

Argentaffin Carcinoid Tumors of the Rectum: An Immunohistochemical and Ultrastructural Study

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Summary. Nine argentaffin carcinoids were found among 94 rectal classical carcinoids (9.6% in frequency), and were systematically investigated regarding histologic, immunohistochemical and ultrastructural characteristics, in addition to the prognosis of patients. The carcinoids were characterized by a trabecular pattern frequently exhibiting an acinar arrangement of tumor cells with intracytoplasmic red-brown granules on routine hematoxylin-eosin (HE) staining. Their incidences of hormone-immunoreactivities were as follows: serotonin, 100%; neurotensin, 33%; somatostatin, 29%; glicentin, 20%; peptide YY, 20%; and pancreatic polypeptide, 14%. Ultrastructurally, the tumors were composed mainly of EC1 and/or ECn cells. Among them, one tumor showed atypical nuclei, a few mitoses, and regional lymph nodal metastasis at the time of primary tumor resection. However, others revealed uniform nuclei, but neither mitosis nor nodal metastasis. Goblet cell differentiation was not found in any of the carcinoids. No patients had any recurrence or metastasis in the course of 1.5 to 7.5 years of follow-up. We conclude that rectal argentaffin carcinoids should be categorized as a distinct group, and that they behave in a low-grade malignant manner except for cases with higher nuclear atypia and mitotic activity.

INTRODUCTION

The rectum is one of the commonest sites for carcinoids in the gastrointestinal tract.¹⁾ With silver reaction, rectal carcinoids are usually thought to be argyrophilic in type and occasionally non-reactive.²⁻⁶⁾ On the other hand, another type, i.e., the argentaffin carcinoid, has been sporadically reported;^{1,2,5,7-10)} its characteristics have not been yet established, however, owing to its rarity of occurrence.^{1,5,7,9,11)} Previ-

ous studies on rectal carcinoids did not differentiate argentaffin carcinoids from argyrophil carcinoids with a few argentaffin cells,^{3,4,6)} although these two types of carcinoids differ in nature. In the appendix, argentaffin carcinoids and argyrophil, non-argentaffin ones have been already confirmed to differ not only in histologic patterns and immunohistochemical hormone-products,¹²⁾ but also in the mRNA level of hormone-products using *in-situ* hybridization.¹³⁾ The application of such new analyses urges the classification of rectal carcinoids into distinct subgroups.

This study reports the incidence, histologic, immunohistochemical and ultrastructural characteristics, and clinical course of rectal argentaffin carcinoids, and concludes that rectal argentaffin carcinoids can be categorized as a distinct group among the gastrointestinal carcinoids.

MATERIALS AND METHODS

Nine primary argentaffin carcinoids of the rectum were examined histologically, immunohistochemically, and ultrastructurally. The materials were selected from 94 primary rectal carcinoids registered in the First Department of Pathology, Niigata University School of Medicine. They were histologically defined as endocrine cell tumors composed of uniform, low-grade atypical endocrine cells. Endocrine cell carcinomas (small cell carcinomas)^{1,5,7)} were excluded. Each patient was Japanese and had a single tumor. Eight carcinoids were clinically detected and one (Case 1 in Table 2) was incidentally found on histologic examination. No clinical symptoms or abnormal laboratory data due to the excess of gut hor-

mones were noticed in any of the nine patients. Eight tumors were completely resected by surgery and endoscopic polypectomy. One tumor (Case 4) was obtained by endoscopic biopsy; re-biopsy confirmed no tumor remnant. Follow-up data were obtained in six patients. The average length of follow-up period was 5.3 ± 1.8 (1.5 to 7.5) years.

All materials were fixed in 10% formalin, cut and then embedded in paraffin. The tumors were cut through their center. Each of the serially cut $2 \mu\text{m}$ sections was stained with hematoxylin-eosin (HE) stain, Grimelius' argyrophil and Fontana-Masson's argentaffin stains for endocrine granules, alcian blue-periodic acid-Schiff stain for mucous substances, Victoria-blue stain for elastic fibers of venous wall, and immunostains.

The term "argentaffin carcinoid" is used here to indicate a tumor composed almost totally of argentaffin cells (argyrophil-and argentaffin-positive cells). Cell arrangements were classified into six types as follows: 1) scattered cells and/or small cluster (smaller than $50 \mu\text{m}$ in length); 2) trabecule (longer than $50 \mu\text{m}$ in length); 3) anastomosing-ribbon alveolus; 4) solid alveolus (50 to $99 \mu\text{m}$ in short axis); 5) large solid alveolus (larger than $100 \mu\text{m}$ in short axis) and acinus.

Immunohistochemical Studies: After deparaffinization and blocking of endogenous peroxidase activity

by 0.3% hydrogen peroxide/methanol, the sections were incubated with the primary antisera (Table 1). The sections were then incubated with goat anti-rabbit IgG (Japan Immunoresearch Laboratory, Takasaki, Japan) and rabbit PAP (Dako, Copenhagen, Denmark), or with peroxidase-conjugated rabbit anti-guinea pig IgG (Dako) and were reacted in diaminobenzidine (Sigma, St. Louis, U.S.A.) with hydrogen peroxide.

Ultrastructural Study: Six tumor tissues were obtained from formalin-fixed, paraffin-embedded blocks and then deparaffinized. One tumor tissue was obtained from formalin-fixed material. The materials were postfixed in 1% buffered osmium tetroxide and embedded in Epon. Ultrathin sections, poststained with uranyl acetate and lead citrate, were examined by transmission electron microscopy.

Individual secretory granules were divided into round and irregular-shaped granules.¹²⁾ Tumor cells with irregular-shaped secretory granules were regarded as EC cells, and those with round secretory granules alone as non-EC cells. To determine the average diameter of secretory granules in each cell, all secretory granules (mean \pm SD; 97 ± 43 granules per cell) were measured on prints magnified 23,000 times. For this purpose, 10 to 20 cells were selected in each tumor.

Table 1. Antisera applied for immunohistochemical study.

Antisera against	Abbreviation	Antigen	Directed against	Source
Serotonin	Ser	Serotonin-creatinin phosphate	—	J. Uwo, Kyoto, Japan
Neurotensin (R3512)	Neu	Synthetic bovine neurotensin	N-terminus	N. Yanaihara, Shizuoka, Japan
Somatostatin	So	Human somatostatin	—	S. Ito, Niigata, Japan
Glicentin (R4804)	Gli	Glicentin (49-69)	N-terminus	N. Yanaihara, Shizuoka, Japan
Glucagon	Glu	Human pancreatic glucagon (19-29)	C-terminus	Japan Immunoresearch Lab, Takasaki, Japan
Peptide YY	PYY	Synthetic peptide YY	—	S. Ito, Niigata, Japan
Pancreatic polypeptide	PP	Porcine pancreatic polypeptide	—	S. Ito, Niigata, Japan
Gastrin/cholecystkinin	Ga/CCK	Human gastrin	—	CEA-IRE-SORIN, France
Adrenocortico-tropic hormone	ACTH	Synthetic adrenocortico-tropic hormone (1-24)	—	S. Ito, Niigata, Japan
β -endorphin	β -end	Synthetic β -endorphin	—	Japan Immunoresearch Lab, Takasaki, Japan
Motilin (R1105)	Mo	Synthetic porcine motilin	—	N. Yanaihara, Shizuoka, Japan
Gastrin releasing peptide (R6902)	GRP	Synthetic porcine gastrin releasing peptide	C-terminus	N. Yanaihara, Shizuoka, Japan
Insulin	Ins	Synthetic insulin	—	Japan Immunoresearch Lab, Takasaki, Japan

Anti-peptide YY and anti-insulin serum were raised in guinea pigs and were applied by indirect immunoperoxidase method. All others were raised in rabbits and were by peroxidase-antiperoxidase method. Detailed characteristics of antisera have been presented previously.^{1,12)}

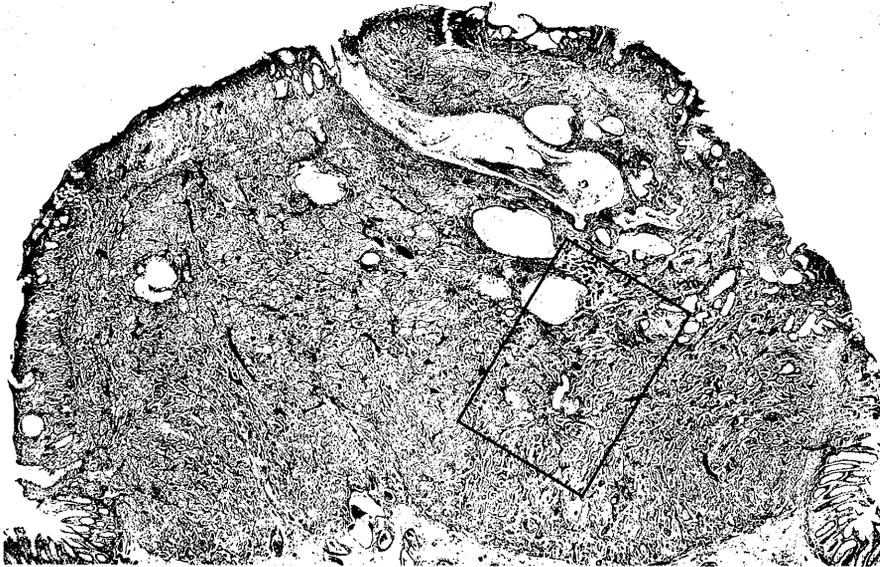


Fig. 1. Argentaffin carcinoid with polypoid shape. The section cut along the center of tumor shows many tubules in the submucosal tumor tissue. Some of them continue to the overlying non-neoplastic epithelium. (Case 9, $\times 11$)

RESULTS

1. Histologic and histochemical findings

Argentaffin carcinoids showed a predominance of trabecular and solid patterns, frequently associating with an acinar arrangement (Table 2, Figs. 1 and 2). The large solid pattern was rarely found. The nine tumors essentially had similar cytologic features. The tumor cells contained abundant, a slightly granular, slightly eosinophilic to clear cytoplasm, and a uniform oval nucleus (nuclear size: 5 to 6 μm along the minor axis at a magnification of $\times 1000$) with finely dispersed chromatin and an indistinct nucleolus (Fig. 3). Their nuclear/cytoplasmic ratio was lower than 50%. One tumor (Case 8) showed a slight nuclear atypia (Fig. 3) and a few mitoses. Nuclear size varied from 5 to 8 μm along the minor axis in this tumor. This tumor invaded the submucosal lymphatic vessels and nerve plexus, while the other eight tumors did not. Four tumors contained many tumor cells with intracytoplasmic red-brown granules; the other

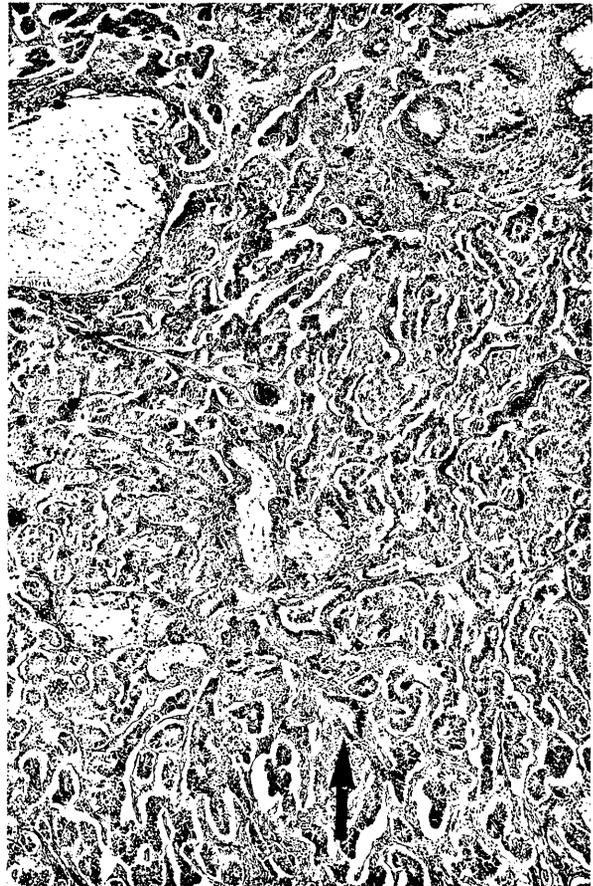


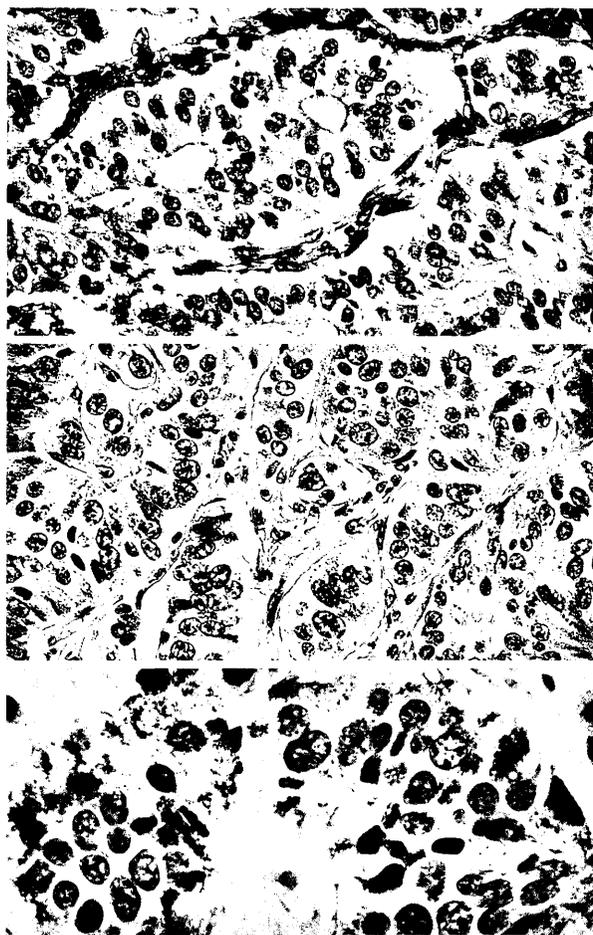
Fig. 2. Part shown by square in Fig. 1. Carcinoid cells are arranged in a trabecular pattern with acinar arrangement. Among them, non-neoplastic tubules composed of columnar cells and goblet cells are immersed. ($\times 33$)

Table 2. Clinical and histologic findings of rectal argentaffin carcinoid tumors.

Case No.	Age (yr)/Sex	Size (mm)	Macroscopic type	Depth of invasion	Histological structure						Population of cell with red-brown granules in HE section
					Scattered cell and/or small cluster	Trabecular	Anastomosing ribbon	Solid	Large solid	Acinar	
1	40/M	1.5	Polypoid	m	+	+++	-	+	-	-	+
2#	63/M	3	Flat	sm	+	++	-	++	+	++	+
3	71/M	3	Polypoid	sm	++	+	-	++	-	++	+++
4	52/M	3	Polypoid	sm	+	+++	-	-	-	-	-
5	57/M	5.5	Polypoid	sm	+	+++	+	+	-	+	+
6	47/M	7.5	Polypoid	sm	+	+++	+	-	-	-	+++
7	52/M	8	Polypoid	sm	+	+++	+	+	-	+	+++
8	51/M	8.5	Polypoid	sm	+	+++	+	+	+	++	+++
9#	62/M	13	Polypoid	sm	+	++	+	+	+	+++	+

Grades of cell type: occupying more than 50% of the total tumor cells in a section examined (+++); 50% to 25% (++); less than 25% (+); or absent (-). Ratio of area of each cell arrangement in tumor was also divided into four grades.

M: male, m: mucosa, sm: submucosa, nt: not tested, nr: not recorded.



four tumors contained small numbers of these cells in the HE section (Fig. 3). Argentaffin reaction was more intense in the former than in the latter. Neither venous permeation nor tumor necrosis was seen in any of the tumors examined. Acidic mucin was seen along the luminal surface of the acinar and tubular arrangements of carcinoid cells in six tumors, but mucous droplets were not seen in any carcinoid cell examined. No tumors coexisted with other epithelial or non-epithelial neoplasia within themselves.

There were tubular structures composed of goblet cells and columnar cells among the submucosal carcinoid tissues in five polypoid tumors, which were examined using a section cut along the center of the tumor (Figs. 1 and 2). Such tubules were present in groups, often associated with degenerated tubules and mucous retention, and frequently continuous to the overlying non-neoplastic epithelia in serially cut sections. These goblet cells and columnar cells showed a clear border against genuine carcinoid cells (Fig. 4). Their cellular and nuclear features were

Fig. 3. An argentaffin carcinoid has a uniform oval nucleus (top, Case 9, $\times 333$). One tumor in this series shows nuclear pleomorphism (center, Case 8, $\times 333$). Argentaffin carcinoid cells are characterized by intracytoplasmic red-brown granules in HE section. (bottom, Case 8, $\times 666$)

Population of positive cell								Population of ultrastructural cell type			Granule size		Metastasis at resection of primary tumor	Prognosis (follow-up)
Argyr	Argent	Ser	Neu	So	Gli	PYY	PP	ECI	ECn	Non-EC	EC (nm)	Non-EC (nm)		
+++	+++	+++	-	+	-	-	-	+++	+	-	260±56*		-	Alive and well (5.5 yr)
+++	+++	++	nt	+	-	nt	-	+	+++	+	200±20	150±2	-	Died of rectal cancer (1.5 yr)
+++	+++	+++	nt	-	-	-	-	+++	-	-	240±29		-	Alive and well (6 yr)
+++	+++	nt	nt	-	-	nt	+	-	+++	+	180±16	170	nr	nr
+++	+++	+++	-	-	-	+	-	+++	-	-	300±54		-	Alive and well (6 yr)
+++	+++	nt	nt	nt	nt	nt	nt		nt				nr	nr
+++	+++	nt	nt	nt	nt	nt	nt		nt				nr	nr
+++	+++	+++	nt	-	+	-	-	+++	+	-	270±50		Regional lymph node	Alive and well (5 yr)
+++	+++	++	+	-	+	-	-	+++	+	-	260±32		-	Died of ileus (7.5 yr)

* Mean ± standard deviation.

#Case 2 had three simultaneous rectal carcinomas in preexisting ulcerative colitis and Case 9 had a simultaneous rectal carcinoma and a heterochronous gastric carcinoma.

similar to those of overlying non-neoplastic epithelial cells as follows. Compared with genuine carcinoid cells, they showed a more distinct cell border, a clear to slightly basophilic, non-granular cytoplasm occasionally displaying condensed dark-eosinophilia, and a large, ellipsoid to polygonal nucleus with uneven outline, minute dotted to scanty chromatin and an occasional small distinct nucleolus.

2. Immunohistochemical findings

Six carcinoids could be immunostained with anti-Ser serum, and all of these were diffusely positive for Ser (Fig. 5). Neu, So, Gli, PYY, and PP cells were sporadically found in 33%, 29%, 20%, 20%, and 14% of tumors stained, respectively. Ga/CCK, Glu, Mo, ACTH, β -end, Ins, and GRP were not found.

3. Ultrastructural findings

Seven carcinoids examined ultrastructurally were

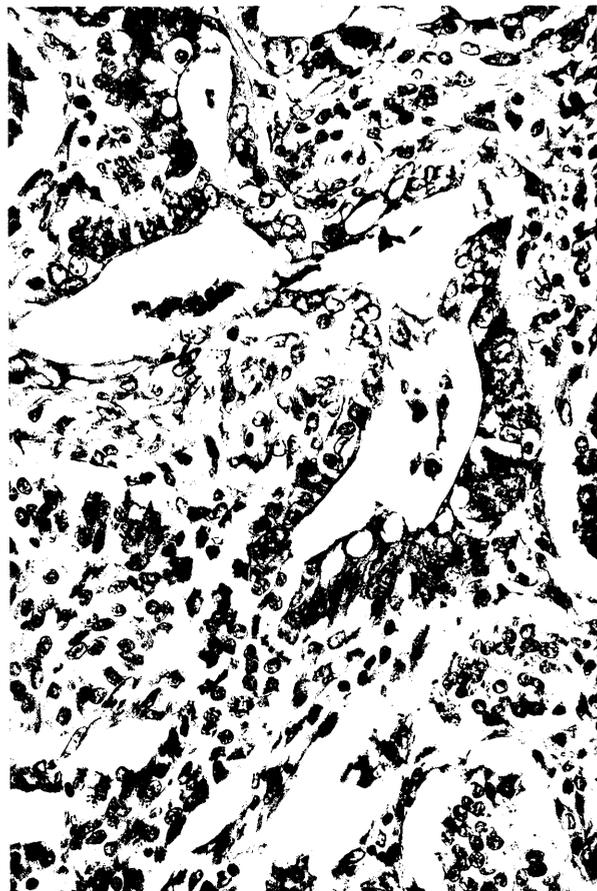


Fig. 4. Magnified figure indicated by long arrow in Fig. 2. Immersed non-neoplastic tubules composed of columnar to cuboidal cells and goblet cells are seen in the upper half. These cells have a larger, ellipsoid to polygonal nucleus with uneven outline and dotted to scanty chromatin as compared with the genuine carcinoid cells in the lower half. ($\times 333$)

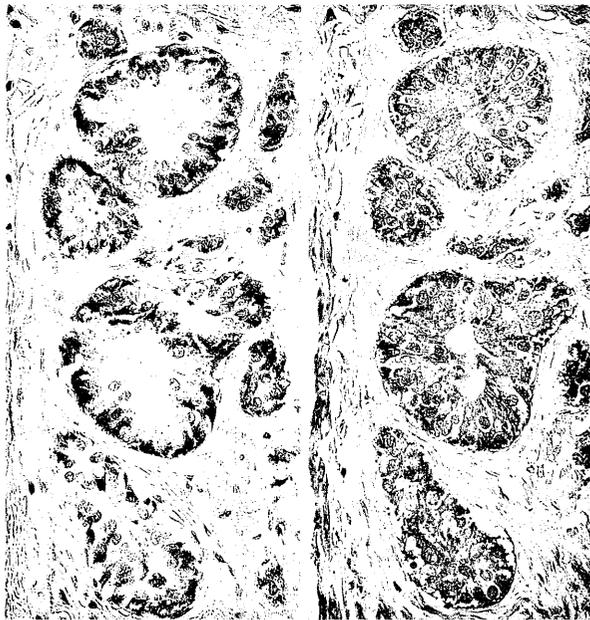


Fig. 5. Carcinoid cells are diffusely positive for argentaffin (left) and serotonin stain (right). (Case 3, $\times 220$)

composed mainly of EC cells; five were of EC1 cells and two of ECn cells (Table 2, Fig. 6). EC1 cell granules were homogeneous in electron-density and ECn cell granules were occasionally mottled (Fig. 7). Small populations of non-EC cells were found in two ECn tumors.

4. Clinical course

One large tumor (Case 8) showing slight nuclear atypia, mitoses, and invasion into submucosal lymphatic vessels and the nerve plexus metastasized to a regional lymph node at the time of primary tumor resection. However, there was no significant difference in immunohistochemical and ultrastructural findings between this tumor and the others. There was no local recurrence or distant metastasis during the follow-up period in all cases. None of the patients died of a carcinoid tumor.

DISCUSSION

The incidence of argentaffin carcinoids among rectal

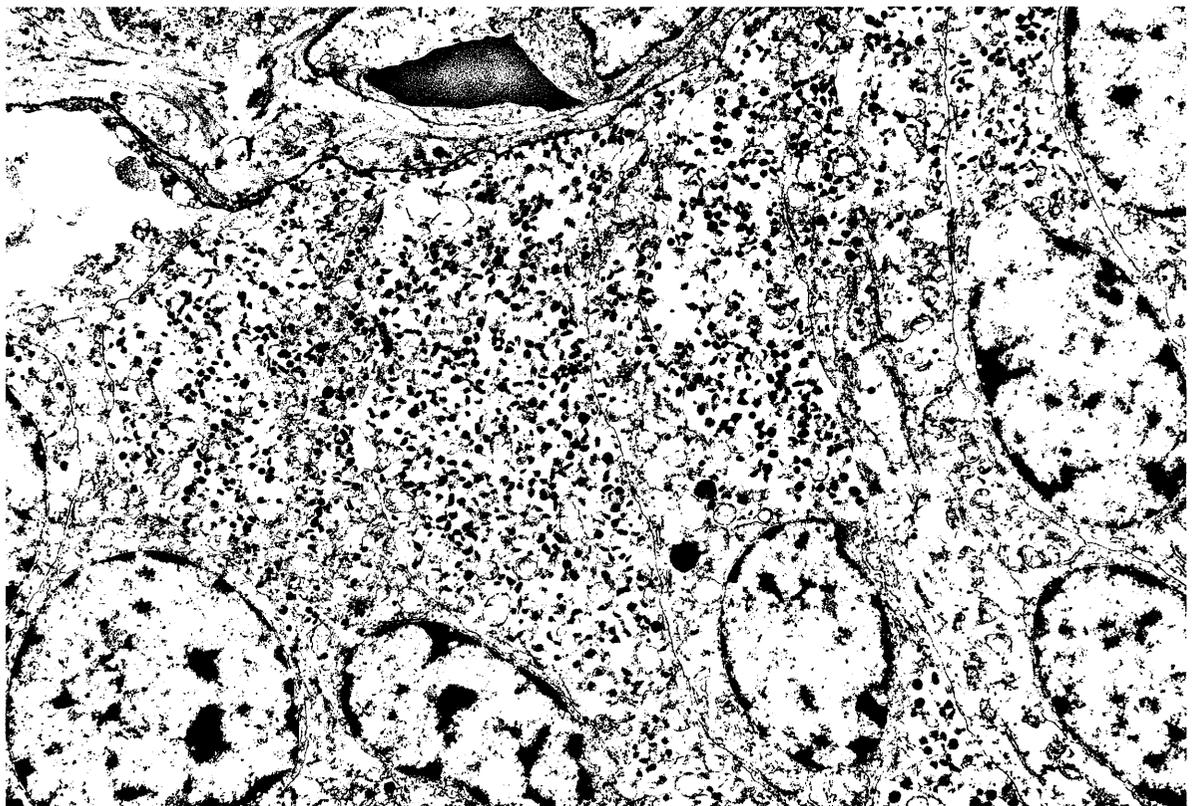


Fig. 6. Rectal argentaffin carcinoid composed homogeneously of EC cells. (Case 9, $\times 4600$)

carcinoids is 7.4%,⁵⁾ 10.1%,¹⁾ 13.0%,⁹⁾ and 14.3%⁷⁾ in an individual series, 8.3% in review of 108 reported tumors¹¹⁾ and 9.6% in the present study. Rectal argentaffin carcinoids may occur more frequently than generally thought.

This study confirmed that rectal argentaffin carcinoids were characterized by trabecular and solid patterns in frequent association with an acinar arrangement of carcinoid cells with intracytoplasmic red-brown granules on histologic examination with HE section.

Immunohistochemical and ultrastructural characteristics of rectal argentaffin carcinoids remain to be definitively determined. Hormone-immunoreactivity was reported only in three tumors: one with substance P,⁸⁾ one with So, Glu, and Ga,⁵⁾ and one with Ser.⁹⁾ Ultrastructurally, two distinctive cell types, one with pleomorphic granules and another with small round granules, were found in three tumors.^{2,9,10)} Our study concluded that rectal argentaffin carcinoids are characteristically composed predominantly of Ser-immunoreactive, EC1 and/or ECn cells with or without small numbers of peptide-positive cells and non-EC cells. The infrequent association of carcinoid syndrome in patients with rectal carcinoid can be explained by the rare occurrence of argentaffin carcinoids in the rectum.

The midgut (jejunal, ileal, and appendiceal)—derived argentaffin carcinoids are characterized by solid and large solid patterns of Ser-positive EC cells.¹²⁾ Rectal argyrophil carcinoid with or without a few argentaffin cells is known to grow predominantly in trabecular and anastomosing ribbon-like patterns with rare acinus,^{3,5,11)} to be composed mainly of non-EC cells without distinct intracytoplasmic red-brown granules, and to be frequently positive for PP, PYY and Gli and infrequently for Ser.^{1,3,4,6)} Based on these histologic, immunohistochemical and ultrastructural findings, rectal argentaffin carcinoids should be categorized into a distinct group of gastrointestinal carcinoids.

As for carcinoid cell differentiation, Shimoda et al.⁵⁾ and Arai et al.¹⁴⁾ reported goblet cell differentiation of rectal carcinoid cells. As described in detail in the result of this study, goblet cells and columnar cells found in carcinoid tissues were apparently diagnosed as non-neoplastic cells immersed in carcinoid tissues by careful examination of their cellular and nuclear appearance. We did not find goblet cell differentiation of carcinoid cells in the rectum or in other sites of the gastrointestinal tract.^{1,12)} Rectal polypoid carcinoids frequently inter-

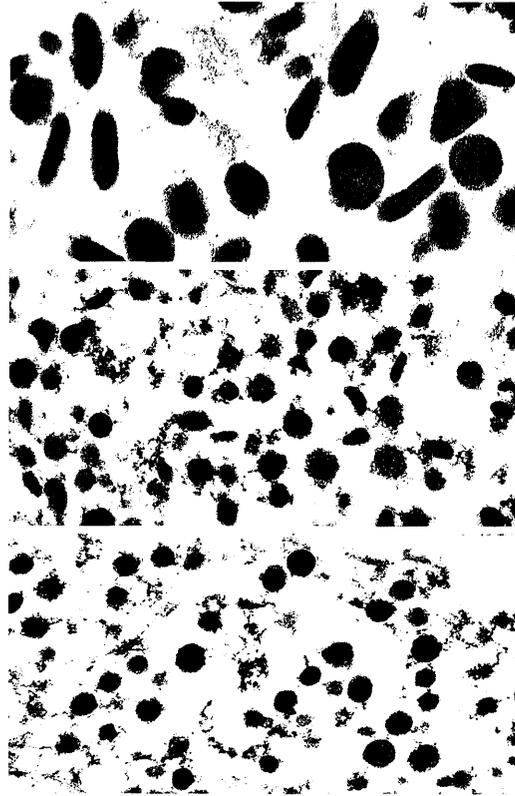


Fig. 7. Secretory granules of each cell type in rectal argentaffin carcinoid ($\times 23,000$): EC1 cell (top, Case 5), ECn cell (center, Case 2) and non-EC cell. (bottom, Case 2)

mixed non-neoplastic tubules and fibromuscular stroma among tumor tissue as shown in this study. Both changes are thought to occur in the slow-growing course of carcinoid tumors.

We have already reported that gastrointestinal carcinoids composed of uniform low-grade atypical endocrine cells behave in an indolent, low-grade malignant manner and have a good prognosis,^{1,6)} and that endocrine cell carcinomas, a high-grade malignant counterpart of the endocrine cell tumor, progress rapidly, have an unfavorable prognosis, and are characterized by cellular and nuclear atypia, frequent mitoses and tumor necrosis.^{1,5,7)} This study demonstrated that nuclear atypia and mitotic activity well indicate the biological behavior of rectal argentaffin carcinoids.

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