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Changes of gastrointestinal argyrophil endocrine cells in the COLO205 tumor-implanted Balb/c-nu/nu mice

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The regional distributions and frequencies of argyrophil endocrine cells in gastrointestinal (GI) tract of Balb/c-nu/nu mouse were studied using Grimelius silver stain after abdominal subcutaneous implantation of COLO205. The experimental animals were divided into two groups, one is non-implanted group (Sham) and the other is COLO205-implanted group. Samples were collected from GI tract (fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum) at 21 days after implantation of COLO205 cells (1×10^6 cell/mouse). In this study, argyrophil cells were detected throughout the entire GI tract with various frequencies regardless of implantation. Most of these argyrophil cells in the mucosa of GI tract were generally spherical or spindle in shape (open type cell) while cells showing round in shape (close type cell) were found occasionally in gastric and/or intestinal gland regions. The regional distributions of argyrophil cells in COLO205 were similar to those of Sham. However, significant decreases of argyrophil cells were detected in COLO205 compared to those of Sham except for the jejunum and ileum. In the jejunum and ileum, argyrophil cells in COLO205 showed similar frequencies compared to those of Sham. In the pylorus, the most dramatically decreasement of argyrophil cells were detected in COLO205 compared to that of Sham. Implantation of COLO205 tumor cell line induced severe quantitative changes of argyrophil cell density, and the abnormality in density of GI endocrine cells may contribute to the development of gastrointestinal symptoms such as anorexia and indigestion, frequently encountered in patients with cancer.

Key words: argyrophil, COLO205, endocrine, nude mice, tumor

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Introduction

Gastrointestinal (GI) endocrine cells dispersed in the epithelia and gastric glands of the digestive tract synthesized various kinds of gastrointestinal hormones, and played an important role in the physiological functions of the alimentary tract [2]. Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenic study [6] and the endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and a change in their density would reflect the change in the capacity of producing these hormones [8]. Silver techniques have been regarded as a general method for detecting GI endocrine cells and Grimelius positive cells are classified as argyrophil cells [11,12]. The changes of distribution and frequency of GI argyrophil endocrine cells in some diseases are also well demonstrated especially in some cancer [10,17,21], gastritis including *Helicobacter pylori* [1,18], and inflammatory bowel disease [7]. These GI argyrophil cells are also changed after treatment of some drugs such as ebrotidine [20] and omeprazole [5]. In addition, a significantly decrease of GI endocrine cells in the ovariectomized osteoporotic rats using silver techniques was previously reported [15,16], and the distribution and frequency of GI endocrine cells were varied with feeding habits [24].

In the patients with cancer, the most frequent and distressing symptoms are gastrointestinal disorder, and Komurcu *et al.* [14] reported that dry mouth, weight loss, early satiety, taste changes, constipation, anorexia, bloating, nausea, abdominal pain and vomiting were 10 most common gastrointestinal symptoms in patients with lung, breast and prostate cancer. Although nearly one-half of the most frequently reported and most distressing symptoms in patients with cancer are gastrointestinal in nature [14], the study about changes of gastrointestinal endocrine cells was restricted to the region of endocrine carcinoid tissues or nonneoplastic mucosa around the carcinoids [10,17,21]. In addition, there was no report dealing with changes of gastrointestinal argyrophil cell profiles after subcutaneous implantation of tumor.

The purpose of this study is to clarify the changes of the GI argyrophil cells in the Balb/c-nu/nu mouse after subcutaneous implantation of COLO205, non-metastatic human colonic adenocarcinoma cell line, by silver stain. In the present study, samples were collected from 8 parts of GI tract at 21 days after implantation of COLO205 cells (1×10^6 cell/mouse).

Materials and Methods

Experimental animals

Ten SPF Balb/c-nu/nu female mice (6-wk old upon receipt; Charles River, Japan) were used after acclimatization for 7 days. Animals were allocated 5 per polycarbonate cage in a temperature (20-25°C) and humidity (30~35%) controlled room. Light: dark cycle was 12 hr: 12 hr and feed (Samyang, Korea) and water were supplied free to access. Animals were divided to two groups, COLO205-implanted group (COLO205) and non-implanted Sham (Sham) group. Each of 10 mice was used in this study.

Implantation of COLO205 cells

COLO205 human colon cancer cells were inoculated intradermally at abdominal skin with viable tumor cells (3×10^6 cells). In Sham, saline was intradermally injected at abdominal skin instead of inoculation.

Histology and quantity analyses

After phlebotomy, each region of GI tract, the fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum was collected from all experimental animals at 21 days after implantation and/or Sham, after 18hrs fasting to GI empty. Collected samples were fixed in Bouin's solution, embedded in paraffin, sectioned (3~4 μm), and stained with hematoxylin-eosin stain for confirming normal architecture of each region of GI tract. For observing the regional

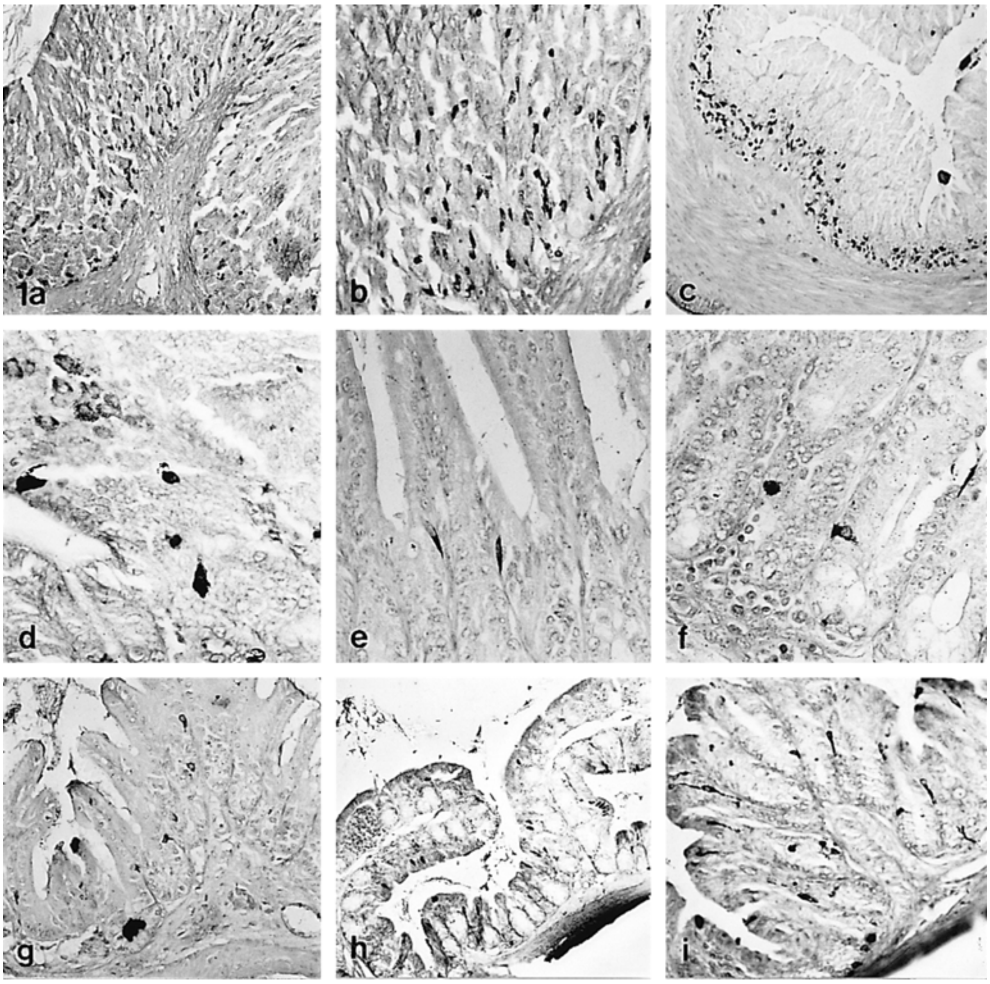

Fig. 1. Argyrophil endocrine cells in the GI tract of Sham; Most of argyrophil cells were dispersed in the mucosa of the fundus (a, b), pylorus (c), duodenum (d), jejunum (e), ileum (f), cecum (g), colon (h) and rectum (i). a, c and h: ×75; b, g and i: ×150; d~f: ×300, Silver stain.

distribution and frequency of argyrophil endocrine cells in each region of GI tract, silver stain was conducted [12].

Quantity analysis

The frequency of argyrophil cells was calculated using automated image analysis (Soft Image System; GmBH, Germany) under microscope (Carl Zeiss, Germany) in the uniform area of GI mucosa among 1,000 parenchymal cells according to the osteoporetic SD rats [15,16]. Argyrophil cell numbers were calculated as cell numbers/1,000 parenchymal cells.

Statistical analysis

Results are expressed as the mean ± standard deviation. Mann-Whitney U-Wilcoxon Rank Sum W test (M-W test) was used to analyze the significance of data with SPSS for Windows (Release 6.1.3; SPSS, USA) and a *p*-value of less than 0.05 was considered a significant difference.

Results

In this study, argyrophil endocrine cells were detected throughout the entire GI tract of rats in both COLO205 and Sham. Most of these argyrophil cells in the mucosa of GI tract were generally spherical or spindle in shape (open type), while occasionally round in shape (close type cell) cells were also found in the gastric and intestinal gland regions. According to the location of the GI tract, different regional distributions and frequencies of argyrophil cells were observed. Argyrophil cells were mainly dispersed in the basal portions of gastric and intestinal mucosa rather than surface epithelia regions and they were more numerously detected in the stomach regions compared to that of the intestinal regions. In the large intestine, more numerous cells were detected compared to that of the small intestine regardless of Sham (Fig. 1) and COLO205 (Fig. 2).

Among 1,000 parenchymal cells, argyrophil cells in Sham

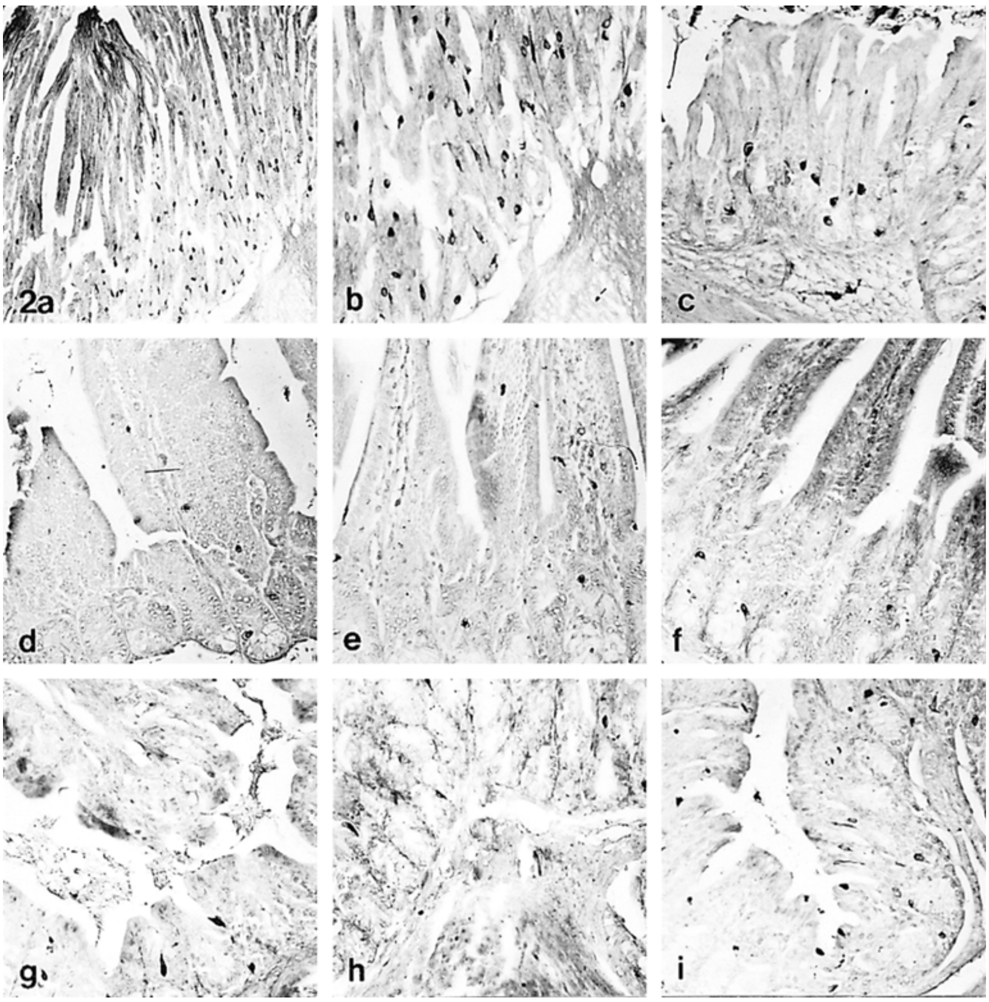

Fig. 2. Argyrophil endocrine cells in the GI tract of COLO205; Most of argyrophil cells were dispersed in the mucosa of the fundus (a, b), pylorus (c), duodenum (d), jejunum (e), ileum (f), cecum (g), colon (h) and rectum (i). a: ×75; b~i: ×150. Silver stain.

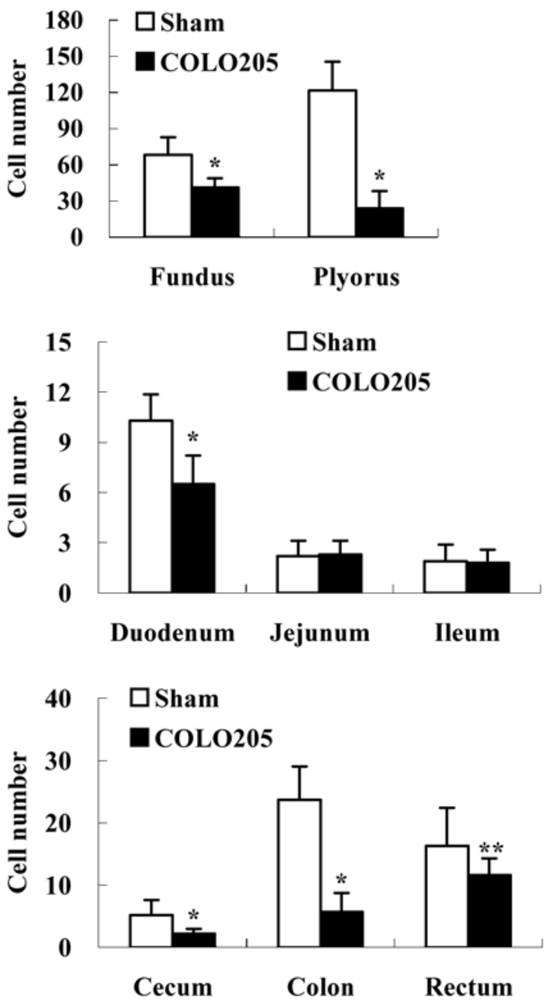

Fig. 3. Number of argyrophil cells in GI tract of sham (□) and their changes after COLO205-implantation (■). *$p < 0.01$ compared to that of Sham (n = 10); **$p < 0.05$ compared to that of Sham (n = 10).

were detected in the fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum with 68.30 ± 14.65, 121.60 ± 23.81, 10.30 ± 1.57, 2.20 ± 0.92, 1.90 ± 0.99, 5.20 ± 2.39, 23.70 ± 5.36 and 16.30 ± 6.13 cells, respectively. In COLO205, argyrophil cells were detected with 41.20 ± 7.52, 24.00 ± 14.26, 6.50 ± 1.72, 2.30 ± 0.82, 1.80 ± 0.79, 2.20 ± 0.79, 5.70 ± 3.02 and 11.60 ± 2.72 cells/1000 parenchymal cells in the fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum, respectively. Throughout the whole regions of GI tract, argyrophil cells showed significant ($p < 0.01$ or $p < 0.05$) decrease in COLO205 compared to that of Sham except for the jejunum and ileum. Argyrophil cells in the jejunum and ileum of COLO205 showed similar frequency compared to that of Sham (Fig. 3).

Discussion

COLO205 is a non-metastatic human colonic adenocarcinoma cell line and one of the most prevalently used cancer cell line in anti-cancer research [4,19]. Balb/c-nu/nu is an outbred Balb/c background mouse. The original was first reported in 1966 as a hairless mouse occurring as a spontaneous mutation. This strain of mouse is T cell-deficient immunodifficient mice and used excessively in cancer research [9,13]. This strain is essential mouse for study xenograft models in anticancer research especially in tumor cell lines originated from human [23]. In addition, it also used in human hepatitis C virus fields [22], and somewhat different profiles of pancreatic endocrine cells in this strain were already reported [27].

In the present study, the changes of the argyrophil cells in the GI tract of Balb/c-nu/nu mouse after subcutaneous implantation of COLO205 were observed by silver stain. The general distribution of the argyrophil cells in the GI tract of COLO205 showed quite similar patterns compared to that of Sham. However, as results of COLO205-implantation, argyrophil cells were significantly ($p < 0.01$ or $p < 0.05$) decreased in the entire intestinal tract except for the jejunum and ileum. In the pylorus, the most dramatical changes were demonstrated. These changes might be inducing gastrointestinal disorder observed in patients with cancer [14]. Silver techniques have been regarded as a general method for detecting GI endocrine cells and Grimelius positive cells are classified as argyrophil cells [11,12]. The GI endocrine cells were generally divided into two types, one was round to spherical shaped close type cells which were located in the stomach regions, and the other was spherical to spindle shaped open type cells which were situated in the intestinal regions. In addition, the endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and a change in their density would reflect the change in the capacity of producing these hormones [8].

It was generally accepted that the changes of argyrophil cells were clearly related to digestive status of animals. In *Helicobacter pylori* infection, hyperplasia of argyrophil cells were demonstrated [18] and they also increased in patients with ulcerative colitis and Crohn's disease [7], atrophic gastritis [1], hypergastrinemia [3], and pernicious anemia [26]. In addition, a significantly decrease of GI argyrophil cells were reported in the ovariectomized SD rats [15] and in there, it has been postulated that the changes in the GI endocrine cells are a selective process to meet the new demands exerted by the dramatic decrease in intestinal absorption and the decrease of GI argyrophil endocrine cells may be responsible for the malabsorption of calcium and lipids that occur in patients with postmenopausal osteoporosis [15,16]. Therefore, the decreases of GI argyrophil cells detected in the present study also considered that it directly related to the clinically reported gastrointestinal symptoms [14] detected in the patients with cancer.

In conclusion, implantation of tumor cell mass (COLO205) should be induced severe quantify changes of the intestinal endocrine cell density and the abnormality in density of endocrine cells may contribute to the development of gastrointestinal symptoms such as anorexia and indigestion, frequently encountered in patients with cancer. However, the target or individual changes of GI endocrine cells are not clear. Elucidation of the changes of individual GI endocrine cells using immunohistochemistry [25] will provide mechanisms for understanding GI disorder that occurs in various diseases. Further detailed studies with immunohistochemical techniques will be needed.

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