Twenty pairs of pancreatic cancerous and non-cancerous tissues were analyzed by western blotting with anti-vinculin antibody, and the different intensities of the bands between cancerous and non-cancerous tissues were analyzed by the Student's t-test (Fig. 3A). The mean intensities of the bands of cancerous and non-cancerous tissue samples were 125.2 and 66.4, respectively (Fig. 3A). Four pairs of cancerous and non-cancerous tissues were used for western blotting, to demonstrate the upregulation of α-enolase (14) as a positive control in cancerous tissues, compared to non-cancerous tissues (Fig. 3B). The appearance of vinculin on the 2-DE gels was located by 2-D western blotting (Fig. 4).

Discussion

We identified six upregulated proteins, calreticulin, glutathione synthetase, stathmin, vinculin, α-enolase and glyceraldehyde-3-phosphate dehydrogenase, in pancreatic cancerous tissues, compared to non-cancerous tissues. In this study, we reported only on those increased in cancerous tissues because many of the decreased proteins may have been replaced by stromal cells. To the best of our knowledge, this is the first report suggesting that vinculin is a candidate biomarker of PC.

Vinculin is a highly conserved intracellular protein (~123.8 kDa) with an important role in the regulation of cell adhesion and migration (12,13). Bakolitsa *et al* have explained how vinculin regulates cell adhesion by their detailed protein structural analysis (15). Highly metastatic cells have been reported to lack vinculin expression (16,17). Vinculin inhibits cell metastasis when transfected back into vinculin-null cells (17). Evidence reveals that apoptosis is related to cell motility (18,19), and that vinculin regulates cell apoptosis and motility via controlling the ERK pathway (18).

Paradoxically, our study demonstrated that vinculin, which usually behaves as a potent inhibitor to the survival and motility of cells (16-18), was significantly overexpressed in pancreatic cancerous tissues. Our findings indicate that vinculin could be a useful biomarker of PC for its high specificity. Vinculin is well characterized by its intracellular connecting component within adhesion complexes (16), but its functions remain unclear. A new report suggests that vinculin is a main driver gene of the 10q22 amplification in 10q22-amplified prostate carcinomas and that overexpression of vinculin may play an enhancing role in tumor cell proliferation during prostate cancer progression (20). This may be explained by the alternative splicing of vinculin gene, resulting in the alteration of the vinculin function during prostate carcinogenesis (21). Further studies are required to clarify whether vinculin overexpression contributes to PC progression by enhancing tumor cell proliferation, and to elucidate vinculin's action in PC. Additional studies must be conducted in order to identify post-transcriptional modifications of vinculin in PC. Our data sheds light on a new facet of vinculin; its function in PC progression.

A previous report demonstrated that vinculin is related to tumor-suppressing properties (22). However, our findings revealed a different property of vinculin in PC and suggest that vinculin may play a significant role in the diagnosis or prognosis of PC.

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Glyoxalase I (GLO1) is Up-regulated in Pancreatic Cancerous Tissues Compared With Related Non-cancerous Tissues

YUFENG WANG[1], YASUHIRO KURAMITSU[1], TOMIO UENO[2], NOBUAKI SUZUKI[2], SHIGEFUMI YOSHINO[2], NORIO IIZUKA[2], JUNKO AKADA[1], TAKAO KITAGAWA[1], MASAAKI OKA[2] and KAZUYUKI NAKAMURA[1]

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Abstract. *Background: Glyoxalase I (GLO1), an enzyme involved in the detoxification of methylglyoxal in glycolysis pathway, was found to be frequently overexpressed in various types of cancer. Recent studies showed that GLO1 is related to proliferation and apoptosis in human cancer cells. However, expression of GLO1 in pancreatic cancer (PC) has not been precisely defined. Since PC is one of the most malignant types of cancer, we investigated the level of GLO1 in tissues from patients with PC. Methods: We examined the expression of GLO1 in tumor from patients with PC and adjacent normal tissue by Western blotting. Results: Western blotting demonstrated that GLO1 was significantly overexpressed in pancreatic cancerous tissues compared with adjacent non-cancerous tissues (n=20, p<0.05). Conclusion: GLO1 could be a clinically useful target in therapy of PC.*

Glyoxalase I (GLO1) is a ubiquitous enzyme in all mammalian cells that plays a role in the detoxification of methylglyoxal, tissue maturation and cell death; glyoxalase components, including GLO1 and GLO2, reduce glutathione and transform electrophilic reactive α-oxoaldehydes including methylglyoxal into the corresponding non-cytotoxic α-hydroxy acids (1). Overexpression of GLO1 has been reported in various tumor tissues and cells, including colon, breast, prostate, lung, stomach, ovary, brain and renal cancer (2-5). Moreover, GLO1 was found to be frequently overexpressed in antitumor agent-resistant human leukemia cells, and the overexpression of GLO1 enhances resistance to antitumor agents such as etoposide and adriamycin (6). A recent study showed that GLO1 was related to proliferation and apoptosis in human malignant melanoma (7). Pancreatic cancer (PC) is one of the most malignant types of cancer, and the median survival period is less than 12 months, with an overall 5-year survival rate of less than 5% (8). The mechanisms of rapid spread and high chemotherapy resistance in PC are not completely clear. However, expression of GLO1 in pancreatic cancerous tissues has not been defined. In this study, we investigated the expression of GLO1 in cancerous tissues compared with paired non-cancerous tissues from 20 patients with PC by western blotting.

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Key Words: Western blotting, pancreatic cancer, glyoxalase I.

Materials and Methods

Tissues. Twenty pairs of non-cancerous and cancerous pancreatic tissues were collected from 20 patients with pancreatic cancer (Table I), and resected pancreas at the Department of Surgery II, Yamaguchi University Hospital. None of the patients had received any preoperative therapy. Written informed consent was obtained from all patients before surgery. The study protocol was approved by the Institutional Review Board for human use of the Yamaguchi University School of Medicine.

Sample preparation. Tissues were homogenized in lysis buffer [1% NP-40, 1 mM sodium vanadate, 1 mM phenylmethanesulfonyl fluoride (PMSF), 10 mM NaF, 10 mM EDTA, 50 mM Tris, 165 mM NaCl, 10 μg/ml leupeptin, and 10 μg/ml aprotinin] on ice (9-11). Supernatants were incubated for 1 h at 4oC and stored at –80°C until use (12-14). Protein concentration was determined by the Lowry method.

Western blotting. Proteins of samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transfered onto polyvinylidene fluoride (PVDF) membranes at 90 mA for 78 min. The membrances were blocked with Tris-buffered saline (TBS) containing 5% skimmed milk at room temperature for 1 h (15). Membranes were incubated with primary antibody against GLO1 (anti-glyoxalase I mouse polyclonal antibody, diluted 1:1000; Abnova, Taipei, Taiwan) or anti-extracellular regulated protein kinases (ERK1/2) (anti-ERK1/2 rabbit polyclonal antibody, diluted 1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4°C overnight, and then incubated with the secondary antibody conjugated with horse

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Table I. *Clinicopathological parameters of patients with pancreatic cancer included in this study.*

No.	Age (year)	Gender	TNM stage	Tumor grade[a]
1	54	Male	IVa	Moderately differentiated
2	57	Male	IVb	Moderately differentiated
3	54	Male	III	Moderately differentiated
4	74	Female	IVa	Poorly differentiated
5	72	Male	III	Moderately differentiated
6	72	Male	IVa	Well differentiated
7	76	Female	IVa	Moderately differentiated
8	73	Female	III	Papillary carcinoma
9	53	Male	IVb	Well differentiated
10	69	Female	III	Moderately differentiated
11	79	Male	IVb	Mucinous carcinoma
12	34	Male	IVb	Acinor carcinoma
13	71	Female	III	Moderately differentiated
14	67	Female	IVa	Moderately differentiated
15	68	Male	III	Moderately differentiated
16	60	Male	IVb	Moderately differentiated
17	67	Male	IVa	Well differentiated
18	60	Female	III	Moderately differentiated
19	48	Female	IVa	Moderately differentiated
20	73	Male	IVa	Moderately differentiated

[a]Tumor was graded according to the degree of histologic differentiation, as follows: Well differentiation, 5% or less of a nonsquamous or nonmorular solid growth pattern; Moderate differentiation, 6% to 50% of a nonsquamous or nonmorular solid growth pattern; Poor differentiation, more than 50% of a nonsquamous or nonmorular solid growth pattern.

radish peroxidase (1:10,000) for 1 h at room temperature after washing three times with TBS containing Tween-20 and once with TBS. Membranes were then treated with a chemiluminescent reagent (ImmunoStar Long Detection; Wako, Osaka, Japan) and proteins detected by using Image Reader LAS-1000 Pro (Fujifilm Corporation, Tokyo, Japan) (16).

Statistical analysis. Statistical significance was calculated by Student's t-test.

Results

Western blot analysis of GLO1 in tumor from patients with PC and adjacent normal tissue. Twenty pairs of pancreatic cancerous and non-cancerous tissues were analyzed by western blotting with primary antibody against GLO1 and ERK1/2. The protein expression levels were elevated significantly (n=20, $p<0.05$) in cancerous tissues compared with paired non-cancerous tissues (75%) (Figure 1A). The different intensities of the bands between cancerous and non-cancerous tissues were analyzed by Students *t*-test. The intensity of the bands in non-cancerous and cancerous tissue samples was 853.9 and 1598.5 units, respectively (Figure 1B).

Discussion

Overexpression of GLO1 in various types of cancer have been reported (2-5). Research showed that GLO1 may play an important role in malignant transformation, tumor progression, cancer cell survival and resistance to chemotherapeutic agents (6, 17-20). Recent studies indicate that GLO1 may be a useful molecular target for cancer chemotherapy, and pharmacological inhibitors of GLO1 have shown anticancer activity (21, 22). The mechanism of multidrug resistance (MDR) and tumor progression associated with GLO1 overexpression is not fully understood, but it may be linked to increased formation of methylglyoxal by anticancer drugs and related toxicity (23). Overexpression of GLO1 inhibited methylglyoxal-induced tumor growth arrest and toxicity; silencing of GLO1 in cancer cells with high rates of glycolysis and methylglyoxal formation leads to a high level of accumulation of methylglyoxal and cytotoxicity (24). Methylglyoxal induces apoptosis and indirectly stimulates the release of cytochrome *c* from mitochondria and subsequent apoptosis (25-27). Activation of c-Jun *N*-terminal protein kinase 1 (JNK1) and p38 mitogen-activated protein kinases (p38MAPK) may also be involved in GLO1-associated MDR in lung cancer cells (5).

Recently, *GLO1* gene was found to exhibit altered expression in cases of liver metastasis, but not in lymph node metastasis of PC cells (28). This indicates a possible role of GLO1 in PC progression. However, the differences in GLO1 expression between pancreatic cancerous and related non-cancerous tissues have not been defined, although these may be necessary for understanding the mechanisms of rapid spread and chemotherapy resistance of PC (1, 29). In this study, we investigated the expression of GLO1 in tissues from patients with PC. The results indicate that GLO1 was significantly overexpressed (n=20, $p<0.05$) in pancreatic cancerous tissues compared with related non-cancerous tissues (75%). The intensity of GLO1 was more than 1.8-fold increased in PC tissues. These results suggest that GLO1 may play a role in tumor progression and resistance to chemotherapeutic agents of PC. Our study also indicates that GLO1 could be a new clinically useful target for therapy of PC.

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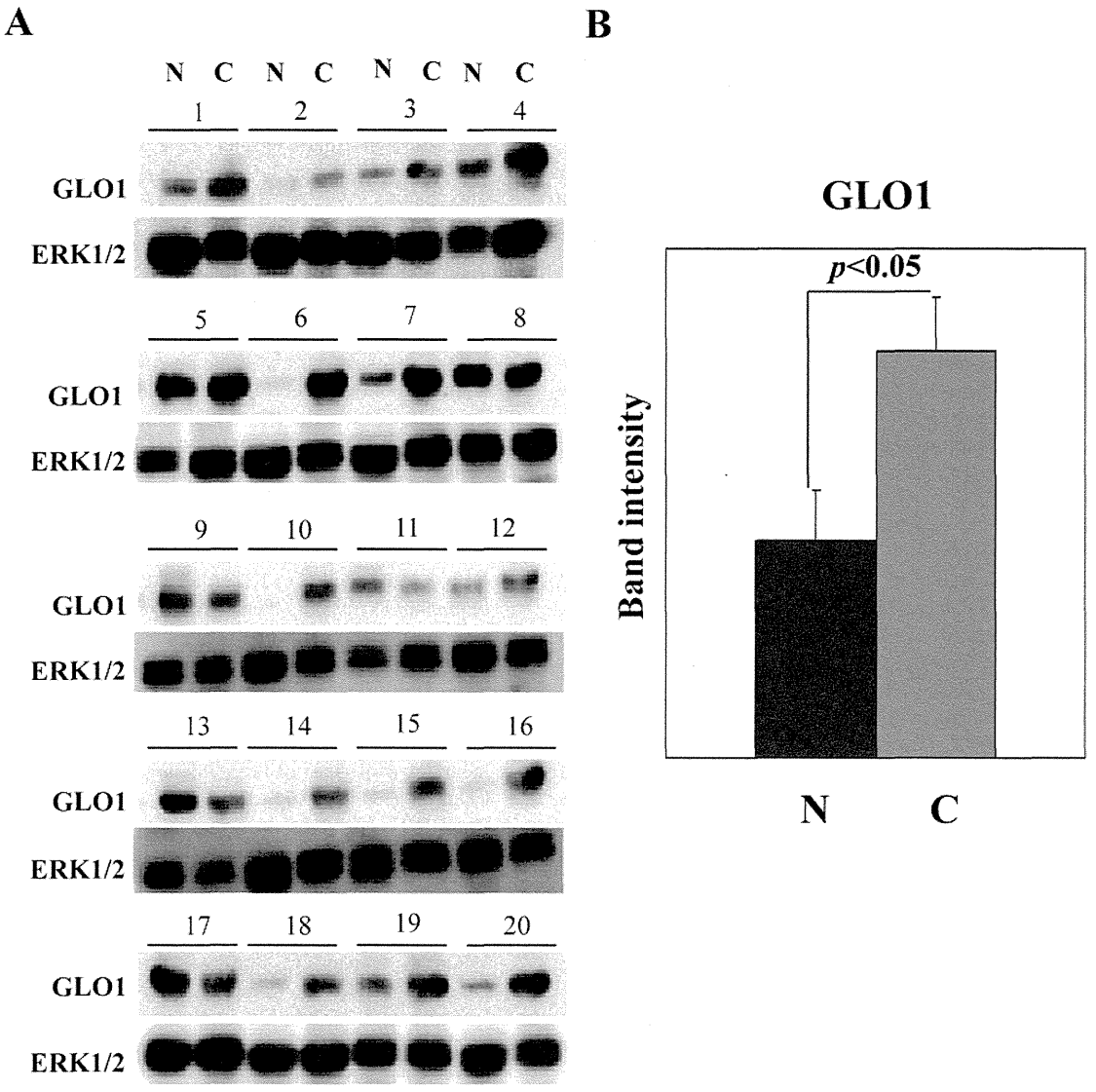

Figure 1. *Western blot analysis of glyoxalase I (GLO1) in PC. A: Tissues from 20 patients with pancreatic cancer (C) and paired non-cancerous tissues (N) were used for western blotting with anti-GLO1 and anti-extracellular regulated protein kinases (ERK1/2) antibody. The expression of GLO1 was confirmed to be increased in pancreatic cancerous tissues (75%). Each patient number (1-20) is the same as that in Table I. B: Comparison of the intensity of the bands between cancerous and non-cancerous tissues by Students t-test (n=20, p<0.05). The relative standard errors (SE) of cancerous and non-cancerous tissues samples were 198.2 and 213.3 units, respectively.*

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Review

Heat-shock Protein 27 Plays the Key Role in Gemcitabine-Resistance of Pancreatic Cancer Cells

YASUHIRO KURAMITSU[1], YUFENG WANG[1], KUMIKO TABA[1,2], SHIGEYUKI SUENAGA[1,2], SHOMEI RYOZAWA[2], SEIJI KAINO[2], ISAO SAKAIDA[2] and KAZUYUKI NAKAMURA[1]

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Abstract. *Pancreatic cancer is one of the most fatal types of cancer in developed countries. Most patients have locally advanced or metastatic cancerous lesion when they are diagnosed because of the progressive, invasive and metastatic capacity of this disease to liver, lymph nodes and distant organs during early stages. Although the only curative therapy is complete surgical resection, the disease has usually already progressed by the time of diagnosis, and the majority of patients have metastatic disease. Therefore, palliative chemotherapy remains the only therapy for these patients with progressive disease. Gemcitabine has been used for pancreatic cancer as the most effective anticancer drug. However, there are many cases resistant to gemcitabine. Thus, a better understanding of the molecular mechanisms of resistance to gemcitabine is essential to allow it to be used more effectively. Our previous proteomic studies demonstrated that the expression of heat-shock protein 27 (HSP27) was increased in gemcitabine-resistant pancreatic cancer cells and this might play a role in determining the sensitivity of pancreatic cancer to gemcitabine. Increased HSP27 expression in tumor specimens was related to higher resistance to gemcitabine and shorter survival period in patients of pancreatic cancer. Furthermore, it was shown that treatment strategies combining the HSP inhibitor KNK437 or interferon-γ with gemcitabine were effective for gemcitabine-resistant pancreatic cancer cells in vitro. Furthermore, combined therapy of gemcitabine with interferon-γ (IFN-γ) of gemcitabine-resistant pancreatic cancer-bearing nude mice showed synergistic therapeutic effects on gemcitabine-resistant pancreatic cancer bearers. In this review, we summarize the current understanding of HSP27 and the roles that it plays in gemcitabine resistance.*

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Key Words: Two-dimensional gel electrophoresis, LC-MS/MS, pancreatic cancer, gemcitabine (GEM), proteomics, heat-shock protein 27, review.

Pancreatic cancer is very aggressive and difficult to diagnose at an early stage, and to treat with effective therapies. Therefore the prognosis of pancreatic cancer patients is still very poor (1, 2). When diagnosed as having pancreatic cancer, most patients have locally advanced or metastatic cancerous lesions and these who survive more than five years are very few. At this point, the surgical resection of all of the tumor tissues is the only curative therapy. However, the number of patients who can be treated by complete surgical resection is very limited. Thus patients with progressive pancreatic cancer have no choice but to depend on palliative chemotherapy.

Gemcitabine (2'-deoxy-2'-difluorodeoxycytidine: Gemzar) is a deoxycytidine analog with structural and metabolic similarities to cytarabine. Gemcitabine is currently the drug of choice to treat patients with advanced pancreatic cancer. The use of gemcitabine is expected to prolong survival of patients with advanced pancreatic cancer. However, intrinsic or acquired resistance of pancreatic cancer impacts the therapeutic effect of gemcitabine (3). Since it is important to understand the molecular mechanisms of resistance to gemcitabine, we have carried out proteomic analysis of gemcitabine-resistant pancreatic cancer cells.

Proteomics is a useful tool to identify proteins which are differently expressed, or have different post-translational modifications or functions. Proteomic differential display is a powerful method to analyze protein expression or carry out post-translational modification profiling of two or more groups. For the quantitative comparison of protein expression among samples, two-dimensional gel electrophoresis (2-DE)

has been commonly used. For characterizing the sequence of the spots, mass spectrometry (MS) has been commonly used. The technique of 2-DE is able to separate proteins according to both their charge in isoelectoric focusing (IEF) gels and their weight in sodium dodecyl sulfate (SDS) gels. Two-DE has unique advantages for examining the expressions of hundreds of proteins simultaneously and for examining post-translational modifications of the protein spots. After in-gel trypsin digestion of the spots, digested peptides are ionized from the sample by matrix-assisted laser desorption/ionization (MALDI) or by electrospray ionization directly from the samples. In MS, ionized peptides are separated on the basis of their m/z and analyzed. For protein identification, peptide-mass fingerprinting and peptide sequencing are usually used. Peptide-mass fingerprinting is usually used for MALDI–time-of-flight (MALDI-TOF) MS and peptide sequencing is used for tandem mass spectrometers (MS/MS).

By using these proteomic technologies our previous studies identified heat-shock protein 27 (HSP27) as a key molecule playing an important role in gemcitabine resistance. We investigated protein expression in gemcitabine-resistant and -sensitive human pancreatic adenocarcinoma cell lines by proteomics. Two-DE showed proteins up-regulated and down-regulated in gemcitabine-resistant cell lines compared with gemcitabine-sensitive cell lines, and these were identified by LC-MS/MS. Three isoform spots of HSP27 on 2-DE were found to be increased in the resistant cell lines compared with sensitive cell lines. The knock-down analysis for HSP27 in KLM1-R pancreatic cancer cells restored sensitivity to gemcitabine, and increased HSP27 expression in tumor specimens was related to higher resistance to gemcitabine in patients with pancreatic cancer (4, 5). Further experiments showed the treatment of KLM1-R cells with interferon-γ (IFN-γ) or the HSP inhibitor KNK-437 down-regulated expression of HSP27 and increased the cytotoxic effect of gemcitabine on gemcitabine-resistant KLM1-R cells (6, 7). The up-regulated isoforms were identified as phosphorylated HSP27 in gemcitabine-resistant pancreatic cancer cells. This suggested that the phosphorylation of HSP27 plays an important role in gemcitabine resistance (8).

Identification of Gemcitabine-resistance-related Protein by Proteomic Differential Display

To identify the gemcitabine-resistance-related protein proteomic differential display by using 2-DE and MS was performed. The protein spots whose expression was different between gemcitabine-resistant and -sensitive pancreatic cancer cells were selected by means of commercial software for 2-DE image analysis. Many kinds of software have been used by researchers. In our study Progenesis PG240 and Progenesis SameSpot (Nonlinear Dynamics Ltd. Newcastle upon Tyne, UK) were used. Both software packages decide spot positions on the gels and measure spot intensities automatically. After statistical analysis for the selected spots, the candidate proteins which may play important roles in gemcitabine-resistance can be obtained. By proteomic differential display analysis, some proteins, including HSP27, were identified. From the candidate proteins, the expression of HSP27 in gemcitabine-resistant and -sensitive cells was investigated, and the results showed up-regulation of HSP27 in gemcitabine-resistant cells compared to gemcitabine-sensitive cells, not only in those with acquired gemcitabine resistance, but also in those which were intrinsically resistant (4).

HSP27 and Drug Resistance

Many reports about HSP27 being an important protein in cancer cell drug resistance have been published. Garrido *et al.* showed that HSP27 inhibited cytochrome *c*-dependent activation of procaspase-9 and prevented etoposide-induced apoptosis (9). Hansen *et al.* reported that HSP27 overexpression inhibited doxorubicin-induced apoptosis in human breast cancer cells by altering the expression of topoisomerase II (10). Richards *et al.* showed that the HSP27-overexpressing human testicular tumor cells were more resistant to cisplatin and doxorubicin, and this was associated with modest increases (17-30%) in population doubling times and a small reduction in the number of S-phase cells (11). Why does HSP27 induce chemo resistance in cancer cells? The cause of HSP27-induced chemo resistance seems to be less the specific inhibition of various anticancer drugs and more the common inhibition of apoptosis. Anti-apoptotic pathways are induced by HSP27, and these pathways lead to cancer cells being resistant to apoptosis. How does HSP27 induce resistance to apoptosis in cancer cells? It was reported that HSP27 protects the cells from apoptosis by associating with death-associated protein 6 (DAXX), truncated BH3 interacting domain death agonist (tBid), Bcl2 associated X-protein (Bax), cytochrome *c*, I-kappa-B kinase (IKK), caspase-3 and others (12, 13). The stress signals which are caused by chemotherapy activate c-Jun N-terminal kinase (JNK). This activation induces the activation of procaspase-9. In turn, active caspase-9 activates procaspase-3. Active caspase-3 catalyzes death substrates, and induces the cells to undergo apoptosis. HSP27 might be one of the inhibitors of this pathway. HSP27 was also reported to interact with Ak-thymoma (Akt), and to increase its stability (14). For chemotherapies which aim at the induction of apoptosis in cancer cells, it is very important to control such factors concerning HSP27.

Effect of HSP27 Inhibition on Chemotherapy

In considering the success of chemotherapy for pancreatic cancer patients, it seems crucial important to control HSP27

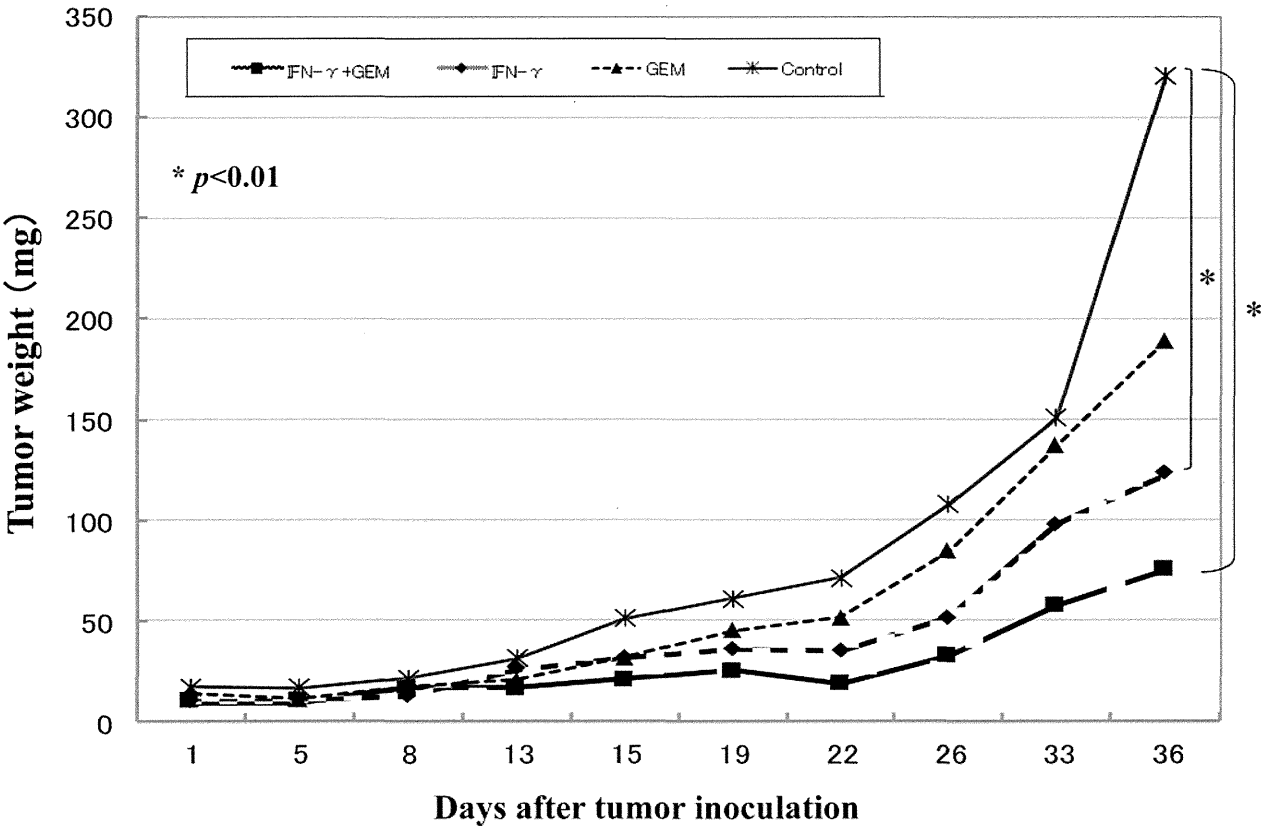

Figure 1. *The growth curves of gemcitabine(GEM)-resistant pancreatic cancer PK59 cells in nude mice treated with or without GEM and/or interferon-γ (IFN-γ). GEM-resistant PK59 cells (1×10^7) were transplanted subcutaneously in nude mice on the day 0, and they were treated with or without IFN-γ and/or GEM. IFN-γ (1×105U) was injected intratumorally into mice twice a week. GEM (80 mg/kg) was injected intraperitoneally once a week. The tumor diameters were measured twice a week. Each group had 5 mice. The graphs show the average tumor weight of the 5 mice from each group.*

expression. Our previous studies on the *in vitro* synergistic effects of gemcitabine and HSP27 inhibitors (siRNA for HSP27, IFN-γ, KNK-437) showed that down-regulation of HSP27 changed the gemcitabine-resistant pancreatic cancer cells to being gemcitabine sensitive. Recently, we performed combined therapy of gemcitabine with IFN-γ for gemcitabine-resistant pancreatic cancer-bearing nude mice in vivo. Gemcitabine-resistant PK59 cells (1×10^7) were transplanted subcutaneously in nude mice on day 0. One group of mice was then treated 1 had 5 mice treated with PBS only; another was treated intraperitoneally with gemcitabine at 80 mg/kg once per week only; another was treated intratumorally with IFN-γ at 1×10^5U twice a week only; and the final group was treated with both gemcitabine and IFN-γ. The tumor diameters were measured twice a week. Figure 1 shows the growth curves of gemcitabine-resistant PK59 cells in nude mice treated with or without gemcitabine and/or IFN-γ. Although the treatment with IFN-γ only had a good suppressive effect on the tumor growth, the combined treatment of gemcitabine with IFN-γ showed significant synergistic effect on the growth curve of gemcitabine-resistant PK59 pancreatic cancer cells. This shows that the combined therapy of GEM and HSP27 suppressor can be expected to be effective chemotherapy of gemcitabine-resistant pancreatic cancer cells.

Some agents and proteins which down-regulate HSP27 have been reported. Tumor necrosis factor-α and IFN-γ are cytokines, and they were reported to down-regulate HSP27 (15, 16). KNK-437 is a benzylidene lactam compound, and this reagent has shown cytotoxic activity towards gemcitabine-resistant cells treated with GEM synergistically (7). Quercetin is one of the most widely distributed bioflavonoids. This flavonoid was reported to inhibit the expression of HSP27 in tumor cells (17). Tanshinone IIA is a phenanthrene quinine extracted from the roots of *Salvia miltiorrhiza* Bunge. This reagent down-regulates expression of HSP27 in cancer cells (18). Triptolide is a diterpene triepoxide from the plant *Triptergium wilfordii*. Westerheide *et al.* showed triptolide abrogated the transactivation function of heat-shock transcription factor HSF1, so it is expected to

use them for combinatorial therapy with gemcitabine in order to down-regulate expression of HSP27, and up-regulate the sensitivity to gemcitabine of pancreatic cancer cells (19).

In order to down-regulate HSP27 expression, we need to try the combined therapy of gemcitabine with HSP27. KRIBB3 {[5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl) isoxazole]} is a synthetic agent. This agent inhibits HSP27 phosphorylation (20). Since phosphoryration of HSP27 is increased in gemcitabine-resistant cells, inhibition of phosphorylation by using KRIBB3 may be useful for combined therapy with gemcitabine for gemcitabine-resistant pancreatic cancer cells.

Besides the role of HSP27 in drug resistance, increased levels of HSP27 in cancer tissues including gastric, head and neck, renal and prostate cancer have been reported (21-24). What is the role of increased HSP27 expression in cancer tissues? Anti-apoptosis activity of HSP27 is necessary for cancer cells suffering from stress including anticancer drugs, oxidative stress and irradiation. Cancer cells are defended from apoptosis induced by stress by up-regulation of HSP27. HSP27 also has a role in the progression of cancer. Song *et al.* showed increased HSP27 expression in metastatic hepatocellular carcinoma tissues (25). Cancer cells overexpressing HSP27 had increased metastatic capacity (26, 27). On the other hand, HSP27 depletion induces the cells to undergo apoptosis and down-regulates tumor progression in prostate cancer cells (28). Not only mature cancer cells, but also cancer stem cells were reported to show increased HSP27 expression. Wei *et al.* reported up-regulation and phosphorylation of HSP27 in breast cancer stem cells, and silencing of HSP27 in these cells reduced cancer stem cell-like features, including the epithelial–mesenchymal transition (29).

These reported features of HSP27 show that the control of HSP27 is very important in the treatment of cancer cells, not only from the view of gemcitabine-resistance, but also with regard to cancer progression and cancer stem cell-like features.

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Heat Shock Protein 27 (HSP27) and Anti-Apoptosis Activity in Cancer Cells

A molecular chaperone, heat shock protein 27 (HSP27, heat-shock 27-KD protein 1, HSPB1) is one of the small heat shock protein family. It modulates the ability of cells to respond to several types of injury, heat shock, oxidative stress and other stresses. HSP27 is expressed in almost all organisms from prokaryotes to mammals. It interacts with many proteins and can prevent a wide variety of apoptotic agents from causing cell death. HSP27 regulates apoptosis by interacting with key components of the apoptotic signaling pathway [1]. It was reported that HSP27 inhibited cytochrome c and dATP triggered activation of procaspase-9 and prevented etoposide-induced apoptosis [2], and HSP27 altered the expression of topoisomerase II and inhibited doxorubicin-induced apoptosis [3]. Furthermore, it was reported that over-expression of HSP27 in prostate cancer cells rendered cells resistant to etoposide-, diethylmaleate-, cycloheximide- or radiation-induced apoptosis, which may be mediated by the production of survival factors [4]. From our recent studies, up-regulation of HSP27 in pancreatic cancer cells has been clarified to be linked to the resistance to gemcitabine (GEM), and the down-regulation of HSP27 by using HSP27 inhibitors; siRNA for HSP27, interferon γ or KNK437 in GEM-resistant cells showed the increasing sensitivity for GEM [5-8]. They showed that the up-regulation of anti-apoptotic pathways induced by HSP27 enhanced the resistance of cancer cells to apoptosis. Enhanced resistance to apoptosis in cancer cells induced by HSP27 may be caused by many factors. HSP27 protects the cells from apoptosis by concerning with DAXX, tBid, cytochrome c, IKK, caspase-3 and etc. [9,10]. For the chemotherapies which aim at the induction of apoptosis in cancer cells, it is very important to control such factors concerning with HSP27 or itself.

HSP27 and Cancers

In these days many reports about the up-regulation of HSP27 in cancer tissues of stomach, head and neck, renal, prostate and etc, have been published [11-14]. Why is HSP27 up-regulated in cancer cells? Song et al. [15] showed that constitutively activated signal transducer and activator of transcription 3 (STAT 3) up-regulated HSP27 in breast cancer cells. What does up-regulated HSP27 do in cancer cells? We should clarify the roles of HSP27 in cancer cells. As described above, increased HSP27 prevents the induction of apoptosis by anti-cancer drugs, oxidative stress, irradiation or etc in cancer cells. Over-expressed HSP27 helps cancer cells to survive. Does HSP27 play a role as only an outside player? Increased levels of HSP27 were observed in metastatic HCC tissues compared with non metastatic tissues [16]. Furthermore, in vitro studies have shown that over-expression of HSP27 increased the metastatic capacity on several factors in human melanoma and prostate cancer cells [17,18], and HSP27 depletion induced apoptosis and inhibited tumor progression in prostate cancer cells [19]. These suggest that HSP27 plays a key role in metastasis formation as an offensive player, too.

Furthermore, in these days some groups reported up-regulation of HSP27 in cancer stem cells. Wei et al. [20] showed increased expression and phosphorylation of HSP27 in breast cancer stem cells, and knockdown of HSP27 in breast cancer stem cells decreased characters of them. They concluded that HSP27 regulated the epithelial-mesenchymal transition process to contribute the maintenance of breast cancer stem cells. Hsu et al. [21] demonstrate that lung cancer stem cells have elevated levels of activated HSP27 upon treatment with superoxide and traditional chemotherapy.

Therefore, it seems to be the most important to control HSP27 in cancer cells to conquer the cancers.

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Proteomic Analysis Showed Down-regulation of Nucleophosmin in Progressive Tumor Cells Compared to Regressive Tumor Cells

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Abstract. *Important strategies against cancer are based on the understanding of the mechanisms of tumor progression. To elucidate alterations regarding tumor progression, we have performed proteomic differential display analysis for the expression of intracellular proteins in the regressive murine fibrosarcoma cell clone QR-32 and the progressive malignant tumor cell clone QRsP-11, derived from QR-32, by means of combination of two-dimensional gel electrophoresis (2-DE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), and we have previously reported on relevant results. However, besides the protein spots which we already reported, we identified three more particular spots of interest. In the present study, two-dimensional western blot analysis demonstrated a significantly lower expression of three isoforms of nucleophosmin in progressive, compared to regressive cell clones. These results suggest that the down-regulation of the identified nucleophosmin proteins in QRsP-11 cells compared to QR-32 cells is possibly related to tumor malignant progression.*

The most crucial features of malignant tumors are unpredictable development and progression. Progressive tumor cells show rapid growth, unrestricted proliferation activity, serious invasiveness and disorderly metastatic capacity, compared to regressive benign tumor cells. Okada *et al.* have established progressive and regressive murine fibrosarcoma tumor models (QR-32 clone and QRsP-11 clone) (1, 2). The regressive clone QR-32 is a weakly tumorigenic and non-metastatic cell clone. The progressive clone QRsP-11, on the other hand, is a progressive malignant tumor cell clone derived from QR-32. After injection of 1×10^6 cells intravenously, or up to 2×10^5 cells subcutaneously, in normal syngeneic mice, QR-32 cells regress spontaneously. However, when they are subcutaneously co-implanted with gelatin sponge, they grow progressively. After these progressively-growing cells were established as cell lines (QRsP), they had the ability to progressively grow in mice, even in the absence of gelatin sponge. The characteristic feature of QRsPs as malignant tumor cell clones is that they are more tumorigenic and metastatic, and QRsP-11 is one such QRsP clone.

The aim of this study was to identify the differentially expressed proteins between the clones QR-32 and QRsP-11. The comparison of the differential expression of proteins between benign tumor cells of single-cell origin and their derived malignant tumor cells is beneficial in detecting various important factors in inflammation-associated tumor progression. We have reported many proteomic studies of QR-32 and QRsP-11 cells by using two-dimensional gel electrophoresis (2-DE) (3-5). The differential display analysis for the expression of nuclear proteins between QR-32 and QRsP-11 showed eight nuclear proteins to be differentially-regulated, including zing finger protein ZXDC, in QRsP-11 compared with QR-32 cells (4). The proteomic differential display analysis for the expression of cytoplasmic proteins in QR-32 and QRsP-11 cells showed 11 spots for differentially regulated proteins, including heat-shock protein (HSP)-90 in QRsP-11 compared with QR-32 cells (3). Our recent 2-DE analysis of QR-32 and QRsP-11 cells showed three spots for down-regulated proteins in the progressive malignant tumor cell clone QRsP-11, compared to QR-32 cells, which were not identified in previous studies. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) identified these

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