—**Original**—

Overexpression and Localization of Heat Shock Proteins mRNA in Pancreatic Carcinoma

Masao Ogata[1,2,3], Zenya Naito[1], Shigeo Tanaka[2], Yuhkichi Moriyama[3] and Goro Asano[1]

[1]Department of Pathology, [2]Second Department of Surgery, [3]The Center for Digestive Diseases, Nippon Medical School

Abstract

In the present study we examined the localization and overexpression of heat shock proteins (hsps), mainly hsp90, in pancreatic carcinoma tissue compared with control tissue (including chronic pancreatitis and normal pancreas tissue), with the aid of immunohistochemical staining, *in situ* hybridization and reverse transcriptase polymerase chain reaction. Hsp90 α mRNA was overexpressed more highly in pancreatic carcinoma than in the control tissue. The proliferating-cell-nuclear-antigen labeling index was also high in pancreatic carcinoma tissue compared with the other tissue. These findings suggest that the overexpression of hsp90 α mRNA in carcinomas may be correlated with cell proliferation. However, hsp90 β was constitutively overexpressed almost equally in all groups of pancreatic tissue including pancreatic carcinoma, chronic pancreatitis and normal pancreas tissue. Immunohistochemical staining demonstrated a differentiation in the expression of hsp90 between histological types of pancreatic carcinoma. These findings suggest that hsp90 α is involved in carcinogenesis and that hsp90 β is correlated to structural conformation. Hsp90 α and hsp90 β seem to perform different functions in tissue containing malignant cells.

P53, MDM2 and WAF1, that were cell-cycle-related oncogene product were more strongly expressed in the nuclei of the cancer cells of the cancer tissue. Especially, MDM2 was more strongly expressed in mucinous carcinoma and the mucin secreting tissues surrounding pancreatic carcinoma tissue. The expression of MDM2 protein might also be correlated to secretion systems during structural conformation and be correlated to hsp90 β. (J Nippon Med Sch 2000; 67: 177—185)

Key words: heat shock proteins, immunohistochemistry, ISH (*In situ* hybridization), RT－PCR, pancreatic carcinoma

Introduction

The synthesis of heat shock proteins (hsps) in cells is induced by various stresses including heat shock[1]. The hsps have been grouped into several families of differing molecular weight, and the members of the different families are characterized not only by their size, but also by a number of functions at specific locations. The major functions of the hsps are based mostly upon the formation of complexes with other proteins, thus changing the functional status of these proteins[1]. In recent studies, it has been suggested that hsps are closely related to cell proliferation, cell cycle regulation and the metabolism of gene products, and that hsps play important roles in various processes of

Correspondence to Masao Ogata, Department of Pathology, Nippon Medical School, 1−1−5 Sendagi, Bunkyo-ku, Tokyo 113−8602, Japan. e-mail, masao-66@dd.iij4u.or.jp

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carcinogenesis. In particular, hsp90, which is associated with several proteins including pp60 v-src[2,3], casein kinaseII[4], elF2 α kinase[5], cytoskeletal proteins (actin[6] and tubulin[7]), and steroid receptors for estrogen, glucocorticoids, and progesterone[8] appears to contribute to regulation of the cell cycle[9,10] and processes of carcinogenesis[11-13]. Hsp70, which forms complexes with the mutant p53 of gene products[14] and binds to steroid receptors, also seems to be involved in the control of the cell cycle[15] and to play important roles in the process of carcinogenesis[16,17]. However, the relationship between the expression and localization of hsps, in situ, of human pancreatic carcinoma, and tumor growth and differentiation has not yet been clarified. We have therefore examined the expression of hsps, especially hsp90, and p53, MDM2 and WAF1 that were thought to play important roles in carcinogenesis and their relationship with cell proliferation in pancreatic disease, especially carcinomas, using the techniques of immunohistochemical staining, *in situ* hybridization and reverse transcriptase polymerase chain reaction (RT-PCR).

Materials and Methods

(1) *Tissue preparation*

The human pancreatic tissue used in this study was provided by the Department of Surgery of Nippon Medical School. Thirty-three specimens of pancreatic tumor tissue (20 cases of well to moderately differentiated ductal adenocarcinoma, 4 cases of poorly differentiated adenocarcinoma, 3 cases of mucinous cystadenocarcinoma, 2 cases of solid and cystic tumors, 2 cases of endocrine tumors, and 1 case each of mucinous carcinoma, and hamartoma) and 5 cases each of both normal pancreas tissue that exhibited chronic pancreatitis were examined. The specimens were fixed with 10% neutral formalin and embedded in paraffin for immunohistochemical staining. After extirpating the tumors, a small piece of each of these specimens was fixed immediately with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 6～8 h at 4℃. The specimens were then immersed successively in 10% (w/v) sucrose in PBS, 20% (w/v) sucrose in PBS, and then 30% (w/v) sucrose in PBS continuously for 12 h at 4℃ each, frozen in OCT compound (Tissue Tek, Elkhart, USA), and stored at −80℃ for *in situ* hybridization. A small piece of each of these specimens was also stored, without fixation, at −80℃ for RT-PCR.

(2) *Immunohistochemical staining*

The specimens were sliced into 3 μm thick sections and mounted onto 3-aminopropyltriethoxy silane-coated slides. Immunohistochemical staining was performed using the streptavidin biotin complex method (sABC kit, Nichirei, Tokyo, Japan). The following primary antibodies were visualized using an sABC kit: anti-hsp90, and anti-hsp70 antibodies (Affinity Bioreagents, Neshanic Station, NJ, USA, diluted to ×500, and ×300, respectively), anti-hsp60 antibody (Stress Gen Biotechnologies Corp., diluted to ×60), anti-ubiquitin antibody (Chemicon International, Temecula, CA, USA diluted to ×300), anti-wild and mutant p53 antibody (PAb1801, Oncogene Science, Cambridge, M. A, USA, diluted to ×20, and CM-1, Novocastra Laboratories, diluted to ×800, respectively), anti-mutant p53 antibody (PAB240, Cambridge Research Biochemicals, UK, diluted to ×150), anti-murine double minute 2 (MDM2) antibody (Oncogene Science, Cambridge, M. A, USA, diluted to ×20), and anti-wild type p53 activated fragment 1 (WAF1) antibody (Oncogene Science, Cambridge, M. A, USA, diluted to ×30). The sections were deparaffinized with xylene for 15 min and then washed in ethanol (100% to 70% for 30 s each). After washing in water, these sections were timely retrieved to expose the antigens by microwave treatment in 0.1 M citrate buffer (pH 6.0) for 15 min and endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in absolute methanol at room temperature (RT) for 30 min. The sections were washed three times with PBS (pH 7.2) for 5 min each, and then incubated with 10% normal rabbit or mouse serum for 10 min. The sections were incubated with the primary antibodies listed above, in a closed humid box at RT overnight. After washing the sections in PBS (three times for 5 min each), they were incubated with either biotinylated rabbit anti-mouse IgG or goat anti-rabbit IgG for 20 min. The sections were washed again in PBS (three times for 5 min each) and then incubated with streptavidin peroxidase conjugate for 10 min. After washing

in PBS (three times for 5 min each), the antibodies were visualized by treating the sections with diaminobenzidine tetrahydrochloride containing hydrogen peroxide for up to 10 min. The sections were counterstained with either hematoxylin or methyl green and then coverslipped.

(3) *Probe preparation*

For *in situ* hybridization, the following oligonucleotide antisense DNA probes for hsp90 α and hsp90 β were prepared based on nucleotide sequences reported by Hickey (1432～1491)[18] and Rebee (789～839)[19], respectively: antisense DNA probe for hsp90 α: 5'TTCAGGTTTGTCTTCCGACTCTTTCTCTTCTT-TTTCTTTTTCTTCTTCTTTGTCTTCTT-3'); antisense DNA probe for Hsp90 β (5'CTTGGGCTTTTC-TTCATCATCTTTATCTTCCTCTTCTTTCTCAC CTTTCTC-3'). These probes were labeled with digoxigenin dUTP (Boehringer Mannheim, Bedford, MA, USA) by the 3' terminal tailing method. In addition, a complementary sequence for each probe was prepared and similarly labeled as a sense probe. Reverse transcription was carried out on the total RNA extracted from frozen specimens. Polymerase chain reaction (PCR) amplification was performed using two synthetic oligonucleotide primers each, that were prepared as primers for hsp90 α (5'-ACCCAGACCCA-AGACCAACCG-3' and 5'ATTTGAAATGAGCTCT-CTCAG-3') based on nucleotides 13～33 and 133～153, respectively[18], and as primers for hsp90 β (5'-GT-GCACCATGGAGAGGAG-3' and 5'-ATTAGAGATC-AACTCCCGAAG-3') based on nucleotides 105～122 and 210～230, respectively.[19]

(4) *In situ hybridization*

All solutions for *in situ* hybridization were made using 0.1% diethyl pyrocarbonate-treated water added to distilled water and autoclaved after incubation overnight. All glassware was heat sterilized (at 220℃ for 2 h) after diethl pyrocarbonate treatment. The *in situ* hybridization was performed essentially as described previously by Ishiwata et al.[20] In brief, the frozen specimens were sliced into 5 μm thick sections and mounted onto 3-aminopropyltriethoxy silane-coated slides. After drying with cold air for 30 min, circles were drawn around each individual sections using white pap pen, rinsed in PBS (pH 7.2) (three times for 5 min each), and then acid treated in 0.2 N HCl for 20 min. This was followed by incubation with proteinase K (1～2 μg/m*l* PBS) for 15 min at 37℃, washing with PBS for 5 min, and postfixation in 4% (w/v) paraformaldehyde in PBS for 5 min. After washing in PBS for a further 5 min, the sections were immersed twice in glycine in PBS (2 mg/m*l*) for 15 min, each and then washed in PBS for 5 min. The sections were prehybridized with 50% formamide in 2 X standard saline citrate (SSC) for 1 h at 37℃. A digoxigenin-labeled DNA probe and hybridization buffer were mixed and denatured for 10 min at 95℃, and then immediately cooled on ice. Hybridization was then performed using the same hybridization buffer which was dropped (100 μ*l*) onto each section and incubated at 37℃ overnight in a closed, humid box filled with distilled water. After hybridization, the sections were washed twice with 50% formamide/2 X SSC for 15 min each, and then with 2 X SSC for 15 min at RT. The digoxigenin labeled oligonucleotide probe was detected using a DIG nucleic acid detection kit (Boehringer Mannheim GmbH, Best, Germany). After rinsing in buffer 1 (0.15 M NaCl, 0.1 M Tris-HCl, pH 7.5) for 30 min, the sections were incubated with buffer 2 (1.0% (w/v) blocking reagents in buffer 1) for 1 h at RT. The sections were then washed again with buffer 1 for 1 min, and then incubated with a 1: 2000 dilution of polyclonal sheep anti-digoxigenin Fab fragments conjugated with alkaline phosphatase in buffer 1 containing 0.2% Tween 20 for 30 min at RT. This was followed by washing twice with buffer 1 containing 0.2% Tween 20 for 15 min each, and then finally washed with buffer 3 (0.1 M NaCl, 0.05 M MgCl 2, 0.1 M Tris-HCl, pH 9.5) for 2 min at RT. The sections were incubated with a color solution containing nitroblue tetrazolium, 5-bromo-4-chloro-3-indolyl phosphate and buffer 3 in a dark, closed and humid box for 6－8 h. After stopping the reaction with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), the sections were mounted in aqueous mounting medium (Daido Sangyo, Tokyo, Japan). Sense probes were used as negative controls.

(5) *Reverse transcriptase polymerase chain reaction (RT-PCR)*

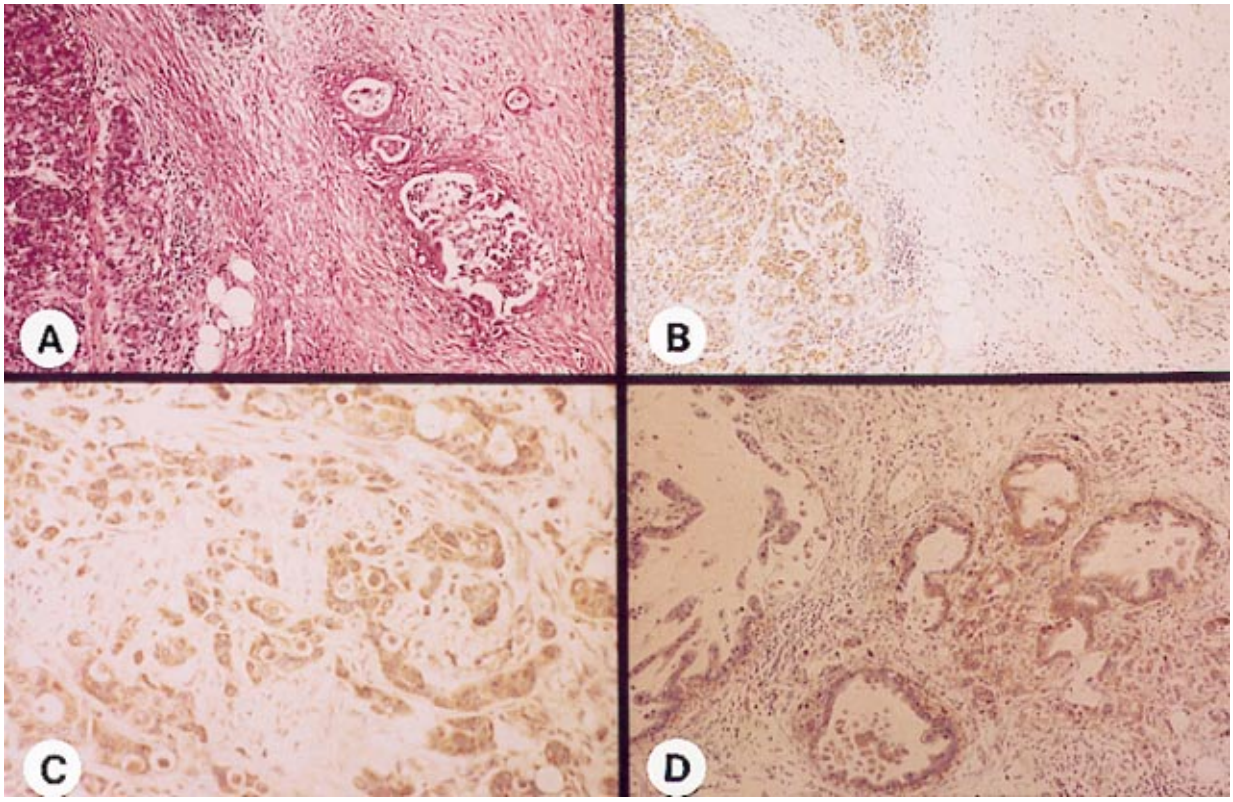

Fig. 1 Immunohistochemical staining in pancreatic carcinoma tissues. (A) HE staining in cancer and in adjacent to the cancer. ×100, (B) Hsp90 is localized nucleus and cytoplasm not only in cancer cells, but also in the cells adjacent to the cancer cells. ×100, (C) Hsp90 is highly expressed in the poorly differentiated adenocarcinoma. ×300, (D) Hsp90 is expressed in mucinous type carcinoma. ×300

Total RNA was extracted from fresh frozen specimens using a mRNA isolation kit (ISOGEN, Nippon Gene Corp., Toyama, Japan) according to the manufacturer's instructions, and cDNA was synthesized using a 1st strand cDNA synthesis kit (Boehringer Mannheim, Bedford, MA, USA). Using this cDNA as a template, the RT-PCR method was performed. Two primers (5'-TACATGGCTGGGGTGTTGAA-3' and 5'-AAGAGAGGCATCCTCACCCT-3') were used as internal controls for β-actin[21]. PCR was performed using a DNA Terminal Cycler (Perkin-Elmer Cetus, Norwalk, CT, USA) under the following conditions: template DNA (50－100 ng), dNTP (200 uM), primer (2 pM), Taq DNA polymerase (2 U, Takara Shuzo, Tokyo, Japan); denaturation, at 95℃ for 1 min; annealing, at 55℃ for 1 min; primer extention, at 72℃ for 2 min; 30 cycles for each. Electrophoresis of the PCR products through 2% agarose gel revealed amplification of hsp 90 α, hsp 90 β, and β-actin bands of about 141 base pairs (bp), 126 bp, and 218 bp, respectively. The analysis of each band was performed semiquantitatively using NIH image analysis software version 1.56, and the rations of hsp90 α and hsp90 β to β-actin were calculated[21,22]. Thirty cycles of PCR was selected because in a preliminary experiment we found that exponential amplification was maintained for all PCR products through 30 cycles (data not shown). As a negative control, specimens processed without adding avian myeloblastosis reverse transcriptase at the time of cDNA synthesis were used.

Results

1. Immunohistochemical staining

Hsps were expressed in the cancer cells and non-neoplastic cells of the pancreas. Hsps were strongly expressed in the acinar cells, ductal cells and inflammatory cells of the non-neoplastic tissue adjacent to the cancer as well as chronic pancreatitis, and the PCNA L.I. of non-neoplastic cells was high at the same location. As to histological types of ductal adenocarcinomas, hsps, especially in hsp90, were more highly expressed in the poorly differentiated adenocarcinoma and mucinous carcinoma than in the well to moder-

Table 1a Immunohistochemical study of Hsps, MDM2 and p53 in pancreatic tissues

Tissue	hsp90	hsp70	hsp60	ubiquitin	MDM2	p53	PCNA L.I.
Normal							
acinar cell	+	−	−	−	−	−	
ductal cell	+	+	+	+	−	−	38.3 ± 3.98
Langerhans island	+	+	+	++	−	−	
chronic pancreatitis	1/3	2/3	1/3	3/3	0/3	0/3	
acinar cell	+	+	+	+	−	−	
ductal cell	+	+	−	+	−	−	56.5 ± 1.89
Langerhans island	−	−	−	+	−	−	
benign pancreatic neoplasm	2/2	2/2	2/2	2/2	1/2	0/2	61.8 ± 1.01

− : negative (MDM2 localized in only cytoplasm), + : positive (MDM2 localized in nucleus and cytoplasm), ++ : strongly and diffusely positive

Table 1b Immunohistochemical study of Hsps, MDM2, p53 and WAF1 in pancreatic tissues

Tissue	hsp90	hsp70	hsp60	ubiquitin	MDM2	p53	WAF1	PCNA L.I.
non neoplastic tissue	27/31	19/28	15/28	20/28	11/28	4/16	5/10	
acinar cell	21/28	15/28	12/28	19/28	8/28	0/16	3/10	
pancreatic duct	22/28	15/28	11/28	17/28	4/28	0/16	2/10	62.5 ± 1.78
Langerhans island	24/31	14/28	12/28	18/28	2/28	4/16	4/10	
malignant tumor								
ductal carcinoma	17/20	19/20	17/20	18/20	7/20	5/16	9/16	
well to moderately differentiated	14/17	16/17	14/17	15/17	6/17	4/14	8/14	59.1 ± 1.14
poorly differentiated	3/3	3/3	3/3	3/3	1/3	1/2	1/2	62.6 ± 2.19
mucinous type carcinoma	2/3	2/3	2/3	2/3	2/3	2/3	1/1	
mucinous cystadenocarcinoma	1/2	1/2	1/2	1/2	1/2	1/2	1/1	61.3 ± 2.90
mucinous carcinoma	1/1	1/1	1/1	1/1	1/1	1/1	—	
endocrine tumor	1/3	3/3	0/2	3/3	1/1	1/1	0/1	56.6 ± 2.94

・hsp positive; there are over 10% strongly expresed epithelial components, ・MDM2, WAF1 positive; granular expression in cytoplasm and over one expression in nucleus of epithelial components ・p53 positive; there are over three expressions in nucleus of epithelial components.

ately differentiated ductal adenocarcinoma. Hsp90 which was localized throughout the nucleus and cytoplasm of the cancer cells, was observed not only in cancer cells, but also in the cells adjacent to the cancer cells (**Fig. 1**). P53, MDM2 and WAF1 were more strongly expressed in the nuclei of the cancer tissue. MDM2 was expressed to a greater extent than p53 and WAF1 in the nuclei and also localized in the cytoplasm of the tissue adjacent to the cancer tissue. Moreover, the tissue in which MDM2 was expressed quite prominently exhibited a high proliferating-cell-nuclear-antigen labeling index (PCNA L. I. **Table 1a** and **1b**). MDM2 was more strongly expressed in the cytoplasm of ductal cells of normal and chronic pancreatitis, and in the cytoplasm and nucleus of mucinous carcinoma cells and mucin hypersecreting non-neoplastic ductal cells surrounding pancreatic carcinoma. The localization of MDM2 in these tissues was predominantly in the cell nucleus and the cytoplasm that seemed to be compatible with Golgi apparatus area (**Fig. 2**).

2. *In situ* hybridization

Hsp90 α and hsp90 β mRNA, visualized as brown reaction products, were expressed mainly in the cytoplasm around the nucleus of cancer cells: hsp90 α mRNA was much more strongly expressed than hsp 90 β mRNA using the anti-sense probe. As a negative control, no specific signals were detected using the sense probe (**Fig. 3**).

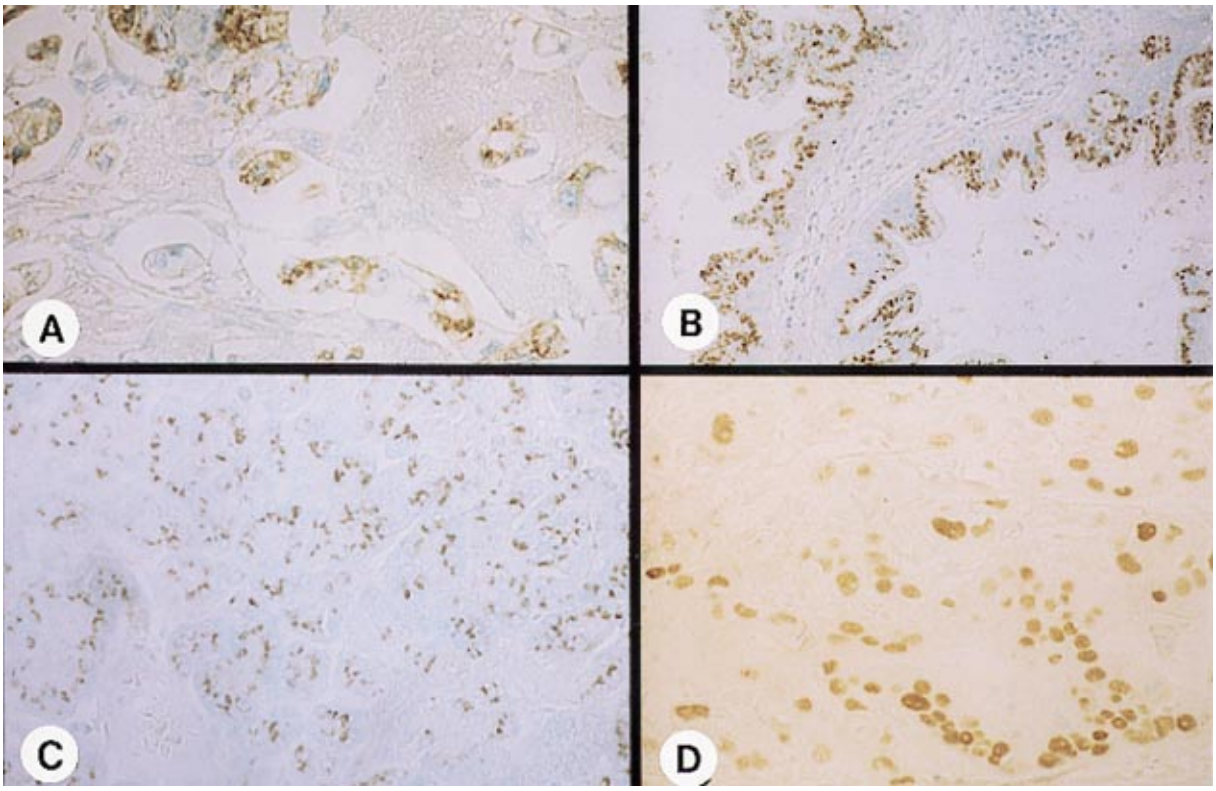

Fig. 2 Immunohistochemical staining in cancer and non cancer cells of pancreas. (A) MDM2 is expressed in the mucinous carcinoma. ×400, (B) MDM2 is localized in the cytoplasm of mucin hypersecreting ductal cells of normal tissue. ×200, (C) MDM2 is localized in the cytoplasm of mucin producing hyperplastic ductal lesion surrounding the pancreatic carcinoma. ×300, (D) p53 (CM-1) is localized in nucleus of the mucinous cyst adenocarcinoma. ×400

3. Reverse transcriptase polymerase chain reaction

In neoplastic and non-neoplastic tissues the products of PCR amplification with RT-PCR were detected at about 141 bp for hsp90 α mRNA, at about 126 bp for hsp90 β mRNA, and at about 218 bp for β-actin mRNA. Hsp90 α mRNA was expressed in all cancer tissues, but was not expressed in any of the chronic pancreatitis tissue. Hsp90 β and β-actin mRNA were expressed in all specimens (**Fig. 4**). The expression levels of hsp90 α and hsp90 β mRNA, which were evaluated by RT-PCR using β-actin as an internal control, were analyzed using NIH image analysis software, and compared with PCNA L. I.s (**Table 2**). Hsp 90 α mRNA expression was greater in the neoplastic tissue than in the non-neoplastic tissue, and was significantly correlated with the PCNA L.I.. Hsp90 β mRNA expression was not correlated with the PCNA L. I. (analysis of variance test).

Discussion

The prognosis of pancreatic carcinoma is not good. The therapies of surgery, radiation and chemotherapy are usually combined to treat the disease, but the rate of recurrence and liver metastasis is very high. To improve the prognosis, early detection and studies to define the biological behavior of pancreatic carcinomas are needed. Recent studies have reported that hsps (in paticular hsp90 and hsp70) are closely related to cell proliferation, cell cycle regulation and the metabolism of gene products, and that they play important roles in various processes of carcinogenesis[9-13,15-17]. We have examined the expression of hsps, especially hsp90, and gene products of p53, MDM2 and WAF1, and their relationship to cell proliferation in pancreatic disease, especially carcinomas. In immunohistochemical study, hsp90 of hsps which was localized throughout the nucleus and cytoplasm of the cancer cells, was observed not only in cancer cells, but

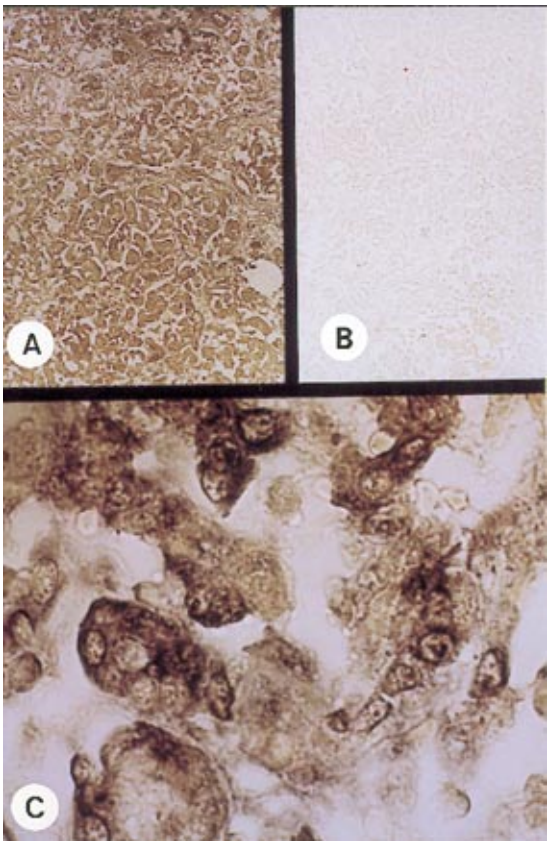

Fig. 3 Expression of hsp90 α mRNA in pancreatic carcinoma. (A) *In situ* hybridization for hsp 90 α with anti-sense probe × 100, (B) *In situ* hybridization for hsp90 α with sense probe × 100, (C) *In situ* hybridization for hsp90 α with anti-sense probe × 600

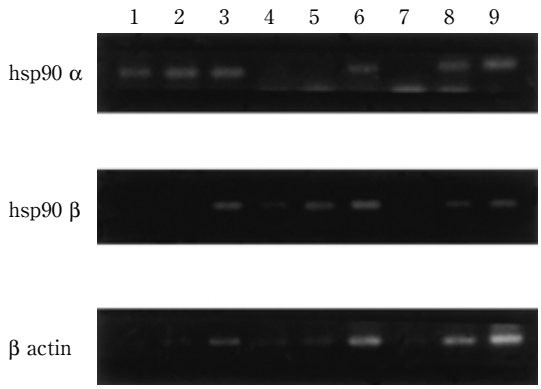

Fig. 4 Expression of hsp90 α, β mRNA in pancreatic tissues by RT-PCR. Lane 1～3; pancreatic cancer, lane 4; benign pancreatic tumor, lane 5～7; chronic pancreatitis, lane 8, 9; normal pancreas

also in the cells adjacent to the cancer cells.

Hsps would be strongly expressed in the acinar cells, ductal cells and inflammatory cells of the non-neoplastic tissue adjacent to the cancer and the PCNA L.I. of non-neoplastic cells was high at the same location. Moreover, hsps are well known to be induced by various kinds of stresses and cytokines. It is suggested that this result is due to the reaction of the non-neoplastic tissue according to the infiltration of cancer cells. The hsps and p53 were expressed more highly in poorly differentiated adenocarcinomas and mucinous carcinomas than in well to moderately differentiated ductal adenocarcinomas. It is thought that the differences in this expression are related to the histological differentiation of the cancer tissue. The expression of MDM2 was predominant in mucinous carcinomas and mucin secreting tissue around the carcinoma, and it was thought that the expression of MDM2 would be correlated not only to histological differentiation and proliferation, but also the functions of secretion. MDM2 is considered to be a proto-oncogene that is frequently overexpressed in malignant tumors. Immunohistochemical studies in which the quantity of MDM2 protein in human sarcomas has been investigated have revealed that this protein was localized in 30% of the specimens[23]. MDM2 protein can form a stable complex with p53 and inhibit the ability of wild type p53 to transactivate other genes. However, MDM2 expression is induced by wild type p53[23,24]. Whereas wild type p53 causes the cell cycle to stop at the G1 stage, the MDM2-p53 complex is thought to allow the progression to the S stage in the cell cycle. The overexpression of p53 promotes the transcription of WAF1. The product of this transcription, p21, causes growth arrest through the inhibition

Table 2 Relation between expression of hsp90 mRNA and PCNA in pancreatic disease

	hsp90 α/β actin (mean ± S.D.)	hsp90 β/β actin (mean ± S.D.)	PCNA L.I. (mean ± S.D.)
cancer	5.30 ± 2.77	2.72 ± 1.29	70.3 ± 2.08
chronic pancreatitis	0.17 ± 0.29	2.95 ± 2.68	56.5 ± 1.89
control	0.73 ± 0.10	0.94 ± 0.20	38.3 ± 3.98

of cyclin-dependent kinases (Cdks), which are required for the G1 to S transition in the cell cycle[25-28]. Moreover, it has been shown that WAF1 is directly connected with PCNA in vitro and inhibits DNA replication[29]. The results of our immunohistochemical studies appear to concur with these findings. MDM2 was strongly expressed in mucinous carcinomas and mucin hypersecreting tissues surrounding pancreatic carcinomas. In addition, MDM2 seemed to be localized strongly in the nucleus and cytoplasm of the Golgi apparatus area of the cells in these lesions and in the cytoplasm of mucin hypersecreting ductal cells of normal and chronic pancreatitis. These findings suggested that MDM2 might be localized within the Golgi apparatus or the endoplasmic reticulum, though ultrastructural studies were necessary. Therefore, MDM2 protein may have an intranuclear function as well as a role in the secretion system. MDM2 protein may play a role in structural conformation as well as in transformation or inflammation.

Mammalian hsp90 is comprised equally of two isoforms, hsp90 α and hsp90 β, the differences in the primary structure of the two being only (about 14%)[30], hsp90 α and hsp90 β are encoded by different gene chromosomes 14q32[31] and 6p12[32], respectively. The two isoforms mainly form homo-dimers, although hsp 90 β also exists as monomers[33]. We have investigated the distribution of hsp90 α and hsp90 βmRNAs using the in situ hybridization method. The anti-sense probe revealed that both hsp90 α and hsp90 β mRNAs were overexpressed in the cytoplasm around the nucleus of cancer cells. We analyzed the bands produced by RT-PCR using NIH image analysis software and recognized a significantly more marked overexpression of hsp90 α mRNA and a significantly high PCNA L.I. in the cancer tissue. The PCNA L.I. was correlated with the overexpression of hsp90 α,but not with the overexpression of hsp90 β,suggesting the involvement of hsp90 α in cell proliferation. Recent studies have reported that hsp90 α contributes to cell proliferation by controlling the cell cycle of yeast cell cultures[10] or chicken cell cultures[9], and hsp90 α is a poor biological prognostic factor for human breast cancer[34].

Other studies have reported a high level of hsp90 α overexpression in human leukemia cells[12] and pancreatic carcinomas[13], and that hsp90 βmRNA overexpression is not correlated with malignancies or with the PCNA L.I. However, hsp90 β may be correlated to structural conformation, by forming complexes with the actin and tubulin that constitute the cytoskeleton. In addition, hsp90 β has been reported to inhibit apoptosis and cell differentiation[35]. Therefore, hsp90 α and hsp90 β seem to have at least some differing functions in tissues containing malignant cells. The results of our study suggest that hsp90 α is, either directly or indirectly, involved in carcinogenesis and cell proliferation, and that hsp90 β is correlated to structural conformation. We suggest that regulation of the expression of hsps is important in the therapy of pancreatic carcinomas. These findings also indicate that thermotherapy is available to treat pancreatic carcinoma cells by increasing hsps, mainly hsp90. The synthesis of hsps is inhibited by quercetin and several other flavonoids which may be useful to the treatment of pancreatic carcinomas.

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