Claudin expression in different pancreatic cancers and its significance in differential diagnostics

Ph.D. Thesis Synopsis

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1. INTRODUCTION

Neuroendocrine (NE) tumors make up 1-2 % of pancreatic tumors, frequently appearing as part of the MEN-1 and von Hippel-Lindau syndrome. Based on size and proliferation activity, the well differentiated tumors can be divided into groups of benign tumors and those of uncertain behaviour. Well differentiated NE carcinomas spread to the neighbouring organs and/or give metastases. Poorly differentiated NE carcinomas show high proliferation activity. Among functioning tumors, insulinomas show benign behaviour in 90 % of cases. Other hormone producing tumors (gastrin, glucagon, VIP, somatostatin, serotonin, ACTH, GH, PTH) and non-functioning tumors are malignant in nature in almost every case.

Ductal adenocarcinomas are the most frequent malignant tumors of pancreatic origin. Apart from smoking, diabetes and alcoholism, a number of chromosomal alterations also play part in their development (K-ras, p16, p53, SMAD4/DPC4, cyclin D1, MUC1, MUC5, PSCA, mesothelin). Ductal adenocarcinomas have expressedly bad prognosis, with a 5 years survival rate of only 15-20 %.

Mucinous cystic pancreatic tumors are most frequent, occurring predominantly in perimenopausal women. Based on dysplasia and the presence of invasion, they can be divided into groups of mucinous cystadenomas, uncertain (borderline) mucinous cystic tumors and mucinous cystadenocarcinomas.

Acinar cell carcinomas make up 1-2 % of pancreatic exocrine tumors and similar to pancreatoblastomas, they show acinar differentiation and produce trypsinogen and AFP. Acinar cell carcinomas mostly develop in adulthood, whereas the literature makes mention of only a few childhood cases. Diagnosis of pancreatoblastomas includes the so-called squamoid cell groups, which are missing in case of acinar cell carcinomas.

Claudins (CLDNs) are members of the tight junction (TJ). This molecule family was discovered in 1998, with currently 24 known members in humans. Each CLDN molecule consists of 4 transmembrane segments, has two extracellular loops and the N and C terminal regions are located in the cytoplasm. CLDNs play primary role in the sealing of intercellular spaces, controlling of selective paracellular diffusion, functioning as selective ion channels, in addition to maintaining the varying protein and

lipid composition between the apical and basolateral membranes. Further, CLDNs also take part in the regulation of cell growth and differentiation and in tumorgenesis. The lack or decreased presence of CLDNs as well as the altered ratio of CLDNs imply decreased cell adhesion, tumor infiltration and metastasis formation. The enhanced expression of certain CLDNs has been noticed in a number of tumor cases in which the changed structure of TJs possibly played role in invasion.

CLDNs are organ- and tissue specific molecules. For example, CLDN-2 protein is highly expressed in gastrointestinal epithelial cells and the tumors deriving from these. Endothelial cells and tumors originating from them show CLDN-5 positivity. The enhanced CLDN-4 expression of bile duct carcinomas separates these tumors from hepatocellular carcinomas.

CLDN-4 has also proved to be highly expressed in pancreatic adenocarcinomas and intraductal papillary mucinous neoplasias (IPMN), as well as in several other tumors of varying origin, e.g. in tumors of the prostate, ovarium, breast, stomach, endometrium; in carcinomas of the lungs, colon, kidneys, thyroid gland; oesophagus adenocarcinomas; cervix tumors and bile duct tumors. Small cell carcinomas are marked by the presence of high CLDN-3 expression in the lungs, making separation easy from atypical carcinoids, adenocarcinomas and epithelial carcinomas. In certain tumors the degree of expression refers to prognosis, e.g. the expression of CLDNs-1, -3, -4 and -5 is lower in diffuse type gastric tumors, which types usually have bad prognosis as opposed to intestinal tumors. In case of breast tumors, CLDN-1 and -7 proteins display decreased expression in the invasive, metastatic tumors**.** In cervical carcinomas the decreased expression of CLDNs-1 and -2 was observed with the development of invasion.

Based on the above, it is suggested that the expression of TJ proteins exhibits significant changes in pancreatic tumors of various cellular origin. Further, that endocrine and exocrine tumors differ in regard to the expression of TJ proteins. There are no data in the literature in respect to CLDN expression in endocrine tumors of the pancreas. In case of other organs, like lung tumors, however, it has become evident that the carcinoids of the organ differ essentially from glandular epithelial tumors from the aspect of cell junctions. On the other hand, the ductal and acinar cell junctions of the pancreas as well as the cell junction changes in the tumors deriving from them are still

mostly unclear. The aim of our studies was to examine the expression of CLDNs in normal pancreatic cells and under pathological conditions, primarily in case of carcinomas. It is assumed that in the course of detecting the changes in TJ proteins and their components, knowledge on these changes might provide data for the better understanding of the pathogenesis of several tumors. Furthermore, identification of the CLDN patterns characteristic to certain pancreas tumors might also serve as a diagnostic tool for the differentiation of the endocrine and exocrine tumors of the pancreas. CLDNs-3 and -4 are the receptors of the Clostridium perfringens enterotoxin (CPE) and based on data in the literature, they cause the lysis of protein-containing tumor cells (pancreas adenocarcinoma, prostate carcinoma). This fact might suggest the use of CPE in the development of new, target-oriented therapy.

2. THE AIM OF THE STUDY

During the course of our studies, our aim was the mapping of TJ proteins located among the various cell types of the pancreas. Further, to map the characteristics of these proteins and their possible role in the development of various tumors of the pancreas. According to our assumption, the ductal, acinar and endocrine cells show significant differences in the composition and distribution of certain TJ proteins, of CLDNs in particular. Furthermore, it is assumed that the characteristic „CLDN patterns" observable in tumors originating from the given cells remain unchanged, while the degree of expression might exhibit changes. Such changes, as well as the possible appearance or disappearance of new CLDN proteins might aid our knowledge of both the origin of certain tumors of the pancreas and the derivation from a common stem cell. In order to give answers to these assumptions, the following goals were raised:

2.1. To investigate CLDN expression of different cell types of normal pancreas as in ductal, acinar and endocrine cells.

2.2. To examine CLDN expression patterns in endocrine pancreatic tumors.

2.3. To determine CLDN expression in exocrine ductal adenocarcinomas.

2.4. To compare CLDN expression patterns in endocrine tumors and exocrine adenocarcinomas.

2.5. To examine the correlation of CLDN expression in different prognostic groups of cystic mucinous pancreatic tumors.

2.6. To study CLDN expression in acinar cell carcinoma.

2.7. To compare CLDN expression in pancreatic tumors and normal pancreatic cells of different origin.

3. MATERIAL AND METHODS

3.1. Samples

Retrospective series of 74 formalin fixed, paraffin embedded tissues were obtained from the archives of the 2nd Department of Pathology of the Semmelweis University with the permission of the Regional Ethical Committee of the Semmelweis University of Budapest (#172/2003).

Samples from five normal pancreas were studied to analyse the expression pattern of different CLDNs in nontumoros tissue. Pancreatic adenocarcinoma tissue samples were obtained from 20 patients who underwent surgery for surgically resectable disease. The mean age of adenocarcinoma patients was 60 (31-84), (13 males, 7 females). The majority of adenocarcinomas infiltrated the duodenal wall (pT3:11), while 8 did not (pT1:2, pT2:6). One of the 20 tumors was found to be extended to the spleen (pT4). Sixty five percent of patients had lymph node metastases (13/20) with no organ metastases present at the time of diagnosis. Seventy five percent (15/20) of tumors were moderately differentiated (Grade II.), however, 2 tumors were well- (Grade I.) and the remaining 3 poorly (Grade III.) differentiated, respectively. Eight tumors were selected for detection of mRNA expression.

Twenty endocrine tumors were studied, which were localised as follows: head: 8, body: 4, tail: 8. The mean age of these patients was 55 (28-78), with 6 males and 14 females comprising the group. According to WHO classification 4 endocrine tumors were benign, 11 proved to be borderline and 5 were malignant tumors. Two benign and three borderline tumors were functional, producing insulin. Eight tumors were selected for detection of mRNA expression.

Twelwe mucinous cystadenomas, 11 borderline tumors and 5 mucinosus cystadenocarcinomas were studied by immunohistochemistry, from which 5 benign, 5 borderline and 5 malignant tumors were investigated for detection of mRNA expression.

Acinar cell carcinoma of a 10 years old boy with Cushing syndrome was examined by immunohistochemistry.

3.2. Methods

3.2.1. Histology and histochemical reactions

Formalin fixed and paraffin embedded tissues from normal pancreas and tumors were stained with hematoxylin and eosin. To detect mucin production sections were stained with periodic acid Schiff (PAS), PAS-diastase and mucicarmin in case of acinar cell tumor.

3.2.2. Immunohistochemistry

3.2.2.1. Immunohistochemical reactions with CLDN-1, -2, -3, -4 and -7 antibodies

Paraffin embedded, 3-4 µm thick sections were used for immunohistochemistry. After endogen peroxidase blocking and antigen retrieval the following primary antibodies were used: CLDNs-1, -3 and -7 were polyclonals, CLDN-2 and -4 antibodies were monoclonals (Zymed, San Francisco, CA, USA).

The biotinylated secondary antibody and avidin-streptavidin-enzyme conjugate were used according to the protocol of automated Ventana system. Reagents and secondary antibodies were produces of Ventana (Tuscon, AZ, USA). Diaminobenzidine (DAB: Ventana, Tuscon, AZ, USA) was used for visualisation, and counterstained with hematoxylin.

For negative controls, the appropriate antibody was omitted and either the antibody diluent alone or isotype matched IgG serum was used. Positive controls recommended by the manufacturer (Zymed Inc, San Francisco, CA, USA) were used to confirm correct immunohistochemical staining for CLDNs, that is normal skin epithelium for CLDN-1, normal colon mucosa for CLDNs-2, -3, -4 and normal ductal cells in breast for CLDN-7.

Reactions were scored positive where linear membrane staining was seen, except for CLDN-2, where cytoplasmic granular reaction was detected as well. Immunreaction was evaluated by the percentage of cells staining positively. The following values were given for semiquantitative evaluation: 0 score (0-5% positivity), 1 score (6-20%

positivity), 2 score (21-40% positivity), 3 score (41-60% positivity), 4 score (61-80% positivity), 5 points (81-100% positivity). Tumors scored from 1 to 5 were regarded as positive, while 0 scores were handled as negative. Immunohistochemical reaction of mucinous cystic tumors was evaluated by combining two scoring systems which made possible tissue multoblocks in cases of these tumors: the percentage of positively stained cells (described above) and the intensity of staining (mild: 1; medium: 2; high intensity: 3 points). These two values were multiplied in each scoring and used for further statistical analysis.

3.2.2.2. Other immunohistochemical reactions in case of acinar cell carcinoma

The following primary antibodies were used by Ventana state machine: mouse monoclonal (**β-cathenin:** BD Bioscience–610154, **CEA:** DAKO-M7072, **chromogranin:** DAKO-M0869, **CK 20:** DAKO-M7019, **CK 7:** DAKO-M7018, **CK AE1-AE3**: DAKO-M3515, **CK MNF116**: DAKO-M0821, **Ki-67** DAKO-M7240, **NSE** (DAKO-M873, **p53:** DAKO-M7001, **serotonin:** DAKO-N1530, **synaptophysin** DAKO-M0776, **vimentin** DAKO-M0725), rabbit polyclonal (**AFP** DAKO-A0008, **alfa-1-antitrypsin:** DAKO-A012, **lysosyme:** DAKO-A0099, **somatostatin.** DAKO-N1551, **gastrin**: DAKO-N1540, **glucagon**: DAKO-N1542) and guinea-pig polyclonal (**insulin**: DAKO-N1542).

Labeled streptavidin-biotin 2 (LSAB 2) system (DAKO Cytomation, Ghostrup, Denmark, DK) with chromogen diaminobenzidine tetrachloride (DAB: Ventana, Tucson, AZ, USA) was used for the detection of trypsinogen, CRF and ACTH. For nuclear staining hematoxylin was used. Polyclonal rabbit anti-rat trypsinogen was used to demonstrate acinar differentiation in the tumor. This antibody was prepared and affinity purified at the Department of Biochemistry, Eötvös University, Budapest and was proved by Western-blot analysis to crossreact with human trypsinogen. Two corticotropin releasing factor (CRF) antibodies were used; one (Peninsula Laboratories, Inc., Belmont, CA, USA) for frozen sections; the other prepared and characterized by Görcs TJ (Semmelweis Univesity, Department of Anatomy, Histology and Embryology) for paraffin sections. For CRF and ACTH (DAKO-N1531A) immunohistochemical reactions, antibodies were used in carefully experienced titres,

and the assays were repeated on frozen sections using indirect immunofluorescence technique to avoid unspecific labeling. Immunohistochemical reactions for secretin (Accurate Chemical and Scientific Corporation, Westbury, N.Y.) and vasoactive intestinal polypeptide (VIP: Accurate Chemical and Scientific Corporation, Westbury, N.Y.) were prepared using indirect immunofluorescence technique (secundary antibody labeled with fluorescent isothiocyanate: Vector Laboratories, Burlingame, CA, USA).

For control reactions, chronic pancreatitis and pancreatic ductal adenocarcinoma samples were obtained from pancreatectomy specimens of adult patients**.** In case of ACTH immunohistochemistry, positive control reactions were prepared on a hypophysis obtained at autopsy and on an ACTH-secreting carcinoid tumor of the lung from a 50-year-old patient.

3.2.3. Tissue microarray

Mucinous cystic tumors were studied immunhistochemically on slides from tissue microarray.

3.2.4. Statistical analysis

Statistical analysis of the immunohistochemical scores was achieved by the Mann-Whitney U test. Probability values of $P < 0.05$ were accepted as being significant.

3.2.5. Electronmicroscopy

For electron microscopy, tissue pieces were fixed in 2.5% glutaraldehyde and osmium tetroxide. Ultrathin sections were cut and double contrasted with uranyl acetate and lead citrate.

3.2.6. Immunochemiluminometric assay (ICMA)

Direct determination of ACTH content in the tumor was done by means of an immunochemiluminometric assay. For this, pieces of frozen tumor tissue were

homogenized in Krebs–Ringer bicarbonate buffer containing ethylene-diaminetetraacetic acid (EDTA), and after centrifugation, the supernates were used for the assay. For comparison, pieces of pancreatic ductal adenocarcinoma and chronic obstructive pancreatitis were similarly processed. The ACTH concentration in supernates was determined by a commercial kit according to the manufacturer's instructions (Nichols Institute Ltd., San Juan, CA, USA).

3.2.7. Real-time (RT-) PCR

Total RNA from formalin fixed paraffin embedded tumor tissues was isolated with High Pure RNA Paraffin Kit (Roche, Indianapolis, Indiana, USA). Samples were microdissected to select the areas containing purely exocrine or endocrine pancreatic tumors. Proteinase K digestion time was 16 hours for each sample. All purifications were performed in accordance with the manufacturer's protocol. Total RNA (500 ng) was reverse transcribed for 50 minutes at 42°C in 30 μl with MulV reverse transcriptase (Applied Biosystems, Foster City, CA, USA) in the presence of RNAse inhibitor (Applied Biosystems) using Random Hexamers (Applied Biosystems).

Real-time PCR was performed with 2 μl cDNA template in a total volume of 25 μl, using the ABI Prism 7000 sequence detection system (Applied Biosystems). Each PCR was conducted in 25 μl volume of SYBR Green Supermix (Bio-Rad, Hercules, CA, USA, 1708851). Real-time PCR was performed in duplicates in 96-well plates, for 2 minutes at 95°C for initial denaturing, then 40 cycles at 95°C for 20 seconds, at 63°C for 30 seconds and at 72°C for 1 minute.

Data analysis and statistical evaluation were performed by REST (expression software tool, www.wzw.tum.de/gene-quantification), using the average of GAPDH as reference gene for relative quantification.

4. RESULTS

4.1. CLDN expression in normal, tumor free pancreas. In addition to the well-known CLDN-1 and -4 expressions, CLDNs-2, -3, and -7 were demonstrated in ductal cells, CLDN-3 and -7 proteins showed expression in acinar cells. Expression of CLDNs-3 and -7 was manifest in endocrine cells.

4.2. CLDN expression pattern in endocrine pancreatic tumors by immunohistochemistry. CLDN-1 and -4 immunoreactions proved to be negative in all endocrine tumors, whereas, only one endocrine tumor (1/20) was positive for CLDN-2. On the other hand, high CLDN-3 and -7 expressions were detected in all endocrine tumors. No statistically significant differences were detected between benign, borderline and malignant endocrine tumors.

4.3. CLDN expression in ductal adenocarcinomas by immunohistochemical reactions. CLDNs-1 and -4 definitely showed high, diffuse expression in all adenocarcinomas as published in the literature. As a new result, intracellular granular staining was noted for CLDN-2 in 45 percent of ductal adenocarcinomas. No CLDN-3 was detected. CLDN-7 was positive in all cases with focal, variable positivity. There was no statistically significant difference between grade I, II, and III tumors in CLDN expression.

4.4. CLDN expression in endocrine pancreatic tumors compared with ductal adenocarcinomas by immunohistochemical reactions and RT-PCR. Significantly higher percentage of endocrine tumor cells expressed CLDNs-3 and -7, while a considerably higher percentage of adenocarcinomas expressed CLDNs-1, -2 and -4. mRNA expression comparing ductal adenocarcinomas and endocrine carcinomas revealed the mRNA expression of specific CLDNs to reflect parallel changes with the protein expression of these tumors.

4.5. Correlation of CLDN expression with prognosis in mucinous cystic tumors. Higher CLDN-1, -2, -4 and -7 expressions were detected with immunohistochemical

reactions and RT-PCR analysis in mucinous cystadenocarcinomas as compared with adenomas, but significant difference was found in CLDN-4 expression by immunohistochemistry and with both analyses regarding expression of CLDNs-4 and -7.

4.6. CLDN expression pattern in acinar cell carcinoma. Based on morphological and immunohistochemical features acinar cell carcinoma was diagnosed. This is the first published case to detect acinar cell carcinoma in a child with Cushing syndrome. The expression of CLDNs-1 and -2 and the lack of CLDN-3 and -7 expressions could rather underline the exocrine origin of this tumor.

4.7. CLDN expression pattern in pancreatic tumors and cells with different origin and its significance in differential diagnostics. According to our investigations on pancreatic endocrine tumors, pancreatic ductal adenocarcinomas, mucinous cystic tumors and acinar cell carcinomas, CLDN expression patterns showed characteristic features. The presence of CLDN-3 referred to endocrine differentiation. Exocrine adenocarcinomas, mucinous cystic tumors showed CLDN-1, -2, -4 and -7 positivity, whereas in acinar cell carcinomas only CLDN-1 and -2 positivity was detected.

5. CONCLUSIONS

5.1. In addition to the well-known CLDN-1 and -4 expressions, CLDN-2, -3 and -7 proteins were demonstrated in ductal cells, CLDN-3 and -7 proteins showed expression in acinar cells. Expression of CLDNs-3 and -7 was manifest in endocrine cells.

5.2. CLDN-3 and -7 proteins showed high expression in endocrine tumors, while CLDN-1, -2 and -4 proteins were detected in exocrine tumors. This is the first report of CLDN protein expression in endocrine tumors.

5.3. The level of CLDN-1, -4 and -7 protein expressions in borderline cystic tumors is inbetween that of benign and malignant tumors. This supports the sequential development theory regarding mucinous cystic tumors.

5.4. This is the first report on childhood acinar cell carcinoma with Cushing syndrome.

5.5. The presence of CLDN-3 refers to endocrine differentiation. The adenocarcinomas and cystic mucinous tumors of exocrine origin denoted CLDNs-1, -2, -4 and -7 positivity, whereas acinar cell carcinomas expressed only CLDN-1 and -2 positivity. Considering the CLDN expression observed in normal pancreas cells, it can be established that CLDN-1, -2 and -4 proteins are definitely markers of ductal differentiation, CLDN-1 protein of acinar differentiation, and CLDN-3 of endocrine differentiation.

5.6. The increased CLDN-4 expression in adenocarcinomas and mucinous cystic tumors, as well as the high CLDN-3 expression in endocrine tumors may open up new prospects in the targeted therapy of these tumors.

5.7. The CLDN expression pattern of pancreas tumors may be employed in the differential diagnosis of these tumors and may be of help in deciding dignity.

6. LIST of PUBLICATIONS

Cummulative impact factors: 8.482

Publications related to the Dissertation

Borka K, Kaliszky P, Szabó E, Lotz G, Kupcsulik P, Schaff Z, Kiss A. Claudin expression in pancreatic endocrine tumors as compared with ductal adenocarcinomas. Virchows Arch. 2007. 450: 549-57.

IF:2.029

Illyés G, Luczay A, Benyó G, Kálmán A, **Borka K**, Köves K, Rácz K, Tulassay T, Schaff Z. Cushing's Syndrome in a Child with Pancreatic Acinar Cell Carcinoma. Endocr Pathol. 2007. 18: 95-102.

IF:1.837

Publications not related to the Dissertation

Corradi G, Kiss F, **Borka K**, Füle T, Szabó K. Current viewpoints on the evaluation of testicular biopsy. Orv Hetil. 1998. 139: 2243-2246.

Borka K, Illyés G, Zsirka K. A, Flautner L, Schaff Z. Acute pancreatitis with few symptoms and extensive necrosis. Orv Hetil. 2001. 26: 1403-1404.

Kaliszky P, Jámbor G, Nagy Z, **Borka K**, Regáli L. Early results after aortoiliac reconstruction in our department in the past six years. Magy Seb. 2003. 56: 103-107.

Nádas G, Bánfi G, **Borka K**, Romics I. A pénisz Kaposi-sarcomája. Uroonkológia, 2004. 3: 81-83.

Vojnisek Z, Arató A, Bodánszky H, Dezsőfi A, Veres Gábor, **Borka K**, Glasz T, Nyitrai A, Kis É, Verebély T, Kálmán A, Kiss I, Szőnyi L. Cholangitis sclerotisans és haemobilia együttes előfordulása. Gyermekgyógyászat 2005. 3: 275-283.

Sobel G, Halasz J, Bogdanyi K, Szabo I, **Borka K**, Molnar P, Schaff Z, Paulin F, Banhidy F. Prenatal diagnosis of a giant congenital primary cerebral hemangiopericytoma. Pathol Oncol Res. 2006. 12: 46-49.

IF:1.241

Borka K, Patai K, Rendek A, Sobel G, Paulin F. Pleomorphic rhabdomyosarcoma of the uterus in a postmenopausal patient. Pathol Oncol Res. 2006. 12: 102-104.

IF:1.241

Kelemen Z, Nyirády P, Bánfi G, Joós L, **Borka K.** A hímvessző vastagítása vazelinnel – következmények és azok ellátása. Magyar Urológia. 2006. 1: 16-27.

Nyirady P, **Borka K**, Banfi G, Kelemen Z. Lichen sclerosus in urological practice. Orv Hetil. 2006. 44: 2125-2129.

Borka K, Bánfi G, Kelemen Z, Nyirády P. Romics I. Lichen sclerosis és hímvesszőrák összefüggése. Magyar Urológia. 2007. 1: 15-18.

Nyirády P, Kelemen Z, Kiss A, Bánfi G, **Borka K**, Romics I. Treatment and outcome of vaseline-induced sclerosing lipogranuloma of the penis. Urology. 2008. 71: 1132-1137.

IF:2.134

Quotable abstracts related to the Dissertation

Borka K, Kiss A, Kaliszky P, Kupcsulik P, Schaff Z. Lack of claudin 4 expression and high expression of claudin 3 and claudin 7 differentiate pancreatic endocrine tumors form pancreatic ductal carcinoma. 20th European Congress of Pathology, September 3-8, 2005, Paris, France. Virchows Arch. 2005. 447: 404.

Quotable abstracts not related to the Dissertation

Fischer M, **Borka K**, Illyés G, Hermányi Z, Szalay F. Venoocclusive disease after restrart of long-term oral contraceptive use following cesarean section for twin pregnancy. 41th Annual Meeting of the Hungarian Society of Gastroenterology, June 8-12, 1999, Balatonaliga, Hungary. Z. Gastroenterol. 1999. 37:122.

Borka K, Illyés G, Fischer M. Hepatic veno-occlusive disease caused by oral contraceptives. European Congress of the International Hepato-Pancreato-Biliary Association (IHPBA), May 24-28, 1999, Budapest, Hungary. Dig Surg. 1999. 16: 1-127.

Borka K, Horányi J, Sipos B, Klöppel G. Endocrine tumors of the pancreas: benign or malignant? 18th European Congress of Pathology, September 8-13, 2001, Berlin, Germany. Virchows Arch. 2001. 439: 251.

Borka K, Horányi J, Sipos B. Endocrine Tumors of the Pancreas: Benign or Malignant? Zeitschrift für Gastroenterologie, 43th Annual Meeting of the Hungarian Society of Gastroenterology, June 4-8, 2001, Balatonaliga, Hungary. Z. Gastroenterol. 2001. 39: 382.

Kaliszky P, **Borka K**. The role of CD44 variants and C-erbB-2 gene expression in the progression of pancreatic tumors. 45th Annual Meeting of the Hungarian Society of Gastroenterology, June 3-8, 2003. Balatonaliga, Hungary. Z. Gastroenterol. 2003. 41: 430.

Borka K, Kiss A, Tőkés A, Kaliszky P, Kupcsulik P, Schaff Z. HER-2 gene amplification in pancreatic cancer. 46th Annual Meeting of the Hungarian Society of Gastroenterology, June 1-5, 2004. Balatonaliga, Hungary. Z. Gastroenterol. 2004. 42: 414.

Kaliszky P, Gyurkovics E, Regáli L, Lukovics P. **Borka K**. Gastrointestinal complications requering reoperation after aorto-iliac reconstructions. 46th Annual Meeting of the Hungarian Society of Gastroenterology, June 1-5, 2004. Balatonaliga, Hungary. Z. Gastroenterol. 2004. 42: 414.

Borka K, Szijjarto A, Kaliszky P, Kiss A, Schaff Z, Kupcsulik P. Role of immunohistochemical and molecular identification of her-2 gene in human pancreatic cancer. European Congress of the International Hepato-Pancreato-Biliary Association (IHPBA), May 25-28. 2005. Heidelberg, Germany. HPB (Oxford). 2005. 7: 91–102.