ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2009, **57**, 295–301
PL ISSN 0004-069X
DOI 10.1007/s00005-009-0033-x

Immunohistochemical localization of metallothionein and p53 protein in pancreatic serous cystadenomas

Mariola Śliwińska-Mossoń[1], Halina Milnerowicz[1], Jerzy Rabczyński[2] and Stanisław Milnerowicz[3]

[1] Department of Biomedical and Environmental Analyses, Wrocław Medical University, Wrocław, Poland
[2] Department of Pathological Anatomy, Wrocław Medical University, Wrocław, Poland
[3] Department and Clinic of Gastrointestinal and General Surgery, Wrocław Medical University, Wrocław, Poland

Received: 2008.08.14, **Accepted:** 2009.01.30, **Published online:** 2009.07.04

Abstract

Introduction: The objective of this study was to determine the expression levels of metallothionein (MT) and p53 protein, recognized neoplastic transformation markers, in pancreatic serous cystadenomas (SCA) and adenomocarcinomas.
Materials and Methods: Neoplastic pancreatic tissue was taken from 20 patients with diagnosed benign (SCA: 5 cases) or malignant tumors (adenomocarcinomas: 15 cases) and control pancreatic tissue from healthy persons who had died in car accidents. Sections were stained with hematoxylin-eosin. Immunohistochemical localization of MT and p53 protein was carried out by LSAB2-HRP using specific antibodies against MT and p53.
Results: Metallothionein expression was observed only in the epithelial cells of the neoplastic tissue of SCAs. MT expression in the cystadenomas was weaker than in the healthy pancreatic tissue. No tissue was found with p53 protein expression. In the adenomocarcinomas, positive staining for MT was observed in 67% and p53 was positive in the carcinoma cells.
Conclusion: The weak MT expression and lack of p53 protein expression in pancreatic SCAs confirms the lack of local invasive potential of the neoplastic lesion. Increased expressions of MT and p53 were observed in the less differentiated tumors. Thus the expression of MT may be a potential prognostic marker for tumors.

Key words: serous cystadenoma, adenomocarcinoma, pancreas, immunochemistry, metallothionein, p53 protein.

Abbreviations: MT – metallothionein, SCA – serous cystadenomas, PAS – periodic acid-Schiff.

Corresponding author: Halina Milnerowicz, MD, Ph.D., Department of Biomedical and Environmental Analyses, Wrocław Medical University, Grunwaldzka 2, 50-355 Wrocław, Poland, tel.: +48 71-3217470, fax.: +48 71-7840172, e-mail: msm@tox.am.wroc.pl

INTRODUCTION

Metallothionein (MT) is a low-molecular-weight metal-binding protein (Coyle et al. 2002; Kagi 1991) which plays important roles in the detoxification of heavy metals, homeostasis of essential metals, and scavenging of free radicals (Kagi 1991; Klassen et al. 1999; Nath et al. 1988). Physiologically, MT level is low and increases in response to the presence of heavy metals, hormones (e.g. glucagon, glucocorticoids), as well as cAMP, interleukins 1 and 6, interferon, and tumor necrosis factor (Davis and Cousins 2000). Metallothionein is present in many healthy tissues, such as hepatic parenchymatous cells, kidneys, lungs, thymic epithelial cells, erythrocytes, lymphocytes, and many exo- and endocrine pancreatic cells (Cherian 1994; Danielson et al. 1982; Tekada et al. 1997; Tomita 2000a; Tomita 2000b). A number of studies have demonstrated the presence or enhanced synthesis of MT in rapidly proliferating normal cells, regenerating cells, and cancer cells (Abdel-Mageed and Agrawal 1998). MT has been considered a potential prognostic marker in invasive ductal carcinomas of the breast (Schmid et al. 1993), skin (Zelger et al. 1993), and pancreas (Ohshio et al. 1996). Irregular cell growth due to increased cell proliferation or failure of cells to undergo apoptosis is recognized as a major contributory factor to the malignant process (Fan and Cherian 2002; Janssen et al. 2002; Madej et al. 1999; Ohshio et al. 1996; Oyama et al. 1996; Saga et al. 2002). However, the mechanism of tumor MT expression is not fully understood.

There are mechanisms in healthy and tumor cells

allowing for interaction between MT and p53 protein and for the growth cycle of dividing cells (Fan and Cherian 2002). *In vitro* investigations have shown that cell exposure to chelating metals induces changes in the p53 gene's three-dimensional structure, probably predisposing to gene mutation (Cherian and Apostolova 2000). Irregularities in p53 gene are the most frequent genetic disturbance in tumor cells. They are typical of pancreatic adenocarcinoma (p53 gene mutation frequency is estimated at 21–70%) and influence its biology and prognosis. Previous studies showed that p53 protein expression in pancreatic tumors was related to poor prognosis and worse histological grading of the tumors (Pellegata et al. 1994).

Adenomas are very rare tumors, accounting for 0.5–1% of all pancreatic tumors, and serous cystadenomas (SCAs) constitute 25% of them (Gerdes et al. 2003). SCAs, also known as glycogen-rich adenomas, are rare benign tumors of the pancreas (Alrefaie et al. 2004; Compton 2000; Gerdes et al. 2003; Ishikawa et al 1998; Perez-Ordonez et al. 1996; Rabczynski et al. 2006; Santos et al. 2002; Strobel et al. 2003). Increased SCA morbidity occurs in patients over 60 years of age, with an evident female preponderance (92%) (Compton 2000; Ishikawa et al. 1998; Pellegata et al. 1994; Santos et al. 2002; Strobel et al. 2003). The histogenesis of pancreatic SCA remains unknown and its detection is difficult at each stage of the disease's progression. An SCA is usually discovered incidentally or due to nonspecific abdominal symptoms. Gastrointestinal symptoms, biliary tract obstruction, and diabetes mellitus, when normal islets of Langerhans are destroyed by the tumor, are the first signs observed in SCA patients. The majority of SCAs are located in the pancreatic head. The rare occurrence, unclear histogenesis, and differentiated clinical symptoms of this disease result in even individual cases being subjected to a series of studies (Compton 2000; Ishikawa et al. 1998; Santos et al. 2002; Strobel et al. 2003).

Serous cystadenoma of the pancreas usually occurs as a solitary and well-formed tumor with neoplasm. It may sometimes also coexist with other pancreatic tumors, such as adenocarcinomas or mucinus cystadenoma (Alrefaie et al. 2004; del Vecchio et al. 2002; Montag et al. 1990; Nitta et al. 2008; Posniak et al. 1991). The localization and role MT in SCA of the pancreas have not yet been reported in the literature. The aim of this study was to identify immunohistochemically the localization of MT and p53 protein and to determine their expressions in pancreatic SCA.

MATERIALS AND METHODS

The study material was obtained from 20 patients with diagnosed benign (serous cystadenoma: n=5) or malignant tumors (adenomocarcinomas: n=15) hospitalized at the Department and Clinic of the Gastroenterological and General Surgery of Wrocław Medical University between 1992 and 2001. The tumors of the pancreas were classified histologically according to World Health Organization criteria (Klöppel et al. 1996).

All the patients with SCA (five females 39–71 years old, mean age: 55.2 years) 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, esophagogastroduodenoscopy, and endoscopic retrograde cholangiopancreatography. In case of diagnostic doubts, magnetic resonance or magnetic resonance cholangiopancreatography and Doppler USG were made. Cystic changes in the pancreas were found in all patients during the preoperative examination. No family history of changes and no characteristics of von Hippel Lindau syndrome were detected in any case. Changes were identified in two cases 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 applied in all cases (Table 1).

The 15 cases of adenomocarcinoma consisted of 10 female and 5 male patients with a mean age of 50.6 years (range: 29–77 years). Only 6 of the patients (40%) underwent curative surgical resection and 3 (20%) had palliative surgery. In the remaining cases, only tissue sections of the pancreas were taken for histopathological diagnosis. Changes were found in the pancreas head in eleven cases and in the pancreas head and body in

Table 1. Characteristics of patients with serous cystadenoma of the pancreas (n=5)

No./ /gender/ /age	Location in the pancreas	Type of surgery	Post-surgery observation	Coexisting diseases
1/F/39	head	pancreatodudenectomy Whipple procedure	5 years	chronic cholecystitis
2/F/46	head	pancreatodudenectomy Whipple-Traverso procedure	6 years	chronic cholecystitis
3/F/64	body	left-side resection of the pancreas	11 years	none
4/F/56	body tail	enucleation of the cyst	4 years	chronic cholecystitis
5/F/71	tail	left-side resection of the pancreas	3 years	chronic mucosal gastritis

F – female.

four. None of the patients had been treated with chemotherapy and/or radiation therapy prior to pancreatic surgery. In one case the patient died after the operation, in eleven cases the time of post-operative observation was about three months, and in three cases about nine months.

Control pancreatic tissue originated from healthy persons who had died in car accidents. All tissue sections were routinely fixed in a phosphate-buffered 10% formaldehyde solution and embedded in paraffin. Serial 4-μm-thick sections were stained with hematoxylin and eosin and periodic acid-Schiff (PAS) and MT and p53 protein were localized immunohistochemically using the method described earlier (Gerdes et al. 2003; Madej et al. 1999; Pillai et al. 2003).

This study was approved by the local institutional committee (No. KB 257/2000) authorized to approve research with human subjects and was funded by Wrocław Medical University.

The serial tissue sections, deparaffinized and hydrated in an alcohol series, were incubated with a 3% hydrogen peroxide solution to block any intracellular peroxidase activity. For p53 antigen retrieval, the sections were boiled in citrate-buffered saline. Nonspecific bonds were blocked using antibody diluent (DAKO, Carpinteria, CA, USA; code: S0809). The tissue sections were subsequently incubated for 30 min at room temperature with specific monoclonal antibodies against MT (DAKO Carpinteria, CA, USA; code: E09) and p53 (DAKO, code: N 1581). After washing in 0.05 M Tris-HCl (Bio-Rad Laboratories; Hercules, CA, USA; code: 170-6435) with 0.1% Tween 20 (Sigma Chemical, St. Louis, MO, USA; code: P1379) the antibody-hormone complexes were visualized using the LSAB2-HRP test (DAKO, Carpinteria, CA, USA, code: K0673). The peroxidase activity was located against 3,3'-diaminbenzidine in an imidazole-HCL buffer, pH 7.5. Then the sections were washed in distilled water, contrasted with hematoxylin (Chem Mate™, DAKO Glostrup, Denmark; code: S2020), closed in glycerin gel, and left to dry. A negative control was performed for each tissue section by replacing the primary antibody with an anti-rabbit IgG antibody immunoglobulin control (Negative Control DAKO, Carpinteria, CA, USA; code: X0903).

The incubation times of the individual stages of the test were matched experimentally under laboratory conditions. The sections were stained with hematoxylin-eosin and PAS stains. The stained tissue sections were viewed under high power with an Olympus BX41 light microscope (Olympus Optical Co. Ltd., Japan) interfaced to an Olympus DP70 digital camera (Olympus Optical Co. Ltd., Japan) that digitized the microscopic image.

Assessment of immunohistochemical staining

A semi-quantitative method was used to assess histopathologically the intensity reaction of tissue specimen staining: 0 no immunohistochemical reaction, + weak reaction, ++ moderate reaction, +++ strong reaction, and ++++ very strong reaction.

RESULTS

Tissue of normal pancreas

The exocrine and endocrine pancreatic cells were normal. Clearly isolated islet cells showed a weak but positive (+) dispersed expression of MT in the pancreas on their whole surface. In all four control cases, no immunohistochemical reaction (0) to MT was observed in the pancreatic duct cells. A dispersed moderate (++) reaction in all parts of the organ was found in few acinar cells (Fig. 1). The immunohistochemical localization of p53 was negative in normal pancreas (Fig. 2).

Tissue of pancreas with serous cystadenoma

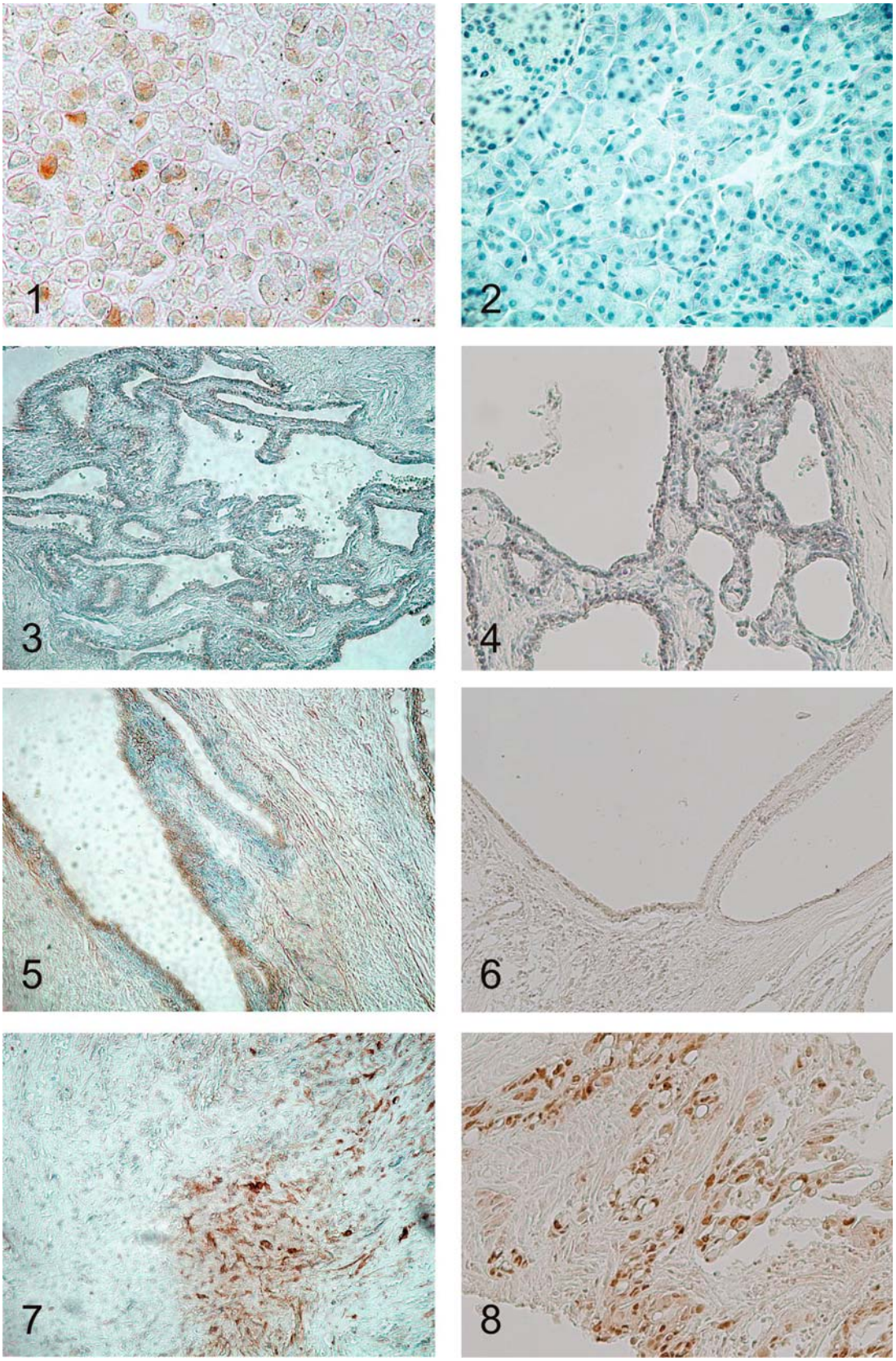
The histopathological examination revealed features characteristic of serous cystadenoma in all the SCA cases. The tumor was composed of multilocular areas of various diameters. A single layer of cuboidal epithelium lined the cyst. Cells of similar size with a clear, slightly granular cytoplasm with a single round or oval, slightly hyperchromatic nucleus were visible. Some single, fine nerve trunks and vessels were visible in the connective septa.

The immunohistochemical localization of p53 was negative in all SCA cases (Figs. 4 and 6). In four cases, MT expression was weak (+) and in one case moderate (++). This is the first unusual case of a serous cystadenoma that produces islet hormones, somatostatin, and pancreatic polypeptide (Rabczynski et al. 2006). The hormone expression was focal and limited to the lining epithelium of a neoplastic cyst, similarly to the expression of MT (Fig. 5).

Tissue of pancreas with adenocarcinoma

Microscopically, an infiltrating ductal adenocarcinoma of the pancreas is an invasive malignant epithelial neoplasm with ductal differentiation and without a predominant component of any of the other carcinoma types. The tumor is composed of diverse (anaplastic) cells with eosinophilic cytoplasm and a condensed hyperchromatic nucleus with large conspicuous nucleoli. Many tumor cells contain unusually large nuclei with unequally disposed chromatin. Less differentiated pancreatic adenomocarcinomas were detected in all the pancreatic sections of the patients.

MT immunostaining was diffusely or focally strong positive (+++) in 10 of the cases (67%) of pancreatic carcinoma (results described earlier) (Milnerowicz et al. 2006). In most cases both nuclear and cytoplasmic staining were observed (Fig. 7). The immunohistochemical localization of p53 was positive in the carcinoma cells (Fig. 8).



Figs. 1–8. Immunohistochemical localization of MT and p53 in normal pancreas and tumors of the pancreas. **Fig. 1.** Positive staining for MT in a few acinar cells of the normal pancreas (×210). **Fig. 2.** Negative staining for p53 in tissues of normal pancreas (×210). **Figs. 3–4.** Patient no. 4: negative staining for MT and p53 in tissues of benign (SCA) tumor (×137, ×210). **Figs. 5–6.** Patient no. 3: moderate immunohistochemical MT reaction limited to the lining epithelium of a neoplastic cyst and no reaction for p53 protein (×257; ×210). **Figs. 7–8.** Malignant tumors of the pancreas: strong MT and p53 immunohistochemical reaction in the carcinoma cells (×210).

The prognoses of the patients with positive expression of MT and p53 were significantly poorer than of those with negative expressions of these parameters.

DISCUSSION

The tumor suppressor protein p53 is able to respond to different cellular stresses, such as DNA damage, hypoxia, oxidative stress, and oncogene activation. Upon activation, p53 initiates a number of cellular activities that can ultimately culminate in G1 or G2 cell-cycle arrest and DNA repair, apoptosis, or other cellular changes (Schwartz and Rotter 1998). As a critical cellular mediator of the response to genotoxic damage, p53 has a direct role in maintaining genome integrity. Loss of p53 activity has been associated with tumor progression and unfavorable prognosis (Symonds et al. 1994). It was recently found that there is an association between MT and p53 expression in lung and breast cell carcinomas (Fan and Cherian 2002; Joseph et al. 2001). However, the mechanism of tumor MT and p53 expression is not fully understood.

Metallothionein plays an important role in the homeostasis of zinc, a metal important for tumor growth and progression (Cherian 1994; Fan and Cherian 2002). There are mechanisms whereby MT and p53 may interact in the control of cell division. *In vitro* studies have shown that exposure to a metal chelating agent induces a reversible conformational change in wild-type p53 to a mutant form. It was thought that the binding of zinc ions to cysteinyl residues stabilizes the tertiary structure of p53 (Hainaut and Milner 1993). MT also has a high affinity to zinc ions and could thus act as an intracellular sequester of zinc. Ioachim et al. reported that cells containing MT in quantities sufficient to reduce intranuclear zinc ion levels and thus induce functional inactivation of p53 would acquire growth advantages and thus be able to proliferate and accumulate mutational events (Ioachim et al. 1999). Furthermore, Zeng et al. reported that mutation-induced MT overexpression may interfere with the function of DNA-binding zinc finger transcription factors involved in controlling the expressions of a wide range of genes regulating cell proliferation and apoptosis, such as p53, and conferring growth advantage on the mutated cells (Zeng et al. 1991).

Recent studies have demonstrated a negative relationship between MT expression and apoptosis (Hadama et al. 2002). MT-null cells have been shown to be more sensitive to apoptosis induced by anticancer drugs than MT-expressing cells. Furthermore, the transduction of an MT-antisense oligomer decreases bcl-2 expression and augments apoptosis (Kondo et al. 1997). These findings suggest that MT itself acts as an anti-apoptotic molecule and also upregulates other anti-apoptotic molecules. The overexpression of MT genes and reduction in apoptotic cells have been found to be associated in various human tumors (Hadama et al. 2002). A significant positive correlation between MT expression and poor clinical outcome has been found in breast carcinomas, colorectal carcinomas, and malignant melanomas (Fan and Cherian 2002; Ioachim et al. 1999; Schmid et al. 1993; Zelger et al. 1993).

Ohshio et al. showed that positive staining for MT in pancreatic carcinomas was related to the incidence of metastasis, worse histological grading of the tumors, and poor prognosis (Ohshio et al. 1996). These results are compatible with our study (Fig. 7). MT staining was frequently positive not only in metastatic regions, but also in samples obtained from primary pancreatic regions. These results suggest that MT-positive pancreatic cancers acquire an enhanced ability to produce MT as their malignant potential increases (Milnerowicz et al. 2006; Ohshio et al. 1996). In our study, MT staining was generally observed in the cytoplasm, but more than half of the MT-positive cases of pancreatic carcinomas showed nuclear as well as cytoplasmic staining. It is now known that the subcellular localization of MT is regulated in cells undergoing proliferation, developmental progression, and tumorigenesis (Cherian and Apostolova 2000). Cancer cells with nuclear MT may be more aggressive than cells with only cytoplasmic staining for MT because patients with nuclear staining survived for only two months. Our study shows that positive staining for MT in pancreatic carcinomas is related to the p53 expression of the tumor cells and to poorer prognosis.

The localization and role of MT in serous cystadenoma of the pancreas have not yet been not reported in the literature. In our study a weak MT expression was observed in SCA epithelial cells. The MT expression in SCA was similar to that in cells of normal pancreas. In this study we also employed immunohistochemical techniques to detect the alteration of p53 in SCAs. It has been shown that in various neoplasms and human pancreatic carcinoma cells lines, high-level expression of immunohistochemically detectable p53 correlates with p53 gene mutation (Bartsch et al. 1998; Satoh et al. 1996). In carcinoma lesions, the occurrence of K-ras and p53 alterations was as high (90 and 70%, respectively) as that shown in pancreatic carcinoma (Bartsch et al. 1998; Satoh et al. 1996). Recent genetic studies demonstrated that serous cystoadenomas are benign tumors of the pancreas. K-ras mutations have not been detected in serous cystadenoma of the pancreas (Bartsch et al. 1998; Gerdes et al. 2003; Ishikawa et al. 1998). The immunohistochemical localization of p53 was negative in all SCA cases. These results correlate with good prognosis and benign tumor lesions. The immunohistochemical investigation of the presence of mutated p53 gene showed its lack in SCA cells, which demonstrates correct function of the suppressor p53 gene in pancreatic SCA. The weak MT expression in SCA confirms the lack of a potentially invasive, local character of the neoplastic lesion; MT may therefore be considered a pancreatic neoplastic marker. Its degree of expression will indicate the type of tumor change. However, further studies are necessary to prove this claim.

In conclusion, in this study MT and p53 expression were observed in the majority of pancreatic carcinomas and were not observed in benign tumors.

REFERENCES

Abdel-Mageed AB, Agrawal KC (1998) Activation of nuclear factor kappaB: potential role in metallothionein-mediated mitogenic response. Cancer Res 58:2335–2338

Alrefaie W, Katz MH, Easter DW et al (2004) Simultaneous serous cystadenoma of the pancreas and mucinous cystadenoma of the appendix. J Pancreas 5:97–100

Bartsch D, Bastian D, Barth P et al (1998) K-ras oncogene mutations indicate malignancy in cystic tumors of the pancreas. Ann Surg 228:79–86

Cherian MG (1994) The significance of the nuclear and cytoplasmic tumor of metallothionein in human liver and tumor cells. Environ Health Perspect 102(suppl 3):131–135

Cherian MG, Apostolova MD (2000) Nuclear localization of metallothionein during cell proliferation and differentiation. Cell Mol Biol 46:347–356

Compton CC (2000) Serous cystic tumors of the pancreas. Semin Diagn Pathol 17:43–55

Coyle P, Philcox JC, Carey LC et al (2002) Metallothionein: the multipurpose protein. Cell Mol Life Sci 59:627–647

Danielson KG, Ohi S, Huang PC (1982) Immunochemical detection of metallothionein in specific epithelial cells of rat organs. Proc Natl Asad Sci USA 79:2301–2304

Davis SR, Cousins RJ (2000) Metallothionein expression in animals: A physiological perspective on function. J Nutr 130:1085–1088

del Vecchio MT, Pergola L, Tripodi SA et al (2002) Microcystic adenoma associated with a mucinous cystadenocarcinoma: a "collision tumor" of the pancreas. Pancreas 24:106–108

Fan LZ, Cherian MG (2002) Potential role of p53 on metallothionein induction in human epithelial breast cancer cells. Br J Cancer 87:1019–1026

Gerdes B, Wild A, Wittenberg J et al (2003) Tumor-suppressing pathways in cystic pancreatic tumors. Pancreas 26:42–48

Hadama T., Kinoshita M., Kobayashi D et al (2002) Endogenous tumor necrosis factor promotes resistance to cellular stresses by inducing the metallothionein-1A gene. Anticancer Res 22:4059–4063

Hainaut P, Milner J (1993) A structural role for metal ions in the "wide-type" conformation of the tumour suppressor protein p53. Cancer Res 53:1739–1742

Ioachim EE, Goussia AC, Agnantis NJ et al (1999) Prognostic evaluation of metallothionein expression in human colorectal neoplasms. J Clin Pathol 52:876–879

Ishikawa T, Nakao A, Nomoto S et al (1998) Immunohistochemical and molecular biological studies of serous cystadenoma of the pancreas. Pancreas 16:40–44

Janssen AM, van Duijn W, Kubben FJ et al (2002) Prognostic significance of metallothionein in human gastrointestinal cancer. Clin Cancer Res 8:1889–1896

Joseph MG, Banerjee D, Kocha W et al (2001) Metallothionein expression in patients with small cell carcinoma of the lung. Correlation with other molecular markers and clinical outcome. Cancer 92:836–842

Kagi JH (1991) Overview of metallothionein. Methods Enzymol 205:613–626

Klassen CD, Liu J, Choudhuri S (1999) Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 39:267–294

Klöppel G, Solcia E, Longnecker DS et al (1996) Histological typing of tumours of the exocrine pancreas, second edition. Springer-Verlag, Berlin-Heidelberg-New York, pp 7–18

Kondo Y, Rusnak JM, Hoyt DG et al (1997) Enhanced apoptosis in metallothionein null cells. Mol Pharmacol 52:195–201

Madej JA, Milnerowicz H, Dzimira S (1999) Immunohistochemical localization of metallothionein (MT) in epithelial tumors in animals. Med Wet 55:806–808

Milnerowicz H, Sliwinska-Mosson M, Bizon A et al (2006) Expression of metallothionein and p53 in pancreatic tumors in relation to prognosis. Gut 55:A324

Montag AG, Fossati N, Michelassi F (1990) Pancreatic microcystic adenoma coexistent with pancreatic ductal carcinoma: a report of two cases. Am J Surg Pathol 14:352–355

Nath R, Kambadur R, Gulati S et al (1988) Molecular aspects, physiological, functions, and clinical significance of metallothioneins. Crit Rev Food Sci Nutr 27:41–85

Nitta H, Hirota M, Ohkado A et al (2008) Coexistence of serous cystadenoma and ductal adenocarcinoma in the pancreas: a case report. Pancreas 36:218–219

Ohshio G, Imamura T, Okada N et al (1996) Immunohistochemical study of metallothionein in pancreatic carcinomas. J Cancer Res Clin Oncol 122:351–355

Oyama T, Take H, Hikino T et al (1996) Immunohistochemical expression of metallothionein in invasive breast cancer in relation to proliferative activity, histology and prognosis. Oncology 53:112–117

Pellegata NS, Sessa F, Renault B et al (1994) K-ras and p53 gene mutations in pancreatic cancer: ductal and nonductal tumors progress through different genetic lesions. Cancer Res 54:1556–1560

Perez-Ordonez B, Nassem A, Lieberman PH et al (1996) Solid serous adenoma of the pancreas: the solid variant of serous cystadenoma? Am J Surg Pathol 20:1401–1405

Pillai G, Roberts H, Gatter K et al (2003) p53 expression in normal paraffin-embedded tissue using different antibodies and antigen retrieval buffer systems. Histopathology 42:83–87

Posniak HV, Olson MC, Demos TC (1991) Coexistent adenocarcinoma and microcystic adenoma of the pancreas. Clin Imaging 15:220–222

Rabczynski J, Milnerowicz H, Sliwinska-Mosson M et al (2006) Hormone-producing serous cystadenoma of the pancreas. Neoplasma 53:495–499

Saga Y, Hashimoto H, Yachiku S et al (2002) Immunohistochemical expression of metallothionein in human bladder cancer: correlation with histopathological parameters and patient survival. J Urol 168:2227–2231

Santos LD, Chow C, Henderson CJ et al (2002) Serous oligocystic adenoma of the pancreas: a clinicopathological and immunohistochemical study of three cases with ultrastructural findings. Pathology 34:148–156

Satoh K, Shimosegawa T, Moriizumi S et al (1996) K-ras mutation and p53 protein accumulation in intraductal mucin-hypersecreting neoplasms of the pancreas. Pancreas 12:362–368

Schmid KW, Ellis IO, Gee JM et al (1993) Presence and possible significance of immunocytochemically demonstrable metallothionein over-expression in primary invasive ductal carcinoma of the breast. Virchows Arch A Pathol Anat Histopathol 422:153–159

Schwartz D, Rotter V (1998) p53-dependent cell cycle control: response to genotoxic stress. Semin Cancer Biol 8:325–336

Strobel O, Z'graggen K, Schmitz-Winnenthal FH et al (2003) Risk of malignancy in serous cystic neoplasms of the pancreas. Digestion 68:24–33

Symonds H, Krall L, Remington L et al (1994) p53-dependent apoptosis suppresses tumor-growth and progression in-vivo. Cell 78:703–711

Tekada A, Hisada H, Okada S et al (1997) Tumor cell growth is inhibited by suppressing metallothionein-I synthesis. Cancer Lett 116:145–149

Tomita T (2000a) Metallothionein in pancreatic endocrine neoplasms. Mod Pathol 13:389–395

Tomita T (2000b) New markers for pancreatic islets and islet cell tumors. Pathol Int 52:425–432

Zelger B, Hittmair A, Schir M et al (1993) Immunohistochemically demonstrated metallothionein expression in malignant melanoma. Histopathology 23:257–263

Zeng J, Heuchel R, Schaffner W et al (1991) Thionein (apometallothionein) can modulate DNA binding and transcription activation by zinc finger containing factor Sp1. FEBS Lett 279:310–312