Frequent Presence of Lymphovascular Invasion in Small Rectal Neuroendocrine Tumors on Immunohistochemical Analysis

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Key Words

Lymphovascular invasion · Rectal neuroendocrine tumors · Endoscopic resection · Immunohistochemical analysis

Abstract

Rectal neuroendocrine tumors (RNETs) have become common in recent years and are good candidates for endoscopic resection (ER). To achieve clear resection margins, more advanced techniques such as endoscopic submucosal dissection, endoscopic submucosal resection with a ligation device, and cap-assisted endoscopic mucosal resection are available for ER. After ER, lymphovascular invasion (LVI) is regarded as an important predictor of nodal metastasis. Previous studies have shown that small RNETs with LVI were uncommon (0–8.3%). However, using immunohistochemical analysis, a recent study revealed the frequent occurrence of LVI in small RNETs in a systematic manner (46.7%). There is a possibility that the actual detection rate of LVI in small RNETs is not always evaluated accurately because of the limited detection sensitivity of conventional hematoxylin-eosin staining. In addition, the correlation between LVI detected using immunohistochemical analysis and the development of me-

tastasis remains unclear. Further prospective studies are required to clarify the role of LVI detected using immunohistochemical analysis.

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Treatment Strategy for Small Rectal Neuroendocrine Tumors

Rectal neuroendocrine tumors (RNETs) have become common in recent years, and the number of RNETs has been increasing because of the widespread use of screening endoscopy for colorectal cancer [1]. RNETs have a higher incidence among the Asian population. In contrast, the prevalence of colon NETs is much higher among the Caucasian population. In the Japanese study, 302 of 332 (92%) cases of colorectal NETs were found to have originated from the rectum [2]. RNETs demonstrate a broad range of clinical behavior from benign and asymptomatic to disseminated and metastatic. Assessment of the size and depth of invasion is thought to be the simplest method to predict the behavior of RNETs; RNETs measuring ≤10 mm rarely metastasize [3–5]. Previous studies have

Fig. 1. ESMR-L of a RNET. **a** Endoscopic macroscopic appearance. **b** Saline injection into the submucosa to create a voluminous submucosal swelling beneath the lesion. **c** An elastic band was deployed. **d** Endoscopic appearance after resection.

reported that the risk of metastasis is 2–3% for tumors ≤ 10 mm, 10–15% for tumors 11–20 mm, and 60–80% for tumors > 20 mm [3, 6]. Meanwhile, survival analysis of nearly 5,000 cases in the SEER database has demonstrated a 5-year survival rate of 97% among patients with tumors ≤ 20 mm that were confined to the submucosal layer [7]. Based on these studies, RNETs ≤ 10 mm are good candidates for endoscopic resection (ER) and no further treatment is recommended if R0 resection is achieved [8, 9].

ER Techniques

Given that most RNETs involve the submucosal or deeper layers of the rectal wall [6], standard polypectomy or endoscopic mucosal resection (EMR) may not assure

sufficient resection margins. The complete resection rate pooled from 14 studies has been found to be 59.1% with polypectomy or EMR for RNETs (range 17–90%) [10]. To achieve clear resection margins, more advanced techniques such as endoscopic submucosal dissection (ESD), endoscopic submucosal resection with a ligation device (ESMR-L; fig. 1), and cap-assisted EMR (EMR-C) are available for ER of small RNETs [11–13]. ESD is a promising procedure that enables en bloc resection and consequently reduces local recurrence [14]. In addition, ESD has been shown to be an effective treatment for RNETs, with a complete resection rate of 90.3% [11]. However, this technique has some limitations. Compared with EMR, ESD requires a longer procedure time, is technically more demanding, and is associated with a higher rate of complications, particularly perforation [15]. Pre-

Fig. 2. Comparison of the demonstration of lymphatic channel involvement in RNET on serial sections. **a** HE stain. **b** D2-40 immunohistochemical stain. Lymphatic invasion is easily identi-

fied because the lymphatic epithelium is stained brown with D2-40 stain. However, it is difficult to identify lymphatic invasion on HE stain (**a**) alone.

vious studies have reported that ESMR-L and EMR-C are lesser time-consuming than ESD and as effective as ESD for the treatment of small RNETs, with a complete resection rate of 95.2 and 94.1%, respectively [12, 13]. Considering these studies, ESMR-L or EMR-C may be a more appropriate ER technique for the removal of small RNETs than ESD.

Lymphovascular Invasion in Small RNETs

In a recent prognostic study on RNETs ($n = 347$), independent factors predictive of metastasis during multivariate analysis have been found to be size >14 mm, increased mitotic rate, and lymphovascular invasion (LVI) [16]. Therefore, recent guidelines recommend additional treatment for RNETs if LVI is presented after ER [10, 17]. Previous studies have shown that small RNETs have a very low incidence of LVI [18–21]. Nonetheless, in our clinical practice, LVI has often been detected using immunohistochemical analysis in addition to hematoxylin-eosin (HE) staining. Recently, immunohistochemical staining techniques for identifying lymphatic channels and vessels have been widely used because of difficulties in recognizing veins and lymphatic channels using HE staining alone [22–25]. For example, immunostaining with the monoclonal antibody D2-40 (D2-40) can highlight lymphatic endothelial cells and distinguish lymphatic channels from small vessels [22, 23]. Similarly, venous walls are often identified using either Elastica van Gieson (EVG) or Victoria blue staining because the elastic fibers

located in the venous wall are stained dark violet using these techniques [24, 25]. In the assessment of LVI in submucosal invasive colorectal cancer using D2-40 and EVG staining, it was reported that there would be a twofold increase in the detection rate compared with that obtained using HE staining [23]. And, previous studies reported that LVI detected using D2-40 and EVG staining is an important predictor of lymph node metastasis in submucosal invasive colorectal cancer [23, 25]. With regard to small RNETs, no studies have examined the correlation between LVI detected in a systematic manner using immunohistochemical analysis and regional lymph node metastasis. Thus, there is a possibility that LVI in small RNETs is not always evaluated accurately [18–21].

Sekiguchi et al. [26] reported the frequent presence of LVI in small RNETs using immunohistochemical analysis (46.7%). Almost all the lesions were smaller than 10 mm in size, fulfilling the criteria for the application of ER. In addition, the Ki-67 index was less than 3% in all the lesions, which were therefore classified as NET G1 according to the WHO 2010 classification [27]. On the other hand, original diagnosis based on routine HE stain identified venous invasion only in one case and lymphatic invasion in none of the cases. This low incidence of LVI using HE stain was similar to the previously reported detection rate of LVI in small RNETs (0–8.3%) [18–21].

The major reason for this evaluation gap in LVI detection between HE and D2-40 stain would be that RNETs predominantly show a trabecular growth pattern and small lymphatic invasion of tumor cells that may be difficult to distinguish using HE staining alone (fig. 2). Sim-

Fig. 3. Comparison of the demonstration of venous invasion in RNET on serial sections. **a** HE stain. **b** EVG special stain. Venous invasion is easily identified because the elastic fiber of the vein is stained dark with EVG stain. However, it is difficult to identify venous invasion on HE stain (**b**) alone.

ilarly, the HE stain cannot stain the elastic fibers of the venous wall, resulting in difficulty in the identification of venous invasion (fig. 3). Moreover, because NET tumor cells exhibit minute cytological atypia, unlike most other malignant neoplasms, tumor cells within small vessels are often difficult to identify [26]. Because of this unique pathological character of RNETs and the limitation of the HE stain, additional immunohistochemical analysis will be required to accurately identify LVI in small RNETs. We speculate that RNETs would potentially have LVI even in their early stage and that the previous reports may have failed to show the actual detection rate of LVI because of the limited detection sensitivity of the conventional staining methods used.

Clinical Outcomes Following ER

The updated European Neuroendocrine Tumor Society consensus guideline and the Japanese Neuroendocrine Tumor Society guideline recommend additional radical surgery combined with lymph node dissection even for RNETs ≤ 10 mm if LVI is detected [10, 17]. In accordance with these guidelines, nearly 50% of patients examined using immunohistochemical analysis would require additional radical surgery because of the presence of LVI following ER. However, these guidelines may be based on data from assessments based on the conventional HE staining. Park et al. [16] reported that the 3-year survival rate was 100% among patients with RNETs ≤ 10 mm following ER. In addition, Sekiguchi et al. [26]

reported that 90 RNETs treated endoscopically were followed up without additional surgery, and no metastasis or recurrence was detected during the median follow-up period of 67.5 months despite the presence of LVI in nearly half of the lesions. These results showed an excellent prognosis following ER in patients with small RNETs. Rectal surgery is more aggressive than colonic surgery. Since the introduction of abdominoperineal resection, this procedure has been the standard treatment for permanent stomas [28]. Recently, advanced anus-preserving low anterior resection and intersphincteric resection have become more common while avoiding colostomies [29, 30]. Nevertheless, previous studies have reported that some patients experience disordered defecation after these resections [31, 32]. Considering the risk of complications, the role of additional radical surgery for RNETs ≤ 10 mm with LVI detected using immunohistochemical analysis would need to be discussed.

There have been a few reported cases that RNETs ≤ 10 mm confined to submucosal may already have node-positive disease and develop recurrence or metastasis after a long latency period [33, 34]. Kasuga et al. [35] found lymph node metastasis in 10 of 35 patients (28.6%) with RNETs ≤ 10 mm treated by surgical resection following ER. In addition, of 10 RNETs with metastasis, only one lymph node metastasis was detected by preoperative CT. From these findings, we cannot exclude the risk of clinically undetectable minute metastatic lesions in patients treated without surgery. Thus, we emphasize that patients should adhere to follow-up surveillance, even after the removal of small tumors.

Conclusion

A recent study has demonstrated the frequent presence of LVI even in small RNETs using D2–40 and EVG staining. The correlation between LVI detected using immunohistochemical analysis and the development of metastasis remains unclear. Additional prospective studies are required to clarify the role of LVIs detected using immunohistochemical analysis.

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