Distribution of Neuroendocrine Marker-Positive Cells in Colorectal Cancer Tissue and Normal Mucosal Tissue: Consideration of Histogenesis of Neuroendocrine Cancer

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Keywords

Neuroendocrine carcinoma · Neuroendocrine marker-positive cells · Colorectal adenocarcinoma · Neuroendocrine tumor

Abstract

Background: Colorectal neuroendocrine carcinoma (NEC) is a rare disease, and mixed cases with colorectal adenocarcinoma also exist. The histogenesis of this disease remains unclear. We studied the numbers of neuroendocrine marker-positive cells in adenocarcinoma tissue and in normal mucosal tissue to investigate the relation between adenocarcinoma and NEC and to discuss the histogenesis of NEC. **Methods:** We studied a total of 354 curatively resected cases of stage II or III colon cancer and 36 cases of rectal cancer treated at the Tokai University Hospital between 2007 and 2012. Adenocarcinoma tissue and normal mucosal tissue were immunohistochemically stained with chromogranin A, synaptophysin, and CD56. Cases in which neuroendocrine marker-positive cells were found in cancer tissue were defined as positive. In normal mucosa, the numbers of positive cells per 15 high-power fields (HPF) were counted. **Results:**

Among the 390 cases, 181 cases had right sided colon cancer, 173 cases had left sided colon cancer, and 36 cases had rectal cancer. The rates of positive staining for chromogranin A, synaptophysin, and CD56 were significantly higher in the right sided colon than in the left sided colon, consistent with the preferred sites of NEC as reported previously. Cells positive for chromogranin A and synaptophysin in normal mucosa were significantly more common in the rectum and the left sided colon than in the right sided colon. No site-specific differences were found for CD56. **Conclusions:** Neuroendocrine marker-positive cells in colorectal cancer tissue are more common in the right sided colon, whereas neuroendocrine marker-positive cells in normal mucosa are more common in the rectum. These results suggest that NEC may arise from preceding adenocarcinomas.

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Introduction

The number of patients with neuroendocrine tumors (NET) has been increasing recently according to the Surveillance Epidemiology and End Results database of

the United States [1–3]. Gastrointestinal NET is classified into NET G1, NET G2, and neuroendocrine carcinoma (NEC) on the basis of the histological differentiation of tumor cells according to the 2010 World Health Organization (WHO) classification and the proliferative activity of tumor cells as assessed using the Ki-67 index [4].

NEC is a rare disease accounting for 0.6–3.9% of all colorectal cancers and is found significantly more frequently in the right sided colon [5–7]. Concurrent and mixed cases of adenocarcinoma and NEC have been reported. Tumors respectively including $\geq 30\%$ of adenocarcinoma components and NEC components are separately classified as mixed adenoneuroendocrine carcinomas (MANECs) [4]. Colorectal MANECs are thought to arise from multi-potential stem cells that have differentiated bi-directionally [8, 9] or from differentiated adenocarcinoma cells with a neuroendocrine phenotype [10], but this remains to be confirmed.

Neuroendocrine marker-positive cells have been identified in adenocarcinoma tissue stained with hematoxylin-eosin in some cases [11–13]; however, the incidence and sites of such cells remain unclear.

To investigate the relation between colorectal adenocarcinoma and NEC and to discuss the histogenesis of NEC, we studied the site-specific frequency of neuroendocrine marker-positive cells in adenocarcinoma tissue and site-specific differences in the numbers of neuroendocrine marker-positive cells in normal mucosal tissue.

Methods

We studied 354 cases of stage II or III colon cancer and 36 cases of rectal cancer that were curatively resected at the Tokai University Hospital from 2001 through 2012. Patients who received preoperative neoadjuvant chemotherapy or neoadjuvant chemoradiotherapy were excluded.

The cecum, ascending colon, and transverse colon were defined as the right sided colon, and the descending colon and sigmoid colon (including the rectosigmoid) were defined as the left sided colon. Cases of cancer in the splenic flexure, corresponding to the border between the right sided colon and the left sided colon, were excluded.

Immunostaining

One paraffin block of normal mucosa located at least 5 cm from adenocarcinoma tissue and that of adenocarcinoma were selected in each case. Neuroendocrine markers were immunohistochemically stained with anti-chromogranin A antibody (DAKO M0869, mouse monoclonal antibody), anti-synaptophysin antibody (Leica Biosystems; NCL-L-SYNAP-299, mouse monoclonal antibody),

and anti-CD56 antibody (Leica Biosystems; NCL-CD56-1B6, mouse monoclonal antibody). Immunostaining was performed using a BOND RX Fully Automated Research Stainer (Leica Biosystems, Melbourne, Vic., Australia).

After deparaffinization, antibody activation was performed by allowing the specimens to react with BOND Epitope Retrieval Solution (ER1) at 99°C for 15 min. Chromogranin A was allowed to react with a 1:100 dilution of DAKO M0869 for 15 min, and synaptophysin was allowed to react with a 1:100 dilution of Leica SYNAP-299. CD56 was stained by allowing to react with a 1:50 dilution of Leica 1B6.

Evaluation of Neuroendocrine Marker-Positive Cells

Immunostained tumor tissue and normal mucosa were examined under a light microscope (Fig. 1). Cases in which even small numbers of neuroendocrine marker-positive cells were found in adenocarcinoma tissue were defined as positive. The number of neuroendocrine cells in normal mucosa was counted in the deep mucosal layer along the muscularis mucosa, and the numbers of positive cells were summed per 15 high-power fields (2.1 mm²) (Fig. 2).

Statistical Analyses

Statistical analyses were performed with the use of JMP13.2.1 software (SAS Institute Inc.). Two groups were compared with the use of χ^2 tests, and 3 or more groups were compared with the use of Kruskal-Wallis tests. *p* values <0.05 were considered to indicate statistical significance.

Results

Gender, age, tumor site, and histologic type of the 390 patients are shown in Table 1. A total of 181 patients (46.4%) had right sided colon cancer, 173 (44.4%) had left sided colon cancer, and 36 (9.2%) had rectal cancer.

As for the numbers and the proportions of cases that were positive for neuroendocrine markers, chromogranin A was positive in 72 cases (18.4%), synaptophysin was positive in 111 cases (28.5%), and CD56 was positive in 61 cases (15.6%). The relations of the positive neuroendocrine marker rates in cancer tissues to gender, tumor site, and histologic site are shown in Table 2. In right sided colon cancer, the positive staining rates were 23.7% for chromogranin A, 35.3% for synaptophysin, and 22.6% for CD56, as compared with the positive staining rates of 13.2, 21.9, and 8.0%, respectively, in left sided colon cancer. The positive staining rates for each marker were significantly higher in right sided colon cancer ($p = 0.0115$, $p = 0.0054$, and $p = 0.0062$, respectively). The positive staining rates of each marker in rectal cancer were intermediate between the rates in right sided and left sided colon cancer. There was no significant difference in

Fig. 1. Immunostaining of colorectal adenocarcinoma tissue. **a** Hematoxylin-eosin staining. **b** Chromogranin A. **c** Synaptophysin. **d** CD56. Atypical cells forming a ductal structure and showing findings of highly differentiated adenocarcinoma (**a**). Cytoplasm of

cells in adenocarcinoma tissue clearly forming ductal structures, which is evaluated to be positive (**b**, **c**). In **d**, there is no staining of adenocarcinoma tissue, and the evaluation is negative.

the positive staining rates between left sided colon cancer and rectal cancer. All three markers were positive in 22 cases (12.1%) of right sided colon cancer, 5 cases (2.8%) of left sided colon cancer, and 4 cases (11.1%) of rectal cancer. The positive staining rates of all cases with three positive markers were significantly higher in right sided colon cancer and rectal cancer than in left sided colon cancer, respectively ($p = 0.0004$, $p = 0.0123$). At least one marker was positive in 76 cases (41.9%) of right sided colon cancer, 42 cases (24.2%) of left sided colon cancer, and 11 cases (30.5%) of rectal cancer. The positive staining rate of cases with one of three positive markers was significantly higher in right sided colon cancer than in left sided colon cancer ($p = 0.0004$), but did not differ

significantly when comparing with rectal cancer ($p = 0.201$).

Table 3 shows the numbers of neuroendocrine marker-positive cells in normal mucosa of the right sided colon, the left sided colon, and the rectum. The numbers of cells positive for chromogranin A and synaptophysin were significantly higher in the rectum and the left sided colon than in the right sided colon. Table 4 shows the relation between the positivity of the neuroendocrine marker cells in cancer tissues and the numbers of positive cells in normal mucosa. There was no relation between the presence of neuroendocrine marker-positive cells in adenocarcinoma tissues and the numbers of neuroendocrine marker-positive cells in normal mucosa.

Fig. 2. Immunostaining of normal mucosa. Neuroendocrine marker-positive cells are seen primarily in deep regions of the mucosa. **a** Chromogranin A. **b** Synaptophysin. **c** CD56.

Discussion

Iwafuchi et al. [14] proposed that there may be four routes for the histogenesis of NEC: (1) NEC can arise from general adenocarcinomas, (2) carcinoid cells, (3) multi-potential stem cells, or (4) immature neuroendocrine cells [15]. Jesinghaus et al. [16] reported that colorectal NEC and colorectal MANEC are intimately related to colorectal adenocarcinoma genetically [17, 18]. This suggests that NEC and MANEC may arise from stem cells with multipotential properties shared by adenocarcinomas.

Shafqat et al. [7] studied the distribution of NECs in 629 cases arising in the right sided colon, 230 cases in the left sided colon, and 362 cases in the rectum and reported that many cases arose in the right sided colon and the rectum. The distribution of MANEC remains unclear

owing to the low number of cases and the lack of reports summarizing large numbers of cases. As for NET, most cases arise in the rectum, and the incidence in the colon is low [3]. If neuroendocrine marker-positive cells residing in adenocarcinomas are related to pluripotent stem cells or young neuroendocrine cells, the distribution of these neuroendocrine marker-positive cells is expected to be similar to the distribution of NEC. As for the histogenesis of NEC, the results of the present study suggest that NEC is derived from adenocarcinomas rather than from NET.

As for the distribution of neuroendocrine marker-positive cells in healthy mucosa, Muta et al. [19] reported that the distribution of neuroendocrine marker-positive cells in normal colon tissue is highest in the rectum, followed by the sigmoid colon and the duodenum. These results are consistent with our findings. The lack of a relation be-

Table 1. Clinical characteristics

	Number	Ratio (%)
Sex		
Male	216	55.4
Female	174	44.6
Age, years	68.6±11.2	
Tumor location		
Right-sided	181	46.4
Cecum	41	10.5
Ascending	88	22.5
Transverse	52	13.3
Left-sided	173	44.4
Descending	20	5.1
Sigmoid	153	39.2
Rectum	36	9.2
Histologic type		
Well	180	46.2
Moderate	171	43.8
Poor	23	5.9
Mucinous	16	4.1

Table 2. Neuroendocrine marker-positive rates in colorectal adenocarcinoma tissue

	Chromogranin A-positive cases	<i>p</i> value	Synaptophysin-positive cases	<i>p</i> value	CD56-positive cases	<i>p</i> value
Sex, <i>n</i> (%)						
Male	44/216 (21.0)	0.291	65/216 (29.6)	0.426	31/216 (14.4)	0.434
Female	28/174 (16.7)		46/174 (28.0)		30/174 (17.2)	
Tumor location, <i>n</i> (%)						
Right	43/181 (23.7)	0.0412	64/181 (35.4)	0.0143	41/181 (22.6)	0.0008
Left	23/173 (13.3)		37/173 (21.4)		14/173 (8.1)	
Rectum	7/36 (19.4)		10/36 (27.8)		6/36 (16.7)	
Histologic type, <i>n</i> (%)						
Well	36/180 (20.0)	0.292	52/180 (28.9)	0.898	22/180 (12.2)	0.320
Moderate	32/171 (18.7)		49/171 (27.7)		33/171 (19.3)	
Poor	1/23 (4.4)		5/23 (21.7)		4/23 (12.5)	
Mucinous	4/16 (25.0)		5/16 (31.3)		2/16 (17.4)	

Table 3. Numbers of neuroendocrine marker-positive cells according to the sites of normal mucosal tissue

	Chromogranin A	<i>p</i> value	Synaptophysin	<i>p</i> value	CD56	<i>p</i> value
Right-sided colon	62.2±20.5	<0.001	47.7±23.5	<0.001	2.8±2.0	0.295
Left-sided colon	131.9±44.7		95.3±35.1		2.5±1.9	
Rectum	243.7±60.2		156.9±56.8		3.0±2.5	

Table 4. Comparison of the numbers of neuroendocrine marker-positive cells in normal mucosal tissue between neuroendocrine marker-positive cases and negative cases in cancer tissue

	Number of neuroendocrine marker-positive cells	<i>p</i> value
Chromogranin A		
Positive	100.3±7.7	0.165
Negative	112.1±3.7	
Synaptophysin		
Positive	75.0±4.4	0.301
Negative	80.5±2.8	
CD56		
Positive	3.2±0.3	0.083
Negative	2.7±0.1	

tween the numbers of neuroendocrine marker-positive cells in the healthy mucosa and the most common sites of NEC also suggests that the histogenesis of NEC does not involve NET.

Conclusions

Neuroendocrine marker-positive cells in cancer tissues are commonly found in the right sided colon. Although this is consistent with the preferred site of NEC, many neuroendocrine marker-positive cells reside in the normal mucosa of the rectum. These results

suggest that NEC may arise from preceding adenocarcinomas.

Statement of Ethics

This study was approved by the institutional review board of Tokai University (15R-216).

Disclosure Statement

Takashi Ogimi, Sotaro Sadahiro, Yutaro Kamei, Lin Fung Chan, Hiroshi Miyakita, Gota Saito, Kazutake Okada, Toshiyuki Suzuki, and Hiroshi Kajiwarara have no potential conflicts of interest to declare.

Author Contributions

Conception and design: T. Ogimi, S. Sadahiro. Provision of study materials or patients: T. Ogimi, Y. Kamei, L.F. Chan, H. Miyakita, G. Saito, K. Okada, T. Suzuki. Collection and assembly of data: T. Ogimi, S. Sadahiro. Data analysis and interpretation: T. Ogimi, S. Sadahiro, H. Kajiwarara. Manuscript writing: T. Ogimi, S. Sadahiro. Final approval of the manuscript: T. Ogimi, S. Sadahiro, Y. Kamei, L.F. Chan, H. Miyakita, G. Saito, K. Okada, T. Suzuki, and H. Kajiwarara.

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