

Endocytoscopic findings of colorectal neuroendocrine tumors (with video)

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Bibliography

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ABSTRACT

Background and study aims Gastrointestinal neuroendocrine tumors (NET) are generally submucosal in location. Because these tumors are covered with normal mucosa, biopsy is necessary to confirm histological diagnosis before treatment. We explored the diagnostic capabilities of the endocytoscope, which can perform ultra-high magnification in vivo, for staining and diagnosing submucosal tumors in situ.

Introduction

Submucosal tumors (SMTs) are occasionally noted incidentally while performing colonoscopy. When an SMT is discovered, we often perform a biopsy. Gastrointestinal neuroendocrine tumors (NET) are an uncommon SMTs usually detected during endoscopic examination [1]. NETs develop from neuroendocrine cells, which are widely present in the gastrointestinal tract, and grow into the submucosa and sometimes mucosa. In the lower digestive tract, NETs are often found in the rectum during colonoscopy [2–4]. According to the histological classification of tumors developed by the World Health Organization in 2010, NETs are classified as G1 to G3 based on the rate of mitosis and the Ki-67 labeling index, and to determine the histological grade, pathological evaluation of the entire tumor is necessary [5–8]. It is necessary to distinguish NETs from other SMTs such as myoma or lipoma, which are usually not treated. It would be ideal if it were possible to diagnose SMTs from endoscopic findings to avoid excisional biopsy.

An endocytoscope is an ultra-high magnifying endoscope used to visualize living gastrointestinal cells in situ, to enable real-time endoscopic assessment of histology. Reports support the usefulness of endocytoscopy in diagnosis of epithelial neoplasia [9, 10]. The technique has the potential to visualize tumor

cells in the submucosa, because its depth of focus is 50 µm. We hypothesized that endocytoscopy would be useful for diagnosis of NETs because these tumors sometimes extend toward the mucosal surface layer; thus, tumor cells are likely to be observable on the surface of these lesions.

Case reports

Patients and methods

We retrospectively evaluated endocytoscopic images and pathological findings of 13 incidentally identified NETs from 13 patients evaluated endoscopically at Showa University Northern Yokohama Hospital from February 2010 to June 2014. ► **Table 1** shows the characteristics of the patients and lesions. Ultra-high magnification images had been acquired with an integrated-type endocytoscope (CF Y-0020-I, Olympus, Tokyo, Japan) using a video endoscopic system (Evis Lucera Spectrum or Evis Lucera Elite, Olympus). To get the endocytoscopic images, 0.05% crystal violet and 1.0% methylene blue were sprayed onto the mucosa covering the lesion to stain the cytoplasm and nuclei, respectively. The lesions were resected endoscopically, with additional endocytoscopic guidance in cases with positive staining and sent for pathological diagnosis. On the basis of the pathological characteristics of NETs, cord-like

► **Table 1** Characteristics of patients and neuroendocrine tumors.

Case	Gender	Chief complaint	Location	Size (mm)	Ki-67 index	Tumor growth in the intramucosa	Distance from the surface (µm)	Tumor visibility
1	F	Fecal occult blood	Ra	5	<3%	–	374.2	–
2	M	Fecal occult blood	Rb	3	<3%	+	10.5	+
3	M	Fecal occult blood	Rb	10	<3%	+	12.5	+
4	M	Constipation	Rb	8	<3%	+	26.3	+
5	F	Screening	Rb	6	<3%	–	290.7	–
6	F	Fecal occult blood	Rb	7	<3%	+	48.3	+
7	M	Constipation	Rb	11	<3%	+	21.6	+
8	M	Fecal occult blood	Rb	6	<3%	+	13.0	+
9	M	Fecal occult blood	Ra	6	<3%	+	18.4	+
10	F	Constipation	Ra	6	<3%	+	36.4	+
11	M	Fecal occult blood	Rb	9	<3%	+	16.2	+
12	F	Fecal occult blood	Rb	10	<3%	+	187.6	+
13	M	Constipation	Rb	5	<3%	–	250.6	–

or honeycomb arrangements of cells with small round nuclei were defined as NETs on endocytoscopy. We assessed the diagnostic usefulness of endocytoscopy for NET by comparing endoscopic and pathological findings. The Ethics Committee of Showa University Northern Yokohama Hospital approved this study (No.17H028).

Results

All 13 tumors were in the rectum. Pathology graded all lesions as G1. In 10 lesions, tumor cells had been confirmed with endocytoscopy, but three lesions could not be confirmed using the endocytoscope and were localized based on morphology and resected using the endoscope. In the 10 endocytoscopically-confirmed lesions, cells with compact, homogeneous, circular nuclei were arranged in a cord-like or honeycomb array around the epithelial ducts. The cytoplasm did not stain with crystal violet. Vessels running between the tumor cell arrays were observed on a video image generated by the endocytoscopic system (► **Video 1**). Endoscopic findings were consistent with characteristic pathological signs of NET. Pathology confirmed the findings (► **Fig. 1**).

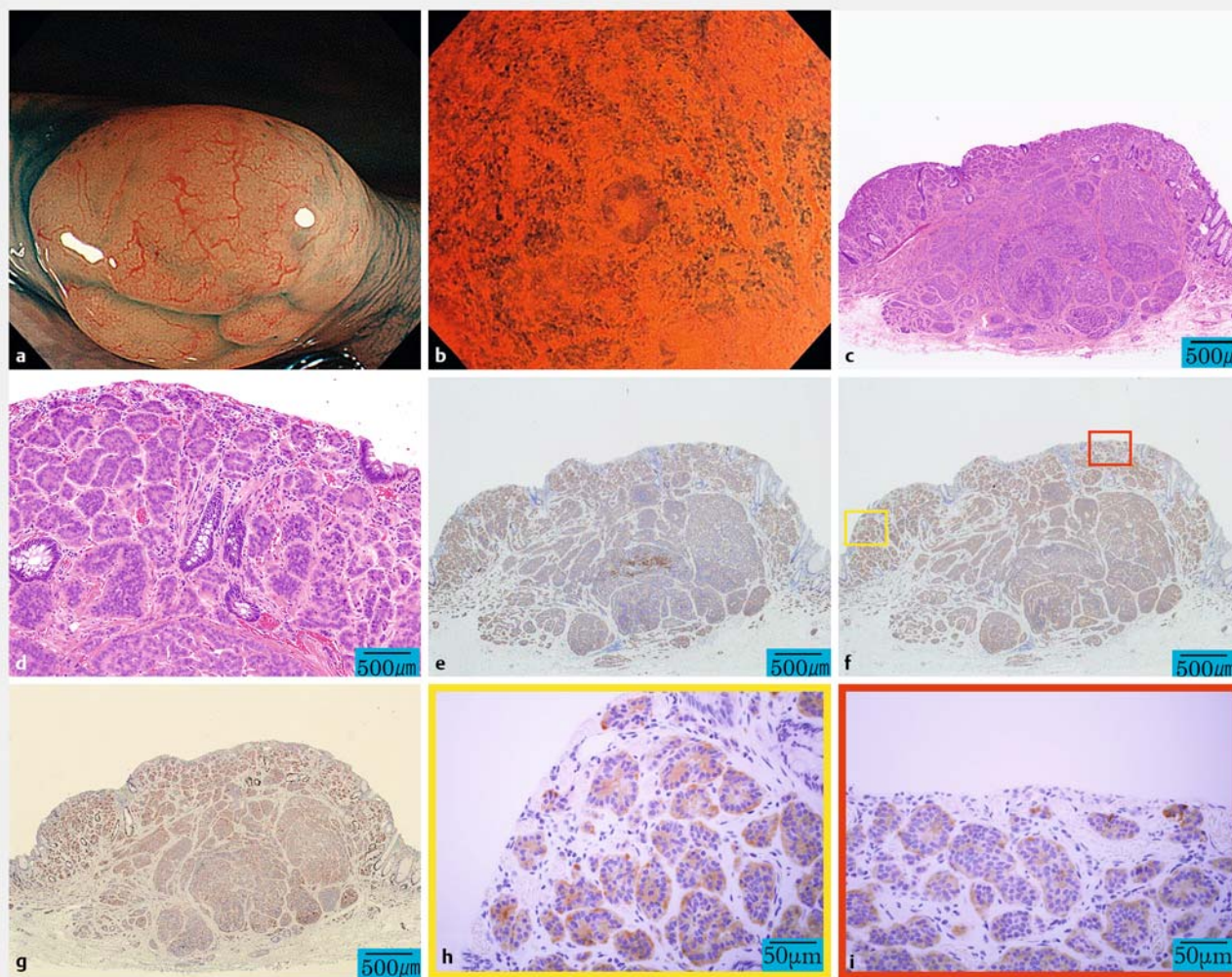
We measured the distance from mucosa to the tumor cells on the pathological sections (► **Table 1**). Of 10 lesions in which the tumor could be observed with endocytoscopy, there was tumor within 50 µm of the mucosal surface in nine lesions. In one lesion, tumor deeper than 50 µm was observed. In the three lesions in which tumor cells were not observed with endocytoscopy, the depth from the mucosa to the tumor cells was >50 µm.



► **Video 1** An endocytoscopic video observing neuroendocrine tumor. The lesion was a submucosal tumor exhibiting a yellowish-white color, 6 mm in diameter. Blood flow is apparent in the vessels stretched over the surface layer. In endoscopic observation, mucosal defects are not observed in the lesion. After staining the lesion with crystal violet and methylene blue, ultra-high magnifying endoscopy commences. Cells with small, round stained nuclei are arranged in a cord-like array, and blood flow is observed in vessels surrounded by the cord-like structures.

Discussion

In the case of SMTs covered with normal mucosa, the operator can only infer the pathological diagnosis from the color and hardness of the tumor [5]. In this study, we were able to observe cord-like or honeycomb arrays of cells in 10 of 13 lesions using endocytoscopy, and these findings corresponded with



► **Fig. 1** Endocytoscopic and pathological findings in Case 8. **a** Indigo carmine-sprayed endocytoscopic image. **b** Endocytoscopic image stained with 0.05% crystal violet (CV) and 1.0% methylene blue (MB). **c** and **d** Histologic sections stained with hematoxylin & eosin (HE). Tumor cells are arranged in honeycomb- and cord-like patterns around the submucosa. **e** Chromogranin A-positive cells are present. **f** Synaptophysin-positive cells are present. **g** Ki-67 staining. The Ki-67 labeling index was <3%. **h** and **i** Histologic section stained with synaptophysin. A NET is located near the mucosal surface but is not exposed.

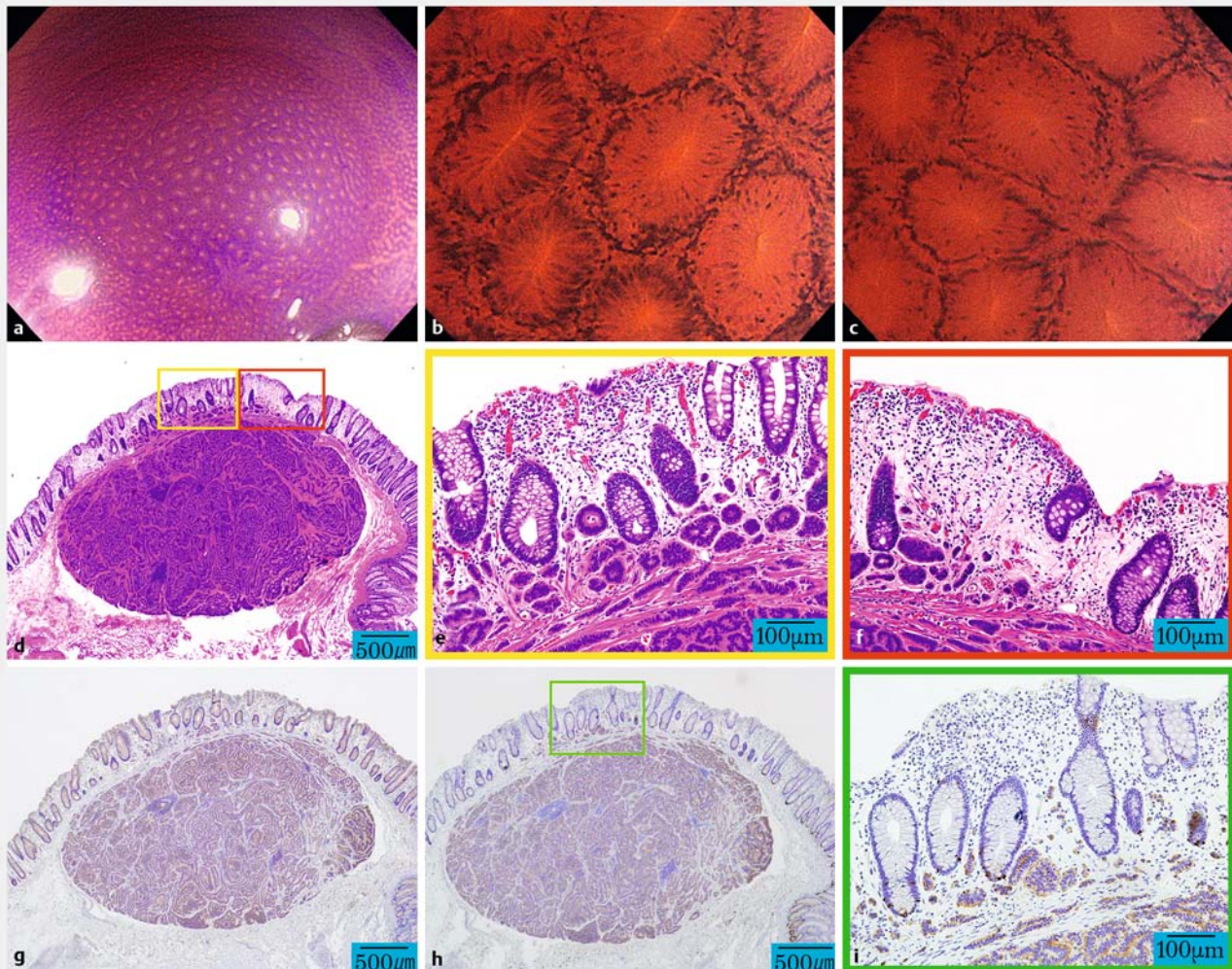
findings on pathology. As NETs enlarge in the submucosa, they extend beneath the superficial mucosa, stretching it and resulting in the crypts becoming sparser and thinning of the surface mucous membrane. We postulated that stretching and thinning of the interstitial epithelium between the crypts would bring these tumors into the depth of focus of the endocytoscope, allowing examination. However, such extension of the tumor to beneath the surface layer was not detected by endocytoscopy or pathological examination of specimens in our study cohort (► **Video 1**, ► **Fig. 1**).

Nine out of 10 diagnosed cases featured NET cells within 50µm of the tumor surface, which can be explained by the fact that the focal depth design of the endocytoscope is 50µm. Among the lesions identified by endocytoscopy, there was one lesion in which tumor cells were >50µm from the surface layer (► **Table 1**, Case 12). In this tumor, the epithelial duct morphology was distorted. Compression of the lesion with the lens

stretched the mucous membrane more thinly, enabling visualization of this tumor. In addition, similarly to the lesions that could be observed with EC, in Case 12, the tumor had extended into the superficial mucosal layer.

Endocytoscopy could not identify tumors in three NETs located much deeper than 50µm from the surface layer, indicating that not all NETs can be detected with endocytoscopy (► **Fig. 2**). In addition, we did not observe tumor growth in the surface layer of the mucosa. It was not possible to examine tumors deep to the epithelial ducts, suggesting that presence of tumors in the surface layer influences their visibility.

This study has some limitations. It was retrospective study and from a single center. We evaluated only NET G1 SMTs; other submucosal tumors were not included because we had no endocytoscopic images of G2, G3, or other submucosal tumors. Because the cytoplasm did not stain with crystal violet, we could visualize only the nuclei, which stained with methylene



► **Fig. 2** Endoscopic and pathological findings in Case 5. **a** An endocytoscopic image of a lesion stained with CV. **b** and **c** Endocytoscopic images. Dense mucosal epithelial cells are apparent. **d**, **e**, and **f** Histologic sections stained with HE. A NET is situated mainly in the submucosal layer, most of its cells being deep to the crypts. **g** Synaptophysin-positive cells are visible. **h** and **i** Chromogranin-A positive cells are visible in the deep mucosal and submucosal layers.

blue. The small abnormal nuclei were arranged in a cord-like or honeycomb array, a finding characteristic of NET that was supported by pathological findings. In a biopsy, a section perpendicular to the mucosal surface image can be evaluated by pathological examination, whereas endocytoscopy mainly enables observation of tumors in a plane parallel to the mucosal surface, making it impossible to fully correlate endocytoscopic images and pathological findings.

Conclusion

The possibility that endocytoscopy can be used for examination of NETs suggests a new indication for this methodology. Examination of more lesions in the future may enable diagnosis of them in situ and assessment of the need for treatment without taking invasive biopsies.

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Competing interests

None

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