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Intercalated duct cell is starting point in development of pancreatic ductal carcinoma?

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Abstract

Background: Although it is well known that the pancreatic ductal carcinoma may develop having a relationship to the mucous gland hyperplasia (MGH) with atypia (PanIN-IB by PanIN system), the starting point of this atypical MGH is unclear. To know it, we examined the pancreas tissue using many methods described below.

Methods: 1. Twenty-seven surgically resected pancreas tissue specimens, including pancreatic ductal carcinomas (PDC), chronic pancreatitis and normal pancreas, were investigated using immunohistochemical stainings for MUC1, MUC6, 45M1, Ki67 and p53. 2. DNA extraction and analysis of K-ras mutation at codon 12 using microdissection method: The paraffin blocks with 16 regions including the intercalated duct cell (IC) adjacent to the atypical MGH were prepared for DNA extraction. Mutation of K-ras codon 12 was analyzed and compared in enriched polymerase chain reaction-enzyme-linked minisequence assay (PCR-ELMA).

Results: 1. In the normal pancreas, although no positive cell was seen in 45M1, p53, Ki67, the cytoplasm of IC were always positive for MUC1 and sometimes positive for MUC6. In the pancreas with fibrosis or inflammation, MGH was positive for MUC6 and 45M1. And atypical MGH was positive for MUC1, MUC6 and 45M1. Some IC adjacent to the atypical MGH was positive for Ki67 as well as atypical MGH. The carcinoma cells in all cases of PDC were diffusely positive for MUC1, 45M1, p53 and Ki67, and focally positive for MUC6. 2. In K-ras mutation, we examined the regions including IC adjacent to the atypical MGH, because the immunohistochemical apomucin stainings of these regions resembled those of PDC as described above. And K-ras mutation was confirmed in 12 of 16 regions (75%). All mutations were a single mutation, in 6 regions GTT was detected, in 4 regions GAT was detected and in 2 region AGT was detected.

Conclusion: Some intercalated duct cell may be the starting point of the pancreatic ductal carcinoma, because the exhibitions of mucin expressions, Ki67, p53 and K-ras mutation in some intercalated duct cell resembled those of mucous gland hyperplasia or pancreatic ductal carcinoma.

Background

The pancreatic ductal carcinoma (PDC) is fatal, even if its size is very small [1,2]. Therefore, it is very important to know the characteristics of pre-cancerous lesion of PDC for the preventive medicine and the early detection of PDC.

It has been well known that the mucous gland hyperplasia (MGH) (goblet cell metaplasia) is one of pre-cancerous lesion of PDC [3-5], and today, the histogenesis of PDC has been accepted by a model for a sequence of morphological changes, named the PanIN system [6], in which the lower grade PanIN is thought to exchange to higher grade PanIN and finally to PDC [7-9]. And MGH with no atypia is almost equal to PanIN-1A and MGH with atypia is almost equal to PanIN-1B in this system [6-9].

The main purpose of the present study was to investigate the starting point of MGH as pre-cancerous lesion of PDC.

Methods

Twenty-seven surgically resected pancreas tissue specimens, including 14 cases of PDC, which consisted of moderately differentiated tubular adenocarcinoma, 7 cases of the chronic pancreatitis and 6 cases of the normal pancreas, were assessed at the Department of Pathology, Juntendo University Shizuoka Hospital between 1998 and 2004. Informed consent for the medical examinations described below was obtained from all patients.

The specimens were fixed in 10% formalin solution for 1 – 5 days and prepared by cutting the lesions into 4 – 5 mm sections. Sections were embedded in paraffin, sectioned at a thickness of 4 µm and stained with hematoxylin and eosin (HE) and many immunohistochemical stainings described below. And after these stainings, some paraffin blocks were used for DNA extraction.

1. Immunohistochemical stainings were performed by the avidin-biotin-peroxidase complex method

anti-p53 oncoprotein (p53: DO7, monoclonal antibody, Novocastra Inc., UK), anti-MUC1 glycoprotein (MUC1: human CA 15-3, DF3, monoclonal antibody, DAKO, USA), anti-MUC6 glycoprotein (MUC6: CLH5, monoclonal antibody, Novocastra Inc., UK), anti-human gastric mucin-45M1 (45M1, monoclonal antibody, Novocastra Inc., UK) and anti-Ki-67 (MIB-1, monoclonal antibody, Coulter Japan Inc., Japan) (MUC1 and MUC6 at a dilution of 1:50, 45M1 at a dilution of 1:50, p53 at a dilution of 1:100 and Ki67 at a dilution of 1:100). Every staining was performed with 15-minute microwave treatment.

2. DNA extraction and analysis of K-ras mutation at codon 12

In 16 regions including the intercalated duct cells (IC) adjacent to the atypical MGH (panIN-1B), the existence or nonexistence of the K-ras codon 12 mutation was investigated, because the immunohistochemical expressions of the IC were very interesting, as described in the results, and K-ras codon 12 mutation is well known as one of the popular genetic abnormalities of PDC and MGH [10-13].

Paraffin blocks with the target foci were prepared for DNA extraction. The target foci were microdissected using a 20-gauge needle, comparing the slide with HE staining of same position. Extracted DNA was diluted with 5 ml of TaKaRa DEXPAT (for DNA Extraction from Paraffin-embedded Tissue, TaKaRa Biomedical Inc.).

Mutation of K-ras codon 12 was analyzed and compared in enriched polymerase chain reaction-enzyme-linked minisequence assay (PCR-ELMA) [14,15]. In PCR-ELMA, upstream for the first and second PCR were 5'-TAAACTTGTGGTAGTTGGAAC-3', downstream for the first PCR was 5'-GTTGGATCATATTCGTCAC-3', and downstream for the second PCR was 5'-CAAATGATCTGAATTAGCTG-3'. The first PCR reaction was performed containing 1 µL of DNA lysate, 100 µM dNTP, 1.5 mM MgCl₂, 1 µM each primers, 0.625 U Taq DNA polymerase and 1 × PCR buffer [containing 10 mM Tris-HCl (pH 8.3 at 25°C), 50 mM KCl and 0.001%(w/v) gelatin] in thermal cycler. And 10 µL of the denatured second PCR product was hybridized with probes for detecting the K-ras codon 12 wild-type (GGT) and six mutants (GAT, GCT, GTT, AGT, CGT and TGT) DNA were immobilized, at 55°C for 30 minutes, and 100 µL of biotinylated A and 0.01 U of TdqdNA polymerase were added and incubation was continued at 55°C for 30 minutes.

In development, 100 µL of avidine-horseradish peroxidase conjugate was contained and the mixture was performed at room temperature for 30 minutes, and 100 µL of tetramethylbenzidine (TMB) substrate was contained and the plates were performed in the dark at room temperature for 20 minutes. And 100 µL of stop solution was contained and the light absorbance of each sample was measured by spectrophotometry (Multiskan Multisoft, Labsystems, Tokyo) with a 450 nm filter wavelength (Figure 1).

Results

1. Immunohistochemical studies of pancreas tissue (Table 1)

In the normal pancreas (acinal cell: 100 regions, IC: 100 regions, duct: 100 regions), although no positive cell was seen in 45M1, p53, Ki67, the cytoplasm of IC were always

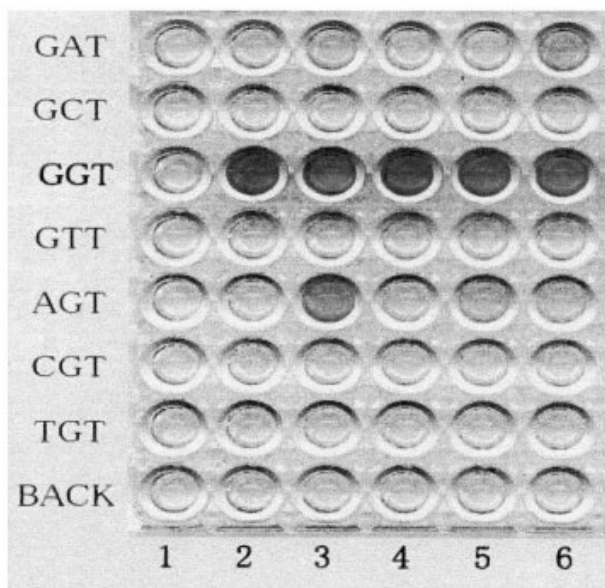


Figure 1
Enriched polymerase chain reaction – enzyme linked mini-sequence assay (PCR-ELMA), showing K-ras codon 12 mutation in mucous gland hyperplasia of the pancreas. AGT – type mutation was seen in lane 3.

Table 1: Positivity of Pancreatic Epithelium for Several Stainings

	MUC1	MUC6	45M1	Ki67	P53
Acinal cell (100 regions)	-	±	-	-	-
Intercalated duct (100 regions)	+	±	-	-	-
Duct (100 regions)	-	±	-	-	-
MGH with no atypia (50 regions)	-	+	+	±	-
Atypical MGH (20 regions)	+	+	+	±	-
Ductal carcinoma (15 lesions)	+	±	+	+	+

MGH: Mucous gland hyperplasia -: negative, +: positive, ±: positive or negative

positive for MUC1 (Figure 2) and sometimes positive for MUC6.

In the pancreas with fibrosis or inflammation, the cytoplasm of the epitheli in MGH [16-18] with both no atypia (50 regions) and atypia (20 regions) was positive for 45M1 (Figure 3) and MUC6. And these atypical MGH (20 regions), which were PanIN-1B by PanIN system [1], were also positive for MUC1. No positive cell for p53 was seen in MGH with atypia or no atypia. Some IC adjacent to the atypical MGH (PanIN-1B) was positive for Ki67 (Figure 4).

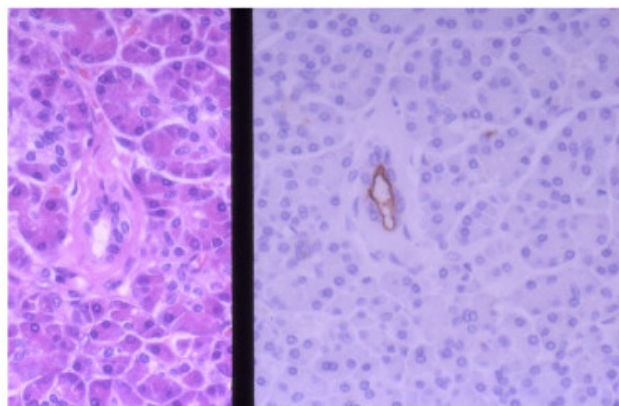


Figure 2
In normal pancreas, only the intercalated duct cells were positive for MUC1. (Left: HE, Right: MUC1, original magnification × 400)

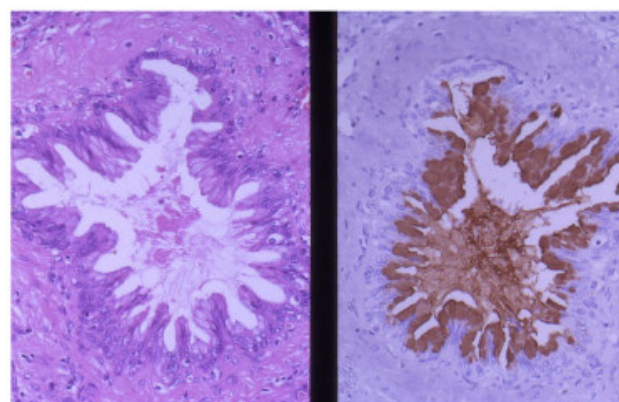


Figure 3
The epithelium of the mucous gland hyperplasia was positive for 45M1. (Left: HE, Right: 45M1, original magnification × 200)

The carcinoma cells in all cases (14 cases) of PIDC were diffusely positive for MUC1, 45M1, p53 and Ki67, and focally positive for MUC6. Namely, in all cases, the nuclei of many carcinoma cells were positive for p53 and Ki67. And in all cases, the expression of MUC1, MUC6 and 45M1 apomucins was found in the cell membranes of many carcinoma cells and the cytoplasm of some carcinoma cells.

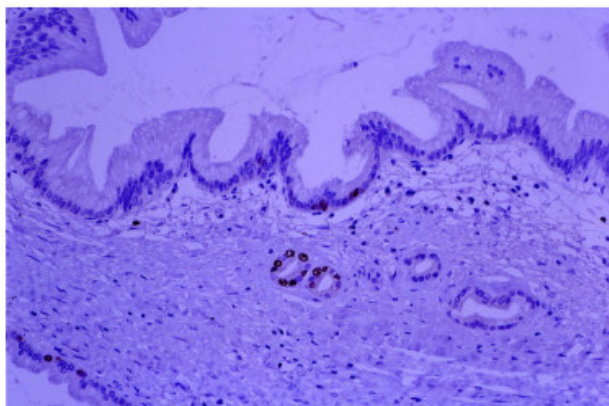


Figure 4
The intercalated duct cells adjacent to the atypical mucous gland hyperplasia were positive for Ki67. (Ki67, original magnification $\times 200$)

2. K-ras mutation

In K-ras mutation, we examined the regions including IC adjacent to the atypical MGH, because the immuno-histochemical apomucins stainings of these regions were almostly equal to those of PDC described above. And K-ras mutation was confirmed in 12 of 16 regions including IC adjacent to the atypical MGH (75%). All mutations were a single mutation, in 6 regions GTT was detected, in 4 regions GAT was detected and in 2 region AGT was detected.

Discussion

Generally, the neoplasia or pre-neoplasia will have some characteristics of its original cell and may develop from the proliferative cell in the organ. Thus, it is important to find out the normal cells which have the characteristics of the neoplasia or pre-neoplasia for detecting the starting point of the neoplasia.

Therefore, in the present study, the mucin expressions which are known as one of the characteristics of PDC [19-24] and K-ras mutation which is very famous gene abnormality in PDC [10,11] were investigated the non-neoplastic tissue or the neoplasia.

Although the results in the current study may be little novel and supportive of the known facts, these findings should reveal that the present study was correct.

That is to say, in the current study, the intercalated duct cells (IC) were positive for MUC1, although the other normal epitheli of the pancreas were negative for it. In the

other reports, the positivity for MUC1 in the normal pancreas lack consistency, positive [22] or negative [21,23]. The reason of this discrepancy is unknown, however, in this study, the normal pancreas tissues were obtained from surgical resection due to non-pancreatic diseases and these specimens were quickly fixed in formalin solution. Thus, these specimens were suitable for the detailed histological examination in the normal pancreas and the results in the current study should be reliable.

And the results in the present study indicated that the mucin phenotypes of the atypical MGH (PanIN-1B by PanIN system) had a diffusely positive reaction for both anti-MUC1 staining and anti-45M1 staining, and had a sparsely positive reaction for anti-Ki-67 staining, and these findings resembled those of the pancreatic ductal carcinoma (PDC), except for the expression of p53 (MGH: negative, PDC: positive). The expressions of the apomucins, Ki67 and p53 in MGH and PDC in the present study matched mostly those of other reports [20,21,23-26].

It has been well known that the K-ras mutation may confirm in MGH and PDC [10-13] and the results in the current study also showed that the K-ras codon 12 mutations were confirmed in the regions including IC adjacent to the atypical MGH of human pancreas (GGT \rightarrow GTT in 6 regions, GGT \rightarrow GAT in 4 regions and GGT \rightarrow AGT in 2 region). And some IC adjacent to the atypical MGH was positive for Ki67.

Namely, the IC adjacent to the atypical MGH (PanIN-1B), which is sometimes proliferative cell, had the mucin phenotypic expression and high frequency of K-ras mutation as well as PDC.

Thus, we think that some IC is the starting point of MGH and may be thought to be the starting point of PDC, considering the histogenesis of PDC (from lower grade PanIN to higher grade PanIN, to PDC).

Further molecular studies concerning the intercalated duct cell in various pancreatic disease should be warranted.

Conclusion

Some intercalated duct cell may be the starting point of the pancreatic ductal carcinoma, because the exhibitions of mucin expressions, Ki67, p53 and K-ras mutation in some intercalated duct cell resembled those of mucous gland hyperplasia or pancreatic ductal carcinoma.

Abbreviations

IC, intercalated duct cell ; PDC, pancreatic ductal carcinoma ; MGH, mucous gland hyperplasia

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