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

YASUHIRO KURAMITSU1, YUFENG WANG1, KUMIKO TABA1,2, SHIGEYUKI SUENAGA1,2, SHOMEI RYOZAWA2, SEIJI KAINO2, ISAO SAKAIDA2 and KAZUYUKI NAKAMURA1

Departments of 1Biochemistry and Functional Proteomics, and 2Hepatology and Gastroenterology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan

Abstract. Pancreatic cancer is one of the most fatal types of cancer in developed countries. Most patients have locally advanced or metastatic cancerous lesions when they are diagnosed, due to 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 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 resistance to gemcitabine and a shorter survival period in patients with pancreatic cancer. Furthermore, it has been shown that treatment strategies combining the HSP inhibitor KNK437 or interferon-γ (IFN-γ) with gemcitabine, were effective in gemcitabine-resistant pancreatic cancer cells in vitro. Furthermore, combined therapy of gemcitabine with 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 its role in gemcitabine resistance.

Pancreatic cancer is very aggressive and thus 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 with pancreatic cancer, most patients have locally advanced or metastatic cancerous lesions and those 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 effects of gemcitabine (3). Since it is important to understand the molecular mechanisms of resistance to gemcitabine, we have carried out proteomic analyses of gemcitabine-resistant pancreatic cancer cells.

Proteomics is a useful tool to identify proteins that are differently expressed, or have different post-translational modifications or functions. Differential proteomic 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, LC-MS/MS, pancreatic cancer, gemcitabine (GEM), proteomics, heat-shock protein 27, review.
electrophoresis (2-DE) has been commonly used. For characterizing the sequence of the protein spots, mass spectrometry (MS) has been commonly used. The technique of 2-DE is able to separate proteins according to both their charge in isoelectric focusing (IEF) gels and their molecular weight in sodium dodecyl sulfate (SDS) gels. Two-DE has unique advantages in examining the expressions of hundreds of proteins simultaneously and in 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 the 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 to the 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 and was thus related to higher resistance to gemcitabine in patients with pancreatic cancer (4, 5). Further experiments showed that the treatment of KLM1-R cells IFN-γ or the HSP inhibitor KNK-437 down-regulated the 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

In order to identify the gemcitabine-resistance-related protein proteomic differential display, 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. Several 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 detect spot positions on the gels and measure spot intensities automatically following statistical analysis for the selected spots, the candidate proteins that 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 an 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 that were intrinsically resistant (4).

HSP27 and Drug Resistance

Many reports pointing at HSP27 as 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's 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 attributed the specific inhibition of various anticancer drugs and more to 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 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 aiming at the induction of apoptosis in cancer cells, it is very important to control such factors concerning HSP27.
Effect of HSP27 Inhibition on Chemotherapy

The success of chemotherapy for pancreatic cancer patients, seems to depend on control of HSP27 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. Gemicitabine-resistant PK59 cells (1×10^7) were transplanted subcutaneously in nude mice on day 0. One group of mice was then 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 had only a good suppressive effect on the tumor growth, the combined treatment of gemcitabine with IFN-γ showed a significant synergistic effect on the growth curve of gemcitabine-resistant PK59 pancreatic cancer cells. This shows that the combined therapy of gemcitabine and of the HSP27 suppressor can be expected to be an effective chemotherapy of gemcitabine-resistant pancreatic cancer cells.

Some agents and proteins that down-regulate HSP27 have been reported. Tumor necrosis factor-α and IFN-γ are two of the cytokines that have been 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 gemcitabine synergistically (7). Quercetin, one of the most widely distributed bioflavonoids has been 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 the expression of HSP27 in cancer cells (18).
Triptolide is a diterpene triepoxide from the plant Tripterium wilfordii. Westerheide et al. showed that triptolide abrogated the transactivation function of heat-shock transcription factor HSF1, so it is expected to use this for combinational 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 phosphorylation of HSP27 is increased in gemcitabine-resistant cells, inhibition of phosphorylation by 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 the 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 the 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.

References


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