CHARACTERISATION OF OESTROGEN RECEPTOR, PROGESTERONE RECEPTOR, TREFOIL FACTOR 1, AND EPIDERMAL GROWTH FACTOR AND ITS RECEPTOR IN PANCREATIC CYSTIC NEOPLASMS AND PANCREATIC DUCTAL ADENOCARCINOMA

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Background and aims: The pancreatic cystic neoplasms, including solid pseudopapillary tumour (SPT), mucinous cystic neoplasm (MCN), and intraductal papillary mucin producing tumour (IPMT), have their characteristic clinicopathological features. A systematic investigation of oestrogen receptor (OR), progesterone receptor (PR), trefoil factor 1 (TFF1), and epidermal growth factor and its receptor (EGF and EGFR) expressed in pancreatic cystic neoplasms and pancreatic ductal adenocarcinoma was determined to elucidate their corresponding sex and age predilection, cell origin, and pathway of malignant transformation.

Methods: Surgical specimens of SPT (n=10), MCN (n=12), IPMT (n=10), and ductal adenocarcinoma (n=20) were studied. The expression of OR, PR, TFF1, EGF, and EGFR were each determined in each disease entity using monoclonal antibodies by immunohistochemical method. The results were correlated with the clinicopathological data.

Results: PR was expressed in all 10 SPT, whereas OR was expressed in none of 10 SPT. TFF1 was not or weakly expressed in SPT. Although EGF was strongly expressed in seven of 10 SPT, synchronous expression of EGF and its receptor was expressed in none of 10 SPT. Of the 12 MCN, six had PR expression in the stroma cells but not in the neoplastic epithelium, seven had a moderate or strong expression of TFF1, and 10 had no or weak EGFR expression, irrespective of their benignity or malignancy. Synchronous expression of EGF and EGFR was observed in only one of 12 MCN. Among 10 IPMT, TFF1 and EGFR were moderately or strongly expressed in all six malignancies, whereas TFF1 and EGFR were not or weakly expressed in three of four benignity. Of 20 ductal adenocarcinomas, TFF1 and EGFR were moderately or strongly expressed in 16 and 12, respectively. Synchronous expression of EGF and EGFR was observed in six of 10 IPMT and nine of 20 ductal adenocarcinoma, respectively.

Conclusion: PR was uniquely expressed in SPT, and OR and PR were expressed in stroma of MCN, reflecting their sex and age predilection. TFF1 expression was related to EGFR such as in IPMT and ductal adenocarcinoma, not related to EGFR such as in MCN, and not related to hormonal receptors such as in SPT. EGF and its receptor might play a part in the malignant transformation of IPMT and ductal adenocarcinoma, but not of SPT and MCN.

Abbreviations: OR, oestrogen receptor; PR, progesterone receptor; TFF1, trefoil factor 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; SPT, solid pseudopapillary tumour of the pancreas; MCN, mucinous cystic neoplasm of the pancreas; IPMT, intraductal papillary mucin producing tumour of the pancreas; PBS, phosphate buffered saline.
Based on the unique characteristic clinicopathological features of the each disease entity aforementioned, we systematically determined the expression of oestrogen receptor (OR), progesterone receptor (PR), trefoil factor 1 (TFF1), and epidermal growth factor and its receptor (EGF and EGFR) in pancreatic cystic neoplasms and ductal adenocarcinoma, which might elucidate their corresponding sex and age predilection, cell origin, and pathway of malignant transformation.

METHODS

Patients

Patients with SPT (n=10), MCN (n=12), IPMT (n=10), and pancreatic ductal adenocarcinoma (n=20) who had undergone curative resection from January 1993 to December 1998 in Chang Gung Memorial Hospital were recruited for this study. All 10 patients with SPT were women with a mean age of 34 years (range, 18 to 47 years). The mean size of the 10 SPT was 7.8 cm in maximal dimension (range, 6 to 10.5 cm). Four patients underwent Whipple’s operation, whereas the remaining six patients underwent distal pancreatectomy. Among the 12 patients with MCN, there were five men and seven women with a mean age of 45 years (range, 19 to 70 years). Five patients underwent Whipple’s operation, and the remaining seven patients underwent distal pancreatectomy. Among the 12 patients with MCN, there were five men and seven women with a mean age of 45 years (range, 19 to 70 years). Five patients underwent Whipple’s operation, and the remaining seven patients underwent distal pancreatectomy. For those 10 patients with IPMT, there were six men and four women, with a mean age of 68 years (range, 54 to 76 years). Whipple’s operation,
nearly total pancreatectomy, and distal pancreatectomy were performed in five, two, and three patients, respectively. Among the 20 patients with pancreatic ductal carcinoma, there were 14 men and six women with a mean age of 58 years (range, 39 to 72 years). Eighteen of the 20 patients underwent Whipple’s operation and the remaining two underwent distal pancreatectomy.

**Immunohistochemical stainings for OR, PR, TFF1, EGF, and EGFR**

Formalin fixed, paraffin wax embedded tissues were cut into 4 µm sections and mounted on glu coated slides. A modification of the avidin-biotin-peroxidase complex immunohistochemical method was performed. Slides were heated at 60°C for 60 minutes, then deparaffinised in xylene, and rehydrated in graded alcohols. Endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxidase in methanol, and slides were rehydrated and washed in phosphate buffered saline (PBS), pH 7.4 for 15 minutes. The tissue sections were reacted with 0.06% diaminobenzidine (Sigma Chemical) for five minutes, rinsed, counterstained with haematoxylin, dehydrated with graded alcohols, cleared xylene, and coverslipped with permount.

PBS rinse, avidin DH: biotinylated horseradish peroxidase complex (Vector Labs) was applied for 30 minutes. After a final PBS rinse, the tissue sections were reacted with 0.06% diaminobenzidine (Sigma Chemical) for five minutes, rinsed, countered stained with haematoxylin, dehydrated with graded alcohols, cleared xylene, and coverslipped with permount.

**RESULTS**

The expression of OR, PR, TFF1, and EGFR in SPT (n=10), MCN (n=12), IPMT (n=10), and ductal adenocarcinoma (n=20), respectively, were tabulated (table 1). OR was expressed in none of 10 SPT, whereas PR was unexceptionally expressed in all 10 SPT (fig 1A). TFF1 and EGFR were not or weakly expressed in SPT. Of the 12 MCN, four had OR expression (fig 1B) and six had PR expression (fig 1C) in the nuclei of the stroma cells rather than in their neoplastic epithelium. All these six patients with a PR expression in the stroma cells were women. Seven had a moderate or strong expression of TFF1 (fig 1D), whereas 10 had no or weak EGFR expression, irrespective of their benignity or malignancy (fig 1E). Of 10 IPMT, TFF1 and EGFR were moderately or strongly expressed in six malignancies (fig 1F and fig 1G), whereas TFF1 and EGFR were not or weakly expressed in three of four benign IPMT, and in eight malignant MCN. Synchronous expression of EGF and its receptor was observed in five of six malignant IPMT, and in eight malignant MCN. Synchronous expression of EGF and its receptor was observed in only one of four benign IPMT, which was considered severe dysplasia histologically, respectively. Synchronous expression of EGF and its receptor was observed in nine of 20 ductal adenocarcinomas.

**DISCUSSION**

EGF has a growth promoting or trophic effect on many tissues, including the pancreas. An overexpression of EGFR has...
been identified in pancreatic ductal adenocarcinoma, along with increased amounts of EGF, which suggests the establishment of an autocrine loop. In this study, EGF receptor represented an important tumourigenic factor in the group of IPMT and ductal adenocarcinoma. All six histologically malignant IPMT had a moderate or strong EGF expression, whereas three of four benign IPMT had either no or weak EGF expression. Of significance, one benign IPMT with synchronous expression of EGF and its receptor was considered severe dysplasia histologically, which might progress to frank malignancy, if not appropriately treated. From this point of view, EGF and its receptor can be clinically regarded as an index of malignant potential of IPMT. On the other hand, EGFR seemed not to be involved in the malignant transformation of MCN. Ten of the 12 MCN, including seven malignant MCN, had no or weak EGF expression. These data conflict with the series of Kirby et al. In their series, eight (61.2%) of 13 malignant MCN exhibited increased expression of EGF, whereas EGF was not detected in any of the 13 benign MCN. This discrepancy needs further clarification. However, the fact that only one of eight malignant MCN had the synchronous expression of EGF and its receptor in our study provided additional evidence that EGF and its receptor had little role in malignant transformation in MCN. Interestingly, although seven of 10 SPT had a strong expression of EGF, all 10 SPT, including two malignancies, had no or weak EGF expression. This phenomenon explains the diverse histopathological features and cellular origin, and less malignant potential of SPT compared with other pancreatic cystic neoplasms.

Treoil factor family (TFF) are abundantly expressed wherever mucin secretion occurs, most predominantly in the mucin secreting cells of the gastrointestinal mucosa. TFF consisted of three trefoil factors (TFF1, TFF2, and TFF3). It is shown that all TFF peptides are motogens; they stimulate epithelial cell migration in a variety test systems. Of them, TFF1, a single trefoil peptide originally found in breast cancer cell line but not of SPT and MCN.

In SPT, the expression of TFF1 was not detected in 67% of breast tumours that were OR positive and 4% of carcinomas that were OR negative. Thus, TFF1 might participate in a functional oestrogen regulatory system. However, the expression of TFF1 can be either oestrogen dependent (as in breast cancer) or oestrogen independent (as in normal gastric epithelium). Welter et al reported a study of TFF1 expression in human pancreatic cancer. Of 23 tumours, 17 exhibited significant expression, and the remainder exhibited weak but detectable TFF1 immunoreactivity. They concluded that the TFF1 expression in these tumours was significantly linked to the molecular steps leading to tumorgenesis. Our data supported part of this statement. In the current series, 16 of 20 ductal adenocarcinoma had a strong or moderate expression of TFF1. The high prevalence of TFF1 expression in ductal adenocarcinoma in our series might have been induced by an increased expression of EGF and its receptor, instead of by hormonal stimuli. The same phenomenon can be observed in patients with IPMT. The highly increased expression of TFF1 in IPMT was accompanied by an up-regulation of EGF in malignant IPMT. In contrast, seven of the 12 MCN had a moderate or strong expression of TFF1 that, however, was unrelated to both the hormonal receptors and EGFR. According to our data, TFF1 expression seemed not to be directly associated with malignant transformation in MCN. Five of eight malignant MCN and two of four benign MCN had moderate or strong expression of TFF1. These data were in accordance with the series of Kirby et al. Again, TFF1 played no part in SPT. Based on our data, TFF1 was not induced in SPT, even though PR was remarkably up-regulated in SPT. Collectively, we speculate that the expression of TFF1 could be enhanced by EGF (as in ductal carcinoma and IPMT), independent of EGF (as in MCN), and independent of hormonal receptors (as in SPT).

A hormonal influence on the pathogenesis of SPT has been suggested in view of its high prevalence in women. There have been some previous attempts to study the role of OR and PR in SPT, but conflicting results have emerged. Nearly all the studies showed no evidence of OR in pancreatic SPT. The only positive findings was that of Landanyi et al, who found OR by biochemical assay, in one woman. This discrepancy occurred because SPT was reported as immunohistochemically negative for the presence of nuclear OR, whereas a specific binding of H-E2 to cytosol from SPT was performed by Landanyi et al. The expression of hormonal receptors in stroma beneath the neoplastic epithelium is not related to malignant potential of SPT and described as an “ovarian-like stroma.” In our study, half of 12 MCN had an expression of hormonal receptors in the corresponding stroma cells but not in the neoplastic epithelium, and all these patients were women. Based on a literature review, in which we found that MCN occurred prevalently in middle aged women, we believe that sex hormone should be deeply involved in the pathogenesis of MCN.

In summary, PR was uniquely expressed in SPT, and OR and PR were expressed in stroma of MCN, reflecting their sex and age predilection. TFF1 expression might be related to EGFR such as in IPMT and ductal adenocarcinoma, not related to EGFR such as in MCN, nor related to hormonal receptors such as in SPT. EGF and its receptor might play a part in the malignant transformation of IPMT and ductal adenocarcinoma, but not of SPT and MCN.

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