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• *BASIC RESEARCH* •

Changes of the gastric endocrine cells in the C57BL/6 mouse after implantation of murine lung carcinoma: An immunohistochemical quantitative study

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Abstract

AIM: The regional distributions and relative frequencies of some gastric endocrine cells of C57BL/6 mice were studied by immunohistochemical method using seven types of specific antisera against chromogranin A (CGA), serotonin, somatostatin, gastrin, cholecystokinin (CCK)-8, glucagon and human pancreatic polypeptide (HPP) after subcutaneous implantation of murine lung carcinoma (3LL) cells.

METHODS: The experimental animals were divided into two groups, one is non-implanted sham and the other is 3LL-implanted group. Samples were collected from the two regions of stomach (fundus and pylorus) at 28 d after implantation of 3LL cells (1×10^5 cell/mouse).

RESULTS: In this study, all the seven types of immunoreactive (IR) cells were identified except for HPP. Most of these IR cells in the gastric portion were generally spherical or spindle in shape (open-type cell) while cells showing round in shape (closed-type cell) were found occasionally. The regional distributions of gastric endocrine cells in the 3LL-implanted group were similar to those of non-implanted sham. However, significant decreases of some types of IR cells were detected in 3LL-implanted group compared to those of non-implanted sham. In addition, the IR cells showing degranulation were numerously detected in 3LL-implanted group. CGA-, serotonin- and somatostatin-IR cells in the fundus and pylorus regions, and gastrin-IR cells in the pylorus regions of 3LL-implanted groups significantly decreased compared to those of non-implanted sham. However, no changes on frequencies of CCK-8- and glucagon-IR cells were demonstrated between 3LL-implanted and non-implanted groups.

CONCLUSION: Endocrine cells are the anatomical units responsible for the production of gut hormones, and the change in their density would reflect a change in the capacity of producing these hormones. Implantation of tumor cell mass (3LL) induced severe quantitative changes of gastric endocrine cell density, and the abnormality in density of gastric endocrine cells may contribute to the development of gastrointestinal symptoms such as anorexia and indigestion, frequently encountered in patients with cancer.

Key words: Gastric endocrine cells; Gut hormones; 3LL

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INTRODUCTION

3-Lewis lung carcinoma (3LL) is a cancer that aggressively and rapidly spreads to other organs of the body. This cancer is ordinarily the most life-threatening type. There are many *in vivo* tumor models that are available for the development and study of anticancer agents in animals with autochthonic or transplanted tumor lines. A system using the 3LL, a tumor which once arose spontaneously in C57BL/6 mice, is particularly useful because it metastasizes into the lungs. Now, 3LL is considered as one of the widely used tumors in development and study of anticancer agents[1,2]. C57BL/6 mouse is an inbred black mouse and is probably the most widely used of all inbred strains though in many ways it seems to be a typical of inbred strains of laboratory mice. It usually has a good breeding performance, depending on substrain, and has been used as the genetic background for a large number of congenic strains covering both polymorphic and mutant loci. This strain of mice is resistant to chloroform toxicity[3], to induction of cleft palate by cortisone, to lethal effects of ozone[4] and to colon carcinogenesis by 1,2-dimethylhydrazine[5]. In addition, it is also a recommended host for the following transplantable tumors: 3LL, mammary adenocarcinoma BW 10232,

melanoma B16, myeloid leukemia C 1498 and preputial gland carcinoma ESR586. The regional distributions and relative frequencies of the endocrine cells along the entire gastrointestinal tract of normal C57BL/6 mice were also reported[6].

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. Until now, the investigation of GI endocrine cells is considered to be an important part of a phylogenic study[7] and endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and the change in their density would reflect a change in the capacity of producing these hormones[8]. In addition, the regional distributions and relative frequencies of these endocrine cells are varied with animal species and feeding habits[9]. Many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the GI tract of the various vertebrates including various species of rodent, and also the researches or data processing about GI endocrine cells in the mouse strains have been widely executed. The changes of GI endocrine cells in some diseases are also well demonstrated. El-Salhy and Sitohy[8] reported an abnormal density of GI endocrine cells in patients with diabetes, and proliferation of endocrine cells in the epithelium was detected in pernicious anemia[10]. In addition, some quantitative changes of endocrine cells were also demonstrated in celiac sprue[11] and the possibilities of changes on gastric endocrine cells were also reported in pancreatectomy[12].

In the patients with cancer, the most frequent and distressing symptoms are gastrointestinal disorder, and Komurcu *et al*[13] reported that dry mouth, weight loss, early satiety, taste changes, constipation, anorexia, bloating, nausea, abdominal pain and vomiting were 10 most common GI symptoms in patients with lung, breast and prostate cancer. Until now, the change of endocrine cells related with tumor has been the focus on the tumor itself induced by chemicals[14,15] and/or by some side effects of diseases[16]. Therefore, the composition, number and types of endocrine cells in endocrine tumors located in the GI tract are well recognized[17-19]. Although nearly one-half of the most frequently reported and most distressing symptoms in patients with cancer are GI in nature[13], the study about changes of gastrointestinal endocrine cells was restricted to the region of endocrine carcinoid tissues or non-neoplastic mucosa around the carcinoids. In addition, there was no report dealing on the changes of gastrointestinal endocrine cell profiles after subcutaneous implantation of tumor.

The objective of this study was to clarify the changes of endocrine cells in the stomach regions of C57BL/6 mouse after subcutaneous implantation of 3LL by specific immunohistochemistry using seven types of antisera against chromogranin A (CGA), serotonin, somatostatin, gastrin, cholecystokinin (CCK)-8, glucagon and human pancreatic polypeptide (HPP).

MATERIALS AND METHODS

Experimental animals

Twenty adult female C57BL/6 mice (6-wk-old, 21-26 body weight upon receipt) were acquired from the Charles River Laboratories (Yokohama, Japan) and used in this study after acclimatization for 7 d. Animals were allocated 5 per autoclaved filter-capped cages (Nalgene, Rochester, NY, USA) in a temperature (20-25 ℃) and humidity (50-55%) controlled room during acclimatization periods. Light/dark cycle was 12 h/12 h and sterilized feed (Samyang, Korea) and autoclaved water was supplied free to access. Animals were divided into two groups, 3LL-implanted group and non-implanted sham group. All 10 mice was used in this study. Experimental animals were treated according to institutional and National Institutes of Health guidelines for animal use and care.

Implantation of Lewis lung carcinoma (3LL)

3LL was maintained as subcutaneous tumor mass. Maintained subcutaneous tumor mass was excised under sterile conditions and single cell suspensions prepared by collagenase type IV and DNase I (Sigma, USA) in PBS and filtration of the resulting tumor cell suspension through cell strainer (Costar, USA). After counting and adjusting the cell number (1×10^5 cell/mouse), 3LL cell was intradermally implanted at abdominal skin with viable tumor cells.

Sampling

After 28 d of implantation, all experimental animals were anesthetized with ethyl ether. For inducing gastric and/or intestinal emptying, animals were fasted about 24 h. After phlebotomization, samples from the fundus and pylorus were fixed in Bouin's solution.

Histology

After paraffin embedding, 3-4 μm serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal gastrointestinal architecture.

Immunohistochemistry

Each representative section was deparaffinized, rehydrated and immunostained with the peroxidase anti-peroxidase (PAP) method[20]. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was

Table 1 Antisera used in this study

Antisera raised[1]	Code	Source	Dilution
CGA[2]	A430	DAKO Corp., Carpinteria	1:1 000
Somatostatin	PUO421295	BioGenex Lab., San Ramon	1:20
Serotonin	BO68082C	BioGenex Lab., San Ramon	1:20
Gastrin	PUO190796	BioGenex Lab., San Ramon	1:20
CCK-8[2]	750257	DiaSorin, Stillwater	1:500
Glucagon	927604	DiaSorin, Stillwater	1:2 000
HPP[2]	A610	DAKO Corp., Carpinteria	1:600

[1]All antisera were raised in rabbits. [2]CGA: chromogranin A, CCK-8: cholecystokinin-8, HPP: human pancreatic polypeptide.

prepared. The peroxidase reaction was carried out in a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 mol/L, pH 7.6). After immunostained, the sections were lightly counterstained with Mayer's hematoxylin and immunoreactive (IR) cells were observed under light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger[20], including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen.

Frequency

Among 1 000 parenchymal cells, numbers of cells showing immunoreactivities against each antiserum were counted using automated image analysis process (Soft Image System, Germany) attached to light microscope. The frequency was calculated in gastric mucosa including the epithelia and gastric gland regions.

Statistical analysis

The frequencies of IR cells were calculated as mean±SD of total 10 parts (n = 10) of the fundus and pylorus. 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 Inc., USA) and the significant values were represented by P<0.05 and P<0.01.

RESULTS

In this study, six kinds of IR endocrine cells were detected with the antisera against CGA, somatostatin, serotonin, gastrin, CCK-8 and glucagon in the stomach of C57BL/6 mice regardless of implantation (Table 2). However, no HPP-IR cells were demonstrated in this study. According to the location of the stomach, different regional distributions and frequencies of these IR cells were observed, and these differences are shown in Table 2. Most of these IR cells in the epithelia regions were generally spherical or spindle in shape (open-type cell), while occasionally round shaped (closed-type cell) cells were also found in the gastric gland regions. In addition, most of these IR cells significantly (P<0.05 or P<0.01) decreased in 3LL-implantation group compared to those of non-implanted sham group except for CCK-8-, glucagon- and HPP-IR cells, which showed similar frequencies with each others.

Table 2 Regional distributions and frequencies of the endocrine cells in the stomach of the C57BL/6 mouse after implantation of 3LL (mean±SD)

Antisera	Non-implanted Sham		3LL-implanted group	
	Fundus	Pylorus	Fundus	Pylorus
CGA[1]	17.30±2.16	41.20±6.16	5.20±2.20[b]	25.70±6.43[b]
Somatostatin	14.20±3.01	14.20±5.01	10.90±1.79[b]	6.20±1.75[b]
Serotonin	54.50±10.32	18.50±4.22	10.50±3.50[b]	7.90±2.88[b]
Gastrin	ND[2]	48.50±11.10	ND	39.10±8.46[a]
CCK-8[2]	ND	45.70±6.58	ND	46.90±6.92
Glucagon	3.10±0.74	ND	3.00±1.25	ND
HPP[2]	ND	ND	ND	ND

Number/1000 cells (n = 10); [1]CGA: chromogranin A, CCK-8: cholecystokinin-8, HPP: human pancreatic polypeptide; [2]ND, not detected; [a]P<0.05 *vs* non-implanted sham by M-W test; [b]P<0.01 *vs* non-implanted sham by M-W test.

CGA-IR cells

CGA-IR cells were observed in the fundus and pylorus regions of non-implanted sham and 3LL-implanted groups. These IR cells were mainly dispersed in the basal portions of gastric gland regions rather than surface epithelia regions, and more numerous cells were detected in the pylorus compared to that of the fundus, regardless of implantation of 3LL. In addition, cells showing degranulation and having relatively narrowed cytoplasm were more numerous in 3LL-implanted group compared to that of non-implanted sham (Figures 1A-D). The frequency of CGA-IR cells in the fundus was detected in non-implanted sham and 3LL-implanted group with 17.30±2.16 and 5.20±2.20 among 1 000 cells respectively. The numbers in the fundus of 3LL-implanted group significantly (P<0.01) decreased compared to those of non-implanted sham (about 69.94% decreased). The frequency of CGA-IR cells in the pylorus of 3LL-implanted group also significantly (P<0.01) decreased compared to that of non-implanted sham (about 37.62% decreased) and they were demonstrated in non-implanted sham and 3LL-implanted group with 41.20±6.16 and 25.70±6.43 among 1 000 cells respectively (Table 2).

Somatostatin-IR cells

Somatostatin-IR cells were observed in the fundus and pylorus regions of non-implanted sham and 3LL-implanted groups. These IR cells were dispersed in the gastric mucosa and they showed similar cell numbers between the fundus

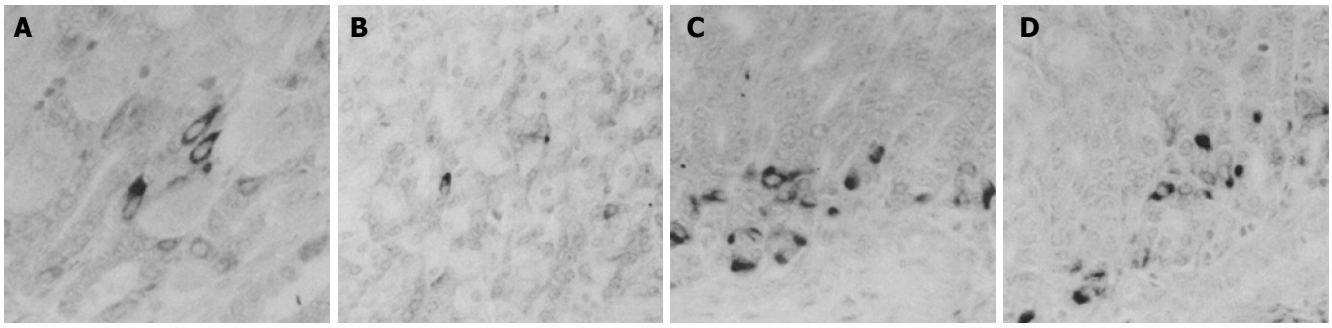

Figure 1 CGA-IR cells in the fundus (A, B) and pylorus (C, D) of non-implanted sham (A, B) and 3LL-implanted group (B, D). In 3LL-implanted group, CGA-IR cells dramatically decreased in the both regions of stomach and cells having relative narrow cytoplasm and degranulation were more numerously detected. PAP methods, scale bar = 40 μm.

and pylorus of non-implanted sham but slightly numerous cells were observed in the fundus of 3LL-implanted group compared to that of the pylorus. In addition, cells showing degranulation and having relatively narrowed cytoplasm were more numerously detected in 3LL-implanted group compared to that of non-implanted sham (Figures 2A-D). The frequency of somatostatin-IR cells in the fundus was detected in non-implanted sham and 3LL-implanted group as 14.20±3.01 and 10.90±1.79 among 1 000 cells respectively. The numbers in the fundus of 3LL-implanted group significantly (P<0.01) decreased compared to those of non-implanted sham (about 23.24% decreased). The frequency of somatostatin-IR cells in the pylorus of 3LL-implanted group also significantly (P<0.01) decreased compared to that of non-implanted sham (about 56.34% decreased) and they were demonstrated in non-implanted sham and 3LL-implanted group with 14.20±5.01 and 6.20±1.75 among 1 000 cells, respectively (Table 2).

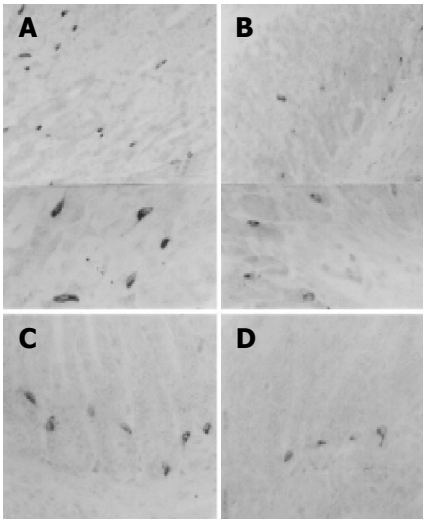

Figure 2 Somatostatin-IR cells in the fundus (A, B) and pylorus (C, D) of non-implanted sham (A, B) and 3LL-implanted group (B, D). In 3LL-implanted group, somatostatin-IR cells dramatically decreased in both regions of stomach and cells having relative narrow cytoplasm and degranulation were more numerously detected. PAP methods, scale bar = 40 μm.

Serotonin-IR cells

Serotonin-IR cells were observed in the fundus and pylorus regions of non-implanted sham and 3LL-implanted groups. Serotonin-IR cells were dispersed throughout whole gastric mucosa of the fundus but they were mainly located in the basal portion of the gastric mucosa in the pylorus, regardless of implantation. More numerous cells were detected in the fundus compared to that of the pylorus in both groups. In addition, cells showing degranulation and having relatively narrowed cytoplasm were more numerously observed in 3LL-implanted group compared to that of non-implanted sham (Figure 3A-D). The frequency of serotonin-IR cells in the fundus was demonstrated in non-implanted sham and 3LL-implanted group as 54.50±10.32 and 10.50±3.50 among 1 000 cells, respectively. The numbers in the fundus of 3LL-implanted group significantly (P<0.01) decreased compared to those of non-implanted sham (about 80.73% decreased). The frequency of serotonin-IR cells in the pylorus of 3LL-implanted group also significantly (P<0.01) decreased compared to that of non-implanted sham (about 57.30% decreased) and they were demonstrated in non-implanted sham and 3LL-implanted group with 18.50±4.22 and 7.90±2.88 among 1 000 cells, respectively (Table 2).

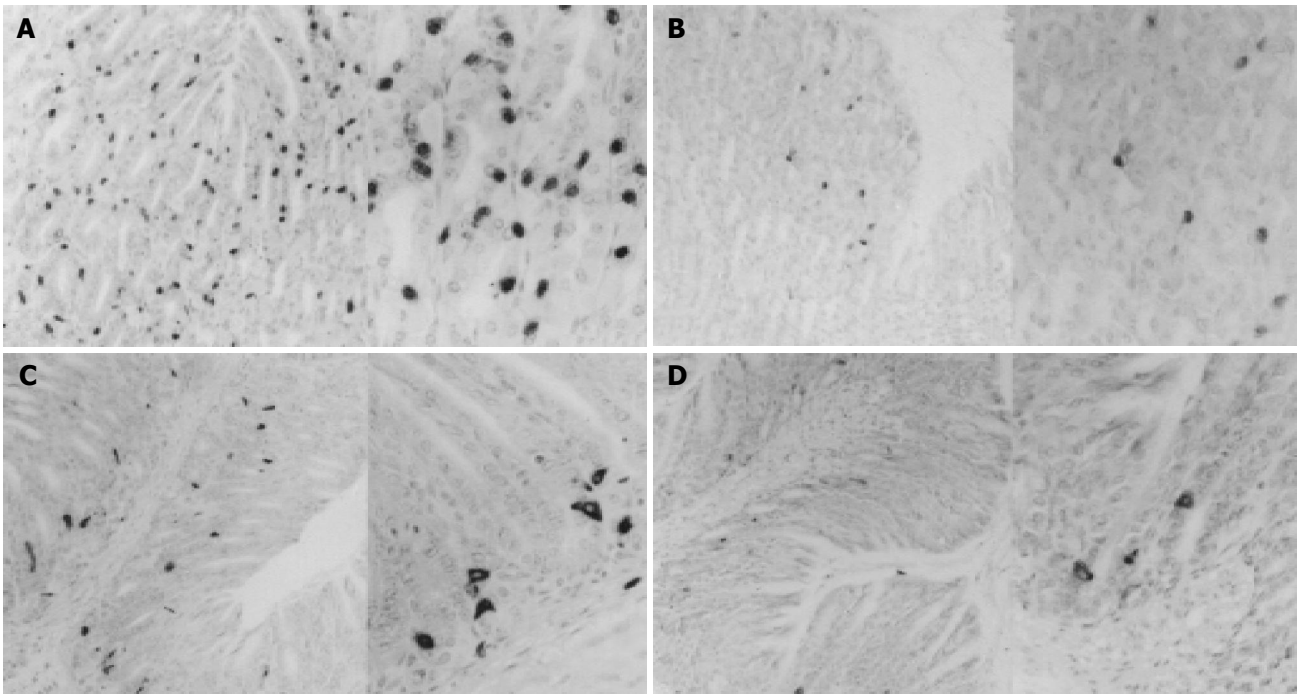

Figure 3 Serotonin-IR cells in the fundus (A, B) and pylorus (C, D) of non-implanted sham (A, B) and 3LL-implanted group (B, D). In 3LL-implanted group, serotonin-IR cells dramatically decreased in the both regions of stomach and cells having relative narrow cytoplasm and degranulation were more numerously detected. PAP methods, Scale bar = 40 μm.

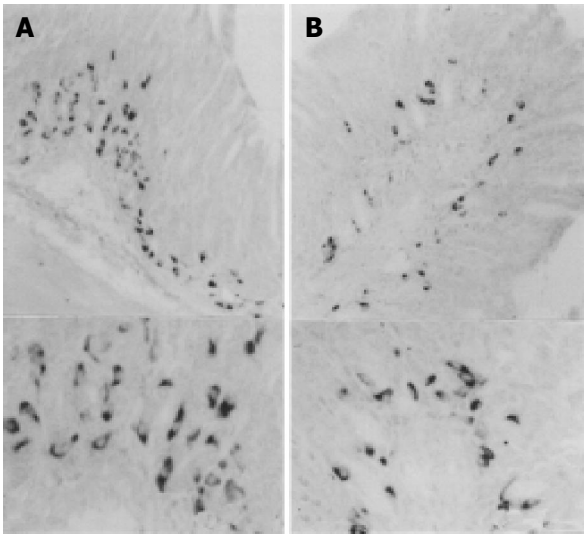

Figure 4 Gastrin-IR cells in the pylorus of non-implanted Sham (A) and 3LL-implanted group (B). In 3LL-implanted group, Gastrin-IR cells were dramatically decreased in the both regions of stomach and cells having relatively narrow cytoplasm and degranulation were more numerously detected. PAP methods, scale bar = 40 μm.

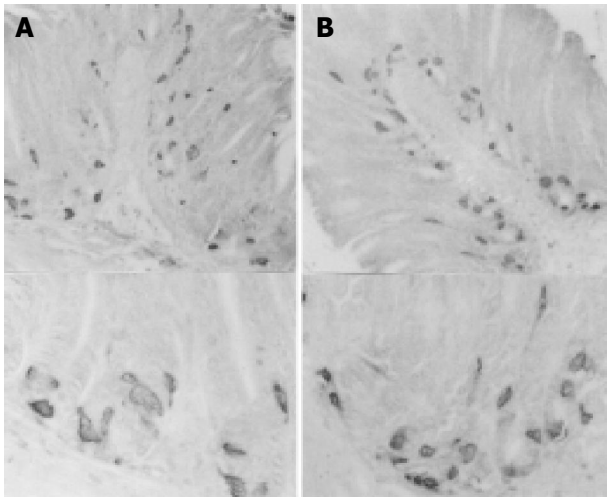

Figure 5 CCK-8-IR cells in the pylorus of non-implanted sham (A) and 3LL-implanted group (B). Similar shaped and distributions of CCK-8-IR cells were demonstrated between non-implanted Sham and 3LL-implanted group. PAP methods, scale bar = 40 μm.

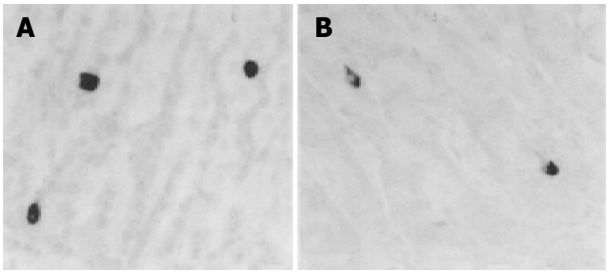

Figure 6 Glucagon-IR cells in the fundus of non-implanted sham (A) and 3LL-implanted group (B). Similar shaped and distributions of glucagon-IR cells were demonstrated between non-implanted Sham and 3LL-implanted group. PAP methods, scale bar = 40 μm.

Gastrin-IR cells

Gastrin-IR cells were restricted to the pylorus regions in both groups. Most of these IR cells were dispersed in the basal portion of the gastric mucosa of the pylorus, regardless of implantation of 3LL. IR cells showing degranulation and having relatively narrowed cytoplasm were more numerously observed in 3LL-implanted group compared to that of non-implanted sham (Figures 4A, B). The frequency of gastrin-IR cells in the pylorus was detected in non-implanted sham and 3LL-implanted group as 48.50±11.10 and 39.10±8.46 among 1 000 cells, respectively. The number of 3LL-implanted group significantly ($P<0.05$) decreased compared to those of non-implanted sham (about 19.38% decreased) (Table 2).

CCK-8-IR cells

CCK-8-IR cells were also restricted to the pylorus regions in both groups. Most of these IR cells were dispersed in the basal portion of the gastric mucosa of the pylorus, regardless of implantation of 3LL (Figure 5A, B) quite similar to that of gastrin-IR cells. The frequency of CCK-8-IR cells in the pylorus was detected in non-implanted Sham and 3LL-implanted group as 45.70±6.58 and 46.90±6.92 among 1 000 cells respectively. The numbers, distributional patterns and shapes of CCK-8-IR cells of 3LL-implanted group showed similar profiles compared to those of non-implanted sham.

Glucagon-IR cells

Glucagon-IR cells were restricted to the fundus of non-implanted sham and 3LL-implanted groups. They were dispersed throughout whole gastric mucosa of the fundus, regardless of implantation of 3LL (Figure 6A, B). The frequency of glucagon-IR cells in the pylorus was detected in non-implanted sham and 3LL-implanted group as 3.10±0.74 and 3.00±1.25 among 1 000 cells, respectively. The numbers, distributional patterns and shapes of glucagon-IR cells of 3LL-implanted group showed similar profiles compared to that of non-implanted sham.

HPP-IR cells

No HPP-IR cells were demonstrated in the fundus and pylorus, regardless of implantation of 3LL (Table 2).

DISCUSSION

It is generally accepted that the 3LL is a cancer that aggressively and rapidly spreads to other organs of the body. This kind of cancer is ordinarily the most life-threatening type, and now it is one of the most frequently used rodent models to research anticancer agents. GI endocrine cells appeared remarkably different depending on the regional distribution, relative frequency and cell types with animal species. In addition, many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the GI tract of the various vertebrates including various species of rodents. Also the researches or data processing about GI endocrine cells in the mice strains have been widely executed[21,22]. Gastrointestinal endocrine cells

were generally divided into two types, one was round to spherical shaped closed-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, endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and the change in their density would reflect a change in the capacity of producing these hormones[8]. In the present study, the changes of endocrine cells in the stomach regions of C57BL/6 mouse after subcutaneous implantation of 3LL were observed by specific immunohistochemistry. The distribution and frequency of the endocrine cells in the stomach regions of non-implantation group showed similar patterns compared to those of already reported of normal C57BL/6 mice[6].

As results of 3LL implantation, CGA-, somatostatin- and serotonin-IR cells significantly decreased in the fundus and IR cells in the pylorus also decreased against chromogranin-, somatostatin-, serotonin- and gastrin. However, no changes were demonstrated against CCK-8-, glucagon- and HPP. HPP-IR cells were not detected in this study. The most dramatically changed cells are serotonin-IR cells, and somatostatin- and serotonin-IR cells were the most dramatically changed ones in the pylorus. These changes might be induced by the gastrointestinal disorder observed in patients with cancer[13].

CGA belongs to a family of large anionic proteins (CG A, B and secretogranin II) and the members, which are known to be present in the secretory granules of a broad spectrum of amine and peptide-producing cells of adrenal medulla and gastrointestinal endocrine system as well as in some neurons of the peptidergic and catecholaminergic nervous system of several mammals[23,24]. CGs have been found in large variety of endocrine organs and cells outside the adrenal medulla, and they have been claimed as common "markers" of all neuroendocrine cells[25]. Although the changes of CGA-IR cells in the GI tract of rodent were seldom, Qian *et al*[26] reported that decrease of CGA-IR cells was demonstrated in the stomach of unilateral cervical vagotomized mouse. In addition, increase of CGA-IR cells was demonstrated in the stomach of proton pump inhibitor treated rat[27] and celiac children[28]. According to these previous reports, it is considered that changes of CGA-IR cells were possible as a sign of diseases and these changes were also considered as a result of changes of other endocrine cells because CGs were regarded as common markers of other endocrine cells[25]. In the present study, CGA-IR cells remarkably decreased in the stomach of 3LL-implanted groups compared to that of non-implanted sham.

Somatostatin consisting of 14 amino acids was isolated from hypothalamus of sheep for the first time and it could be divided into straight form and cyclic form[29]. This substance inhibits the secretion of the other neuroendocrine hormones[30]. It is known that somatostatin-IR cells show the widest distribution in the whole GI tract except for the large intestine of all vertebrate species investigated, including the primitive agnathans with serotonin-IR cells. Somatostatin-IR cells significantly increased in the adjacent mucosa compared to that of distant mucosa in colorectal endocrine cancer patients[31] and decreases of these IR cells were demonstrated in the duodenal ulcer patients with *Helicobacter pylori* but they increased to normality after eradication of *Helicobacter pylori*[32]. In the present study, somatostatin-IR cells remarkably decreased in the stomach of 3LL-implanted groups compared to that of non-implanted sham. These decreases of somatostatin-IR cells were considered that they induced somewhat serious problem to gastrointestinal physiology regarding their functions on digestive tract.

Serotonin, consisting of monoamines was widely distributed in nervous system and GI endocrine cells. Main functions of serotonin were inhibition of gastric acid secretion and contraction of smooth muscle in the GI tract[33]. Serotonin-IR cells dramatically decreased in the duodenum of diabetic mouse[34] and the number of these IR cells and occupied area significantly decreased in the damaged gastro-duodenal regions induced by ethanol in mice[35]. In the present study, serotonin-IR cells are the most significantly changed cells in stomach among observed endocrine cells. These changes considered that it could induce a gastrointestinal problems especially some clinical signs related to gastric motility and gastric acid secretion.

It is generally accepted that gastrin and CCK-8 have originated from same ancestor and in the human duodenum a large fraction of these cells, besides reacting with non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also show immunoreactivity with C-terminal gastrin-34 antibodies, co-localized with CCK in a variable portion of secretory granules[36]. Gastrin secreted by intestinal G cell, promotes the gastric acid secretion and CCK secreted by intestinal I cell stimulates the pancreatic enzyme secretion. Gastrin-IR cells were increased by long-term omeprazole treatment in children[37] but decreased in unilateral cervical vagotomized mice[26] and patients with peptic gastric ulcer[38]. In addition, enteric CCK-8-IR cells significantly increased in celiac sprue[11]. In the present study, after implantation of 3LL, decrease of gastrin-IR cells was demonstrated and these results considered that could induce some gastric disorders. However, CCK-8-IR cells were not changed in the stomach. Therefore, further studies are required to confirm the possibility of changes of CCK-8-IR cell profiles because the changes of enteric CCK-8-IR cell were already reported in celiac sprue[11].

Glucagon is synthesized in the A cells of the pancreas and regulates serum glucose levels. These IR cells have been demonstrated in various mammals. However, there were no reports dealing with the evidence of changes of glucagon-IR cells in any disease case or induced by chemical or manipulation. Since PP was isolated from insulin extraction from pancreas in 1961, the regional distribution of PP-IR cells in the mouse strains was relatively well known but strain-depended differences existed among mice. In case of SKH-1 hairless mouse, they were not detected throughout the whole GIT[39]. Although, very low frequency HPP-IR cells were demonstrated in the fundus of normal C57BL/6 mice of previous study[6] no HPP-IR cells were demonstrated in the stomach regions of C57BL/6 mice of this study.

In conclusion, endocrine cells are the anatomical units responsible for the production of gut hormones, and the change in their density would reflect a change in the capacity of producing these hormones. Implantation of tumor cell mass (3LL) induced severe quantified changes of gastric

endocrine cell density and the abnormal density of gastric endocrine cells may contribute to the development of gastrointestinal symptoms such as anorexia and indigestion, frequently encountered in patients with cancer.

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