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STUDY ON EXPRESS OF α-HCG AND α-HCGmRNA IN PANCREATIC ENDOCRINE TUMORS

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The antigen Alpha-subunit of human chorionic gonadotropin (α-HCG) and α-HCGmRNA in pancreatic endocrine tumors (PET) were investigated by immunohistochemistry and *in situ* hybridization. It was found that α-HCG can be detected in PET buy not in normal islet cells. α-HCG immunoreactivity was expressed by 5 of 28 (18%) in benign PET and 14 of 24 (58.3%) in malignant PET. Using *in situ* hybridization of α-HCGmRNA, a strong signal in PET was obtained. The clinico-pathological significance of α-HCG in PET was discussed.

Key words: Pancreatic endocrine tumor, Alpha-subunit of human chorionic gonadotropin (α-HCG), Immunohistochemistry, *In situ* hybridization.

The histologic criteria of malignancy in most human tumors are unreliable in pancreatic endocrine tumors (PET). The diagnosis of malignancy assessment remain crucial problems. In recent years, the expression of the alpha-subunit of human chorionic gonadotropin (α-HCG) has been related to malignancy. We have applied the immunohistochemistry and *in situ* hybridization technique to observe the α-HCG antigen and α-HCGmRNA in order to assess its value in predicting malignancy of PET.

MATERIALS AND METHODS

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Buin and formalin-fixed, paraffin-embedded tissue of resection specimens of pancreatic endocrine tumors of 52 cases. Serial sections (5 μM) were cut from one block per specimen. Immunohistochemical staining was performed, using the ABC technique. The primary antibodies were shown in Table 1. Control of the specificity of the 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.

A diagnosis of malignancy was based on that it invaded peripheral tissue and vessels or metastasized to regional lymph nodes or liver.

In situ hybridization of α-HCGmRNA was performed in one normal pancreas and five cases of routinely formalin-fixed and paraffin-embedded PET. Synthesis of oligoprobes: the following four oligoprobes were synthesis using a DNA synthesizer:

5'AGCGTGCATTCTGGGCAATCCTGCACA T3'

5'GACCCTGTTATATGATTTAGCTACACA G3'

5'AAGTACTGCAGTGGCACGCCGTGTGGT TCT3'

5'AGTGGAGTGGGATATGCTCTAGAGAAG CAG3'

Each oligoprobe was labeled with digoxigenin-11-dUTP at the 3' end using the Boehringer Mannheim Biochemicals DNA tailing kit. Labeling efficiency was checked with dot blot analysis. The four oligoprobes were used in cocktail in the hybridization mixture.

Table 1. List of antibodies used in the present immunochemical study

Code	Antigens	Working dilution	Positive control
A11	Chromogranin A	1:1000	Pancreas
A31078	α-HCG	1:800	Human placenta
A564	Insulin	1:800	Pancreas
00483	Glucagon	1:8000	Pancreas
211	Pancreatic polypeptide	1:4000	Pancreas
A586	Gastrin	1:200	Normal gastric antrum
A566	Smatostatin	1:40	Pancreas
00495	Vasoactive intestinal polypeptide	1:400	Normal small bowel

In situ hybridization protocol: After rehydration, sections were digested in 10 μg/ml proteinase K at 37 ℃ and postfixed in 0.4% paraformadehyde at 4 ℃. Hybridization was performed in a moist chamber of hybridization solution at 37 ℃ overnight.

Hybridization solution: Oligoprobe cocktails (200 ng/100 μl) were diluted in a solution containing 30% formamide, 4×SSC, 5% Denhardt's solution, 10% dextran sulfate, 1 mM EDTA, 1 μg/μl yeast tRNA, 0.1μg salmon sperm DNA. The stringency washings were performed in 2×SSC and in 0.1×SSC at 37 ℃. Detection of the hybridization was obtained by anti-digoxigenin-alkaline phosphatase procedure using bromochloroindolyl phosphate-nitroblue tetrazolium (BCIP-NBT) as a chromogen. Positive signal are deep blue granular in cytoplasm.

Controls: The positive control is using human placenta and negative control, blank control, replace control were performed.

RESULTS

The types of normal pancreatic endocrine cells in islets were identified by immunohistochemistry into A cells (glucagon positive), B cells (insulin positive), D cells (somatostatin positive) and PP cells (pancreatic polypeptide positive). By the same method, the types of PET were divided by showing hormonal type. α-HCG wasn't found in all pancreatic endocrine cells. 28 cases are benign and 24 cases are malignant. α-HCG positive cells were demonstrated in 5 (18%) of 28 benign PET and in 14 (58.3%) of 24 malignant (Figure 1, 2). In 5 cases benign PET showing α-HCG positive, four cases are insulinoma. All immuno-histochemical results were summarized in Table 2.

Table 2. The α-HCG positive cases in 52 PET

PET type	Cases	*α-HCG positive*
Benign	28	5
Insulinoma	15	4
Glucagonoma	6	1
PPoma	4	0
Non-functioning	3	0
Malignant	24	14
Insulinoma	6	5
Glucagonoma	4	3
PPoma	3	1
Gatrinoma	3	1
VIPoma	2	0
Non-functioning	6	4

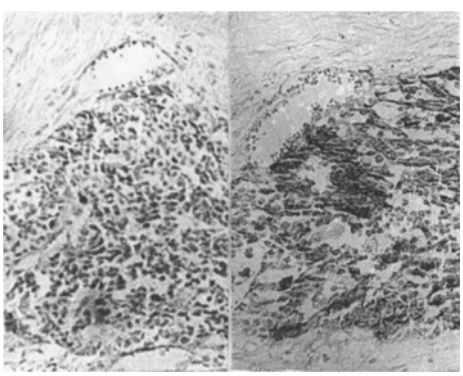

Fig. 1. α-HCG in a malignant insulinoma, positive showed as black. Two are continuous sections. Immunostaining of the insulin (left); α-HCG (right). ABC method × 150

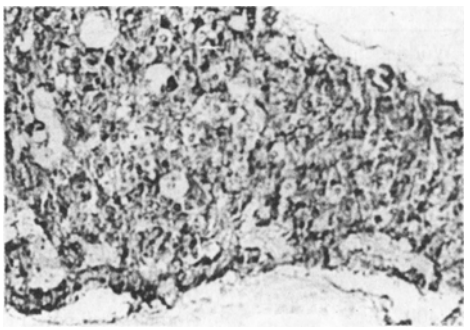

Fig. 2. α-HCG in a malignant glucagonoma, positive showed as black. Immunostaining of the α-HCG. ABC method ×450

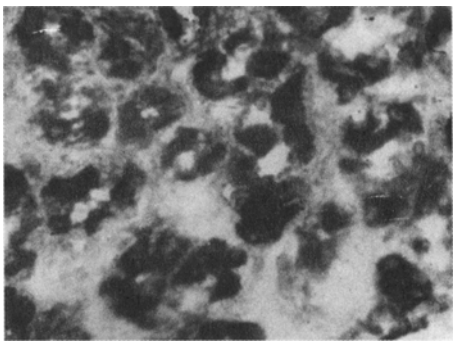

Fig. 4. It is continuous section with Figure 3 tumor. α-HCGmDNA showed by *in situ* hybridization. ×1000

The results of *in situ* hybridization of α-HCGmRNA in one normal pancreas and five cases of PET including tow cases benign and three cases malignant were found in four PET but not in normal islet cells. We have obtained a strong signal in four cases (one is benign and three are malignant) expressing α-HCG antigen by immuohistochemistry (Figure 3, 4).

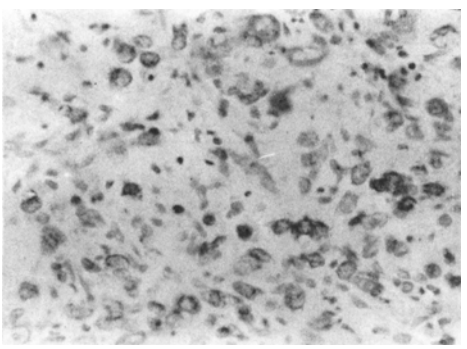

Fig. 3. α-HCG in a malignant insulinoma, positive showed as black. Immunostaining of the α-HCG. ABC method ×450

DISCUSSION

It is long known that α-HCG, the molecular segment structurally similar in all glycoprotein hormones and apparently devoid of any specific hormonal activity, can be immunohistochemically detected in tumoral but not in normal islet cells.[1,2] We have shown by *in situ* hybridization that immuno-reactive PET cells also contain abundant α-HCGmRNA, a finding demonstrating that the protein is synthetized and not merely incorporated in tumor cells. High plasma levels of α-HCG were found by Kahn et al.[3] in 14 of 27 patients with malignant-functioning PET but in none of the patients with benign PET and, consequently, they were regarded as a specific marker of malignancy. Such an assumption was further substantiated by an immunohistochemical study of 155 PET, which revealed a positive α-HCG reaction in 43 of 73 malignant cases (58%) but only in 1 of 84 benign cases (1.2%).[1] In contrast, we found positive α-HCG immunostaining also in 4 of 15 benign insulinoma, a result confirmed by other studies.[3,4] The significance of α-HCG immunoreactivity as marker of malignancy therefore remains questionable especially in insulinomas.[5,6] Some reported[7] that a negative immunohistochemical result combined with a negative AgNOR score provide the highest predictive value of benignity.

REFERENCES

1. Heitz PU, Kasper M, Kloppel G, et al. Glycoprotein hormone alpha-chain production by pancreatic endocrine tumors: a specific marker for malignancy. Immunocytochemical analysis of tumors of 155 patients. Cancer 1983; 51:277.
2. Graeme CF, Nardi G, Compton C. Immunocytotochemical staining for human chorionic gonadotropin subunits does not predict malignancy in insulinomas.

Am J Clin Pathol 1990; 93:373.

3. Kahn CR, Rosen SW, Weintraub BD, et al. Ectopic production of chorionic gonadotropin and its subunits by islet-cell tumors. A specific marker for malignancy. N Engl Jed 1977; 297.
4. Azzoni C, Comintti, Maggioni M, et al. *In situ* hybridization of α-HCGmRNA in routinely fixed tissues. Pathol Res Pract 1993; 189:641.
5. Donnow C, Baisch H, Heitz PU, et al. Nuclear DNA content in 27 panceatic endocrine tumours. Correlation with malignancy, survival and expression of glycoprotein hormone alpha chain. Virchows Arch (A) 1991; 419:463.
6. Bordi C, Pilato FP, D'Adda T. Comparative study of seven neuroendcrine markers in pancreatic endocrine tumours. Virchows Arch (A) 1988; 413:387.
7. Ruschoff J, Wilemer S, Brunzel M, et al. Nucleolar organizer regions and glycoprotein-hormone α-chain reaction as markers of malignancy in endocrine tumors of the pancreas. Histopathology 1993; 22:51.