groups was 1.00. (**b**) Comparison in the group in which the primary tumor was composed of 2 subtypes (≤2 mm: 1.1, > 2 mm: 1.2: $P = 0.14$). (**c**) Comparison of the histologic subtypes in the group in which the primary tumor was composed 3 subtypes (≤2 mm: 1.2, > 2 mm: 1.6: $P < 0.01$). (**d**) Comparison of the histologic subtypes in the group in which the primary tumor was composed of 4 subtypes (≤2 mm: 1.3, > 2 mm: 1.8: $P < 0.01$).

Table 3 Comparison between the predominant histrological subtypes in the primary tumors and the metastatic lymph node tumors

Predominant subtype		
Primary tumor	Metastatic LN tumor	(%)
Lepidic	Papillary	4 (50.0)
	Acinar	4 (50.0)
	Solid	0 (0.0)
Papillary	Papillary	92 (47.4)
	Acinar	25 (12.9)
	Solid	77 (39.7)
Acinar	Papillary	5 (4.6)
	Acinar	77 (72.0)
	Solid	25 (23.4)
Solid	Papillary	6 (3.0)
	Acinar	13 (6.5)
	Solid	181 (90.5)

subtype was the original solid subtype (90.5%), followed by the acinar (6.5%) and papillary (3.0%) subtypes. The solid-predominant subtype was identified at a high frequency in the metastatic lymph node tumors, even in cases in which the primary tumor showed other predominant subtypes.

Predominant histologic subtypes according to the sizes of the metastatic lymph node tumors

Next, we compared the predominant histologic subtype of the primary tumors and matched metastatic lymph node tumors in relation to the sizes of the metastatic lymph node tumors (Fig. 3).

1 Primary tumor showing the lepidic-predominant subtype; one case of the papillary-predominant and one of the acinar-predominant subtype were identified in metastatic

Table 4 The correlation between the metastatic lymph node predominant subtype and metastatic tumor size (≤2 mm and >2 mm) in a) papillary and b) acinar predominant primary tumor

a) in papillary predominant primary tumor

	Predominant subtype		
Metastatic lymph node tumors	Papillary (%)	Non-Papillary (%)	*P*
Size			
≤2 mm	18 (35)	33 (65)	0.04
>2 mm	74 (52)	69 (48)	

b) in acinar predominant primary tumor

	Predominant subtype		
Metastatic lymph node tumors	Acinar (%)	Non-Acinar (%)	*P*
Size			
≤2 mm	12 (55)	10 (45)	0.04
>2 mm	65 (76)	20 (24)	

lymph node tumors that were more than 5 mm in size (data not shown).

2 Primary tumor showing the papillary-predominant subtype; the most frequent subtype in the metastatic tumors that were ≤2 mm in size was the solid-predominant subtype (25/51, 49.0%), followed by the papillary subtype 35.3% (18/51). However, when the metastatic tumors grew to more than 5 mm in size, the most frequent subtype was the papillary subtype in 54.3% (44/81), followed by the solid subtype in 39.5% (32/81) (Fig. 3a).
3 Primary tumor showing the acinar-predominant subtype; the acinar- and solid-predominant subtypes were identified in 54.5% (12/22) and 36.4% (8/22) of metastatic lymph node tumors that were ≤ 2 mm in diameter, respectively. On the other hand, the acinar and solid subtypes were seen in 83.8% (31/37) and 10.8% (4/37), respectively, of metastatic lymph node that were more than 5 mm in diameter (Fig. 3b).
4 Primary tumors showing the solid-predominant subtype; the solid-predominant subtype was identified in 98.0% (48/49) of metastatic lymph node tumors that were ≤2 mm in diameter. The solid subtype was seen in 86.3% (88/102) of metastatic tumors that were more than 5 mm in diameter (Fig. 3c).

Comparison of the predominant histologic subtypes between metastatic lymph node tumors that were 2 cm or under and over 2 mm in diameter

Table 4 shows the correlation between the predominant histological subtype and the metastatic tumor size (≤2 mm vs. >2 mm) in cases in which the primary tumor showed the papillary- or acinar- predominant subtype.

When the predominant histologic subtype of the primary tumor was the papillary, 35% of metastatic tumors that were ≤2 mm in diameter showed the papillary-predominant subtype; however, the frequency of tumors showing the papillary-predominant subtype increased to 52% in the metastatic tumors that were >2 mm in diameter ($P = 0.04$).

In addition, when the predominant subtype of the primary tumor was the acinar subtype, 55% of metastatic tumors that were ≤2 mm in size showed the acinar-predominant subtype, with the frequency of this histologic subtype increasing to 76% in metastatic tumors that were >2 mm in diameter ($P = 0.04$).

DISCUSSION

In the present report, we found that the predominant histologic subtype of metastatic lymph node tumors that were 2 mm or less in size often differed from that of the primary tumor. Actually, even when the primary tumor showed the acinar- or papillary-predominant subtype, metastatic lymph node tumors that were small in size often showed a solid-predominant subtype. However, as the metastatic lymph node tumors increased in size, the original subtype began to appear. These findings suggest that the solid morphology could be the initiating feature of metastasis in the early phase of the lymph node metastatic process. Then, as the metastatic tumor cells engraft and grow in the lymph nodes, the tumor cell populations regain the original morphological features and diversity. This sequence implies that the histological and biological characteristics of tumor cells in the engraftment process are largely different from those of tumor cells in the development process of metastatic tumor formation.[14–16] This is the first study to evaluate the correlation between the sizes of the metastatic tumors and the histologic subtype and tumor cell diversity in cases of adenocarcinoma of the lung.

In a previous report about the histological characteristics of metastatic lymph node tumors, Sica *et al.* reported that the

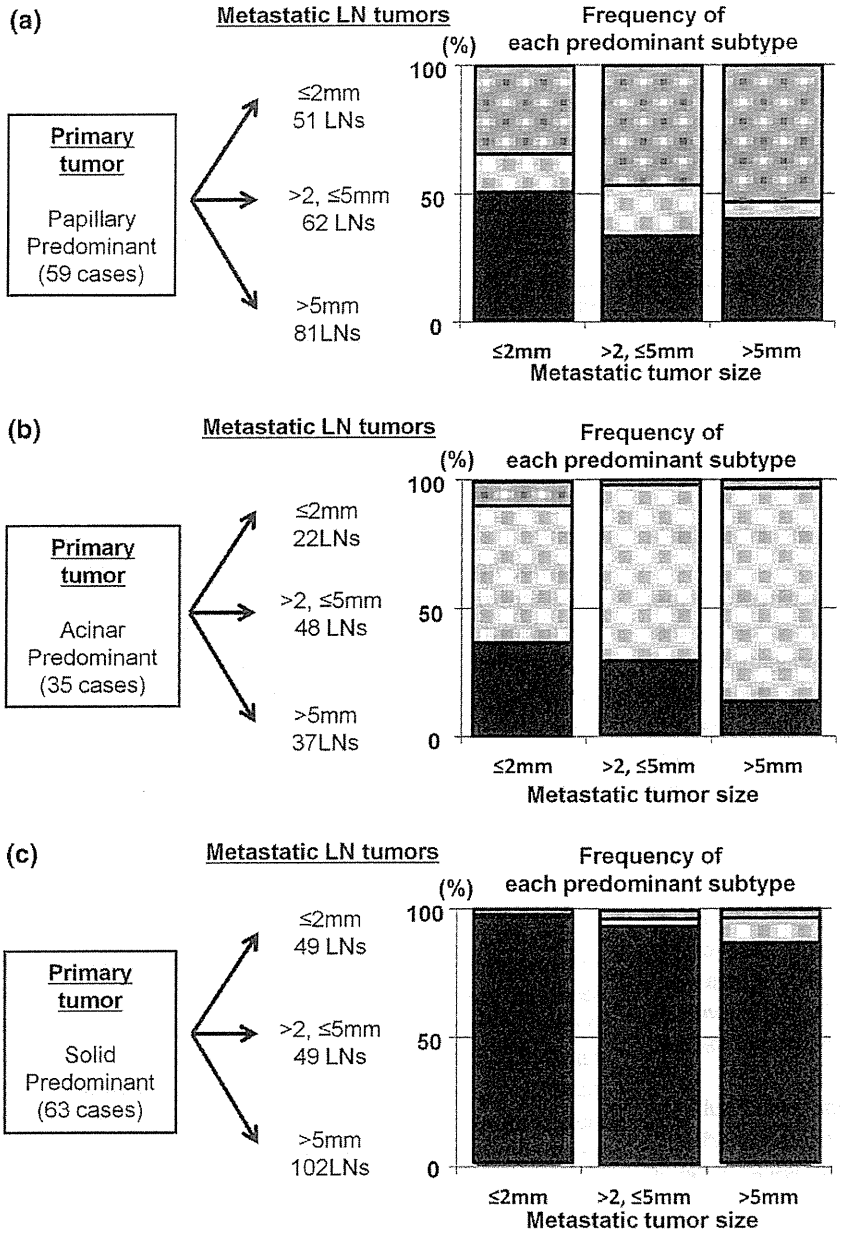

Figure 3 Comparison of the predominant histologic subtypes according to the sizes of the metastatic lymph node tumors. (**a**) Cases in which the primary tumor showed the papillary-predominant subtype. There were 51, 62 and 81 metastatic lymph node tumors that were ≤2 mm, > 2 mm but ≤5 mm and >5 mm in diameter respectively. Of the 51 metastatic tumors that were <2 mm in diameter, 25 (49%) showed the solid-predominant subtype and 18 (35%) showed the papillary-predominant subtype. Of the 81 metastatic tumors that were >5 mm in diameter, 32 (39%) showed the solid-predominant subtype and 44 (54%) showed the papillary-predominant subtype. (**b**) Cases in which the primary tumor showed the acinar-predominant subtype. There were 22, 48 and 37 metastatic lymph node tumors that were ≤2 mm, > 2 mm but ≤5 mm and >5 mm in diameter, respectively. Of the 22 metastatic tumors that were <2 mm in diameter, 12 (55%) showed the acinar-predominant subtype and 8 (36%) showed the solid-predominant subtype. Furthermore of the 37 metastatic tumors that were >5 mm in diameter, 31 (84%) showed the acinar-predominant subtype and 4 (11%) showed the solid-predominant subtype. (**c**) Cases in which the primary tumor showed the solid-predominant subtype. There were 49, 49 and 102 metastatic lymph node tumors that were ≤2 mm, > 2 mm but ≤5 mm and >5 mm in diameter, respectively. Of the 49 metastatic tumors that were <2 mm in diameter, 48 (90%) showed the solid-predominant subtype, and of the 102 metastatic tumors that were >5 mm in diameter, 87 (86%) showed the solid-predominant subtype. ▩, Papillary; □, Acinar; ▩, Solid.

predominant pattern of any primary tumor is more likely to be seen at the metastatic site, and even when the micropapillary and solid patterns are present only at a small percentage in the primary tumor, these patterns are often seen in the metastatic lymph node tumors.[17] While these findings were partly consistent with our current results, there was no reference to the correlation of the metastatic tumor size with the predominant histologic subtype in that study. Taking these observations and our current results into consideration, it may be speculated that the solid morphology may be an important feature reflecting metastasis-initiating cancer cells during the process of development of lymph node metastasis. Takuwa *et al.* reported that solid subtype in lung adenocarcinomas exhibits the invasive immunophenotype, including increased laminin-5 expression, which may, in part, be consistent with our hypothesis.[3]

In this study, the solid predominant subtype in the metastatic lymph node tumor is morphologically defined according to the primary tumor criterion. However, considering cancer stem/initiating cell theory, it is possible to think that biological features of the cancer cells showing 'solid' morphology in metastatic lymph node tumor are different from those of

primary lung tumor showing solid morphology. Further investigation will be needed whether these two morphologically similar tumors display different biological features.

Aokage, one of our colleagues, examined a large number of small intrapulmonary metastases in detail by applying the histological classification of primary adenocarcinoma of the lung to the metastatic tumors.[12] In this report, most intrapulmonary metastatic tumors arising from primary adenocarcinoma exhibited a lepidic-predominant histologic subtype in the early phase and the morphological diversity of the original tumor began to be reproduced as the tumor grew in size. This phenomenon was similar to our finding of recapitulation of the original morphological features as the metastatic tumors grew, but differed in that the major hisotologic subtype in the early phase of pulmonary metastatic tumors was the lepidic-predominant subtype, whereas in our study, it was the solid subtype. This difference suggests the possibility of the histological environment of the metastatic organs decisively affecting the tumor morphological features in the early phase of the metastatic process.

Recent studies have indicated the importance of transient EMT-MET switches in the metastatic process.[16–22] Chaffer *et al.* showed, using a bladder cell line, spontaneous shift from mesenchymal to epithelial characteristics and its association with increased metastatic ability in advanced malignancies.[23] Tsai *et al.* also reported, using a spontaneous squamous cell carcinoma in Twist 1 transgenic mouse model, that activation of Twist1 is sufficient to promote EMT of cancer cells and to disseminate the cells into the circulation, and also that turning off Twist1 is essential to allow reversal of EMT for dissemination of tumors to distant sites.[24] We also previously investigated the immunophenotypes of cancer cells in small metastatic lesions of the lung, and concluded that a dynamic phenotypic change that includes both EMT and MET occurs in the early phase of metastatic tumor formation.[25] Therefore, the solid histologic subtype is the important phenotype corresponding to the transitional phase of EMT-MET in the early stage of the metastatic process.

The cancer stem cell theory may explain the phenomenon of metastatic tumor cells reproducing the morphological diversity of the original tumor as the metastatic tumors grew in size.[26–30] According to the cancer stem cell concept, a single cell or a small number of cancer cells have the potential to reconstitute a primary tumor under favorable conditions and diversity of the tumor cell populations develops during tumor progression.

Successful formation of macroscopic metastatic tumors requires angiogenesis and several extrinsic factors in the microenvironment. Therefore, tumor microenvironment of micrometastases and macrometastasis would be obviously different. To examine the differences of cancer cell phenotype and the stromal reaction between in micrometastases and macrometastases gives us very important information to know the dynamism of tumor metastatic process. For further investigation, we are planning to examine the immunophenotypic differences between lymph node micrometastasis and macrometastasis.

In conclusion, we found a high percentage of cases showing the solid phenotype in the early phase of the lymph node metastatic process, and that the metastatic tumor cells tended to regain the original morphological features and diversity as the tumors grew in size. As a prognostic factor, the small lymph node metastasis is recently noted and prospective cohort surveys have been performed.[31–33] Clarification of the biological features of small lymph node metastases is important for the development of new strategies for early cancer detection and for the development of effective cancer treatment approaches.

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ORIGINAL ARTICLE – COLORECTAL CANCER

Long-term Outcomes after Intersphincteric Resection for Low-Lying Rectal Cancer

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ABSTRACT

Background. As an anus-preserving surgery for very low rectal cancer, intersphincteric resection (ISR), has advanced markedly over the last 20 years. We investigated long-term oncologic, functional, and quality of life (QOL) outcomes after ISR with or without partial external sphincter resection (PESR).

Methods. A series of 199 patients underwent curative ISR with or without PESR between 2000 and 2008, with 49 receiving preoperative chemoradiotherapy (CRT group) and 150 undergoing surgery first (surgery group). Overall survival (OS), disease-free survival (DFS), and local relapse-free survival (LFS) rates were calculated using Kaplan–Meier methods. Functional outcomes were assessed using the Wexner incontinence score. QOL was investigated using the Short-Form 36 questionnaire (SF-36) and modified fecal incontinence quality of life (mFIQL) scale.

Results. After a median follow-up of 78 months (range 12–164 months), estimated 7-year OS, DFS, and LFS rates were 78, 67, and 80 %, respectively. LFS was better in the CRT group than in the surgery group ($p = 0.045$). Patients with PESR or positive circumferential resection margins showed significantly worse survival. The median Wexner incontinence score at >5 years was 8 in the surgery group and 10 in the CRT group ($p = 0.01$). QOL was improved in all physical and mental subscales of the SF-36 at >5 years. Although the mFIQL showed a relatively good score in all groups at >5 years, a significant difference existed between the CRT and surgery groups ($p = 0.008$).

Conclusions. With long-term follow-up, oncologic, functional, and QOL results after ISR appear acceptable, although CRT is associated with disturbance.

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The main therapy for rectal cancer is curative surgical resection. Surgery for rectal cancer has three main objectives: cure of cancer, preservation of anal function, and maintenance of quality of life (QOL). Total excision of the mesorectum as developed by Heald and Ryall[1] has led to improved local control and survival.

Next to radical resection of the tumor, anus-preserving surgery is one of the major goals for lower rectal cancer. Lower rectal cancer located within 5 cm from the anal verge has traditionally been treated using abdominoperineal resection. However, anus-preserving operations for very low rectal cancer have advanced over the last 20 years with the new procedure of intersphincteric resection (ISR). Some authors have proposed ISR to increase the chance of sphincter-saving resection for selected low rectal tumors.[2–14] Combined with neoadjuvant chemoradiotherapy (CRT), ISR has been used to extend the opportunity for sphincter preservation. Although ideal candidates for ISR are limited to patients with the lower edge of T1 and T2 tumors lying >1 cm from the intersphincteric groove, we have extended the indications for ISR to T3 and part of T4 disease by using the technique combined with partial external sphincter resection (PESR) or preoperative CRT.

The aim of this study was to assess long-term oncologic, functional, and QOL outcomes after ISR with or without PESR.

METHODS

Patients

In total, 1,033 consecutive patients with rectal cancer received curative surgical treatment at our institute from

2000 to 2008. Of these, 93 % underwent anus-preserving surgery. Participants in the present study comprised 199 patients who underwent curative ISR with or without PESR for low rectal cancers after being prospectively enrolled.

Informed consent was obtained from each patient, and all study protocols were approved by the hospital ethics committee. ISR with or without PESR was indicated for tumors located within 5 cm from the anal verge. The exact level of the lower edge of the tumor from the anal verge was assessed and measured by digital examination and endoscopy. An exception to selection of ISR with or without PESR was made if the patient showed definitive invasion of the external sphincter or levator ani muscle. Patients with significant clinical fecal incontinence (more than once per week) were also excluded. All patients underwent preoperative imaging of the chest, abdomen, and pelvis, usually with computed tomography and either magnetic resonance imaging or endorectal ultrasonography.

Operative Techniques

The surgical technique included both abdominal and transanal approaches. In the abdominal approach, total mesorectal excision and pelvic lateral node dissection with or without autonomic nerve preservation were performed, although lateral node dissection is not the standard of care outside Japan. The rectum was mobilized as low as possible to the pelvic floor to facilitate the transanal approach. The surgical anal canal was then divided circumferentially from the puborectal muscle and external sphincter. If the tumor adhered to the puborectal muscle and/or external sphincter, those structures were partially resected to obtain adequate safety margins. Fatty tissue of the ischiorectal fossa was thus sometimes visualized. This procedure of PESR has been reported previously.[6,12] Defects in the external sphincter were repaired as much as possible by using manual transanal suturing. Most patients underwent end-to-end coloanal anastomosis. Finally, a diverting stoma was established and closed 3 months postoperatively or after completion of adjuvant chemotherapy.

ISR was classified into three types: total ISR, subtotal ISR, and partial ISR. Total ISR involved complete excision of the internal sphincter for tumors spreading to or beyond the dentate line. The distal cut-end line was at the intersphincteric groove. In partial ISR, the distal cut-end line was just on or slightly above the dentate line, and the distal cut-end line was between the dentate line and intersphincteric groove in subtotal ISR.

Adjuvant Therapy

Preoperative CRT was performed in 49 patients with clinical T3 tumors who agreed to preoperative adjuvant therapy. Other patients underwent surgery without CRT, because preoperative CRT for resectable rectal cancer is still not standard in Japan. Forty-nine patients received 45 Gy of radiotherapy administered with continuous infusion of 5-fluorouracil (250 mg/m^2 day) during the 5-week period preceding surgery. Ninety-five patients with stage IIA, stage IIB, or III (pTMN pathologic classification) received postoperative chemotherapy with 5-fluorouracil and folinic acid or tegafur uracil for 6 months.[15]

Follow-up

Patients were followed up by using a standardized protocol (including clinical examination with digital palpation; computed tomography of the chest, abdomen, and pelvis; and measurement of tumor marker levels) every 4 months for the first 2 years, then every 6 months for 3 years, then annually thereafter. Total colonoscopy was performed at 2 and 5 years after surgery.

Functional and QOL Assessment

Patients who remained alive without recurrence after follow-up for $\geq$60 months were eligible for the functional study. Exclusion criteria were death, pelvic recurrence, definitive stoma for anastomotic trouble or poor function, and psychiatric disorders.

Functional assessment was performed every year after stoma closure by using our functional questionnaire. This questionnaire asked about stool frequency (per 24 h), feces and flatus discrimination, urgency (ability to defer stool evacuation for >15 min), fragmentation ($\geq$2 evacuations in 1 h), soiling during the day and night, use of pads, use of medications, alimentary restriction, and lifestyle alteration. Questionnaires were regularly sent to patients. Incontinence was assessed by using the Wexner score, where 0 represents perfect continence and 20 indicates major incontinence. Functional outcomes were considered "poor" for Wexner scores >10, whereas scores $\leq$10 were considered "good".[16,17]

The Japanese version of the Short-Form 36 questionnaire (SF-36) was applied as a nonspecific, general evaluation of QOL.[18,19] The SF-36 consists of eight multi item scales: physical function, role limitations-physical, bodily pain, general health, vitality, social function, role limitation-emotional, and mental health. On the basis of these subscales, component summary scores can be calculated to provide a global measure of physical function (physical component summary; PCS) and mental function (mental component summary; MCS), respectively. Scale scores ranged from 0 to 100, with higher scores indicating better health status.

TABLE 1 Characteristics of patients undergoing ISR with or without PESR

Variable	Total ($n = 199$)	CRT group ($n = 49$)	Surgery group ($n = 150$)	p value
Age (years) median (range)	59 (27–80)	56 (27–77)	60 (32–80)	0.048
Male/female	144/55	38/11	106/44	0.875
AV mean (cm)	3.8	3.5 (1.5–5)	4.0 (0.6–7)	0.011
Tumor stage (clinical)				0.217
cT1	6	0	6	
cT2	38	9	29	
cT3	136	38	98	
cT4 (prostate, vagina, etc.)	19	2	17	
Operation type (ISR)				
Total	55	23	32	
Subtotal	80	17	63	
Partial	64	9	55	
ISR with PESR	41(20.6 %)	(9)	(32)	
Positive circumferential resection margin (CRM $\leqq 1$ mm)[a]	39 (19.6)	6 (12.2)	33 (22.0)	0.135
Lateral lymph node metastasis	24 (12.1)	7 (14.3)	17 (11.3)	0.582
Pathologic stage (pTNM)				
0	9 (4.5)	8 (16.3)	0 (0.0)	
I	69 (34.7)	15 (30.6)	55 (36.7)	
IIA	44 (22.1)	8 (16.3)	36 (24.0)	
IIB	2 (1.0)	1 (2.0)	1 (0.7)	
IIIA	10 (5.0)	1 (2.0)	9 (6.0)	
IIIB	27 (13.6)	8 (16.3)	19 (12.7)	
IIIC	38 (19.1)	8 (16.3)	30 (20.0)	
Anastomotic dehiscence	20 (10.1)	8 (16.3)	12 (8.0)	0.083
Definitive stoma	20 (10.1)	11 (22.4)	9 (6.0)	0.002
Not closed (diverting stoma)	12 (6.0)	5 (10.2)	7 (4.7)	0.143
Anastomotic trouble	5	3	2	
Recurrence, others	7	2	5	
Reestablished	8 (4.0)	6 (12.2)	2 (1.3)	0.003
Poor bowel function	4	3	1	
Recurrence (APR)	4	3	1	

Data are n (%) unless otherwise noted

CRT preoperative chemoradiotherapy (45 Gy; 5-fluorouracil), *ISR* intersphincteric resection, *PESR* partial external sphincter resection, *APR* abdominoperineal resection

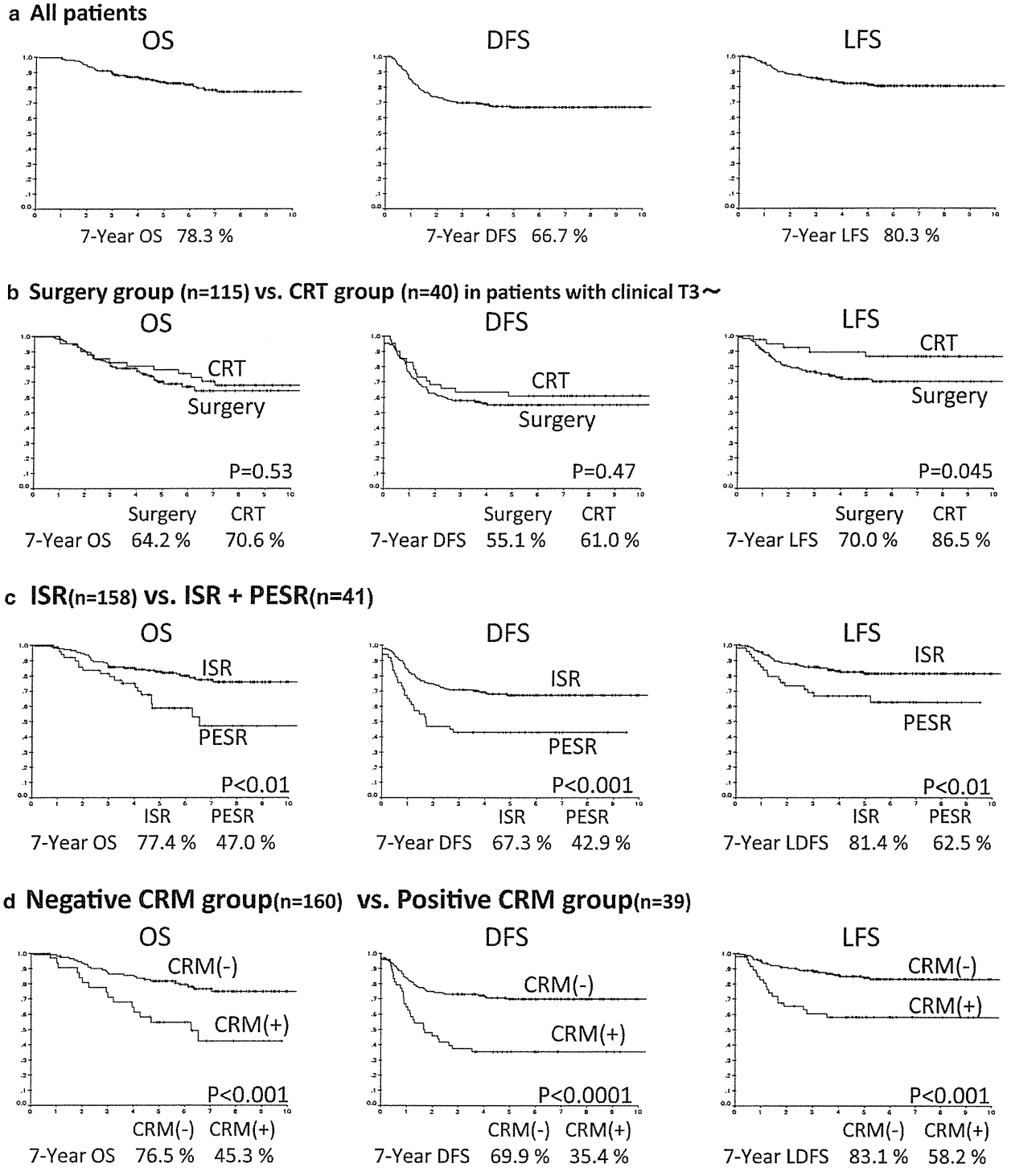
[a] Positive pathologic margin: 0

The Japanese version of the modified fecal incontinence quality of life (mFIQL) questionnaire was used as a specific and sensitive QOL questionnaire.[20] This questionnaire explores 14 items, with each response to a specific item assigned a value from 1 to 4 and summarized in a score. Scale scores ranged from 0 to 100, with higher scores indicating worse QOL.

Statistical Analysis

Patients were divided into groups as follows: ISR without CRT (surgery group) and ISR with CRT (CRT group); ISR only (ISR group) and ISR with PESR (PESR group); and positive surgical circumferential resection margin (CRM) and negative surgical CRM groups. Positive surgical CRM was defined as tumor ≤ 1 mm from the CRM.[21–23]

Overall survival (OS), disease-free survival (DFS), and local relapse-free survival (LFS) rates were calculated by using Kaplan–Meier methods. Differences between curves were evaluated with the log-rank test. Univariate and multivariate analyses were performed to assess the impact of clinical, surgical, and treatment variables on functional



OS: Overall survival, DFS: Disease-free survival, LFS: Local relapse-free survival,
Surgery group: ISR without CRT, CRT group: ISR with preoperative CRT

CRM(-): Negative circumferential margin (>1mm), CRM(+): Positive circumferential margin (≦1mm)

FIG. 1 Kaplan-Meier survival after ISR ± PESR. **a** All patients. **b** Surgery group ($n = 115$) versus CRT group ($n = 40$) in patients with clinical T3～. **c** ISR (n = 158) versus ISR + PESR ($n = 41$). **d** Negative CRM group ($n = 160$) versus positive CRM group ($n = 39$)

TABLE 2 Functional results at 2 or >5 years after stoma closure

Variable	2 Years ($n = 116$)	>5 Years ($n = 104$)
Stool frequency per 24 h (mean ± SD)	4.7 ± 4.6	4 ± 3.5
Fecal urgency (% of n)	30	32
Feces/flatus discrimination (% of n)	16	18
Stool fragmentation (% of n)	43	51
Difficult evacuation (% of n)	17	25
Daytime soiling (% of n)	35	30
Nighttime soiling (% of n)	30	26
Incontinence to gas (% of n)	56	55
Pad wearing (% of n)	50	55
Lifestyle alteration (% of n)	34	21

outcome. Data on postoperative functions and QOL are given as median and range. Differences between groups were tested by using the unpaired t test and the Chi square test or Fischer's exact test, as appropriate. Statistical evaluation was performed with SPSS for Windows version 20.0 software (SPSS, Chicago, IL). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Details of patient characteristics in the CRT and surgery groups are listed in Table 1. Overall, 150 patients underwent curative ISR without preoperative CRT. PESR was performed in 41 patients (20.6 %). Of these, 29 had not undergone preoperative CRT. The CRT and surgery groups were comparable with respect to demographics and treatment characteristics except for tumor location. The median distance between the lower edge of the tumor and anal verge was 3.5 cm (range 1–5.5 cm) in the CRT group and 4.0 cm (range 0.6–6 cm) in the surgery group. The rate of pathologic lateral lymph node metastasis was 12.1 % in all patients.

Oncologic Results

After a median follow-up of 6.5 years (range 12–164 months), pulmonary metastasis, local recurrence with or without distant metastasis, liver metastasis, and combined recurrence occurred in 28 patients (14.1 %), 27 patients (13.6 %), 15 patients (7.5 %), and 9 patients (4.5 %), respectively. Nine patients with only local recurrence received salvage surgery (abdominoperineal resection, n = 4; tumor resection, $n = 5$). Seven-year OS, DFS, and LFS rates in all ISR patients were 78.3, 66.7, and 80.3 %, respectively (Fig. 1a). No significant differences in OS and DFS were identified between the surgery and CRT groups. The CRT group showed a tendency to decrease local recurrences ($p = 0.045$; Fig. 1b). The PESR group showed significantly worse survival than the ISR group, with 7-year OS rates of 77.4 % for the ISR group and 47.0 % for the PESR group ($p < 0.01$) and 7-year DFS rates of 67.3 % for the ISR group and 42.9 % for the PESR group ($p < 0.01$; Fig. 1c). Survival rates in the positive and negative CRM groups are shown in Fig. 1d. The positive CRM group displayed significantly worse OS, DFS, and LFS than the negative CRM group, with 7-year OS rates of 45.3 % for the positive CRM group and 76.5 % for the negative CRM group ($p < 0.001$). Seven-year DFS rates were 35.4 % for the positive CRM group and 69.9 % for the negative CRM group ($p < 0.0001$). Seven-year LFS rates were 58.2 % for the positive CRM group and 83.1 % for the negative CRM group ($p < 0.001$).

Functional Results

Of the 199 patients with curative ISR including PESR, 131 were candidates for evaluation of function and QOL at >5 years, and 104 patients (79.4 %) who responded to the questionnaire were assessed for continence function and QOL.

Mean stool frequency per 24 h was 4.0 ± 3.7. Approximately 50 % of patients had stool fragmentation and incontinence to gas, 30 % still experienced soiling during the day and at nighttime, and a quarter suffered from difficulties in evacuation (Table 2).

The median Wexner score was 8.5 (range 0–20) in all patients at >5 years after stoma closure. The CRT group showed significantly higher scores (median 10; range 4–20) than the surgery group (median 8; range 0–20; $p = 0.01$), although 70 % of patients showed good continence status (Wexner score 0–10; Fig. 2a). Univariate and multivariate analyses of functional outcomes are shown in Table 3. Significant effects on functional outcome were seen for sex and neoadjuvant treatment, although no effects were shown for age, tumor characteristics such as category or distance, or operative type. Female patients and the surgery group showed better continence function (female vs. male, $p = 0.01$; surgery vs. CRT, $p = 0.02$). Furthermore, similar findings were shown in multivariate analyses (Table 3).

QOL Results

QOL was improved in all physical and mental subscales (PCS and MCS) of the SF-36 at >5 years after ISR. No difference in PCS score was seen between the CRT and surgery groups (Fig. 2b-i). The CRT group showed worse MCS scores than the surgery group ($p = 0.02$). In addition, the mFIQL score differed significantly between the CRT and surgery groups (48 vs. 23; $p = 0.008$), although the

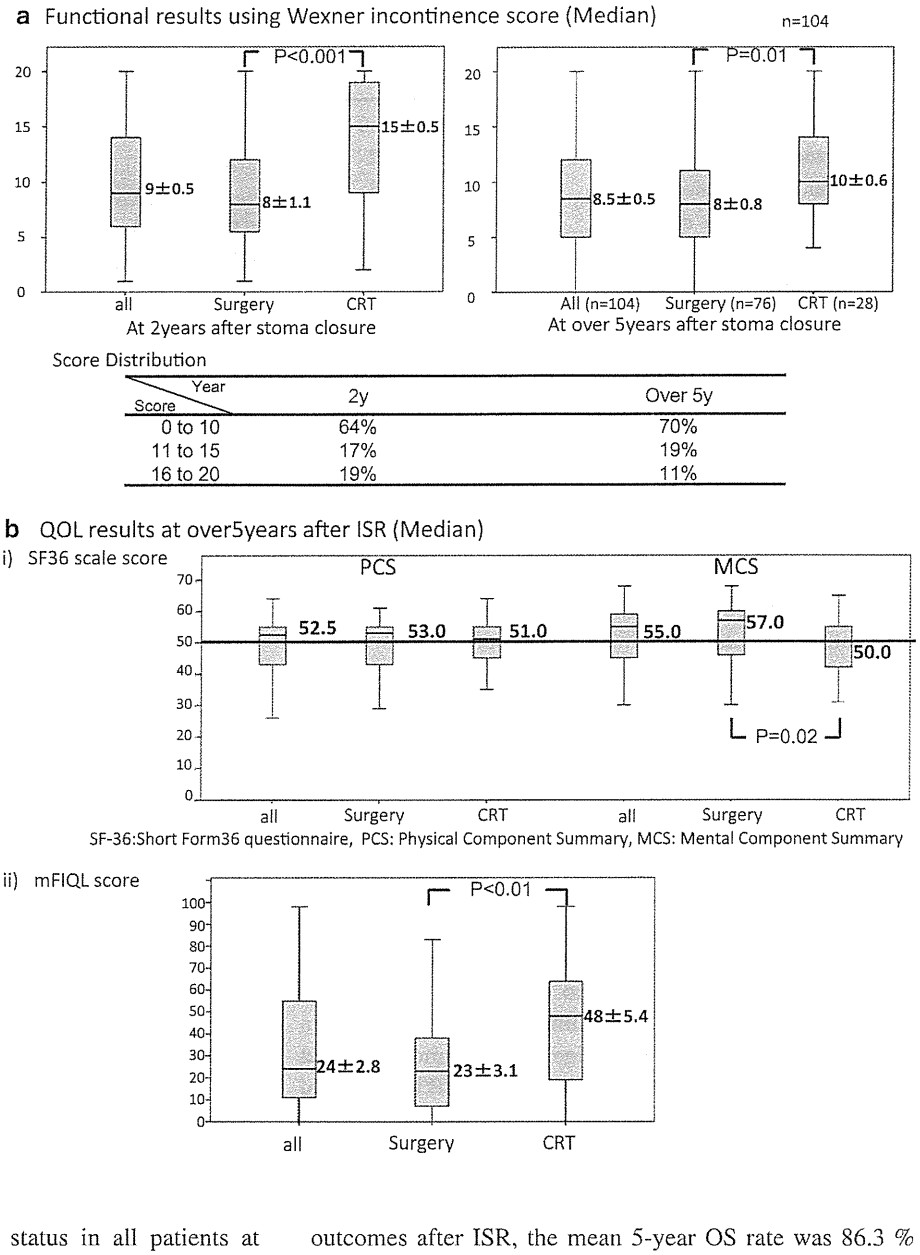


FIG. 2 Functional and QOL results. **a** Functional results according to the Wexner incontinence score (median). **b** QOL results at >5 years after ISR (median). *(i)* SF-36 scale score; *(ii)* mFIQL score

mFIQL showed relatively good status in all patients at >5 years (Fig. 2b-ii).

DISCUSSION

This study was designed to investigate long-term oncologic, functional, and QOL outcomes after ISR under extended indications. Many reports have described acceptable oncologic and functional results of ISR with mid-term observations, but few have examined long-term functional and QOL outcomes after ISR.

Our data with a median follow-up of 78 months showing a 7-year OS rate of 76.6 % were comparable to results from other studies. According to a systematic review of outcomes after ISR, the mean 5-year OS rate was 86.3 % (range 62–97 %), the DFS rate was 78.6 % (range 69–87 %), and the mean local recurrence rate was 6.7 % (range 0–23 %) at a median follow-up of 56 months.[24] In patients with clinical T3 or T4 tumors, neoadjuvant CRT tended to decrease local recurrences after ISR, although no differences in OS or DFS were seen. Conversely, significant differences in OS, DFS, and LFS were identified between ISR and PESR groups. The PESR group showed worse survival outcomes than the ISR group. Candidates for PESR should be selected with great care, and neoadjuvant therapy should be administered, because these patients have extended T3–4 tumors, and surgery alone offers limited efficacy for achieving local control. In the