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STUDY ON PROLIFERATIVE ACTIVITY OF ENDOCRINE CELLS IN GASTRIC CARCINOMA

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Endocrine cell (EC) in 11 cases picked up from 56 cases of gastric carcinoma were observed by double immunohistochemical method using chromogranin A(CgA) and proliferating cell nuclear antigen (PCNA) . The endocrine cells were recognized by a antibody to CgA and the proliferative activity by a monoclonal antibody to PCNA. 11 cases CgA positive GC cells with PCNA incorporation was in 51/4069 (0–3%) with an average 1.25%. By contrast, PCNA reaction was positive in 31.9±14.7% of CgA negative GC cells. It suggests that there were a few cell in S and G_1 phase and most of them in G_0 phase.

Key words: Endocrine cells, Gastric carcinoma, Cell proliferation.

It is known that gastric carcinoma have endocrine cells (EC) which were as tumor component from 3.1–53.3% detected by argyrophilia staining, under electron microscopy or by positive chromogranin A (CgA) reaction in immunohistochemical staining. We have applied a double immunohistochemical staining using CgA and PCNA antibodies to observe the proliferation of EC and carcinoma cells in gastric carcinoma[1] in order to probe the DNA-synthetic activity of EC.

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MATERIALS AND METHODS

The EC in 11 cases of gastric carcinoma were evaluated, which were selected from 56 patients with gastric carcinoma operated. Two cases of normal antral mucosa were observed by same procedure. The EC were distinguished by immunohistochemical method using antibody CgA, then the 11 cases with EC were carried out double immunostaining for PCNA and CgA on same sections[1]

A brief description is as follows. The sections were incubated with a 1:20 dilution of anti-PCNA (DAKO K376, 1:20 dilution) at 4°C for 12 h, then reacted with biotinylated sheep anti-mouse IgG at 37°C for 30 min, followed by visualization with the ABC-alkaline phosphatase system using kit (DAKO, K376) and fast blue as chromogen. The positive reaction yields blue product deposits in the nucleus. Thus sections then were washed by stirring for 1 h at 4°C with glycine-HCL buffer (pH 2.2), followed incubation for 14 h at 4°C with anti-CgA (DACO A434. 1:400 dilution). Then visualization of bound anti-CgA antibody was done with biotinylated sheep anti-rabbit IgG using the BA-immunoperoxidases system and DAB as chromogen. The positive reaction was brown cytoplasm. Control of the specificity of the immuno-

reaction was performed by incubating consecutive sections with noimmune serum instead of the primary antiserum or with the specific antiserum preabsorbed with an excess of respective antigens.

The labeling indexes of the samples stained with anti-PCNA were determined by counting the number of cells with reaction deposits in 20 randomly selected, high-power microscope fields. In each case a mini-mum of 1000 CgA-positive cells were scanned for the presence of a distinct blue nuclear stain, evidence of PCNA expression.

RESULTS

In the normal antral mucosa the cytoplasm of the endocrine cells, scattered deeply within the crypts of pyloric glands, was stained brown and the nucleus in neck zone of glands were staining blue. Normal CgA-positive cells showed no uptake of PCNA staining. CgA-positive brown cytoplasm and PCNA blue were readily distiguished in the tumor tissue. The positive of nuclear staining of cancer cells incubated with anti-PCNA varied from cell to cell. The proportion of stained cells ranged from 21.3±12.6 to 42.3±18.6, with an average value for the 11 cases studied of 31.9±14.7%. It indicates that the percentages of PCNA-positive cells were unevenly distributed within the tumor cell population. Table 1 shows that the 4059 CgA-positive cells of 11 cases of gastric carcinoma was observed to detect the presence of 0–3% in both positive of CgA and PCNA staining. In fact, no PCNA uptake was found in the CgA-positive cells in 5/11 cases. In another cases, we rarely encountered CgA-positive tumor cells with blue nuclear staining.

Table 1. Double immunostaining of gastric cancer cells

Case No.	Histologic subtype	PCNA-labeled cancer cells (%)	PCNA/CgA	Labeling indexes %
1	poor	21.3± 12.6	5/543	0.92
2	poor	27.8± 13.1	0/435	0
3	tubular	42.3± 18.6	3/245	1.2
4	poor	35.6± 13.4	0/513	0
5	poor	28.9± 12.5	0/67	0
6	tubular	34.3± 20.6	28/1300	2.15
7	poor	40.5± 15.3	5/234	2.14
8	tubular	31.5± 14.1	6/200	3.0
9	poor	25.4± 10.9	0/120	0
10	tubular	32.3± 17.8	4/256	1.56
11	poor	31.5± 12.7	0/156	0

DISCUSSION

In the present study, out of 56 cases of gastric carcinoma contained endocrine cells detected by immunostaining using anti-CgA. A double immuno-histochemical staining using anti-CgA and anti-PCNA was used to probe endocrine cells in the S-phase. PCNA is located in the nucleus and is expressed maximally by cell in late G_1-S transition.[2] It is evident from the results of the double immunostaining procedure. A few of PCNA-positive nucleus (0–3%) with definite CgA-positive cells were found, however 21.3–42.3% cells of gastric carcinoma were found PCNA-positive nucleus in our study. The endocrine cells in gastric carcinoma are likely between the G_0 and early G_1 phases of cell cycle.[3, 4] It suggests that endocrine cells in gastric carcinoma appear to be in a

quiescent state, hence do not travel the cell cycle. But some report[5, 6] pointed out the cells arrested in the G_0 or early G_1 phase of the cycle does not necessarily imply that the gastric carcinoma are less malignant. As Cox speculated,[7] since *in vitro* studies using cultured cells show that cells in quiescent state with unduplicated DNA (G_0) can be activated and able to re-enter the cell proliferation cycle during G_1 phase using physiologically active peptides such as insulin, bombesin and EGF. In the digestive organs, gut hormones as well as several growth factor are playing an important role in cellular proliferation of normal and tumor tissues.[8, 9] Although most of the EC in gastric carcinoma were besides cell cycle, in our opinion, it didn't imply that the gastric carcinoma are less malignant, soma physiologically active peptides such as gastrin, glucagon produced by carcinoma can stimulate tumor growth. The further study are required to verify the relation of endocrine cells and behaviour of gastric carcinoma.

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