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Expression of *cripto* in Human Pancreatic Tumors

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The expression of *cripto* gene product was examined immunohistochemically in 45 surgically resected pancreatic tumors, including 32 invasive ductal carcinomas, 4 intraductal papillary adenocarcinomas, 4 intraductal papillary adenomas, 2 mucinous cystadenomas, 2 islet cell tumors, and one solid and cystic tumor, and compared with that in 32 areas of accompanying chronic pancreatitis present in the cases of invasive ductal carcinomas and 5 non-tumorous areas of pancreas without pancreatitis. All pancreatic ductal tumors including adenomas and carcinomas showed positive staining with no difference in terms of staining intensity among intraductal tumors and invasive carcinomas with or without mucin hypersecretion. Islet cell tumors were positively stained but the solid and cystic tumor was negative. Duct epithelial cells and acinar cells were negative but islet cells were positive in the pancreas tissues without pancreatitis. Cells arranged in duct-like structures in areas of accompanying chronic pancreatitis were positively stained. The results suggest that *cripto* expression might be associated with a growth advantage of tumor cells and also with differentiation to form duct-like structures.

Key words: *cripto* expression — Human pancreatic carcinoma — Growth factor

The prognosis of pancreatic duct adenocarcinomas is still poor in spite of the application of advanced diagnostic techniques and it remains the fifth leading cause of cancer death in Japan. The poor prognosis of this cancer is largely a result of its aggressive behavior, invasion and metastasis, and elucidation of the factors involved in the characteristic growth of such cancer cells is required. Imbalance of growth factors has been reported in pancreatic duct adenocarcinomas, with EGF[5] receptors in overabundance and TGF-α overexpressed in cultured human pancreatic cells as well as in carcinoma tissues.[1-4] bFGF was found to stimulate ornithine decarboxylase gene transcription, leading to proliferation, in a pancreatic tumor cell line.[5]

Recently, *cripto*, a novel gene of the EGF family, was cloned from an undifferentiated human teratocarcinoma cell line NTERA2 clone D1 (NT2D1).[6] Human *cripto* gene cDNA is 2.2 kbp long with an open reading frame of 564 bp encoding a protein of 188 amino acids which has a structural homology with other members of the EGF supergene family, such as human EGF, human TGF-α, and human amphiregulin.[6,7] In the present study, we investigated expression of *cripto* gene in human pancreatic carcinomas using an immunohistochemical technique.

Tissue materials were all fixed in 10% formalin followed by routine processing and embedding in paraffin blocks. As a pretreatment for immunohistochemical staining, deparaffinized tissue sections were immersed in 0.01 *M* citrate buffer (pH 6.0) and irradiated in a microwave oven. The sections were then stained immunohistochemically using an LSAB kit(DACO). We raised anti-*cripto* polyclonal antibody C2-1 against a synthetic oligopeptide corresponding to 12 amino acids (116 Gly–127 Lys) predicted from the published nucleotide sequence.[6] The antibody was purified through a *cripto* peptide-specific affinity column.[8] To examine the specificity of this antibody, absorption of the primary antibody by the specific peptide was performed. This antibody failed to give a signal in western blotting, but this is not uncommon when an antibody is raised against a peptide whose structure has been chosen solely on the basis of the DNA sequence. The antibody we used is expected to be specific to *cripto* protein because the synthetic peptide used as the antigen has a unique structure. Peptide 79–111 of *cripto* has sequence homology with EGF and TGF-α, but our peptide, 116–127, has no sequence homology with EGF, TGF-α or any other known molecule.[6]

[5] Abbreviations: EGF, epidermal growth factor; TGF, transforming growth factor; bFGF, basic fibroblastic growth factor.

The positive staining in the gastrointestinal carcinomas, pancreatic carcinomas, and pancreas tissues was abolished by the antibody absorbed with the specific peptide. It was confirmed that in gastrointestinal carcinomas the immunohistochemical staining used the anti-*cripto* antibody was consistent with the expression levels of *cripto* mRNA.[9, 10] Expression levels were divided into the following: tissue not stained at all, negative (−); a part of the tumor tissue stained positively, slightly positive (±); almost all the tumor tissue stained positively, positive (+); and almost all the tumor tissue stained strongly positively, strongly positive (++).

The results on expression of *cripto* are summarized in Table I. Of 8 cases of intraductal tumors, 4 papillary adenomas and 4 papillary adenocarcinomas, all showed slightly positive to positive/strongly positive staining, as shown Fig. 1. In 32 cases of invasive ductal carcinomas, 15 cases of well differentiated tubular adenocarcinomas were positive to strongly positive (Fig. 2), 9 cases of moderately differentiated tubular adenocarcinomas were slightly to strongly positive, 6 cases of poorly differentiated adenocarcinomas were slightly positive to strongly positive, one case of anaplastic ductal carcinoma was positive, and one case of mucinous carcinoma was slightly positive. The expression level of *cripto* tended to be higher in well differentiated adenocarcinomas than in poorly differentiated adenocarcinomas. In cases of mucinous carcinoma derived from intraductal papillary adeno-

Table I. Expression of *cripto* in Human Pancreatic Tumors

Histological type	No. of cases	*cripto* expression[a]			
		−	±	+	++
Intraductal tumor	8	0	2 (25)	4 (50)	2 (25)
papillary adenoma	4	0	1 (25)	3 (75)	0
papillary adenocarcinoma	4	0	1 (25)	1 (25)	2 (50)
Invasive ductal carcinoma	32	0	6 (19)	17 (53)	9 (28)
well differentiated	15	0	0	9 (60)	6 (40)
moderately differentiated	9	0	3 (33)	4 (44)	2 (22)
poorly differentiated	6	0	2 (33)	3 (50)	1 (17)
anaplastic	1	0	0	1	0
mucinous	1	0	1	0	0
Mucinous cystadenoma	2	0	0	0	2 (100)
Islet cell tumor	2	0	1 (50)	1 (50)	0
Solid and cystic tumor	1	1 (100)	0	0	0

a) Criteria are described in the text.

Fig. 1. Immunohistochemical staining with anti-*cripto* antibody. Positive staining of intraductal papillary adenocarcinoma cells. (×80)

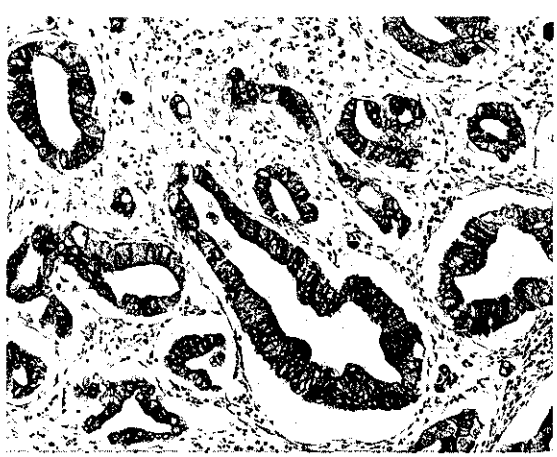

Fig. 2. Immunohistochemical staining with anti-*cripto* antibody. Strongly positive well-differentiatied adenocarcinoma. (×150)

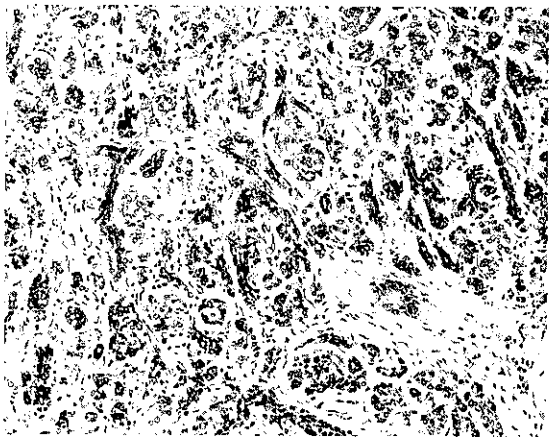

Fig. 3. Immunohistochemical staining with anti-*cripto* antibody. Positive cells arranged in duct-like structures in an area of chronic pancreatitis accompanying an advanced ductal carcinoma. (×80)

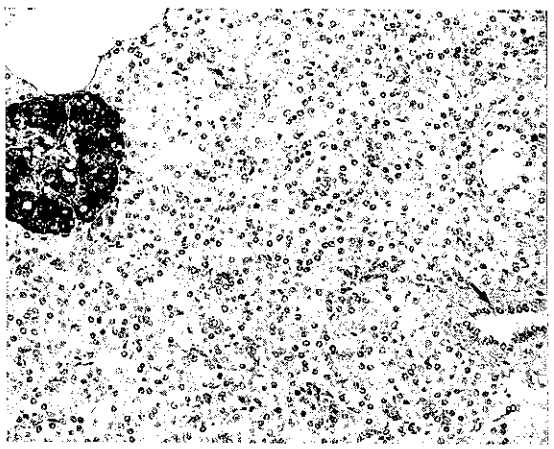

Fig. 4. Immunohistochemical staining with anti-*cripto* antibody. Negative ductal and acinar cells but positive islet cells in a pancreas without disease. The arrow indicates a small duct of the pancreas. (×100)

carcinomas, the tendency was for higher expression in intraductal than in invasive and mucinous components. Islet cell tumors showed slightly positive to positive staining, whereas the solid and cystic tumor was negative. The results of *cripto* staining in pancreatic tissues surrounding tumors were as follows. Duct epithelial cells including major pancreatic duct cells and centroacinar cells showed negative or only slightly positive staining. However, cells arranged in duct-like structures in areas of chronic pancreatitis accompanying advanced ductal carcinomas demonstrated positive *cripto* staining (Fig. 3). Acinar cells, with or without chronic pancreatitis, were negative. Islet cells, with or without chronic pancreatitis, showed strongly positive staining (Fig. 4).

Concerning the possible function of *cripto*, it has been demonstrated that overexpression of EGF or TGF-α cDNA as well as *cripto* by gene transfer can transform mammalian epithelial cells or fibroblasts *in vitro*.[6, 11-16] However, it was reported that *cripto* expression is not in itself sufficient to lead to a tumorigenic phenotype since cells transformed *in vitro* by transfection with *cripto* cDNA could not grow in nude mice.[16] *cripto* expression has been described in gastric[17] and colorectal[17, 18] cancers, with a correlation between level of expression and tumor progression.[17] In the present investigation, *cripto* expression was detected in all ductal tumors, with only a slight tendency for decrease from intraductal to invasive carcinoma component, indicating a lack of any direct relation to cancer progression. Therefore, during development of pancreatic ductal neoplasms, *cripto* expression may occur in a relatively early phase. Whether it endows mutated cells with any advantage for proliferation is a question which requires further detailed study. It is well known that activation of the K-*ras* gene is a common occurrence in human pancreatic ductal tumors[19-23] and the present results suggest that a high incidence of *cripto* expression may cooperate with such K-*ras* activation during pancreatic carcinogenesis.

In pancreas tissue without any disease, duct epithelial cells and acinar cells were not stained with anti-*cripto* antibody, though cells arranged in duct-like glands within areas of pancreatitis were positive. These latter might be de-differentiated acinar cells, suggesting that *cripto* expression could be related to differentiated status. The observed *cripto* expression in normal islet cell populations remains unexplained. The role of *cripto* expression in normal cells and cancer cells is obscure, because the function of *cripto* gene product is not well-known in terms of cell proliferation, and its receptor has not been identified. In conclusion, while the role of *cripto* in the pancreas is obscure, the present study adds weight to the hypothesis that this growth factor may be involved in processes of differentiation and carcinogenesis.

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