Hormone-producing serous cystadenoma of the pancreas

J. RABCZYNSKI[1], H. MILNEROWICZ[2], M. SLIWINSKA-MOSSON[2], M. JABLONOWSKA[2], S. MILNERIWICZ[3*], W. KNAST[3], M. ZABEL[4]

[1]Department of Pathological Anatomy, Wroclaw Medical University, 50-355 Wroclaw, Poland; [2]Department of Biomedical and Environmental Analyses, [3]Department and Clinic of Gastrointestinal and General Surgery, e-mail: hm@tox.am.wroc.pl, and [4]Department of Histology and Embryology, Wroclaw Medical University, Poland

Received March 6, 2006

The clinical and histochemical examination of hormone-producing serous cystadenomas of the pancreas are presented.

The study material was obtained from five female patients. The patients underwent diagnostic examinations, including ultrasonography, computer tomography (CT), magnetic resonance imaging (MRI) and Doppler ultrasonography examination of abdomen. In all cases the presence of serous cystadenoma of pancreas was detected in the histopathologically verified sections. The test applied to immunohistochemically localize paraffin-embedded sections of neoplastic tissues of the pancreas was the LSAB2-HRP test using monoclonal antibodies against epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), synaptophysin, p53 and polyclonal antibodies against insulin, glucagon, somatostatin and pancreatic polypeptide.

In one patient, ultrasonography revealed an irregular space filled with fluid resembling a multicellular cystic lesion. The Doppler ultrasonography examination showed a pathologically vascularized focus in the pancreatic head. In the adenoma sections of this patient, the immunohistochemical techniques revealed a strong positive somatostatin, pancreatic polypeptide and synaptophysin expression in the lining epithelium of neoplastic cysts.

Key words: serous cystadenoma, pancreas, immunochemistry, pancreatic hormones

Serous cystadenomas (SCA), also known as microcystic adenomas or glycogen rich adenomas, are rare benign tumors of the pancreas [1, 2, 4–13]. Increased SCA morbidity occurs in the patients aged over sixty with an evident female preponderance (about 92% of SCA cases occur in women) [1–4, 8, 10, 11]. The histogenesis of pancreatic SCA remains unknown and the detection of this neoplasm is difficult at each stage of the disease progression. SCA is usually discovered incidentally or due to non-specific abdominal symptoms. Gastrointestinal symptoms, biliary tract obstruction and diabetes mellitus (when normal Langerhans' islets are destroyed by the tumor) are the first signs observed in SCA patients. The majority of SCA cases are localized in the pancreatic head. Rare occurance, unclear histogenesis and various clinical symptoms of this disease result in the fact that even individual cases are subject to series of studies [1, 3, 4, 7–11].

Serous cystadenoma of the pancreas usually occurs as a solitary and well-formed tumor. Sometimes it may also coexist with other pancreatic tumors, e.g., adenocarcinomas [14–16]. Recently, a few cases of the coexistence of SCA and an endocrine tumor of the pancreas have been presented. In those cases, the tumor composed of two different structures – a microcyst and a hormone-producing endocrine component – has been observed [17–21].

The extension of routine histopathological examination in which serous cystadenoma of the pancreas was found by immunohistochemical analysis additionally made it possible to identify in one case an endocrinal function of SCA of the pancreas.

No hormone-producing SCA has been reported in the world literature yet. This study describes the first unusual case of serous cystadenoma that produces islet hormones, somatostatin and pancreatic polypeptide (PP).

*Corresponding author

Abbreviations: CEA – carcinoembryonic antigen; CT – computer tomography; EMA – epithelial membrane antigen; F – female; MRI – magnetic resonance imaging; PAS – periodic acid-Schiff; PP – pancreatic polypeptide; SCA – serous cystadenoma; SCAC – serous cystadenocarcinoma; WHO – World Health Organisation.

Material and methods

The study material was obtained from five female patients, aged 39–71 years (mean age 55.2). Patients were treated in the Department and Clinic of Gastrointestional and General Sugery, Wroclaw Medical University, between the years 1992–2001.

All patients were admitted due to persistent abdominal pain and, in two cases, developing jaundice. The diagnosis was made in all cases by means of ultrasonography (USG), computed tomography (CT), esophagogastroduodenoscopy (EGD) and endoscopic retrograde cholangiopancreatography (ERCP).

In case of diagnostic doubts magnetic resonance (MR) or magnetic resonance cholangiopancreatography (MCRD) and USG-Doppler were made.

Cystic changes in the pancreas were found in all patients during pre-operative examination. No specific family history and no characteristics of von Hippel Lindau syndrome were detected in any case.

In two cases changes were identified in the pancreas head, in one case in the pancreas body, in one case at the border between the body and tail and in one case in the pancreas tail. Resection was performed in all cases. Sections were histopathologically verified and a primary serous cystadenoma of the pancreas was detected in all patients (Tab. 1).

Immunohistochemical localization of pancreatic hormones was made in all cases. Clinical examination of patient No. 1, in whom the somatostatin and pancreatic polypeptide-secreting serous cystadenoma of pancreas was identified by immunohistochemical tests, have been described more extensively below.

The patient was admitted to the clinic with a suspected proliferative pathology of the pancreatic head. Ultrasonography revealed a hypoechogenic focus, 5.6 x 4.2 cm, with the fluid-filled spaces. This finding was confirmed by CT and MRI scans that showed an irregular, smooth-contoured space filled with the fluid resembling multicellular cystic lesion. Doppler ultrasonography revealed pathological vascularization of the focus observed in the pancreatic head. Patient underwent surgery and a modified Whipple resection was performed.

Immunohistochemistry was performed on paraffin-embedded tissue, employing the streptavidin-biotin method. Serial tissue sections, deparaffinized and hydrated in an alcohol series, were incubated with a 3% hydrogen peroxide solution to block any intracellular peroxidase activity. The tumors were tested with monoclonal antibodies: epithelial membrane antigen (EMA) (DAKO, M 0804), carcinoembryonic antigen (CEA) (DAKO, M 0803), synaptophysin (DAKO, N 1566) and p53 (DAKO, N 1581). Polyclonal antibodies to insulin (DAKO N1542), glucagon (DAKO N1541), somatostatin (DAKO N1551), pancreatic polypeptide (PP) (DAKO A0619) and chromogranin A (DAKO, N1535) were also performed (Tab. 2). For EMA, CEA, synaptophysin, chromogranin A and p53 antigen retrieval, the sections underwent boiling in citrate-buffered saline. Non-specific bonds were blocked using Antibody Diluent (DAKO, S0809). The tissue sections were subsequently incubated in room temperature with monoclonal and specific polyclonal antibodies. After washing in 0.05 M Tris-HCL saline (DAKO Bio-Rad TBS170-6435), with 0.1% Tween 20 (Sigma P1379), Ab-hormone complexes were visualized using the LSAB2-HRP test (DAKO K0673). The peroxidase activity was located against 3,3'-diaminobenzidine (DAKO DAB K0637) in imidazole-HCL buffer, pH 7.5. Consequently, the sections were washed in distilled water and contrasted with hematoxylin (DAKO Chem Mate^{TM} S2020), and finally were closed in glycerin gel and left until dried.

A negative control was performed for each tissue section, replacing the primary antibody with a anti-rabbit immunoglobulin control IgG antibody (DAKO Negative Control X0903).

Diluted antibodies against EMA, CEA, PP and incubation times of individual stages of the test were matched experi-

Table 1. Characteristics of patients with serous cystadenoma of the pancreas (five cases)

No/ Gender/ Age	Location in the pancreas	Type of surgery	Complications	Observation post surgery – years
1 /F /39	Head	Pancreatodudenectomy m. Whipplea	None	5 years
2 /F /46	Head	Pancreatodudenectomy m. Whipplea-Traverso	Respiratory insufficiency	6 years
3 /F /64	Body	Left-side resection of the pancreas	Pancreatic fistula	11 years
4 /F /56	Body Tail	Enucleation of the cyst	None	4 years
5 /F /71	Tail	Left-side resection of the pancreas	None	3 years

F – female

Table 2. Antibodies used for immunostaining of serous cystadenoma of the pancreas

Antibodies	Source	Working dilution	Polyclonal/ monoclonal	Antigen Retrieval
Epithelial membrane antigen (EMA)	Dako	1:20	Clone E27	Boiling
Carcinoembryonic antigen (CEA)	Dako	1:20	Clone II-7	Boiling
Chromogranin A	Dako	–	Polyclonal	Boiling
Synaptophysin	Dako	–	Clone SY38	Boiling
aInsulin	Dako	–	Polyclonal	–
Glucagon	Dako	–	Polyclonal	–
Somatostatin	Dako	–	Polyclonal	–
Pancreatic polypeptide (PP)	Dako	1:600	Polyclonal	–
p53	Dako	–	Clone DO-7	Boiling

mentally in laboratory conditions. The sections were stained with hemotoxylin-eozin and periodic acid-Schiff (PAS) stains (Fig. 1, 2).

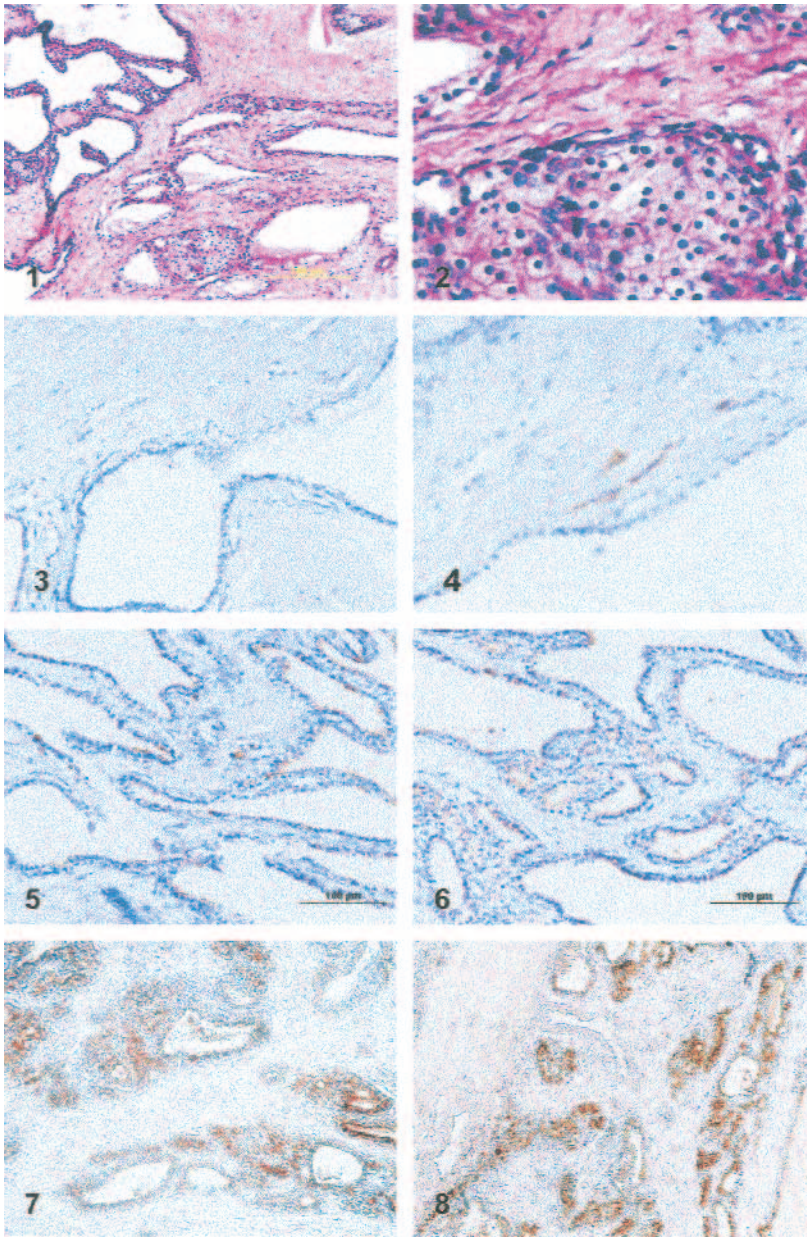

Figure 1–8. Serous cystadenoma, in case No 1.
1. The typical histological features of serous cystadenomas, such as a microcystic architecture with a uniform and bland appearance and a hypocellular, dense collagen stroma. (H-E magnification x 110).
2. The periodic acid-Schiff's stain demonstrates moderate expression of cytoplasmic glycogen (PAS, magnification x 420).
3, 4. Immunostainig for carcinoembryonic antigen (CEA) and chromogranin A was negative for cells of the cystadenoma (magnification x 120, 230, respectively).
5, 6, 7, 8. Immunostainig for epithelial membrane antigen (EMA), synaptophysin, somatostanin and pancreatic polypeptide reveals many positive cells in lining epithelium of the neoplastic cyst (magnification x 120).

Stained tissue sections were viewed under high power by means an Olympus BX41 light microscope (Olympus Optical Co. Ltd, Japan) that was interfaced with an Olympus DP70 digital camera (Olympus Optical Co. Ltd, Japan) that digitized the light microscopic image.

Assessment of immunohistochemical staining. The semi-quantitative method was used to assess histopathologically the intensity of tissue specimen staining: 0 – no immunohistochemical reaction; + weak reaction; ++ moderate reaction; +++ strong reaction; ++++ very strong reaction.

Results

Tissue of serous cystadenoma of the pancreas. The histopathologic examination revealed features characteristic of serous cystadenoma in all studied cases. The tumor was composed of multilocular areas of various different tissue types. A single layer of cuboidal epithelium lined the cyst. The cells of a similar size with a clear, slightly granular cytoplasm with the single, round or oval slightly hyperchromatic nucleus were visible. There were no located lesions. Some single, fine nervous trunks and vessels were visible in the connective septa. The solid cellular formations or structures resembling normal Langerhans' islets were absent (Fig. 1).

Immunohistochemical localization of pancreatic hormones in SCA tissues. The immunohistochemical localization of p53 and pancreatic hormones (insulin, glucagon, somatostatin and PP) was negative in four SCA cases (data not shown). The case No. 1 of the serous cystadenoma showed no p53, CEA, chromogranin A, insulin and glucagon expression, but very strong immunoreactivity to synaptophysin, EMA, somatostatin and PP (Fig. 3–8). The hormone expression was focal and limited to a lining epithelium of a neoplastic cyst. The immunohistochemical localization of somatostatin and PP was evidently related to SCA epithelium – these were not the components of endogenous structures retracted into the adenoma tissue as a result of the tumor growth.

Discussion

Adenomas are very rare pancreatic tumors (they account for 0.5%–1% of all pancreatic

tumors), and serous cystadenomas constitute 25% of them [1, 22].

The World Health Organization (WHO) report published in 1996 contains a classification of endocrine tumors. Adenomas are divided into serous cystadenomas (SCA) (morphology code 1.1.1 in the International Classification for Oncology (ICD-O) and the Systematized Nomenclature of Medicine [SNOMED]), and serous cystadenocarcinoma (SCAC) (morphology code 1.3.4 ICD-O/SNOMED) [2]. The former category, can be subdivided into: serous microcystic adenomas and serous oligocystic adenomas [1, 2].

The coexistence of SCA and the other tumors of the pancreas (e.g. cystadenoma and endocrine tumors) has been recently reported in the literature [14–23]. In the cases described, serous cystadenoma occurred in association with an endocrine tumor that produced different peptide pancreatic hormones. During microscopic examination a tumor area composed of cysts originated from glycogen rich cell lines and a solid formation of endocrine cells were observed. 21 The immunohistochemical reaction for islet hormones and chromogranin A was positive only in cells of the endocrine component of a mixed tumor. Endocrine components of tumors showed a varying immunoreactivity for insulin, glucagon, somatostatin and chromogranin A [18–21].

Earlier immunohistochemical examinations of the pancreatic hormone and synaptophysin expression were negative in SCA with a classic formation [4, 5, 8]. The results of these examinations are in agreement with our findings in the four cases of the serous cystadenoma, with no immunohistochemical reaction for insulin, glucagon, somatostatin, PP and synaptophysin. SCA in case No. 1 revealed a strong positive somatostatin, PP and synaptophysin expression. The tumor cells were positive for epithelial membrane antigen (EMA) and were negative for CEA and p53 [1, 4, 10, 18]. The expression of hormones was focal (not all epithelial areas showed hormone expression), and limited to the lining epithelium of neoplastic cysts. In the immunohistochemical sections stained with hemotoxylin-eozin, the presence of endocrine formation in the form of a tumor or islets incorporated into the cyst structure was excluded. Doppler ultrasonography, performed prior to SCA resection, revealed pathological vascularization. A well developed vascular system and neoplastic changes in the pancreatic head are the most characteristic features of endocrine neoplasm of this organ [21].

The histogenesis of serous cystadenoma is still unknown. Some authors suggest, that SCA originates from acinar or glycogen rich centroacinar cells [1, 3–8, 10, 11, 13, 24]. The presence of mixed tumors composed of serous cystadenoma and neuroendogenous tumor formation may indicate that they originate from the same clone of neoplastic cells manifesting endo- and exocrine differentiation [21].

Experimental studies suggested a significant role of endocrine cells in the development of pancreatic carcinogenesis in Syrian golden hamsters [28]. Other studies revealed that the endo- and exogenous formations develop from ductal cells [26, 27].

In the experimental conditions, it has been shown that a small population of stem cells manifests differentiation along two lines of pancreatic cells: endocrine and exocrine, ones. The exocrine cells may mature consequently into either ductal or vesicular ones. A gradual transformation of acinar cells into tubular structures has also been evidenced. Maturated acinar cells may direct their phenotype change towards ductal cells [31]. This again provides an evidence that there is a continuity between three types of pancreatic tissues originating from the same host cell. A tumor of the classical SCA structure producing hormones, somatostatin and PP is likely to develop from the stem cell during the initiation of the neoplastic process.

The so called "intermediate cells", having the properties of both acinar and endocrine cells are present in the pancreas [32, 33]. They can also be referred to as: transit, acinar-islets, amphophilic, extrainsular or exocrine-endocrine cells. Although their role in a healthy pancreas is not significant, they may occur in pathological conditions, nesidioblastosis could be evidence of this suggestion (34). Moreover some authors suggest, that such intermediate cells may undergo neoplastic transformation [32, 33]. It cannot be excluded that a hormone-producing serous cystadenoma of the pancreas may develop from an intermediate cells showing acinar-endocrine properties.

Pancreatoblastoma appears to be the counterpart of childhood tumors that originate from cells of other organs. Both endocrine and exocrine differentiations have been identified in component cells, therefore ductal, acinar, and islet cells may be seen at the histological, immunohistochemical, and ultrastructural level [35]. The acinar-endocrine cell tumor of the pancreas is a rare mixed tumour reported only in one pediatric and one adult patient. The detection of both zymogen and neuroendocrine granules within the same cell by ultrastructural and enzymatic analyses in acinar-endocrine cell tumors suggests a neoplastic proliferation of intermediate cells. Similarly, simultaneous neuroendocrine granule formation concentrated in basal pole or in the cytoplasmic processes with mucin production have been described in amphicrine carcinomas (mucinous islet cell carcinomas) [18].

Nevertheless, the question concerning the type of cells which might trigger the developmental process of a serous cystadenoma still remains open.

The unusual case of SCA presented in this paper is the first classic SCA formation and hormone-producing neoplasm described.

References

[1] COMPTON CC. Serous cystic tumors of the pancreas. Semin Diagn Pathol 2000; 17: 43–55.

[2] KLÖPPEL G, SOLCIA E, LONGNECKER DS, CAPELLA C, SOBIN LH. In Collaboration with Pathologists in 7 Countries. Sec-

ond Edition. Corrected Rinting. (eds): World Health Organization International Histological Classification of Tumours. Histological Typing of Tumours of the Exocrine Pancreas (ed. 2). Berlin: Springer-Verlag, 1996; 7–18.

[3] STROBEL O, Z`GRAGGEN K, SCHMITZ-WINNENTHAL FH, FRIESS H, KAPPOLER A et al. Risk of malignancy in serous cystic neoplasms of the pancreas. Digestion 2003; 68: 24–33.

[4] SANTOS LD, CHOW CH, HENDERSON CHJ A, BLOMBERG DN, MERRETT ND, KENNERSON AR et al. Serous oligocystic adenoma of the pancreas: a clinicopathological and immunohistochemical study of three cases with ultrastructural findings. Pathology 2002; 34: 148–156.

[5] PEREZ-ORDONEZ B, NASSEM A, LIEBERMAN PH, KLIMSTRA DS. Solid serous adenoma of the pancreas: the solid variant of serous cystadenoma? Am J Surg Pathol 1996; 20: 1401–1405.

[6] MORI K, TAKEYAMA S, HIROSAWA H, WATANABE T, TANIYA T et al. A case of macrocystic serous cystadenoma of the pancreas. Int J Pancreatol 1995; 17: 91–93.

[7] EGAWA N, MAILLET B, SCHRÖDER S, MUKAI K, KLÖPPEL G. Serous oligocystic and ill-demarcated adenoma of the pancreas: a variant of serous cystic adenoma. Virchows Arch Pathol Anat 1994; 424: 13–17.

[8] ALPERT LC, TRUONG LD, BOSSART MI, SPJUT HJ. Microcystic adenoma (serous cystadenoma) of the pancreas: a study of 14 cases with immunohistochemical and electron-microscopic correlation. Am J Surg Pathol 1988; 12: 251–263.

[9] JIN YM, YIM H, CHOI JI. Pancreatic serous cystadenoma mimicking pseudocyst. Yonsei Med J 1997; 38: 63–65.

[10] ISHIKAWA T, NAKAO A, NOMOTO S, HOSONO J, HARADA A et al. Immunohistochemical and molecular biological studies of serous cystadenoma of the pancreas. Pancreas 1998; 16: 40–44.

[11] HODGHINSON DJ, REMINE WH, WEILAND LH. Pancreatic cystadenoma: a clinico-pathologic study of 45 cases. Arch Surg 1978; 113: 512–519.

[12] MARTIN J, HAMMOND P, SCOTT J, REDHEAD D, CARTER DC et al. Cystic tumours the pancreas. Br J Surg 1998; 85: 1484–1486.

[13] KNAST W, MARKOCKA-MACZKA K, RABCZYNSKI J, SZELACHOWSKI P. Cystic neoplasms of the pancreas. Pol Przegl Chir 2003; 75; 633–642.

[14] POSNIAK HV, OLSON MC, DEMOS TC. Coexistent adenocarcinoma and microcystic adenoma of the pancreas. Clin Imaging 1991; 15: 220–222.

[15] MONTAG AG, FOSSATI N, MICHALASSI F. Pancreatic microcystic adenoma coexistent with pancreatic ductal carcinoma: a report of two cases. Am J Surg Pathol 1990; 14: 352–355.

[16] DEL VECCHIO MT, PERGOLA L, TRIPODI SA, COLLINI A, FORZINI L et al. Microcystic adenoma associated with a mucinous cystadenocarcinoma: a “collision tumor” of the pancreas. Pancreas 2002; 24: 106–108.

[17] KEEL SB, ZUKERBERG L, GRAEME-COOK F, COMPTON CC. A pancreatic endocrine tumor arsinig within a serous cystadenoma of the pancreas. Am J Surg Pathol 1996; 20: 471–475.

[18] USTON MO, TUGYAN N, TUNAKAN M. Coexistence of an endocrine tumour in serous cystadenoma (microcystic adenoma) of the pancreas, an unusual association. J Clin Pathol 2000; 53: 800–802.

[19] KIM YW, PARK YK, LEE S. PARK JH, LEE SM et al. Pancreatic endocrine tumor admixed with a diffuse microcystic adenoma – a case report. J. Kor. Med. Sci 1997; 12: 469–472.

[20] JUNG HK, SON HY, LEE HC, YI SY. Microcystic adenoma coexistent with low-grade malignant islet cell of the pancreas. J Clin Gastroenterol 2001; 32: 441–443.

[21] SLUKVIN II, HAFEZ RG, NIEDERHUBER JE, WARNER TF. Combined serous microcystic adenoma and well-differentiated endocrine pancreatic neoplasm: a case report and review of the literature. Arch Pathol Lab Med 2004; 127: 1369–1372.

[22] GERDES B, WILD A, WITTENBERG J, BARTH P, RAMASWAMY A et al. Tumor-suppressing pathways in cystic pancreatic tumours. Pancreas 2003; 26: 42–48.

[23] HERESBACH D, ROBERT I, LE BERRE N, RAOUL JL, SIPROUDHIS L et al. Cystic tumors and endocrine tumor of the pancreas: an unusual association. Gastroenterol Clin. Biol. 1993; 17: 968–971.

[24] YAMAGUCHI K, CHIJIIWA K, NOSHIRO H, TORATA N, KINOSHITA M et al. Ki-ras codon 12 point mutation and p53 mutation in pancreatic diseases. Hepatogastroenterology 1999; 46: 2575–2581.

[25] SCHRON DS, MENDELSOHN G. Pancreatic carcinoma with duct, endocrine, and acinar differentiation: a histologic, immunocytochemical, and utrastructural study. Cancer 1984; 54: 1766–1770.

[26] MONAKI PJ, ANDREN-SANDBERG A, KAZAKOFF K, POUR PM. Pancreatic mixed ductal-islet tumors. Is this an entity? Int J Pancreatol 1992; 11: 23–29.

[27] SZYNAKA B, ZIMNOCH L, PUCHALSKI Z, SZYNAKA P. Endocrine and intermediate cells in pancreatic ductal carcinoma and in chronic pancreatitis. Rocz Akad Med Bialymst 2002; 47: 21–30.

[28] ISHIKAWA O, OHIGASHI H, IMAOKA S, NAKAI I, MITSUO M et al. The role of pancreatic islets in experimental pancreatic carcinogenicity. Am J Pathol 1995; 147: 1456–1464.

[29] POUR PM, RUNGE RG, BIRT D. Current knowledge of pancreatic carcinogenesis in the hamster and its relevance to the human disease. Cancer 1981; 47: 1573–1587.

[30] WAGNER M, LUHRS H, KLÖPPEL G, ADLER G, SCHMID RM. Malignant transformation of duct-like cells originating from acini in transforming growth factor alfa transgenic mice. Gastroenterology 1998; 115: 1254–1262.

[31] HALL PA, LEMOINE NR. Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas. J Pathol 1992; 166: 97–103.

[32] MELMED RN. Intermediate cells of the pancreas. An appraisal. Gastroenterology 1979; 76: 196–201.

[33] BERTELLI E, BENDAYAN M. Intermediate endocrine-acinar pancreatic cells in duct ligation conditions. Am J Physiol 1997; 273: 1641–1649.

[34] TOMASZEWSKA R, NOWAK W, RUDNICKA-SOSIN L, STACHURA J. Nesidioblastosis in an adult man – case raport. Pol J Pathol 1999; 50: 43–46.

[35] MOROHOSHI T, KANDA M, HORIE A, CHOTT A, DREYER T et al. Immunocytochemical markers of uncommon pancreatic tumors. Acinar cell carcinoma, pancreatoblastoma, and solid cystic (papillary-cystic) tumor. Cancer 1987; 59: 739–747.