• CLINICAL RESEARCH •

Neuroendocrine markers in adenocarcinomas: an investigation of 356 cases

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Abstract

AIM: To investigate the incidence of neuroendocrine (NE) cells and their hormone products in adenocarcinomas and evaluate their significance in clinical pathology and prognosis.

METHODS: By using tissue sectioning and immunocyto-chemistry, 356 cases of adenocarcinomas were studied to examine the presence of chromorgranin and polypeptide hormones in adenocarcinoma samples from our hospital.

RESULTS: The positive rate of NE cells and hormone products was 41.5 % (54/130) and 59.3 % (32/54), respectively in large intestinal adenocarcinoma cases; 39.6 % (38/96) and 36.8 % (14/38), respectively in gastric cancer cases; 38.1 % (8/21) and 50.0 % (4/8), respectively in prostatic cancer cases; 21.0 % (17/81) and 17.6 % (3/17), respectively in breasr cancer cases; 17.9 % (5/28) and 60.0 % (3/5), respectively in pancreatic cancer cases. Among carcinomas of large intestine, pancreas and breast, the highly differentiated NE cell numbers were higher than the poorly differentiated NE cell numbers; while the gastric carcinoma cases had more poorly differentiated NE cells than highly differentiated NE cells. The higher detection rate of NE cells and their hormone products, the higher 5-year survival rate among the large intestine cancer cases.

CONCLUSION: Close correlation was observed between NE cells and their hormone products with the cancer differentiations. For colorectal carcinomas, there is a close correlation of the presence of NE cells and their hormone products with the tumor staging and prognosis.

INTRODUCTION

Compared with neuroendocrine cancers, little investigation is carried out on the relationship of neuroendocrine cells and their hormone products in non- neuroendocrine cancers, especially in the common adenocarcinoma cases. By using nine different antibodies and immunocytochemistry, NE cells and their hormone products in 356 adenocarcinamas was observed with the aim of revealing the incidence and distribution of NE cells and the correlation between the cancer differentiation with the biological behaviors was evaluated.

MATERIALS AND METHODS

Materials

All the 365 adenocarcima samples were got from the first affiliated hospital of Zhejiang University Medical College from 1975 to 1994. There were 96 cases of gastric cancer samples (31 samples were got from the clinical biopsy; 65 samples were got from radical operation and 22 samples had lymph nodes metastasis); there were 130 case of large intestine cancer samples got from radical operations (110 cases had the follow-up data); there were 81 and 28 case of breast and panereatic cancer, respectively. The remaining 21 samples were got from prostatic cancer biopsy.

Methods

All the samples were fixed with 10 % formaldehyde with paraffin embedding and continuous sectioning at 4 μM in thickness. Gross pathological observation was made on the HE stain slides followed by immunocytochemistry. All the samples were treated with anti-chromogranin serum fot the primary screening positive cases. Further immunocytochemistry was carried out for those positive NE samples by using peptide hormone antibodies such as ST (diluted at 1:10 000, provided by the 4th Military Medical Academy) and other Dako's antibodies (somatostatin diluted at 1:300; glucagon diluted at 1:800; pancreatic polypeptide diluted at 1:800; gastrin diluted at 1:350; insulin diluted at 1:150; ACTH diluted at 1:800 and calcitonin diluted at 1:150). The immunostains were done by ABC method and coloured with AEC. The antiserum of serotonin and gastrin was used in the gastric mucosa; the pancreas tissue was used to detect the chromogranin, somatostatin, glucagon, insulin and pancreatic polypeptide; calcitonin antiserum was used in the medullary carcinoma of the thyroid gland while ACTH in the pituitary was used as the positive control. The negative control was carried out by using normal sheep serum to replace the 1st antibody. Based on the chromoganin positive NE cell numbers, all the samples were divided into three grades as the following. Negative: there was no NE cells; Positive(+): the number of NE cells was fewer than $5/mm^2$; Super positive (++): the number of NE cells was over $5/mm^2$.

Statistical analysis

The date were analysed by χ^2 test.

RESULTS

Morphology of NE cells and their incidence

Among the five common adenocarcinomas from different tissue sources, the incidence rate was 41.5 % (54/130) for the large intestinal carcinomas; 39.6 % (38/96) for the gastric carcinoma; 38.1 % (8/21) for the prostatic carcinoma; 21.0 % (17/81) for the breast cancer and 17.9 % (5/28) for the pancreatic cancers, respectively. The highest incidence was

seen in large intestinal carcinomas while the lowest in the pancreatic carcinomas. When observing the chromoganin stained slides, clear edges of NE cells and brownish granules could be seen in the cytoplasm under the microscopy. In the low differentiated carcinomas, the NE cells presented as an oval, round or irregular shape without polarizations. Abnormal structural characteristics were observed among these low differentiated NE cells, which was similar to the adjacent tumor cells; while for those highly differentiated carcinomas, the NE cells were pyramid or bar shaped with the apex pointing to the cavity of the gland. A few NE cell processes could be observed reaching the gland cavity surfaces. The distribution of NE cells were scattered or localized infiltrating all the layers with the cancer cells. NE cells could be seen in both of the primary carcinoma and the metastasis sites.

Relationship between NE cells and carcinoma differentiation

No exact correlation between NE cells and carcinoma differentiation was observed among different carcinomas. The highly differentiated NE cell incidences were 41.7 % (5/12) for the large intestinal carcinomas, 42.9 % (3/7) for the pancreas carcinomas and 32.5 % (14/43) for the breast cancers, which was much higher than that of the low differentiated carcinomas. Prostatic carcinomas had the same tendency but there was no statistical significance due to fewer case numbers. In the low differentiated gastric carcinomas, 50 % (27/54) had the positive NE cells, which was significantly higher than that of the highly differentiated carcinomas.

Distribution of hormone products of NE cells in tumors

From Table 1, the number of hormone products types was more in large intestinal and gastric carcinomas (5 types of hormone products); hormone products detected in breast cancers were the fewest (only three in 17 cases). Most of them were the tumor origin tissue hormones, but few of them were ectopic hormones.

Relationship between positive cell of hormone products and tumor differentiation

In the large intestinal carcinomas, 9 cases were low differentiated carcinomas whose positive cell percentage of hormone products against the total NE cells was 27.0 %, which was obviously lower than that in high differentiated carcinomas (15 cases with the percentage of 43.9 %) (χ^2=115.9, $P<0.01$); It was also the same in the highly differentiated large intestinal carcinomas whose percentage was lower than that in the normal mucus membrane (10 cases with the percentage of 83.1 %) (χ^2=212.3, $P<0.01$) and the mucus membranes adjacent to the tumors (25 cases with the percentage of 88.7 %) (χ^2=168.8, $P<0.01$). The gastric carcinoma had the similar results: the positive cell percentage of hormone products against the total

NE cells was 17.5 %. But in the positive cells of hormone products from 5 gastric sinus mucus membranes, the positive cell percentage of hormone products against the total NE cells was 78.6 % (χ^2=1611.8, $P<0.01$); the samples adjacent to the gastric sinus areas had the obviously higher percentage (46.6 %, χ^2=266.4, $P<0.01$). Significant difference was also observed between the percentage of the adjacent mucus membrane tissues of the tumors and the normal mucus membranes (χ^2=242.0, $P<0.01$).

Ectopic hormones and tumor differentiation

Except for the pancreatic carcinomas, ectopic hormones were revealed in other four types of the adenocarcinomas. One of the large intestinal carcinomas cases showed gastrin positive; Six gastric carcinoma cases showed ACTH positive; Two prostatic cancer cases were glucagons positive; One breast cancer case was somatostain positive while another breast cancer case was calcitonin positive. Except for the large intestinal and gastric carcinomas, all the other nine cases were low differentiated carcinomas among the ectopic hormone carcinomas.

NE cells and tumor differentiation

Observed in large intestinal carcinomas, Dukes A stage accounted for 41.7 % of the NE cell (++) cases (12 cases), which was much higher than that in NE(-) group (19.7 %,76 cases). (χ^2=4.668, $P<0.05$). Among the 110 cases with following-up, the 5-year survival rate was 81.8 % in NE cell (++) group, which was obviously higher than that in the NE (+) group (45.7 %, 35 cases) (χ^2=4.000, $P<0.05$) and in NE cell (-) group (42.2 %, 64 cases) (χ^2=4.397, $P<0.05$).

Among the 32 hormone products positive cases with polypeptide hormones (PH), Dukes A stage cases accounted for 44.1 %, which was higher than that of NE cell positive cases with hormone products negative (36.1 %), yet no statistical difference was found between the two groups (χ^2=0.351, $P>0.05$). In hormone products (+) group (17 cases), the 5-year survival rate was 70.6 %, which was higher than that of hormone products (-) group (37.9 %, 29 cases) (χ^2=4.148, $P<0.05$).

DISCUSSION

The commonly used staining methods for revealing NE cells include silver staining, neuron-specific enolase (NSE), synaptophysin (SY) and chromorgranin (CG) immunocytochemistry. Silver staining is the traditional staining method with less specificity and sensitivity. Although NSE, CG and SY are all the common markers, NSE has poor specificity with distributions in different tissues but is localized in the cytoplasm. CG is distributed in neuroendocrine granules. Both CG and SY are good markers and corresponding to

Table 1 Distribution of hormone productions of NE cells in tumors

Type	NE Positive Case	Serotonin Case (%)	Somatostatin Case (%)	Glucagon Case (%)	P P Case (%)	Gastrin Case (%)	Calcitonin Case (%)	ACTH Case (%)
Colorectal carcinomas	54	30(55.6)	14(25.9)	11(20.4)	5(9.3)	1(1.9)	0	0
Gastric carcinomas	38	5(13.2)	5(13.2)	5(13.2)	0	3(7.9)	0	6(15.8)
Pancreatic Carcinomas	5	1(20.0)	0	1(20.0)	2(40.0)	0	0	0
Breast Carcinomas	17	1(5.9)	1(5.9)	0	0	0	1(5.9)	0
Prostatic Carcinomas	8	4(50.0)	0	2(25.0)	0	0	0	0

respective subcellular structures. CG is a specific matrix component of endocrine granules[1-3]. While SY is localized within small capsule membranes related to the secretion granules, whose specificity and sensitivity are less than those of CG. That's why CG is considered as a realistic marker for NE cells[4-8]. Studies have confirmed that CG could be served as a new way of revealing NE cells and for the diagnosis of NE tumors[9].

Our study demonstrated that NE cell numbers were closely correlated to the tumor differentiation in large intestinal, pancreatic, breast and prostatic carcinomas[10]. The higher differentiated tumors had the higher incidence of the NE cells[8]. That was contradicted to our gastric carcinoma observations, but corresponding to the publication reports[11]. Further studies are needed to be conducted to reveal these differences to see if they are related to the embryology, etiology and tissue development of the tumors.

No serial report were seen about the hormone products of NE cells from the common adenocarcinomas. We observed 5 types of adenocarcinomas and found out that large intestinal and gastric carcinomas had the higher hormone products in their NE cells; but in the gastric and highly differentiated carcinomas, they had lower hormone products in NE cell than those of poor differentiated carcinomas. The hormone products were more in the large intestinal and gastric carcinomas than those in normal mucus membranes and tissues adjacent to the carcinomas. Neoplastic NE cells had lower hormone products and they were decreased with anaplasia which may be due to the fact that these cells were in immature state with lower hormone synthesis. Thus, the amount of hormone products in NE cells of the carcinomas can serve as an index for the determination of tumor differentiation and the diagnosis of benign and malignant tumors[12-15].

Based on the study of the large intestinal carcinomas, we found that hormone products and distribution of NE cells were closely correlated to the tumor grade, clinical pathological stage of the tumor and prognosis of the patients[16-23]. Carcinomas with NE (++) releasing PH were the early stage carcinomas. The higher 5-year survival rate may be due to the somatostatin's inhibition of the tumors[24-29]. Zollinger-Ellison syndrome was reported in some gastric carcinoid cases[30-32], but our study only revealed there were only different hormone products but without sign and symptoms of Zollinger-Ellison syndrome as well as other hormone signs and symptoms, which may be the fact that the hormone products produced by NE cells were not enough in the inactivated form or inactivated by the liver enzymes. Further study is required to examine whether these hormone products participate in the immune regulations of the tumor or the hormone products exert the direct effects on the tumor development and growth.

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