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ORIGINAL ARTICLE

p53 expression in invasive pancreatic adenocarcinoma and precursor lesions

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Abstract

Patients with pancreatic adenocarcinoma are known to have a high mortality rate. The 5-year survival rate still remains low even now compared to that of the 1960's despite new advances in management including surgery, chemotherapy, pathological classification and molecular diagnostic technologies. Precursors to invasive pancreatic adenocarcinoma have been identified in the last ten years that include mucinous cystic neoplasm, intraductal papillary mucinous neoplasm and pancreatic intraepithelial neoplasia. p53 protein accumulation in the nuclei is a common molecular event in most human neoplasms. Our objective is to investigate p53 expression in pancreatic adenocarcinoma and precursor lesions and their significance. The selected study material encompassed 31 invasive ductal adenocarcinoma, 15 mucinous cystic neoplasm and papillary mucinous neoplasm, and 27 cases of pancreatic intraepithelial neoplasia including grade 1, 2 and 3. Immunoscore was given for each case based on intensity of staining and percentage of cells positive and compared between precursor lesions and invasive adenocarcinoma. A score of 50 and above was considered significant. The results showed that p53 expression increased progressively and significantly with the grade of pancreatic intraepithelial neoplasia and adenocarcinoma (p-value < 0.001). These findings support the concept of multistep carcinogenesis in pancreatic adenocarcinoma and suggest that p53 inactivation occurs in the progression of precursors to pancreatic adenocarcinoma.

Key words: malignancy, pancreas, immunoperoxidase, p53

INTRODUCTION

Adenocarcinoma of the pancreatic duct constitutes 85% of all pancreatic malignancy.[1] In the United States, it is the fourth most common cause of death from cancer.[2] In Japan, it is the fifth most common cause of cancer death and the five-year survival rate is 5.5%. This is mainly due to late stage at presentation as more than 40% of patients with pancreatic cancers are detected at stage IV B.[3] The Malaysian National Cancer registry data of 2003 showed that pancreatic adenocarcinoma is rare in Malaysia and constituted only 1.2% of all reported cancers.[4] Non-invasive precursor lesions of the pancreatic ducts have been observed in resected pancreata and autopsy tissues.[5,6,7] It was suggested that the detection and prevention of these lesions may offer a cure in early pancreatic cancers. The recent classification of non-invasive epithelial lesions by Hruban *et al*[8] includes pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN). PanIN has been further subdivided into 1A, 1B, 2 and 3 based on morphology and grade of dysplasia. Likewise, IPMN and MCN have been classified into adenoma, borderline and carcinoma *in-situ* based on the grade of dysplasia.[8,9] It is well known that TP53 gene is one of the most common targets for mutation in many cancers. Hermanova *et al*[10] noted that proportion of p53 protein expression increased progressively from normal pancreatic duct, to precursor lesions to invasive adenocarcinoma. The purpose of this study is to investigate the p53 immunohistochemical expression profile in pancreatic adenocarcinoma and precursor lesions.

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MATERIALS AND METHODS

This is a retrospective study. A total of 180 cases of pancreatic resection specimens were received by the Department of Pathology of Selayang Hospital between 2005 to 2010. Histological sections from these cases were reviewed. The selected study material included 31 invasive pancreatic ductal adenocarcinoma and 15 non-invasive IPMN/MCN. Furthermore, 27 PanIN lesions detected in 15 cases of invasive ductal carcinoma, 8 cases of chronic pancreatitis and 4 cases from benign cystic neoplasms were included in the study (Table 1). In the cases of invasive pancreatic cancer, PanIN lesions were selected from outside the invasive cancer nests. PanIN lesions were classified according to Hruban *et al* into PanIN-1A, PanIN-1B, PanIN-2 and PanIN-3 lesions.[8] The PanIN-1A lesions show columnar cells with abundant apical cytoplasmic mucin, basally located nuclei while PanIN-1B lesions in addition have well formed papillae. Since both PanIN-1A and B have no nuclear atypia, they were grouped together as PanIN-1 in this study. In view of the small numbers of IPMN and MCN cases, they were grouped together, but separated according to the grade of dysplasia such as benign (adenoma), moderate (borderline) and carcinoma-*in-situ*.

p53 immunohistochemistry

Histological sections cut at 4μm were made from representative formalin-fixed, paraffin-embedded blocks of the selected lesions. The slides were mounted on polylysine coated glass slides and examined for p53 immunoreactivity against a monoclonal mouse anti-human p53 protein clone DO-7 (1:25 dilution) obtained commercially from DAKO using the standard avidin-biotin complex immunoperoxidase (IP) method with overnight incubation. Sections from ovarian serous adenocarcinoma that revealed strong positivity for p53 were used as positive controls. Negative controls were performed by incubating the sample without the primary antibodies. For each selected PanIN, IPMN, MCN and adenocarcinoma, the entire lesion in the section was analyzed under high power (magnification X400) microscopy and evaluated for p53 expression (Figure 1) and only nuclear staining is considered positive.

The p53 immunoscore was obtained by multiplying the percentage of positive cells with the numeric score based on the previous published criteria by Hermanova et al.[10] The numeric score was given as follows: score 0 - no staining, nuclei stained blue; score 1- weak staining, nuclei blue-brown; score 2 - nuclei brown; and score 3- nuclei deep brown or black. Scores of above 50 were considered significantly raised.

Statistical analysis was done using SPSS version 17.0. As mentioned before, the IPMN and MCN were grouped together as the numbers were very small. PanIN-1A and PanIN-1B were categorized as one group i.e. PanIN-1. Statistical

TABLE 1: Histopathological background of pancreatic intraepithelial neoplasms (PanIN) included in the study

Pathological diagnosis	Number of Pancreatic intraepithelial neoplasm		
	PanIN-1	PanIN-2	PanIN-3
Adenocarcinoma	7	4	4
Chronic pancreatitis	2	5	1
Serous cystadenoma	1	0	0
Mucinous cystic neoplasm	1	0	0
Intraductal papillary mucinous neoplasm	2	0	0
Total	13	9	5

PanIN-1= Pancreatic intraepithelial neoplasia 1; PanIN-2 = Pancreatic intraepithelial neoplasia 2; PanIN-3= Pancreatic intraepithelial neoplasia 3.

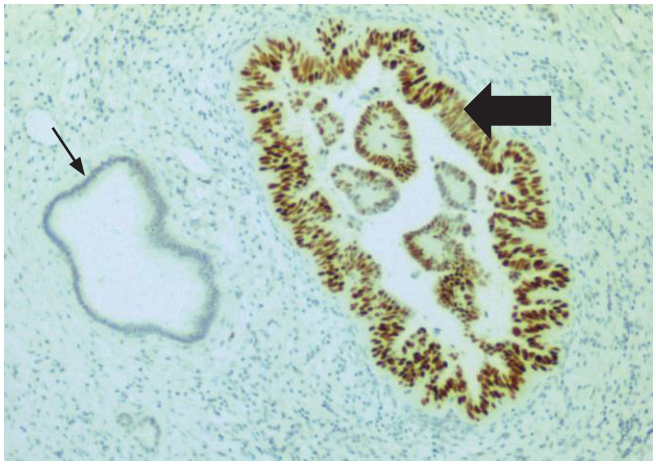

FIG. 1: p53 immunopositivity in pancreatic intraepithelial neoplasia 3 (arrow) and negative in normal gland (narrow arrow). Magnification x 40.

analysis was performed using the cross tabulation and chi square test. A p value of <0.05 was considered statistically significant.

RESULTS

There were 13 cases of PanIN-1 (Figure 2), 9 cases of PanIN-2 (Figure 3), 5 cases of PanIN-3 (Figure 4), 11 cases of MCN (Figure 5) and 4 cases of IPMN. Two cases each of IPMN and MCN were categorized under borderline tumour in view of epithelial dysplasia. There were 31 cases of pancreatic adenocarcinoma. There was no case of carcinoma in situ in the study.

Results of p53 expression in pancreatic adenocarcinoma and precursor lesions are given in Table 2.

A majority of those with adenoma of IPMN/MCN, PanIN-1, PanIN-2 have immunoscores of less than 10, whereas, a majority of those with PanIN-3 and adenocarcinoma have immunoscores of 150-300. However, the minimum expected count is only 0.44, which is very low, due to the small sample size of 73. The chi-square value is 63.121 and the p-value is less than 0.001. Thus, there is a significant association between p53 expression and various precursor lesions.

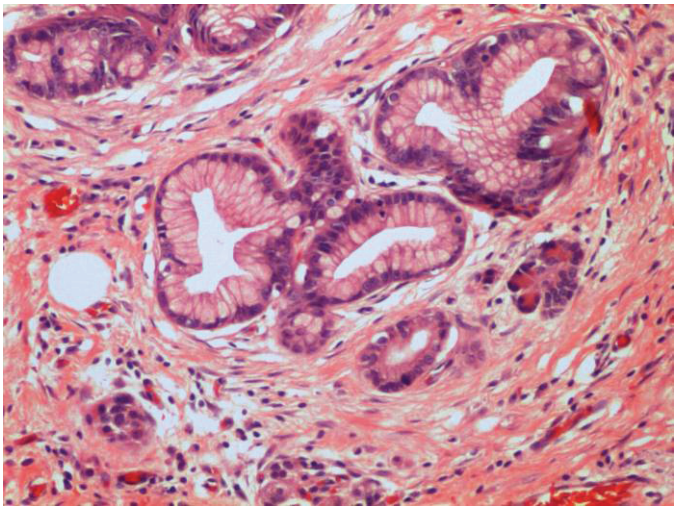

FIG. 2: Pancreatic intraepithelial neoplasia 1. Glands lined by mucinous columnar epithelial cells with basally located nuclei. H&E stain x 100.

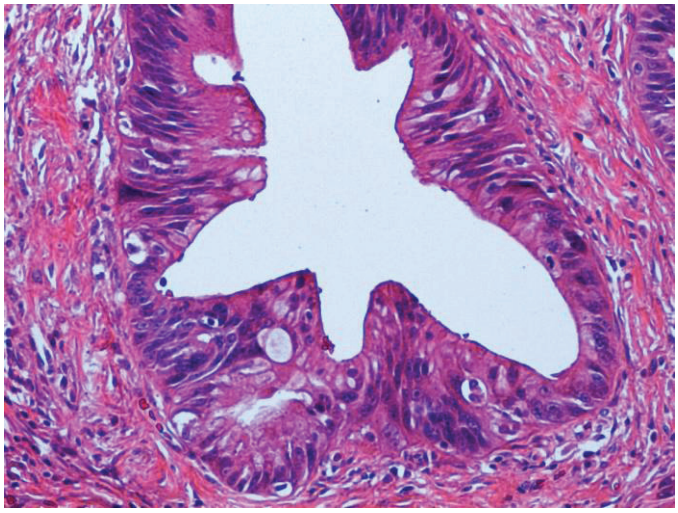

FIG. 3: Pancreatic intraepithelial neoplasia 2. Glands exhibiting nuclear stratification. H&E stain x 100.

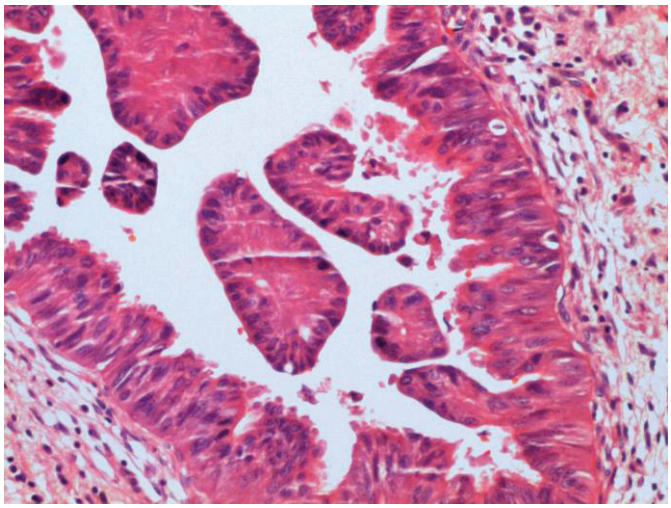

FIG. 4: Pancreatic intraepithelial neoplasia 3. Glands showing budding of cellular tufts with nuclear atypia. H&E stain x 100.

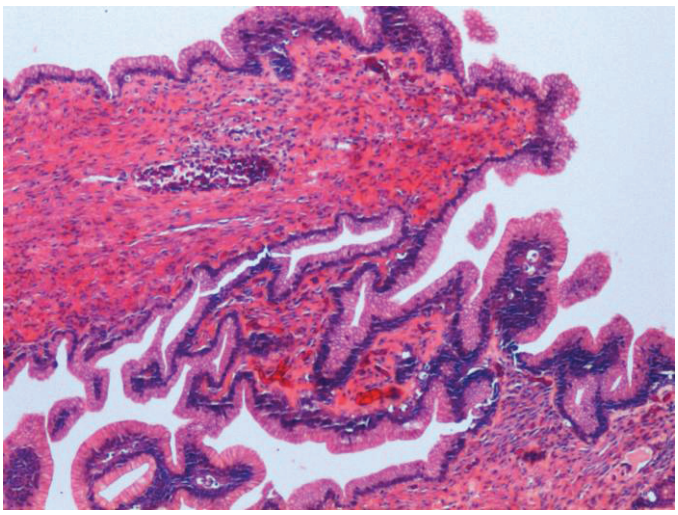

FIG. 5: Benign mucinous cystic neoplasm. H&E stain x 40.

TABLE 2: Expression of p53 in pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasm, mucinous cystic neoplasm and pancreatic adenocarcinoma

Lesion	p53 expression immunoscore				
	<10	10-49	50-149	150-300	Total
IPMN/MCN (adenoma)	10(90.9%)	1(9.1%)	0	0	11(100%)
IPMN/MCN (borderline)	0	0	4(100%)	0	4(100%)
PanIN-1	10(76.9%)	3(23.1%)	0	0	13(100%)
PanIN -2	5(55.6%)	0	3(33.3%)	1(11.1%)	9(100%)
PanIN-3	1(20%)	1(20%)	1(20%)	2(40%)	5(100%)
Adenocarcinoma	2(6.5%)	3(9.7%)	8(25.8%)	18(58.1%)	31(100%)
Total	28(38.4%)	8(11%)	16(21.%)	21(28.8%)	73(100%)

Person Chi-Square = 63.121, df = 15 , p-value < 0.001. Minimum expected count = 0.44
PanIN-1= Pancreatic intraepithelial neoplasia 1. PanIN-2 = Pancreatic intraepithelial neoplasia 2
PanIN-3= Pancreatic intraepithelial neoplasia 3. PMN= Intraductal papillary mucinous neoplasm
MCN = Mucinous cystic neoplasm

DISCUSSION

In this study, it was found that there is a progressive increment in p53 expression from adenoma, to borderline IPMN/MCN, PanIN-1, 2 and 3, and adenocarcinoma. Among the adenoma of IPMN/MCN, all 11 cases showed p53 expression below the immunoscore of 50. Among the PanIN-1 lesions, 77% of p53 expression had immunoscores of <10 and 23% scored between 11 and 49. Among the PanIN- 2 lesions, 56% of p53 expression had immunoscores of < 10. In addition, a good proportion (33.3%) of the scores was between 50 and 149, while 11% were in the 150-300 range, indicating a progression of p53 expression. Among the PanIN- 3 lesions, the lower level immunoscore scores were equal in proportion, while the 150-300 level was twice as much. The progressive p53 expression is most evident in the adenocarcinoma lesion. Overall, there is a progressive p53 expression in the different lesions, though it is less conclusive due to the small numbers of samples in some of the categories. In this study, an alternative would have been the grouping of the Pan IN lesions into one, but this would cause loss of information of the expression of p53 on these precursor lesions. The other alternative would have been the grouping of the immunoscores into smaller number of levels. However, this would not show progressive p53 expression across all the immunoscore ranges. Thus, the best option is to increase the sample size. A bigger sample size would have given a clearer picture of p53 progression. Therefore, it is highly recommended that for future studies, the sample size should be increasd. This can be done by conducting multicentre studies.

The progressive trend of p53 expression in PanIN-1, PanIN-2, PanIN-3 and adenocarcinoma is also in line with other studies.[10,11,12.] Abe *et al*[12] compared p53 expression between IPMN and PanIN adjusted according to the same histological grade. PanIN-1 is thought to be equivalent to IPMN with no dysplasia, PanIN-2 to borderline IPMN that is characterized by moderate dysplasia and PanIN-3 corresponded to IPMN with severe dysplasia/carcinoma in situ. They did not include MCN. Their study showed that there were no significant differences between lower grade IPMN and lower grade PanIN lesions whereas p53 expression was significantly higher among PanIN-3 than IPMN with severe dysplasia. In our study, little difference in p53 immunoscore was observed between PanIN-1 and adenoma of IPMN/MCN. On the other hand, we have only 4 borderline tumours and no carcinoma-in-situ. All the 4 borderline tumours showed (Table 2) scores of 50-149. Fisher exact test was conducted to compare the immunoscore between borderline IPMN/MCN and PanIN-2. After collapsing 50-149 and 150-300 expression levels, the p-value from Fisher's Exact Test was

0.098, which is more than 0.05. Thus, there is no difference in expression between these 2 groups. Comparison between PanIN-3 lesions with carcinoma in situ of IPMN/MCN cannot be made because of the absence of case. Our study is somewhat different from other previous studies because this study also includes MCN. The purpose of this study is to investigate p53 expression in pancreatic adenocarcinoma and its precursor lesions. We found a significant association between p53 expression and the various precursor lesions, suggesting that p53 expressions may be helpful in evaluating the progression of the different precursor lesions. However, the minimum expected count is only 0.44. This is due to the small sample size of only 73. This is one of the limitations of this study.

CONCLUSION

These findings show significant p53 expression in pancreatic adenocarcinoma and the precursor lesions, which support the concept of multistep carcinogenesis in pancreatic adenocarcinoma. It also suggests that p53 inactivation occurs in the progression of preinvasive to invasive lesions. Future studies with large sample sizes should be conducted to strengthen these findings and may help develop a strategy for early recognition and management of pancreatic duct cancer.

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