의학석사 학위논문

Differences in Histologic Characteristics and Oncogenic Protein Expression of Intraductal Papillary Mucinous Neoplasm of Pancreas

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2015년 2월

서울대학교 대학원

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Differences in Histologic Characteristics and Oncogenic Protein Expression of Intraductal Papillary Mucinous Neoplasm of Pancreas

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Abstract

Introduction Intraductal papillary mucinous neoplasm (IPMN) and pancreatic intraepithelial neoplasia (PanIN) are precursors of pancreatic ductal adenocarcinoma (PDAC). The aim of this study was to figure out the histologic characteristics of IPMN, and to document histologic and oncogenic uniqueness of IPMN according to the histologic configuration.

Methods Between January 2001 and December 2009, 98 patients with IPMN satisfying following conditions were analyzed: 1) a cyst of greater than 10mm at preoperative radiologic study, 2) main pancreatic duct dilatation exceeding 5mm at preoperative radiologic study, 3) pathologically diagnosed as IPMN after resection. In order to reassess 98 cases according to the histologic findings excluding the size of cyst, five histologic parameters were evaluated as follows, 1) histologic pattern, 2) glandular type, 3) dysplasia grade of epithelium, 4) transition of normal duct cells to tumor cells, and 5) mucin. With histologic criteria, initial pathologic diagnosis was classified into papillary and mucinous dominant (PM), tubular (T), and flat (F) IPMN. According to the invasiveness, those groups were also classified into noninvasive (NI) and invasive (INV) groups. The expressions of 11 oncogenic proteins (MUC1, MUC2, MUC5ac, SMAD4, S100A4, MSH2, APC, p53, p16, p21, and CD24) in IPMN were analyzed

Results Of the 98 consecutive, mean age was 64.5 years and 66.3% were male. Lesions were commonly found in pancreas head (52.0%) and mean cystic size was

33.0 mm in diameter. Main duct, branch duct, and mixed type of IPMN were found in 15.3%, 61.2%, and 23.5%, respectively. Curative resection was achieved in 94.6%. Of the 46 patients in invasive group, 43.5%, 17.4%, and 39.1% were reclassified as PM-INV, T-INV, and F-INV, respectively. In cellular types, PM-INV showed higher rate of intestinal subtype and T-INV noted pancreatobiliary or oncocytic subtype more frequently ($p<0.001$). In cystic portion, high rate of MUC1 expression ($p=0.009$) and APC loss ($p=0.003$) were noted in T-INV and F-INV. MUC2 was the major differentially expressed protein both in PM-NI and in PM-INV. In invasive portion, MUC2 expression was higher in PM-INV than T-INV and F-INV ($p<0.001$). MUC1 ($p<0.001$), SMAD4 loss ($p<0.001$), S100A4 ($p=0.011$), and p53 ($p=0.029$) showed higher expression in F-INV than PM-INV. In invasive group, F-INV showed poorer prognosis than PM-INV in terms of disease-specific survival ($p=0.002$) and recurrence free survival ($p=0.046$)

Conclusion We demonstrated that IPMNs with flat configuration were different from other types of IPMN in terms of expression of oncogenic protein and histologic characteristics. Flat IPMN showed unique characteristics sharing that of PanIN, and demonstrated poorer prognosis. Thus, flat IPMN can be regarded as PanIN, and this classification can provide clues to proper management of IPMN.

Keywords: intraductal papillary mucinous neoplasm, pancreatic intraepithelial neoplasia, ductal adenocarcinoma of pancreas

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List of Tables

List of Figures

List of Abbreviations

PDAC	Pancreatic ductal adenocarcinoma
IPMN	Intraductal papillary mucinous neoplasm
PanIN	Pancreatic intraepithelial neoplasm
NI	Noninvasive
INV	Invasive
HPD	Hepatopancreatoduodenectomy
PD	Pancreatoduodenectomy
PPPD	Pylorus-preserving pancreatoduodenectomy
5YS	Five-year survival
DS 5YS	Disease-specific five-year survival

Contents

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the USA(1), and the overall five-year survival after surgical resection has been reported to range between 10 and 25%(2, 3). Since most of the patients are diagnosed in advanced tumor stage due to lack of sensitive and specific tools to detect in an early stage, identification and treatment of precursor lesions of PDAC such as intraductal papillary mucinous neoplasm (IPMN) and pancreatic intraepithelial neoplasia (PanIN), can offer potential tools to improve the clinical outcome of PDAC(4-7).

IPMN is characterized by the presence of dilated cystic pancreatic ducts filled with mucin, and predominantly involve the main pancreatic ducts or its major branches(8). PanIN is a microscopic (usually <5 mm) flat or papillary lesion arising in the small intralobular pancreatic ducts, and is composed of columnar to cuboidal cells with varying amounts of mucin and with varying degrees of cytological and architectural atypia(9).

IPMNs and PanINs share many fundamental pathologic characteristics(8-11). Both are intraductal, and are composed predominantly of columnar, mucin-producing cells that may grow in a flat configuration or may produce papillae. Both exhibit a range of cytologic and architectural atypia(12). As a matter of fact, these two precursor lesions of PDAC may be almost impossible to identify by size(13). However, current criteria of differential

diagnosis between PanIN and IPMN are mainly focused on the size of cyst, not on the histologic features(9-11).

Despite these histologic overlaps, mechanisms of oncogenesis or molecular differences between IPMN and PanIN have been reported in several studies(9-11, 14, 15). Moreover, the lesions under the invasive IPMNs are mixture of early ductal cancer arising in prominent preinvasive component (large cystic PanIN), PDAC with prominent papillary and cystic configuration, and IPMN in narrow spectrum which can be defined as unequivocal mural nodule formation or overproducing mucin(8, 13). When IPMNs are accompanied by an invasive carcinoma, histologic types of invasive carcinomas vary from tubular type to conventional type of PDAC(10). Therefore, when invasive carcinoma accompanies cystic component in tumor or transition between two lesions, it is generally diagnosed as IPMN with invasive carcinoma groups by current diagnostic criteria.

Because we hypothesized that size criteria is the cofounding factor of heterogeneity of IPMN, we explored whether IPMN can be classified based on the histologic patterns only, excluding size criteria, and whether this regrouping produced the differences in expression of oncogenic proteins and clinicopathologic implication. To identify these findings, we re-evaluated IPMN and investigated the expression of 11 oncogenic proteins in

noninvasive or invasive IPMN by separate evaluation in intraductal cystic component and invasive component.

Materials and Methods

Patient Selection and Data Collection

Between January 2001 and December 2009, Data of 98 patients with IPMN satisfying following conditions could be retrieved from archives of department of surgery and surgical pathology which were maintained prospectively: 1) a cyst of greater than 10mm at preoperative radiologic study, 2) main pancreatic duct dilatation exceeding 5mm at preoperative radiologic study, 3) pathologically diagnosed as IPMN after resection, 4) presence of available paraffin blocks and medical records. Demographic findings of the patients are summarized in Table 1. Initial surgical indications were as follows: a cyst of greater than 30 mm, main pancreatic duct dilatation exceeding 5mm, the appearance of new mural nodules, or the presence of any symptoms. Surgery was also performed when cyst showed significant growth or when suspicion of invasion was increased(16).

Pathological Assessment and definition of disease groups

Original pathologic diagnosis of 98 cases composed of 5 diagnosis as follows; 1) IPMN with low to intermediate grade dysplasia, 2) IPMN with high grade dysplasia, 3) minimally invasive IPMN, 4) widely invasive IPMN, 5) PDAC with IPMN (Table 1). Diagnostic criteria and terminology followed the general description of WHO classification of tumors of gastrointestinal

tract (8).

In order to reassess 98 cases according to the histologic findings excluding the size of cyst, five histologic parameters were evaluated as follows, 1) histologic pattern, 2) glandular type, 3) dysplasia grade of lining epithelial cells, 4) transition of normal duct cells to tumor cells, and 5) mucin in intra- and extra-cysts. Cutoff point to histologic parameter was 10%. If the mixed patterns were found, each of components which were found in at least 10%, was described as together, such as “tubulopapillary”. Glandular type was assessed according to the predominant type in entire lesions. Degree of dysplasia was graded in the portion showing highest dysplasia of intracystic epithelial cells.

Regardless of cyst size, IPMN of the 98 cases were regrouped as papillary-mucinous dominant IPMN (PM-IPMN), tubular dominant IPMN (T-IPMN), and flat dominant IPMN (F-IPMN). PM-IPMN was defined as follows, 1) papillary histologic pattern without dominant tubular pattern or 2) existence of abundant extracystic mucin pool or intracystic basophilic thick mucin with any histologic pattern (8). T-IPMN was defined when it comes to tubular pattern more than 75% with mural nodule, and F-IPMN was defined as flat or micropapillary epithelium without true papillae or abundant mucin pool (7). Considering invasiveness, each group was divided into two subgroups as noninvasive group including low to high degree of dysplasia and high grade dysplasia, and invasive group including minimally invasive, widely invasive,

and IPMN with invasive carcinoma. Depth of invasion to classify minimally and widely invasive IPMN was 500㎛(17). Invasion more than 500㎛ with mass formation was regarded as widely invasive IPMN. Abbreviations of subgroup were suffixed to the disease group, such as noninvasive papillary-mucinous IPMN (PM-NI), noninvasive tubular IPMN (T-NI), noninvasive flat IPMN (F-NI). In case of invasive tumor, INV was designated rather than NI, such as PM-INV, T-INV, and F-INV, respectively. Representative figures of each group are presented in Figure 1.

Immunohistochemical stain

After identification of representative area from 5 mm-sections which were stained with hematolxylin and eosin, pathologic slides were prepared for the immunohistochemical stain. The antibodies used for immunohistochemistry included mouse human monoclonal antibodies. For all antibodies, heat-induced epitope retrieval was performed using selected buffer solution (Table 2.). The immunohistochemical staining was processed with Leica BOND-MAX^{TM} autostainer. Unequivocal positive staining more than 5% of tumor cell was considered as positive result, and SMAD4, MSH2, and APC were considered as positive result in case of loss of expression more than 25% tumor cells.

Statistical Analysis

IBM SPSS Statistics version 22.0 (IBM Corp, Sommers, NY) was used for the analysis. Nominal data were compared with chi-square test, and continuous data with Student t test or ANOVA or analysis of variance with post hoc test. The Kaplan-Meier analysis was used to compare survival analysis. Two-sided P values of less than 0.05 were considered statistically significant.

Results

Regrouping of intraductal cystic neoplasm by histologic pattern without size criteria

Considering histologic pattern of papillary growth, mucin formation, and presence of mural nodule, regardless of the size of cyst, 50.0%, 7.7%, and 42.3% of noninvasive group (n=52) were classified as PM-NI, T-NI, and F-NI, respectively. Of the 46 patients in invasive group, 43.5%, 17.45%, and 34.6% were reclassified as PM-INV, T-INV, and F-INV, respectively (Table 3).

Comparative analysis of clinicopathologic parameters and survival analysis

Within noninvasive group, mean age, locations of the lesions, CEA (> 5 ng/ml) and CA 19-9 abnormality (> 37 IU/ml), AJCC 7th T stage and N stage, pathologic invasion, and adjuvant treatment showed no statistical differences, whereas the proportion of the male patients is significantly lower in F-NI (Table 5). Within the invasive group, mean age is higher in T-INV and F-INV (p=0.035). The rate of CA 19-9 abnormality was also higher in F-INV (p=0.033). The proportion of the male patients was slightly higher in PM-INV without statistical significance (p=0.835). Perineural and endolymphatic invasion were frequent in F-INV (p=0.005 and p=0.007, respectively). Recurrences were observed in F-INV at high rate (p=0.016),

whereas AJCC 7th T stage and N stage, endovascular invasion, and adjuvant treatment showed no differences (Table 4).

Regarding grade of dysplasia in cystic portion, PM-INV and T-INV showed higher rate of high grade dysplasia (p=0.001), whereas there was no difference within noninvasive groups (p=0.570). In cellular types, PM-INV showed higher rate of intestinal subtype, and T-INV noted pancreatobiliary or oncocytic subtype more frequently (p<0.001). Gastric type was the major cell type in F-INV (p<0.001). There were no differences in cellular subtype within noninvasive groups (Table 5). In terms of histologic pattern, F-NI and F-INV noted flat or micropapillary histologic pattern, and T-NI presented tubular morphology as a main histologic pattern (p<0.001). Intracystic mucin was observed in PM-NI, PM-INV, T-INV, F-INV in high rate, and basophilc intracystic mucin was noted in PM-NI and PM-INV. Extracystic mucin was observed with high rate in PM-NI and PM-INV. Although scanty amount of extracystic mucin was found in F-INV, definite mucin pool was not noted.

For the disease-specific survival analysis of the invasive group, PM-INV and T-INV showed better survival than F-INV (p=0.002 and p=0.061, respectively). Five-year recurrence-free survival of PM-INV, T-INV, and F-INV is 68.4%, 72.9%, and 28.6%, respectively, and statistical significance was noted between PM-INV and F-INV (p=0.046) (Figure 2 and 3).

Expression pattern of oncoproteins in cystic portion

Expression rates of oncogenic protein in cystic portion are summarized in Table 6. Within the noninvasive group, PM-NI and T-NI showed high expression of MUC2 (p=0.001). Although APC loss is more common in F-NI, there was no statistical significance. Within the invasive group, MUC1, MUC2, and APC loss were significantly different. MUC1 expression and APC loss were frequently found in F-INV (p=0.009 and p=0.003, respectively). T-INV had high rate of MUC1 expression and APC loss similar to F-INV, and had unique high expression of S100A4, p53, and p16 without statistical significance. Generally, T-INV had similar expression pattern and higher positive rate than cystic portion of F-INV or PM-INV.

Expression pattern of oncoproteins in invasive portion

Expression rates of oncoproteins in invasive portion are listed in Table 7. MUC1, MUC2, SMAD4 loss, S100A4, and p53 were differentially expressed in invasive portions. MUC2 expression was higher in PM-INV than T-INV and F-INV. MUC1 expression, SMAD4 loss, and S100A4 expression were higher in F-INV than PM-INV.

Discussion

For PDAC, the most important and well-known precursor is IPMN and PanIN. IPMNs belong to the heterogenous group of cystic pancreatic lesions with increasing incidence in recent years (18, 19). IPMNs were initially reported in the 1990s, and the term IPMN was established in the 2000 classification of the World Health Organization(20). IPMN is a tumor of pancreatic ductal epithelium which is characterized by papillary epithelial proliferation and mucin production lead to cystic dilatation of ducts(20).

After first description by Hruban et al in 2001, PanIN scheme has been widely accepted(9). PanIN is a microscopic flat or papillary lesion arising in the small intralobular pancreatic duct, is asymptomatic, and cannot be identified by radiologic modalities because it is usually less than 5mm(9). PanINs are characterized by columnar to cuboidal cells with varying amounts of mucin and varying degrees of cytological and architectural atypia(9). In fact, PanINs are in the stepwise progression from intraepithelial to invasive pancreatic neoplasm(21, 22).

Because there are differences in mechanisms of tumorogenesis and in clinical outcome between PanIN and IPMN(13, 21, 23), precise identification of these two lesions is quite important. However, even with definitions of these two precursor lesions of PDAC, there are many problems in pathologic practice to apply this definition because they share many pathologic characteristics(8, 9). PanIN, although usually manifests in small pancreatic

duct less than 5mm, can involve large pancreatic duct to form large cystic PanIN, and IPMN, although usually presents in relatively large ducts, can involve small duct less than 5mm(9, 13). Therefore, with this current size standard, it is difficult to separate these two conditions(13).

Although IPMNs are less aggressive than conventional PDAC, the overall survival of invasive IPMN is ranging 30-60%(24-26). In invasive IPMN, the invasive portion of IPMN has two types of morphologies, which are a tubular invasive type and a colloid type. The distinction between tubular and colloid types showed prognostic relevance because patients with colloid carcinomas have a better survival outcome than patients with tubular carcinoma(10, 27-29). Therefore, accurate criteria to identify the characteristic of IPMN and PanIN need to be established.

There have been many attempts to establish the clinicopathologic characteristics of IPMN and PanIN. Adsay et al reported the differences in oncogenic mechanism between PanIN and IPMN with using MUC1 and MUC2 immunohistochemistry demonstrating PanIN-Ductal adenocarcinoma pathway in case of MUC1 (+) and MUC2 (-), whereas IPMN-Colloid carcinoma pathway in case of MUC1 (-) and MUC2 (+)(23). Other attempts also have been made to report subtype classification of IPMN with MUC1, MUC2, MUC5ac, MUC6 immunohistochemical stain, and histologic subtype such as gastric, intestinal, pancreatobiliary, and oncocytic types(28, 30, 31). Furukawa et al reported that gastric and intestinal types of IPMN can achieve

better survival than pancreatobiliary type(28), whereas Kang et al. published an article indicating that histological subtypes were associated with the degree of dysplasia, but had of little prognostic value(32).

In this study, we tried to specify the histologic characteristics of IPMN as precusors of PDAC, and then to demonstrated differences of oncogenic protein expression using immunohistochemical markers. At first, initial pathologic diagnosis was classified into PM-NI, T-NI, F-NI, PM-INV, T-INV, and F-INV, according to histologic pattern, glandular type, dysplasia grade of lining epithelial cells, transition of normal duct cells to tumor cells, and mucin in intra- and extra-cysts. This classification of the pancreatic cystic lesions produced clinicopathologic differences successfully, especially when it comes to invasive group. PM-INV and T-INV achieved better survival than F-INV in terms of disease-specific survival and recurrence-free survival.

Immunohistochemical analysis was followed to investigate the difference in oncogenic protein expression under this classification. Besides MUC1, MUC2, and MUC5ac, many molecular markers, which were known to have a role in carcinogenesis, such as SMAD4, S100A4, MSH2, APC, p53, p16, p21, and CD24, were used in experiment. In cystic portion, MUC1 is frequently expressed in T-INV and F-INV, and F-INV showed highest expression of MUC1. MUC2 also showed significant difference in expression among groups, and was not shown in F-INV, whereas MUC2 was the major

differentially expressed protein both in PM-NI and in PM-INV implying that the intestinal type was the major cellular type of PM-IPMN. In cystic portion of invasive group, PM-INV was characterized with low rate of MUC1 expression, SMAD4 loss, and APC loss, and with high rate of MUC2 expression. F-INV had high expression of MUC1 and APC loss, and intermediate expression of SMAD4loss. T-INV had oncogenic protein expression pattern of the intermediate stages between PM-INV and F-INV. In invasive portion, MUC1, SMAD4 loss, S100A4, p53 showed low expression in PM-INV. T-INV, and F-INV were characterized with high expression of MUC1, SMAD4 loss, S100a4, and p53, and with low expression of MUC2.

With these results, initial histologic classification of IPMN as PM-IPMN, T-IPMN, and F-IPMN showed unique differences in oncogenic protein expression. According to histologic type of IPMN regardless of size of cyst, differences in clinicopathologic characteristics and oncogenic protein expression were clearly demonstrated. Moreover, F-IPMN showed similar characteristics to PanIN in terms of histologic configuration and oncogenic protein expression.

Besides immunohistochemical analysis using antibodies to oncogenic protein, GNAS mutation has been recently recognized as a specific genetic alteration in IPMNs among pancreatic neoplasm. One of studies showed that somatic mutations in GNAS were frequently found in IPMNs, and were identified in 48% of cases, whereas were not observed in any of the examined

cases of PDAC(33). Therefore, analysis on somatic mutation in GNAS can provide potential clues to characterize IPMNs more precisely. Because we did not carry out genome sequencing on DMA extracted from IPMN tissues, further study will be necessary to obtain information to specify IPMNs.

In conclusion, it is still hard to distinguish between IPMN and PanIN accurately with current WHO scheme of IPMN in clinical practice. With histological classification of IPMN to describe the lesion more precisely, and with results of immunohistochemical analysis, we can achieve more accurate identification of IPMN. IPMN of flat configuration without mucin pool or mural nodule, showed unique characteristics sharing those of PanIN, and demonstrated poorer prognosis. Thus, F-IPMN can be regarded as PanIN with large cyst, and with this classification, we can provide clues to proper management after surgery in patients with IPMNs.

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Tables

Table 1. Patients Demographics

	Total (n = 98)
Age, mean (SE), years	64.5 (0.9)
Gender, male, n (%)	65 (66.3%)
Location, n (%)	
Head	51 (52.0%)
Body-tail	34 (34.7%)
Diffuse	14 (13.3%)
Cyst size, mean (SE), mm	33.0 (2.0)
Main pancreatic duct size, mean (SE), mm	6.0 (0.5)
Radiological type, n (%)	
Main duct	15 (15.3%)
Branch duct	60 (61.2%)
Mixed	23 (23.5%)
Mural nodule	42 (42.9%)
Surgery	
Whipple's operation	16 (16.3%)
PPPD*	30 (30.6%)
Distal pancreatectomy	31 (31.6%)
Total pancreatectomy	11 (11.2%)
Subtotal pancreatectomy	3 (3.1%)
Enucleation	2 (2.0%)
Duodenum-preserving pancreatic head resection	5 (5.1%)
Curative resection, n (%)	93 (94.9%)
Adjuvant treatment	
Chemotherapy, n (%)	22 (22.4%)
Radiotherapy, n (%)	23 (23.5%)
Dysplasia, n (%)	
IPMN low to intermediate grade dysplasia	39 (39.8%)
IPMC high grade dysplasia	13 (13.3%)
IPMC Minimally invasive (<500㎛)	12 (12.2%)
IPMC Widely invasive (≥500㎛)	18 (18.4%)
PDAC with IPMN	16 (16.3%)
Follow-up, median (range), months	78.3 (21.5-105.2)

* PPPD, pylorus-preserving pancreaticoduodenectomy

Table 2. Immunohistochemical Panel

Antibodies (Clone)	**Clonality**	**Dilution**	**Source**
MUC1 (Ma695)	Mouse monoclonal	1:300	Novocastra
MUC2 (Ccp58)	Mouse monoclonal	1:300	Santa-cruz
MUC5AC (CLH2)	Mouse monoclonal	1:300	Millipore
p21 (187)	Mouse monoclonal	1:200	Santa-cruz
p53 (DO-7)	Mouse monoclonal	1:200	DAKO
S100A4	Mouse monoclonal	1:500	DAKO
SMAD4 (B-8)	Mouse monoclonal	1:200	Santa-cruz
MSH2	Mouse monoclonal	1:200	Oncogene
APC (EP701Y)	Mouse monoclonal	1:100	abcam
p16 (G175-405)	Mouse monoclonal	1:100	BD Biosciences
CD24 (24C02)	Mouse monoclonal	1:200	Neomarkers

Table 3. Pathologic classification of the lesions

n, %	PM-NI (n=26)	T-NI (n=4)	F-NI (n=22)	PM-INV (n=20)	T-INV (n=8)	F-INV (n=18)
IPMN, low to intermediate grade	19 (73.1)	4 (100)	16 (72.7)	0	0	0
IPMN, high grade	7 (26.9)	0	6 (27.3)	0	0	0
IPMN, minimally invasive	0	0	0	10 (50.0)	2 (25.0)	0
IPMN, widely invasive	0	0	0	9 (45.0)	4 (50.0)	5 (27.8)
PDAC + IPMN	0	0	0	1 (5.0)	2 (25.0)	13 (72.2)

Table 4. Clinicopathologic features of total group

Characteristics, n (%)	PM-NI (n=26)	T-NI (n=4)	F-NI (n=22)	P Value	PM-INV (n=20)	TP-INV (n=8)	F-INV (n=18)	P Value
Age, mean (SE), year	61.5 (1.6)	68.5 (2.1)	63.7 (1.8)	0.249	62.3 (2.4)	70.8 (2.8)	68.8 (1.9)	0.035
Gender, male	20 (76.9)	4 (100)	10 (45.5)	0.023	14 (70.0)	5 (62.5)	11 (61.1)	0.835
Location				0.150				0.402
Head	16 (61.5)	3 (75.0)	7 (31.8)		10 (50.0)	5 (62.5)	10 (55.6)	
Body-tail	8 (30.8)	1 (25.0)	9 (40.9)		9 (45.0)	1 (12.5)	6 (33.3)	
Diffuse	2 (7.7)	0	6 (27.3)		1 (5.0)	2 (25.0)	2 (11.1)	
CEA > 5 ng/ml	1 (3.8)	1 (25.0)	2 (9.1)	0.318	1 (5.0)	1 (12.5)	3 (16.7)	0.507
CA 19-9 > 37 IU/ml	2 (7.7)	1 (25.0)	2 (9.1)	0.547	5 (25.0)	3 (37.5)	12 (66.7)	0.033
T stage				0.485				0.090
pT0	19 (73.1)	4 (100)	16 (72.7)		0	0	0	
pTis	7 (26.9)	0	6 (27.3)		0	0	0	
pT1	0	0	0		3 (15.0)	0	1 (7.7)	
pT2	0	0	0		7 (35.0)	3 (37.5)	1 (7.7)	
pT3	0	0	0		10 (50.0)	4 (50.0)	16 (84.6)	
pT4	0	0	0		0	1 (12.5)	0	
N stage								
pN0	26 (100)	4 (100)	22 (100)	>0.999	17 (85.0)	6 (75.0)	11 (61.1)	0.245
pN1	0	0	0		3 (15.0)	2 (25.0)	7 (38.9)	
Pathologic invasion								
Perinueral	0	0	0	>0.999	4 (20.0)	3 (37.5)	13 (72.2)	0.005
Lymphatic	0	0	0	>0.999	0	2 (25.0)	6 (33.3)	0.007
Endovascular	0	0	0	>0.999	0	3 (37.5)	1 (5.6)	0.503
Adjuvant chemotherapy	0	0	0	>0.999	10 (50.0)	3 (37.5)	9 (50.0)	0.732
Adjuvant radiotherapy	0	0	0	>0.999	9 (45.0)	3 (37.5)	10 (55.6)	0.670
Recurrence	1 (3.8)	0	2 (9.1)	0.924	8 (40.0)	2 (25.0)	14 (77.8)	0.016

Table 5. Pathologic characteristics of cystic portion

Characteristics, n (%)	PM-NI (n=26)	T-NI (n=4)	F-NI (n=22)	P Value	PM-INV (n=20)	TP-INV (n=8)	F-INV (n=18)	P Value
Grade of dysplasia				0.570				0.001
Low to intermediate	21 (80.8)	4 (100)	17 (77.3)		0	0	8 (44.4)	
High	5 (19.2)	0	5 (22.7)		20 (100)	8 (100)	10 (55.9)	
Cellular type				0.074				<0.001
Gastric	19 (73.1)	5 (75.0)	19 (86.4)		1 (5.0)	2 (25.0)	13 (72.2)	
Intestinal	6 (23.1)	0	0		15 (75.0)	0	0	
Pancreatobiliary	1 (3.8)	1 (25.0)	3 (16.7)		4 (20.0)	4 (50.0)	5 (27.8)	
Oncocytic	0	0	0		0	2 (25.0)	0	
Intracystic mucin				0.001				<0.001
Present	15 (57.7)	2 (50.0)	5 (22.7)		8 (40.0)	6 (75.0)	13 (72.2)	
Basophilic mucin	6 (23.1)	0	0		10 (50.0)	0	0	
Extracystic mucin	6 (23.1)	0	0	0.034	10 (60.0)	3 (37.5)	2 (11.1)	0.008

Table 6. Expression rate of oncogenic protein in cystic portion

n (%)	PM-NI (n=26)	T-NI (n=4)	F-NI (n=22)	P Value	PM-INV (n=20)	TP-INV (n=8)	F-INV (n=18)	P Value
MUC1	5 (19.2)	1 (25.0)	6 (27.3)	0.801	4 (20.0)	5 (62.5)	12 (66.7)	0.009
MUC2	15 (57.7)	3 (75.0)	2 (9.1)	0.001	18 (90.0)	1 (12.5)	1 (5.6)	<0.001
MUC5ac	26 (100)	4 (100)	18 (100)	>0.999	19 (95.0)	8 (100)	17 (94.4)	0.800
SMAD4 loss	2 (7.7)	0	0	0.368	1 (5.0)	2 (25.0)	6 (33.3)	0.081
S100A4	3 (11.5)	0	3 (13.6)	0.735	6 (30.0)	5 (62.5)	1 (5.6)	0.080
MSH2 loss	4 (15.4)	0	0	0.115	3 (15.0)	2 (25.0)	3 (16.7)	0.839
APC loss	2 (7.7)	0	7 (31.8)	0.056	7 (35.0)	4 (50.0)	16 (88.9)	0.003
p53	16 (64.5)	4 (100)	13 (59.1)	0.283	5 (25.0)	4 (50.0)	3 (16.7)	0.201
p16	8 (30.8)	2 (50.0)	9 (40.9)	0.648	9 (45.0)	4 (50.0)	4 (22.2)	0.245
p21	0	0	0	>0.999	2 (10.0)	2 (25.0)	4 (22.2)	0.503
CD24	4 (15.4)	1 (25.0)	4 (18.2)	0.885	6 (30.0)	4 (50.0)	4 (22.2)	0.236

Table 7. Expression rate of oncogenic proteins in invasive portion

Variables	PM-INV (n=20)	TP-INV (n=8)	F-INV (n=18)	P Value
MUC1	5 (26.3)	7 (87.5)	16 (88.9)	<0.001
MUC2	16 (84.2)	1 (12.5)	3 (16.7)	<0.001
MUC5ac	15 (78.9)	7 (87.5)	17 (94.4)	0.382
SMAD4 loss	1 (5.3)	3 (37.5)	14 (77.8)	<0.001
S100A4	6 (31.6)	5 (62.5)	12 (66.7)	0.011
MSH2 loss	5 (26.3)	2 (25.0)	5 (27.8)	0.987
APC loss	8 (42.1)	4 (50.0)	14 (77.8)	0.107
p53	4 (21.1)	6 (75.0)	8 (44.4)	0.029
p16	7 (36.8)	3 (37.5)	6 (33.3)	0.968
p21	2 (10.5)	0	1 (5.6)	0.588
CD24	8 (42.1)	5 (62.5)	10 (55.6)	0.652

Figures

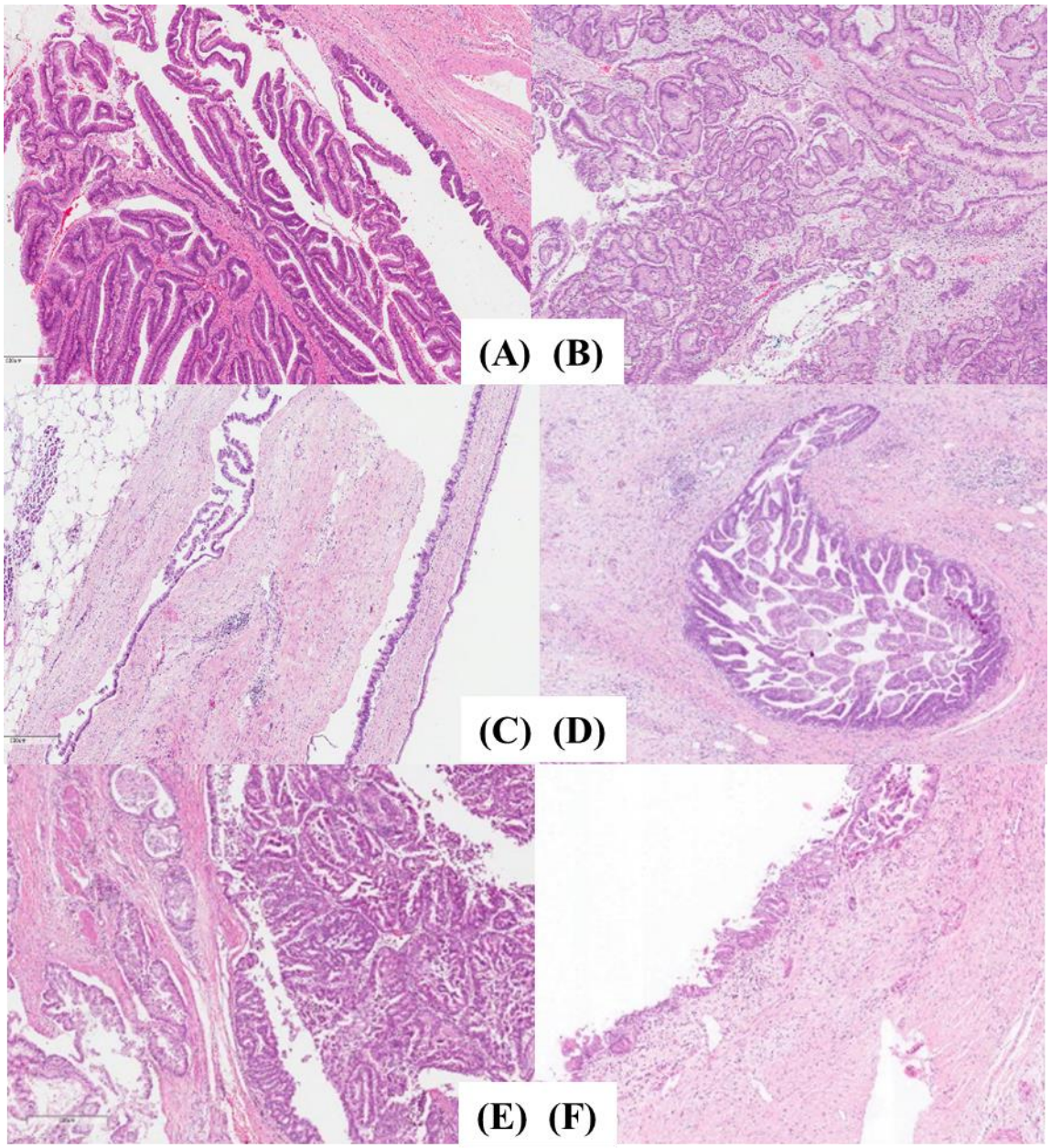

Figure 1. Representative images of (A) noninvasive papillary type (PM-NI), (B) noninvasive tubulary type (T-NI), (C) noninvasive flat type (F-NI), (D) invasive papillary type (PM-INV), (E) invasive tubular type (T-INV), and (F) invasive flat type (F-INV) (Hematoxylin-Eosin, original magnification, X100,)

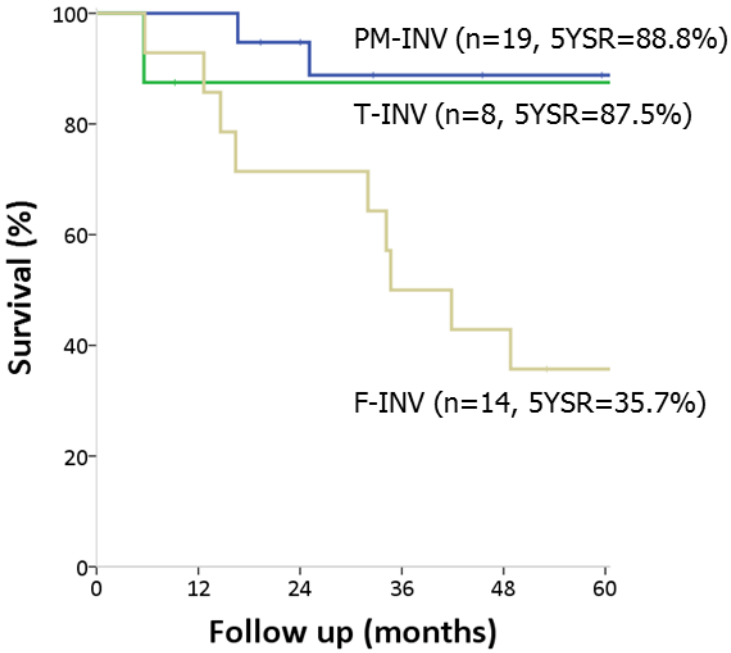

Figure 2. Disease specific survival plot of invasive IPMN according to the morphologic configuration (PM-INV vs. F-INV, p=0.002, T-INV vs. F-INV, p=0.061)

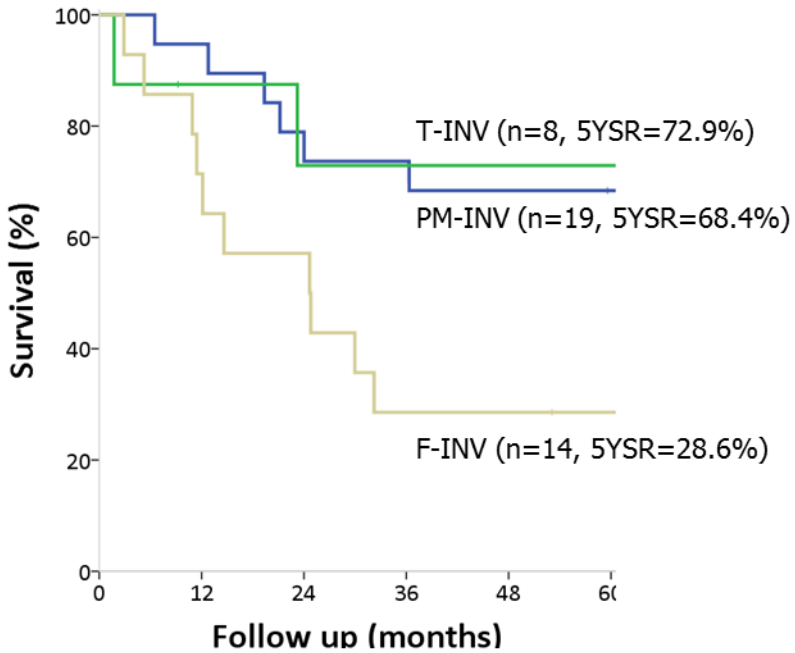

Figure 3. Recurrence free survival plot of invasive IPMN according to the morphologic configuration (PM-INV vs. F-INV, p=0.046, T-INV vs. F-INV, p=0.109)

국문초록

췌관내유두상점액종의 조직학적 특징 및 종양단백질 발현 분석

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장 지 훈

서론: 췌장암의 전구 병변인 췌관내유두상점액종과 췌장상피내종양은 조직학적 유사성으로 인하여 구분이 쉽지 않다. 본 연구에서는 췌관내유두상점액종의 조직학적 및 종양단백질의 발현 특성을 분석하여 그 특징을 확인하고자 하였다.

방법: 2001 년부터 2009 년까지 췌관내유두상점액종으로 수술 한 환자 중, 췌장낭종의 크기가 10mm 이상, 5 mm 이상의 주췌관 확장을 동반, 수술 후 병리검사에서 췌관내유두상점액종으로 확인된 98 명을 분석하였다. 유두상 상피세포의 구조적 모양 및 점액분비 양상, 췌장낭종 벽내결절 유무를 기준으로 유두-점액형, 관형, 편평형 췌관내유두상점액종으로 분류하여 분비샘의 모양, 상피세포 이형성 정도, 상피세포에 종양세포 존재 유무, 점액 존재 유무를 분석하였고, 발현되는 종양단백질에 대한 11 개의 항체(MUC1, MUC2, MUC5ac, SMAD4, S100A4, MSH2, APC, p53, p16, p21, CD24)를 이용해 조직학적 재분류에 따른 종양발생단백질의 발현 정도를 측정하여 비교하였다.

결과: 전체 환자군의 평균나이는 64.5 세, 남자 비율은 66.3%였으며, 병변의 위치는 췌장머리부분 52.0%, 췌장몸통-꼬리부분 34.7%, 미만성 분포는 13.3%였다. 주췌관형은 15.3%, 분지췌관형은 61.2%에서 관찰되었으며 췌십이지장절제술은 46.9%에서 이루어졌다. 전체 환자 중 각각 19.6% 와 21.7%에서 수술후 보조항암화학치료 또는 방사선치료를 받았다. 이형성 정도에 따라 분류했을 때, 저도 또는 중등도 이형성은 39.8%, 고도 이형성은 13.3%, 국소침윤(침윤 깊이 < 500 ㎛)은 12.2%, 광범위 침윤(침윤 깊이 ≥ 500 ㎛)은 18.4%, 췌장암과 동반된 경우는 16.3%였다. 조직학적 특성에 따라

분석했을 때, 위형 세포는 비침습군에서 관찰빈도가 높았다. 침습군 중 유두형에서 장세포형 관찰 빈도가 높았으며(75.0%), 편평형은 위형세포의 빈도가 높았다 (72.2%, p<0.001). 점액은 유두형에서 침습 정도와 상관없이 흔하게 관찰되었으며, 편평형에서는 침습군에서 72.2%에서 보였으나 점액의 양은 적었다. 낭종부분과 침습 부분을 나누어 면역화학염색을 한 결과, 낭종부분의 편평형은 MUC1 과발현, MUC2 저발현을 보였고, 유두형은 정반대되는 발현양상을 보였다. 편평형에서는 높은 빈도의 APC 소실을 보였다. 침습부분의 경우, MUC1, MUC2, SMAD4, S100A4, p53 은 유의한 차이를 보이고 있었으며, MUC2 는 유두형에서 보다 많은 발현을 보였고 (84.2%), 편평형은 SMAD4 소실 (p<0.001), S100A4 과발현 (p=0.011), p53 과발현 (p=0.029)을 보였다. 침습군 중, 완전절제가 이루어진 환자들을 대상으로 한 생존분석 결과, 질병-특이 생존률 및 무병생존률은 편평형이 유두형보다 유의하게 낮았다. (각각 p=0.002, p=0.046),

결론: 췌관내유두상점액종을 유두상 상피세포의 구조적 모양을 기준으로 유두-점액형, 관형, 편평형으로 나누어 조직학적으로 구분했을 때, 각 아형에 따라 조직학적 특징 및 종양단백질 발현에 차이가 있었고, 편평형의 경우 췌장상피내종양과 유사한 특징을 보였으며, 침습군의 경우 편평형이 가장 예후가 좋지 않았다. 이와 같은 분류는 임상적으로 적용함으로써, 췌관내유두상점액종을 세분하여 보다 정확한 예후 예측이 가능하게 할 것으로 예상된다.

주요어 : 췌관내유두상점액종, 췌장상피내종양, 췌장암

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