Chinese Journal of Cancer Research 10(3):211-214,1998.

SIGNIFICANCE OF EXPRESS OF SOME NONHORMONAL ANTIGENS IN PANCREATIC ENDOCRINE TUMORS

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Objective: To study the express of some nonhormonal antigens in pancreatic endocrine tumors. Methods: The nonhormonal antigens including Alpha-subunit of human chorionic gonadotropin (α-HCG), progesterone receptors (PR), 7B2, HISL-19, in normal pancreatic islets and in 52 cases of pancreatic endocrine tumors (PET) were investigated by immunohistochemistry. Results: It was found that HCG can be detected in PET but not in normal islet cells. HCG immunoreactivity was expressed by 3 of 28 (10.7%) benign PET and by 14 of 24 (58.3%) malignant PET. PR was found by 20 of 28 (71.4%) benign PET and by 7 of 24 (29%) malignant PET. 7B2 was detected by 23 of 28 (82.1%) benign PET and by 13 of 24 (54.2%) malignant PET. HISL-19 was appeared by 23 of 28 benign PET and by 11 of 24 (46%) malignant PET. Golgitype persisted in 87.5% malignant tumors. Conclusion: The assay of nonhormonal antigens may be well defined the clinico-pathological characteristics of PET.

Key words: Pancreatic endocrine tumor, Tumor antigens, Immunohistochemistry.

It is difficulty to establish histologic criteria pancreatic endocrine tumors (PET). The nuclear pleomorphism, prominent nucleoli and mitosis count which are the criteria in most human neoplasms are unreliable in PET. In recent years immunohistochemistry has revealed that production of several nonhormonal antigens has been proved to be related to the biological of PET. In this study, Alpha-submit of human chorionic gonadotropin (α-HCG), progesterone receptors (PR), 7B2, HISL-19 are detected in PET and its significance are discussed..

Accepted Apil 25, 1998

MATERIALS AND METHODS

Five specimens of normal human pancreatic tissue were obtained from autopsy. Tissue specimens of 52 PET obtained at surgery. Tissues were fixed in bound's fluid for 24 h, dehydrated and embedded in paraffin. Ten serial sections were cut from each blocks in 5 μm. Except hematoxylin and eosin staging the PET were classified on the basis of their hormonal production, identified by immunohistochemistry using polyclonal antibodies against insulin, glucagon, somatostatin, PP, gastrin and vasoactive intestinal peptide. Nonhormonal antigens including α-HCG, PR, HISL-19 and 7B2 were identified and co-localized on serial sections. Table 1 lists the antibodies used in the study and their respective working dilutions. The immunohistochemical procedure was carried out with overnight incubation at 4℃. The immunoreaction was visualized with the avidin-biotin complex (ABC) procedure (Vectastain ABC kit; Vector Laboratories, Burlinghame. Calif. USA). Control of the specificity of immunoreaction 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. All malignant PET showed lymph node, live metastases and vascular invasion and infiltration of pero-pancreatic fat.

The criteria of nonhormaonal antigens positive in PET as following:

–: positive cells account for 5% of total cells observed;

+: positive cells account for 5–25% of total cells observed;

++: positive cells account for 25–50% of total cells observed;

+++: positive cells account for 50% over of total cells observed;

RESULTS

Expression of Nonhormonal Antigens in Islet

The results of immunohistochemical staining of hormonal antigens including glucagon-producing A cells, insulin producing B cells, somatostatin producing D cells and PP-producing PP cells (PP positive) were compare with the nonhormonal antigens in serial sections. Results are summarized in Table 2. It isn't found that α-HCG was not found positive in each islet cells including A cells, B cells, D cells and PP cells. PR positive cells were found in most islet cells, especially in the A cells. 7B2 and HISL-19 positive cells were found in most islet cells. In general A cell granules were more heavily stained than those of B cells with a preferential localization in the less dense peripheral halo of the alpha granule.

Table 1. Antibodies used in the present study

Code	Antigen	Working dilution	Positive control
A11	CgA	1:1000	Pancreas
A31078	α-HCG	1:800	Human placenta
C-19	PR	1:50	Breast cancer
L-29	7B2	1:200	Pancreas
	HISL*	1:1000	Pancreas
A564	Insulin	1:800	Pancreas
00483	Glucagon	1:8000	Pancreas
211	PP	1:2000	Pancreas
A586	Gastrin	1:200	Gastric antrum
A566	Somatostatin	1:40	Gastric antrum
00495	VIP	1:400	Small bowel

*HISL-19 antibody is from Bordi C laboratory

Table 2. Immunohistochemical expression of nonhormonal antigens in islet

Cells cell type of islet	α-HCG	PR	7B2	HISL-19
A cell	–	+++	+++	+++
B cell	–	+	+++	++
D cell	–	–	+	+++
PP cell	–	+	+	+++

Expression of Nonhormonal Antigens in PET

Results of immunostaining for nonhormonal antigens in 52 PET are summarized in Table 3. Cells immunoreactive for α-HCG were found in 4/18 (14.2%) of benign PET, which the four cases also are insulinomas and 14/24 (58.3%) of malignant tumors. α-HCG positive particles are in cytoplasm. Cells immunoreactive for PR were found in 20/28 (71.4%) of benign PET, and in 7/24 (29%) of malignant PET. Most positive particles of PR are in nucleus. Cells immunoreactive for 7B2 were found in 23/28 (82.1%) of benign PET and in 13/24 (54.2%) in malignant PET. The positive particles of 7B2 are diffuse in cytoplasm. HISL-19 immunostaining are two basic patterns, one is "granular" pattern which were diffuse in cytoplasm and the other is "cluster" pattern which localized around nucleus. The "granular" pattern was seen in 23/28 (82.1%) of benign PET, but in only 11/24 (46%), "cluster" pattern, in contrast, was found in 15/28 (53.6%) of benign PET, but in 21/24 (87.5%) of malignant PET.

Table 3. Immunohistochemical expression of nonhurmonal antigens in pancreatic endocrine tumors

Type of tumors	Number	α-HCG	PR	7B2	HISL-19	
					Granular	Cluster
Benign	28	5	20	23	23	15
Insulinomas	15	4	11	13	10	9
Glucagonomas	6	1	5	6	6	4
PPoma	4	0	3	3	4	1
Non-functioning	3	0	1	1	3	1
Malignant	24	14	7	13	11	21
Insulinomas	6	5	4	5	3	6
Glucagonomas	4	3	2	2	1	4
PPoma	3	1	0	1	0	0
Gastrinomas	3	1	0	1	2	3
VIP-omas	2	0	0	0	2	1
Non-functioning	6	4	1	4	3	0

DISCUSSION

It is difficulty to establish histologic criteria of malignancy. The nuclear plemorphism, prominent nucleoli etc., which predict the prognosis in most human tumors, are unreliable in PET. In recent years, the introduction of valuable methods for the assessment of several nonhormonal antigens including α-HCG, PR, 7B2 and HISL-19 detected by immunohistochemistry has proved to be related to the biological behavior of PET.

α-HCG is not in normal islet cells, but was detected in PET. High plasma levels of α-HCG were found by an et al. in 14 of 27 patients with malignant PET but in none of the patients with benign PET. We found a positive α-HCG reaction in 58.3% but in 18% benign PET ($P<0.05$). It is seem that α-HCG immunoreactivity is as a marker of malignancy, therefore it remains questionable especially in insulinomas. 4 of 15 benign insulinomas showed α-HCG positive in this study. As previously mentioned, a negative immunohistochemical result combined with a negative AgNOR score provide the highest predictive value of benignity.

PR have been immunolocalized in the nuclei of major islet cells. PR immunoreactivity was expressed by 71.4% benign PET and by 29% malignant PET ($P<0.001$). It may be regarded as specific for PET since it was in only 1 of 60 nonpancreatic neuroendocrine tumors. The frequency of PR immunoreactivity of insulinomas was more frequent than that of normal insulin B cells suggesting either preferential origin of tumors from a subset of PR-expressing B cells or acquisition of the antigen during nepotistic transformation.

7B2, a protein with structural characteristics of GTP binding proteins, was isolated from the pituitary gland and found to be widely distributed in the peripheral neuriendocrine system. In our study, the protein was found to be expressed by 82.1% benign and 54.2 malignant PET. 7B2 may be represent a non-permanent component of the tumor cell granular compartment. Thus, it may be involved in the inappropriate hormone secretion that often occurs in PET and deteriorates the patients prognosis.

The HISL-19 was produced after immunization of BALB/c mice with isolated human islet cells. We revealed two basis patterns of immunostaining in PET. One is granular and other is cluster pattern. The granular pattern was seen in 82.1% of benign PET but in 46% of malignant PET. In contrast, cluster pattern was found in 53.6% of benign PET but in 87.5% of malignant PET. The study also documented that a variation from biphasic to cluster pattern may characterize PET dedifferentiation with the passing of time.

The study of several non-hormonal antigens detected by immunohistochemistry in PET may not only represent an adjunct to PET characterization but also provide new insights into the cellular biology of PET resulting in better understanding of the biology as well as of the clinical and pathological associations of PET.

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