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Sex differences in immunohistochemical expression and capillary density in pancreatic solid pseudopapillary neoplasm☆

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ABSTRACT

Solid pseudopapillary neoplasm (SPN) is a rare and low-grade malignant pancreatic neoplasm. Solid pseudopapillary neoplasm is rare in men, and most SPN cases are in young women. This study aimed to investigate sex differences in SPN clinical histopathology including capillary density and expression of immunochemical markers, including glypican 3. A total of 22 resected tumors from pancreatic SPN patients, including 16 women (73%) and 6 men (27%), were analyzed histopathologically and immunohistochemically for synaptophysin, β-catenin, estrogen receptor, progesterone receptor, Ki-67, CD10, CD31, and glypican 3. The median age was 52.5 years in men and 24 years in women ($P = .046$). The median tumor size was 22.5 mm in men and 40 mm in women ($P = .337$). In 11 of the 16 women (69%), but in none of the men, tumors showed complete or incomplete fibrous cap`sules ($P = .006$). Cholesterol clefts were observed in tumors from 10 women (63%) but in none from the men ($P = .012$). No significant sex differences were noted in tumor characteristics, including size, macroscopic cystic degeneration, necrosis, lymphovascular involvement, and perineural invasion. The SPNs were weakly positive for glypican 3, although there was no significant difference between sexes. Capillary density tended to be lower in tumors from men than in those from women, but not significantly. Thus, except for the fibrous capsule and cholesterol clefts often found in tumors and the younger age of the women, there were no significant sex differences in histopathologic or immunohistochemical features of SPN, despite its markedly higher occurrence in women.

1. Introduction

Solid pseudopapillary neoplasm (SPN) is a rare and low-grade malignant pancreatic neoplasm. Solid pseudopapillary neoplasm occurs predominantly in young women (91%) and is rare in men (9%) [1]. Typical SPN is composed of poorly cohesive, monomorphic cells forming solid and pseudopapillary structures with fibrovascular stalks and frequent hemorrhagic-cystic degeneration [2]. In contrast, SPN in men tends to exhibit solid components that lack prominent pseudopapillary or pseudoglandular formations and prominent degenerative changes [3]. Therefore, it is presumed that capillary density of SPN in men is lower than that in women because SPN in men tends to exhibit solid proliferation. However, a comparison of SPN capillary density between sexes has not yet been reported.

Immunohistochemically, SPN usually expresses vimentin, α1-antitrypsin, CD56, progesterone receptor, CD10, and nuclear/cytoplasmic β-catenin [4-6]. β-catenin gene mutation and abnormal nuclear/cytoplasmic accumulation of β-catenin are the most characteristic features of SPN [3,5]. β-catenin associates not only with cadherin cell adhesion molecules but also with components of the canonical Wnt signaling pathway [7–10]. The translocation of β-catenin to the nucleus is a hallmark of the canonical Wnt signaling pathway [8,9]. β-catenin translocated to the nucleus regulates expression of genes such as that encoding cyclin D1 during cancer formation [11]. Glypican 3 is a cell surface, heparan sulfate proteoglycan associated with the canonical Wnt signaling pathway and well known as a valuable marker for hepatocellular carcinoma [12,13]. To date, the comparison of immunohistochemical expression of glypican 3 between sexes in SPNs has not been reported.

In this study, we compared SPN tumors from men and women for clinicopathologic features including capillary density and immunohistochemical expression of several markers including glypican 3.

☆ Conflict of interest statement: The authors have no conflict of interest to declare.

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2. Materials and methods

2.1. Cases and sample preparation

A total of 22 pancreatic SPN patients were examined. These patients had undergone surgical resection between January 1991 and March 2014 at Tokai University Hospital and between April 2002 and June 2014 at Tokai University Hachioji Hospital. All cases had been diagnosed on the basis of routine histologic analysis. Surgically resected tumor samples were fixed in formalin and embedded in paraffin. Sections were cut into 4-μm-thick sections and stained with hematoxylin-eosin according to the standard procedures. Two cases (1 male patient and 1 female patient) included in the study were previously reported as single case reports [14,15].

2.2. Immunohistochemistry

Immunohistochemical staining for synaptophysin (clone 27G12, dilution 1:100; Leica Microsystems, Newcastle upon Tyne, United Kingdom), β-catenin (clone 17C2, dilution 1:100; Leica Microsystems), and glypican 3 (clone 1G12; Nichirei, Tokyo, Japan) was performed using BondMax (Leica Microsystems) according to the manufacturer's manual. Antigen retrieval was performed by treatment with epitope retrieval solution 2 for 20 minutes for glypican 3 and with epitope retrieval solution 1 for 30 minutes for β-catenin and synaptophysin. Assays for estrogen receptor (clone SP1; Ventana Medical Systems, Tucson, AZ), progesterone receptor (clone 1E2; Ventana), Ki-67 (clone 30-9; Ventana), CD10 (clone SP67; Ventana), and CD31 (clone JC70A, dilution 1:30; Dako, Glostrup, Denmark) were performed using BenchMark ULTRA (Ventana Medical Systems) equipped with the Ultra View Detection Kit. Antigen retrieval was performed by treatment with Cell Conditioning 1 (Ventana) for 76 minutes. Appropriate positive and negative tissue control samples were used, and the staining intensity was expressed semiquantitatively as negative (−) or positive (+, ++, or +++).

2.3. Evaluation of capillary density by CD31 staining and digital imaging

The high staining density areas of CD31-positive capillaries ("hot spots") were captured as digital images at magnification ×200 using bright field microscopy. The capillary wall was traced using Photoshop CS4 software (Adobe Systems, San Jose, CA), and capillary density was quantified as a percentage of the entire image using the "Analyze particles" function of Image J software (National Institutes of Health, USA) after setting an appropriate threshold. The capillary density of a given sample is expressed as the mean percentage of 3 highest density "hot spots."

2.4. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics, version 19 (IBM Japan, Tokyo, Japan). The Fisher exact test and Mann-Whitney *U* test were used. $P < .05$ was considered significant.

3. Results

3.1. Characteristics of the SPN patients

The clinical features of the study population were compared between men and women (Table 1). Of the 22 SPN cases studied, 6 were men (27%), and 16 were women (73%), with an overall median age of 27.5 years (range, 12-70 years). The median age for men (52.5 years; range, 12-63 years) was significantly higher than that for women (24 years; range, 13-70 years) ($P = .046$). The overall median tumor size for all 22 cases was 32.5 mm (range, 5-130 mm), whereas the median tumor size in men (22.5 mm; range, 5-75 mm) was smaller than that in women (40 mm; range, 10-130 mm), but not significantly ($P = .337$). The site of the tumor in the pancreas (head or body-to-tail) did not differ significantly between tumors from men and women ($P = .541$). Liver metastasis was observed in 1 woman. In 1 male patient, SPN was detected incidentally during surgery for intraductal papillary mucinous neoplasm.

Table 1
Solid pseudopapillary neoplasm patient and tumor characteristics between women and men

Characteristic	Overall, n = 22	Women, n = 16	Men, n = 6	*P*
Age, y				.046
Mean	33.1	27.6	48	
Median (range)	27.5 (12-70)	24 (13-70)	52.5 (12-63)	
Tumor characteristics				
Size, mm				.337
Mean	41.2	45.6	29.5	
Median (range)	32.5 (5-130)	40 (10-130)	22.5 (5-75)	
Location in the pancreas, n (%)				
Head	6 (27)	4 (25)	2 (33)	.541
Body to tail	16 (73)	12 (75)	4 (67)	
Capsule, n (%)	11 (50)	11 (69)	0	.006
Cholesterol cleft, n (%)	10 (45)	10 (63)	0	.012
Cystic change, n (%)	14 (64)	12 (75)	2 (33)	.096
Calcification, n (%)	13 (59)	10 (63)	3 (50)	.477
Necrosis, n (%)	8 (36)	7 (44)	1 (17)	.255
Local invasion, n (%)	6 (27)	4 (25)	2 (33)	.541
Lymphatic involvement, n (%)	0	0	0	–
Venous involvement, n (%)	1 (5)	0	1 (17)	.273
Perineural invasion, n (%)	7 (32)	5 (31)	2 (33)	.651
Capillary density, %				
Mean	7.1	7.8	5.2	.21
Median (range)	6.1 (0.8-17.3)	7.2 (0.8-17.3)	2.7 (1.2-15.9)	

3.2. Histopathologic features of the SPN tumors

The differences in histologic features in SPN tumors between men and women were analyzed (Table 1). Tumors in 12 of the 16 women (75%) and in 2 of the 6 men (33%) showed macroscopic cystic degeneration, although the difference was not significant ($P = .096$; Fig. 1A and B). Microscopic findings showed that all the tumors analyzed were composed of cells with eosinophilic or clear cytoplasm and a round nucleus. Tumor cells mainly proliferated in a pseudopapillary pattern with a capillary core or sheet-like pattern (Fig. 1B and C). The 2 notable differences were that tumors in 11 of the 16 women (69%), but in none of the men, showed complete or incomplete fibrous capsules ($P = .006$) and tumors in 10 women (63%), but in none of the men, showed cholesterol clefts ($P = .012$). The other histopathologic features of the tumors, including calcification; necrosis; local invasion; and lymphatic, vascular or perineural involvement, were not significantly different between men and women ($P > .2$; Table 1).

3.3. Capillary density of SPN tumors

The median percentage of capillary density of SPNs was 2.7% (range, 1.2-15.9) in men and 7.2% (range, 0.8-17.3) in women (Fig. 2A and B), but the difference was not statistically significant ($P = .21$; Table 1).

3.4. Immunohistochemical staining of SPN tumors

No significant sex differences were observed in the immunohistochemical expression of the different markers (Table 2). All the tumors analyzed showed a diffuse and strong expression of β-catenin in nuclei and cytoplasm, and all were negative for estrogen receptor. Tumors in all 6 men and 10 women were positive for synaptophysin. CD10 was positive in tumors of all 6 men and in 13 of the 16 women. Progesterone