Acta Medica et Biologica
Vol. 46, No.3, 113-120, 1998

Primary Pancreatic Ductal Microcarcinomas Arising from Interlobular Duct and Ductules

Miki YAMANO, Hidenobu WATANABE, Hiroyuki MATSUBAYASHI, Yoshiroh IGA, Kazuo MURAYAMA, Keiji MATSUDA, Ken NISHIKURA, Yoichi AJIOKA and Yoshio SHIRAI

First Department of Pathology, Niigata University School of Medicine, Niigata, Japan

Received September 1 1998; accepted September 7 1998

Summary. **We report an interesting case with a primary ulcerating tumor of the ampulla of Vater associated with multiple primary pancreatic ductal microadenocarcinomas. All of these pancreatic microcarcinomas (number, 69; size, 0.04-1.25mm) were histologically proved to be primary and independent of each other, and were distributed throughout the head, body, and tail of the pancreas. They were all located in the small interlobular ducts and the ductules (including intercalated ducts and centroacinar cells). Sixty-three of 69 microcarcinomas were *in-situ* carcinomas, and the remaining 6 were microinvasive. All microcarcinomas were well-differentiated tubular adenocarcinomas with ordinary cell phenotypes, high-grade cytologic atypia, and adjacent only to normal ordinary pancreatic ductal cells, but not to mucous cell hyperplasia. Immunohistochemically, all of the microcarcinomas showed p53-protein overexpression (p53 positive index: 91.6±8.10% in 12 tumors tested), high proliferative activity (Ki-67 positive index: 64.9±13.3% in the same 12 tumors), and no Ki-ras mutation detected by nested PCR-RFLP in the 12 carcinomas. We concluded that pancreatic microcarcinoma in this case arose from ordinary pancreatic ductal cells of the small ducts and ductules by way of p53 alteration without Ki-ras mutation, and that histogenesis was different from that of the more common Ki-ras-mutated carcinoma arising from metaplastic mucous cells of mucous cell hyperplasia.**

Key Words—pancreas, microcarcinoma, histogenesis, p53-staining, Ki-67 staining, Ki-ras mutation.

Correspondence: Miki Yamano, First Department of Pathology, Niigata University of Medicine, Asahimachi-dori1-757, Niigata 951-8510, Japan.

INTRODUCTION

Solid-type (or common type) pancreatic ductal carcinoma carries a very poor prognosis. Advanced techniques for its early detection have focused on detecting abnormalities in the main pancreatic duct. However, it remains unknown whether or not the primary site of solid-type human pancreatic ductal carcinoma is the main pancreatic duct. Because almost all pancreatic cancers are advanced by the time of clinical diagnosis, the primary sites are difficult to determine. As to the histogenesis of human pancreatic ductal carcinoma, mucous cell hyperplasia is reported to be the most important precursor [1,5,7,10], but there is no precise data on whether or not pancreatic carcinoma arises from ordinary pancreatic epithelium.

Furuta et al. demonstrated using histological reconstruction that 20 of 36 advanced solid-type adenocarcinomas arose not from the main pancreatic duct but from branch ducts [2]. Whereas, Pour et al. reported that 7 solid-type (size not given) *in-situ* carcinomas (CISs) originated from pancreatic ductules (intralobular ducts with their terminal branches), and 1 from ducts (including main pancreatic duct and its first and second interlobular ducts) [8]. Mizumoto et al. reported a surgical case of CIS (size not given) with microinvasion arising both from the main pancreatic duct of the pancreatic head and from the Santorini duct [5]. Fitzgerald and Cubilla described 4 examples of multiple solid-type CISs from surgical and autopsy materials with and without microinvasion. These tumors developed in the main pancreatic duct and its branches in one case, in

large and small ducts in two cases, and in ducts and ductules in another [1].

Most of these human CISs previously reported were accompanied by mucous cell hyperplasia around the cancer tissue and seem to have the same phenotype as that of the mucous cell hyperplasia, judging from diagnostic pictures in their papers [1,8]. However, some of these CISs seem to be of an "ordinary" phenotype with a direct continuity to ordinary pancreatic epithelium [1,5].

In an animal experimental model, Pour et al. reported that multiple CISs resembling human pancreatic duct cell carcinoma developed ubiquitously and simultaneously in pancreatic ducts (main and secondary) and in ductules (interlobular and intralobular) associated with hyperplasia, metaplasia and adenoma around the CISs [7]. Ura et al. reported that CIS arose from large branches of the main pancreatic duct and that atypical hyperplasia arose from small and medium-sized ducts [10]. Scarpelli et al. reported that pancreatic adenocarcinomas closely resembling those of ductal derivation originated from modulated duct-like acinar cells in hamsters [9]. Therefore, experimental data suggests three different possibilities for the origin of pancreatic ductal adenocarcinoma: 1) ductal cells of the main duct and its branch ducts, 2) ductural cells of intercalated duct and centroacinar cells, and 3) acinar cells undergoing duct-like differentiation [9]. In these animal models, there was no precise description on whether the histogenetic cells were of ordinary or metaplasic mucous cells.

We present an interesting case of human primary pancreatic adenocarcinoma composed of 69 microcarcinomas of ordinary phenotype, arising from ordinary epithelium of interlobular, intralobular, and intercalated ducts.

CASE REPORT

A 60-year-old woman presented with pain in the right hypochondrium. She had never suffered from diabetes mellitus or alcohol abuse. Endoscopic and x-ray examinations revealed an ulcerating carcinoma of the ampulla of Vater. Endoscopic retrograde cholangiopancreatography showed a slight dilatation (5mm in diameter) of the main pancreatic duct but no tumor of obstruction. The secondary and peripheral branches were not well demonstrated. Pylorus preserving pancreaticoduodenectomy was performed. Histologically, not only was carcinoma of the ampulla of Vater found but also multiple primary pancreatic ductal microcarcinomas were found in the head of pancreas (Fig. 1). Because of Class V cytology of pancreatic juice taken from the residual pancreas, the residual pancreas was resected 2 months after the first surgery, and also contained multiple microadenocacinomas in the body and tail (Fig. 2).

MATERIALS AND METHODS

A formalin-fixed specimen of the papillary area from the major and minor papilla and the whole pancreas was cut into 4~5mm step-wise tissues and embedded in paraffin. Deparaffinized sections of all paraffin blocks were stained with hematoxylin-eosin (HE) and antibodies against p53 and Ki-67. Histological locations of the carcinoma of the ampulla of Vater and pancreas were mapped on color prints of luminar and cut surfaces (Figs. 1 and 2).

The following stainings were performed for analyses of mucous quality: CA19-9 staining, diastase digestive alcian blue-periodic acid-Schiff reaction (dAB-PAS), high-iron diamine-alcian blue (pH2.5) staining (HID-AB), galactose oxidase-Schiff (GOS) reaction, and paradoxical concanavalin A staining (PCS) for class III mucosubstances. Anti-human endothelial cell CD31 (DAKO) and Victoria blue-hematoxylin eosin (VB-HE) staining were done to confirm lymphatic and venous permeation.

p53 and Ki-67 immunohistochemical study was performed using the avidin biosin peroxidase complex method with a Vectastain Kit (Vector, Burlingame, CA, USA). Immunostaining for PAb 1801 mouse monoclonal antibody against p53 (Oncogene Science, Inc., Manhasset, NY, USA) and Ki-67 antibody MIB-1 (Immnotech, Marseille, France) was performed according to the method we reported previously [4].

As for Ki-ras mutation, the entire regions containing the cancer cells were dissected under a microscope from 5 serial slices of 10 μm xylene deparaffinized-ethanol cleared specimens from 7 areas of carcinoma of the ampulla of Vater, 12 pancreatic microcarcinomas which are chosen at random from all microcarcinomas, 10 foci of mucous cell hyperplasia located apart from microcarcinomas, and 14 foci of ordinary pancreatic duct epithelium. Then, Ki-ras codon 12 mutation was tested with the nested PCR-RFLP method.

As a control, we reviewed 141 cases of carcinoma of the ampulla of Vater from our files to detect metastasis into the resected pancreas. In all cases the pancreas had been cut into 4 to 5mm step-wise tissue.

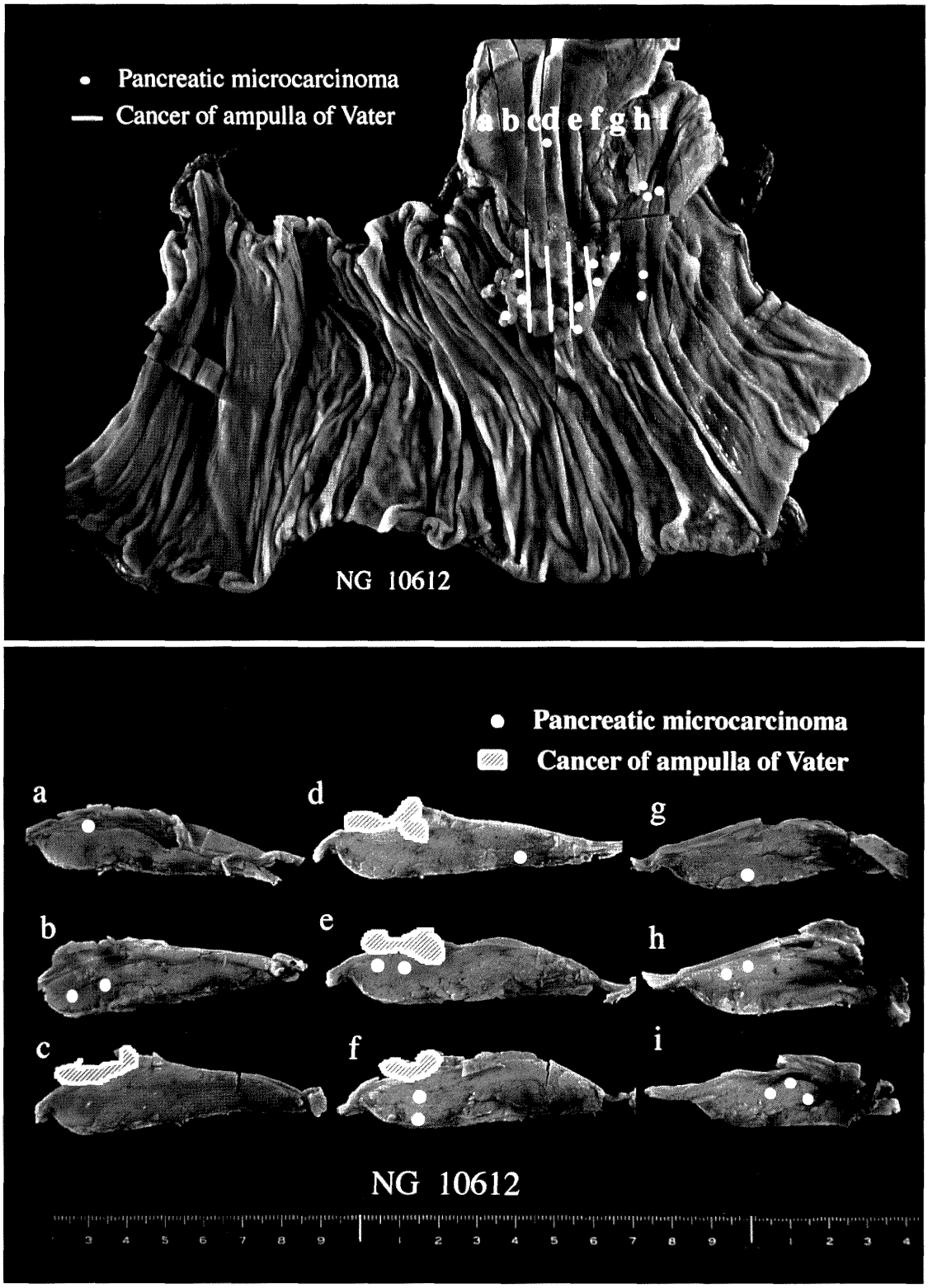

Fig. 1. Mapping of carcinomas in the first resected specimen (**a**, luminar surface view; **b**, cut surface view). Fourteen primary pancreatic microcarcinomas (*white circles*) were histologically detected in the pancreatic head and exsit independently, apart from the carcinoma fo papilla of Vater.

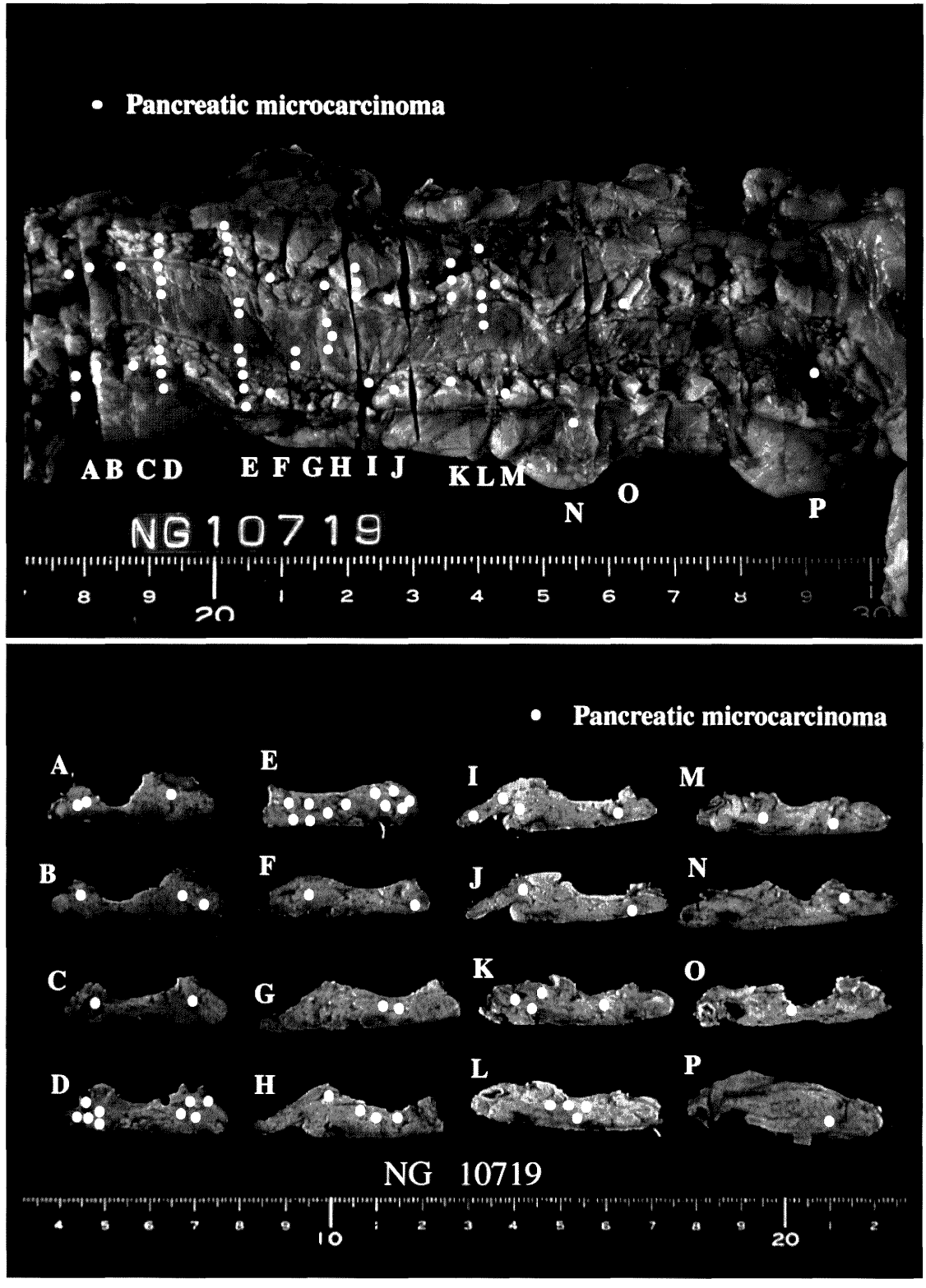

Fig. 2. Mapping of microcarcinomas of the pancreatic body and tail in the second resected specimen (**a**, luminar surface view; **b**, cut surface view). Fifty-five microcarcinomas (*white circles*) were present independently each other.

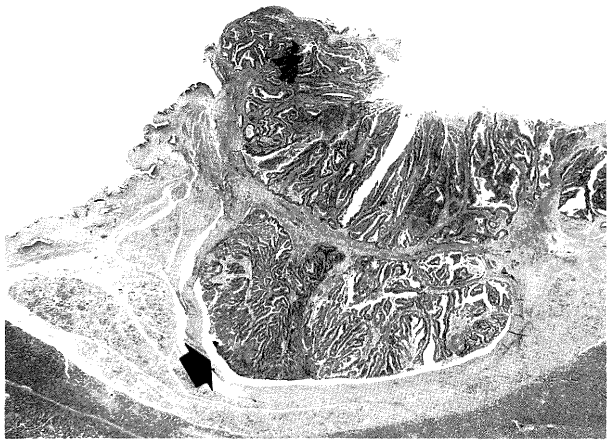

Fig. 3. Carcinoma of the papilla of Vater was located mainly in the ampullary region of the papilla of Vater and extended into the intraduodenal bile and pancreatic ducts and muscle layer of the duodenum (resected specimen was opened along the bile duct). In addition, the tumor extended 5 mm into the epithelium of the main pancreatic duct from the duodenum (*arrows*).

RESULTS

Carcinoma of the ampulla of Vater

The carcinoma of the ampulla of Vater in our patient was an expansive ulcerating tumor, measuring 2.0×1.8×0.4cm, and a well-differentiated tubular adenocarcinoma with a high grade of cytological atypia, which was predominantly positive for CA19-9, rarely positive for sialo-and sulfo-mucin, and negative for GOS and PCS staining. The tumor was mainly located in the ampullary region of the ampulla of Vater, and extended into the intraduodenal bile and pancreatic duct and as well into the muscle layer of the duodenum (Fig. 3). In addition, the tumor extended 5 mm into the mucosa of main pancreatic duct from the duodenum (Fig. 3) but did not have continuity with any microcarcinomas of the pancreas. There was neither lymphatic or venous permeation in this tumor nor metastasis of lymph nodes examined.

Microcarcinomas of the pancreas

Sixty-nine microcarcinomas whose size range from 0.04 mm to 1.2 mm in diameter can be detected under the microscopy with difficulties (Table 1). All of microcarcinomas proved to be independent by histological reconstruction and well-differentiated tubular adenocarcinomas with a high grade of cytological atypia, and were positive for CA19-9, but negative for sialo-and sulfo-mucin as well as neutral mucin (PAS, GOS, and PCS staining). Sixty-three of the 69 microcarcinomas were *in-situ* carcinomas (Fig. 4), and the remaining 6 were microinvasive (Fig. 5). They did not show lymphatic or venous permeation or lymph node metastasis. Microcarcinomas were found adjacent to normal ordinary pancreatic duct cells (Figs. 4 and 5), but never to mucous cell hyperplasias, which were found in a small number apart from the carcinomas.

Table 1. Number and size of pancreatic microcarcinomas by location

	Location of microcarcinoma		
	Ph	Pb	Pt
Number	14[1]	46[3]	9[2]
Size (mm)	0.45±0.34 (0.12~1.25)	0.26±0.18 (0.04~0.9)	0.43±0.45 (0.07~1.2)

Ph, pancreatic head; b, body; t, tail
[ ], Minimal stromal invasion; ( ), Size range.

Microcarcinomas were distributed through the head, body, and tail of the pancreas (Figs. 1 and 2). There was no significant difference in distribution-density and size of the tumors between the head, body, and tail of the pancreas (Table 1). The pancreatic duct levels at which the cancers were located is shown in Table 2. The intralobular (+intercalated) duct level was the most prevalent site for microcarcinomas, and they were not found in the main pancreatic duct. Microinvasions developed in microtumors located in the intralobular acinus duct level.

Main pancreatic duct is slightly dilatated because of the carcinoma of papailla of Vater but none mucus were observed in main pancreatic duct nor in branch of ducts. The findings of chronic pancreatitis, such as interlobular and intralobular fibrosis, atrophy of acinus, hyperplasia of islet cells, are never seen in all of pancreas. A few mucous cell hyperplasia exist in the area apart from microcarcinomas.

p53 and Ki-67 immunostaining and K-ras codon 12 mutation

Carcinoma of the ampulla of Vater and all pancreatic microcarcinomas exhibited a diffuse positive pattern for p53 (Fig. 4b) and Ki-67 staining. The p53 positive index-Ki-67 positive index ratio in the same tumor area in sequential sections was 149% in pancreatic

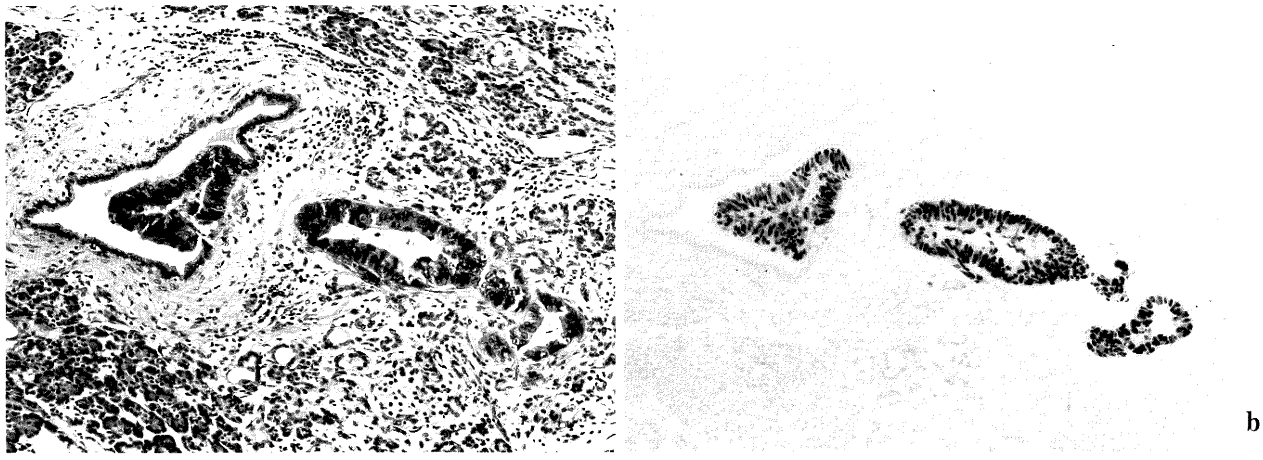

Fig. 4. The *in-situ* microcarcinoma was found at interlobular and intralobular pancreatic ducts level. **a**. The microtumor was a well differentiated tubular adenocarcinoma with high-grade cytological atypia adjacent to normal ordinary duct cells. HE, x 25 **b**. The microcarcinoma was diffusely positive for p53 staining. p53 staining, x 25

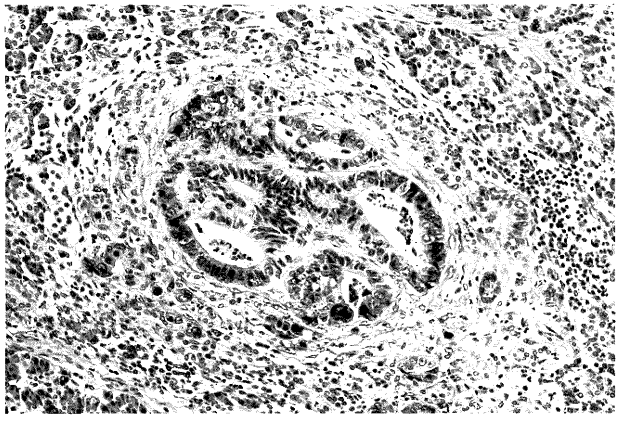

Fig. 5. The microinvasive carcinoma was situated mainly at the intralobular duct and extends into intercalated duct. HE, x 25

microcarcinomas and 129% in Vater's carcinoma. Ki-ras mutation was detected in 2 of 10 foci of mucous cell hyperplasia, but not in ordinary pancreatic epithelium, pancreatic microcarcinoma, and Vater's carcinoma.

Metastasis of Vater's carcinoma into the pancreas

There was no pancreatic metastasis among the 141 cases of carcinoma of the ampulla of Vater we reviewed.

DISCUSSION

Warren and Gates described the following criteria for multiple primary carcinoma: each of the tumors must present a definite picture of malignancy, each must be distinct, and the probability of one being a metastasis of the other (via vascular permeation, or intraductal and intraluminal spread) must be excluded[11].

In our case the carcinoma of the ampulla of Vater and all of the 69 microcarcinomas of the pancreas showed high-grade cytologic atypia, and diffuse Ki-67 and p53 expressions, but not lymphatic or venous permeation nor lymph node metastasis. So these two carcinomas shoud be independant of each other. These microcarcinomas of pancreas were 63 *in-situ* carcinomas and 6 microinvasive lesions. In addition, all of the microcarcinomas (n=69; size, 0.04-1.25 mm) were independent of each other, and distributed non-preferentially throughout the head, body, and tail of the pancreas. They were all located in the small interlobular ducts and ductules (including intercalated ducts and centroacinar cells). We have never seen such a case of Vater's carcinoma metastasizing to the pancreas in the form of multiple microcarcinomas among our 141 cases or in the previous reports. However, a case of synchronous independent intramucosal papillary adenocarcinomas in the main pancreatic duct and the ampulla of Vater was reported[6], but no microcarcinoma in the pancreas as in our case. Therefore, all of the pancreatic ductal microcarcinomas in our case were thought to be primary carcinomas.

Table 2. Location of 69 microcarcinomas according to the level of pancreatic duct

Ductal level	No. of tumors	CIS	Invasive
Interlobular	10(14.5%)	10	0
Intralobular	15(21.7%)	15	0
Intralobular+interlobular	1(1.4%)	1	0
Intralobular+intercalated	32(46.4%)	28	4
Intralobular+interlobular+intercalated	1(1.4%)	1	0
Intralobular+intercalated+acinus	6(8.7%)	4	2
Intercalated	3(4.4%)	3	0
Intercalated+acinus	1(1.4%)	1	0

CIS, carcinoma *in situ*.

Table 3. p53 index, Ki-67 index, and frequency of Ki-ras mutation

	Micro-carcinoma (12 foci)	Ordinary epithelium (14 foci)	Mucous cell hyperplasia (10 foci)	Carcinoma of papilla of Vater (7 foci)
p 53 index	91.6±8.10% (n=5924)	0% (n=4535)	0.3±0.4% (n=4260)	82.5±10.4% (n=2982)
Ki-67 index	64.9±13.3% (n=4299)	2.60±2.50% (n=5587)	11.0±18.4% (n=5488)	63.9±22.8% (n=2996)
p 53-positive index-Ki-67-positive index ratio	143%	0%	2.7%	129%
Ki-ras mutation*	0%(0/12)	0%(0/14)	20%(2/10)	0%(0/7)

*examined using a nested PCR-RFLP method;
n, number of cells counted.

In general, it is very difficult to discover the primary ductal level of solid-type pancreatic carcinoma, because most pancreatic carcinomas are found at an advanced stage. Furuta et al. reported that 20 of 36 solid-type human pancreatic ductal carcinomas might have arisen from branches of pancreatic duct by studying the histological reconstruction of the location of the main pancreatic duct and tumor-mass[2].

Pour et al. reported 7 human autopsy CIS cases (31 lesions). CISs existed in ducts (the main pancreatic duct and its first and second interlobular ducts) and ductules (intralobular ducts with their terminal branches including centroacinar cells) in 1 case, and in ductules in the remaining 6. Three of the 7 occurrences accompanied an advanced pancreatic ductal adenocarcinoma.

Fitzgerald and Cubilla described 5 cases of multiple CISs with microinvasion found in surgical and autopsy materials in the absence of advanced pancreatic carcinoma (we excluded 1 case because of a strong possibility of ductectatic type carcinoma, considering the description)[1]. CISs developed in the main pancreatic duct and its branch in 1 case, in large and small ducts in 2 cases, and in ducts in 1. Mizumoto et al. reported a surgical case of CIS (size not given) with microinvasion arising from both the main pancreatic duct of the pancreatic head and the Santorini duct[5]. As mentioned before, it is very difficult clinically to find out the first originated branch of pancreatic carcinoma.

In animal experimental models, Pour et al. reported that multiple CISs resembling human pancreatic duct cell carcinoma developed ubiquitously and simultaneously in pancreatic ducts (main and secondary) and in ductules (interlobular and intralobular)[7]. Ura et al. reported that CIS arose from large branches of the main pancreatic duct and that atypical hyperplasia arose from of small and medium-sized ducts[10]. Scarpelli et al. reported that pancreatic adenocarcinomas closely resembling those of ductal derivation originated from a modulated duct-like acinar cells in hamsters[9].

In our case, microadenocarcinomas were all located in the peripheral pancreatic ducts, i.e, small interlobular ducts and ductules (including intercalated ducts and centroacinal cells). It is of great interest that the most prevalent site for the microcacinomas in our case was intralobular duct (+ intercalated duct) level as in the animal experimental models.

The histogenesis of pancreatic ductal adenocarcinoma in humans has been considered to be mucous cell hyperplasia (papillary hyperplasia, non-papillary hyperplasia) for the following three reasons. First, mucous cell hyperplasia is closely associated with pancreatic adenocarcinoma[3]. Second, mucous cell hyperplasia and CIS express a similar mucin histochemically, predominantly sialomucin. Third, Ki-ras mutation is equally high between mucous cell hyperplasia and CIS (or invasive carcinoma), but not found in the ordinary epithelium of the pancreas. In the previous reports, almost all CISs, i.e, all 7 occurrences reported by Pour et al.[8], and 3 of 4 occurrences reported by Fizgerald and Cubilla[1] showed the same phenotype as mucous cell hyperplasia. There were 2 cases in which histogenesis was considered to be ordinary epithelium. The first one is a case with 4 CIS foci[1] adjacent to normal ducts lined with ordinary epithelium. The other is a case showing CIS with microinvasion surrounded by ordinary epithelium[5].

Reports on animal models of pancreatic carcinoma have given us no precise description on whether histogenetic cells were of ordinary or metaplasic mucous cells. In our case, all microcarcinomas had the same phenotype as ordinary epithelium mucin. Histochemically, none of them was accompanied by mucous cell hyperplasia, and Ki-ras mutation was negative. Therefore, we conclude that all of the microcarcinomas in our case originated from the ordinary epithelium.

Acknowledgments. We report an interesting case with a primary ulcerating tumor of the ampulla of Vater associated with multiple primary pancreatic ductal microadenocarcinomas. All of these pancreatic microcarcinomas were histologically proved to be primary and independent of each other, and were distributed throughout the head, body, and tail of the pancreas. They were all located in the small interlobular ducts and the ductules (including intercalated ducts and centroacinar cells). We concluded that pancreatic microcarcinomas in this case arose from ordinary pancreatic ductal cells of the small ducts and ductules by way of p53 alteration without Ki-ras mutation, and that histogenesis was different from that of the more common Ki-ras-mutated carcinoma arising from metaplastic mucous cells of mucous cell hyperplasia.

REFERENCES

1) Fitzgerald PJ, Cubilla AL: Pancreas. In: Henson DE, Albores-Saavedra J (eds.) The pathology of incipient neoplasia. WB Saunders Philadelphia: 1986, p217-231.
2) Furuta K, Watanabe H, Ikeda S: Differences between solid and duct-ectatic types of pancreatic ductal carcinomas. *Cancer* **69**: 1327-1333, 1992.
3) Klöppel G, Bommer G, Rückert K, Seifert G: Intraductal proliferation in the pancreas and its relationship to human and experimental carcinogenesis. *Virchow Arch Path Anat* **387**: 221-233, 1980.
4) Matsuda K, Watanabe H, Jass JR, Ajioka Y, Kobayashi M, Saito H, Sasaki M, Yasuda K, Kuwabara A: Frequency of p53-positive cells in differing histological phases of ulcerative colitis. *Acta Med Biol* **43**: 197-203, 1995.
5) Mizumoto K, Inagaki T, Koizumi M, Uemura M, Ogawa M, Kitazawa S, Tsutsumi M, Toyokawa M, Konishi Y: Early pancreatic duct adenocarcinoma. *Hum Pathol* **19**: 242-244, 1988.
6) Nishihara K, Tsuneyoshi M, Shimura H, Yasunami Y: Three synchronous carcinomas of the papilla of Vater, common bile duct and pancreas. *Pathol Int* **44**: 325-332, 1994.
7) Pour P, Althoff J, Takahashi M: Early lesions of pancreatic ductal carcinoma in the hamster model. *Am J Pathol* **88**: 291-308, 1977.
8) Pour P, Sayed S, Sayed G: Hyperplastic, preneoplastic and neoplastic lesions found in 83 human pancreases. *Am J Clin Pathol* **77**: 137-152, 1982.
9) Scarpelli DG, Rao MS, Subbarao V: Augmentation of carcinogenesis by N-Nitrosobis (2-oxopropyl) amine administered during S phase of the cell cycle in regenerating hamster pancreas. *Cancer Res* **43**: 611-616, 1983.
10) Ura H, Makino T, Ito S, Tsutsumi M, Kinugasa T, Kawano T, Ichimiya H, Konishi Y: Combined effects of cholecystectomy and lithocholic acid on pancreatic carcinogenesis of N-Nitrosobis (2-hydroxypropyl) amine in syrian golden hamsters. *Cancer Res* **46**: 4782-4786, 1986.
11) Warren S, Gates O: Multiple primary malignant tumors: A survery of the literature and a statistical study. *Am J Cancer* **16**: 1358-1414, 1932.