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

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Abstract. *Background: Glyoxalase I (GLO1), an enzyme involved in the detoxification of methylglyoxal in the glycolysis pathway, has been 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 levels of GLO1 in tissues from patients with PC. Materials and Methods: We examined the expression of GLO1 in tumors from patients with PC and adjacent normal tissues 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 the therapy of PC.*

Glyoxalase I (GLO1) is a ubiquitous enzyme in all mammalian cells that plays a role in the detoxification of methylglyoxal, in 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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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 4°C 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

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 (dilution 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 were detected by using Image Reader LAS-1000 Pro (Fujifilm Corporation, Tokyo, Japan) (16).

Statistical analysis. Statistical significance was calculated by the 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 a 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 the Student's 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 their 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, the *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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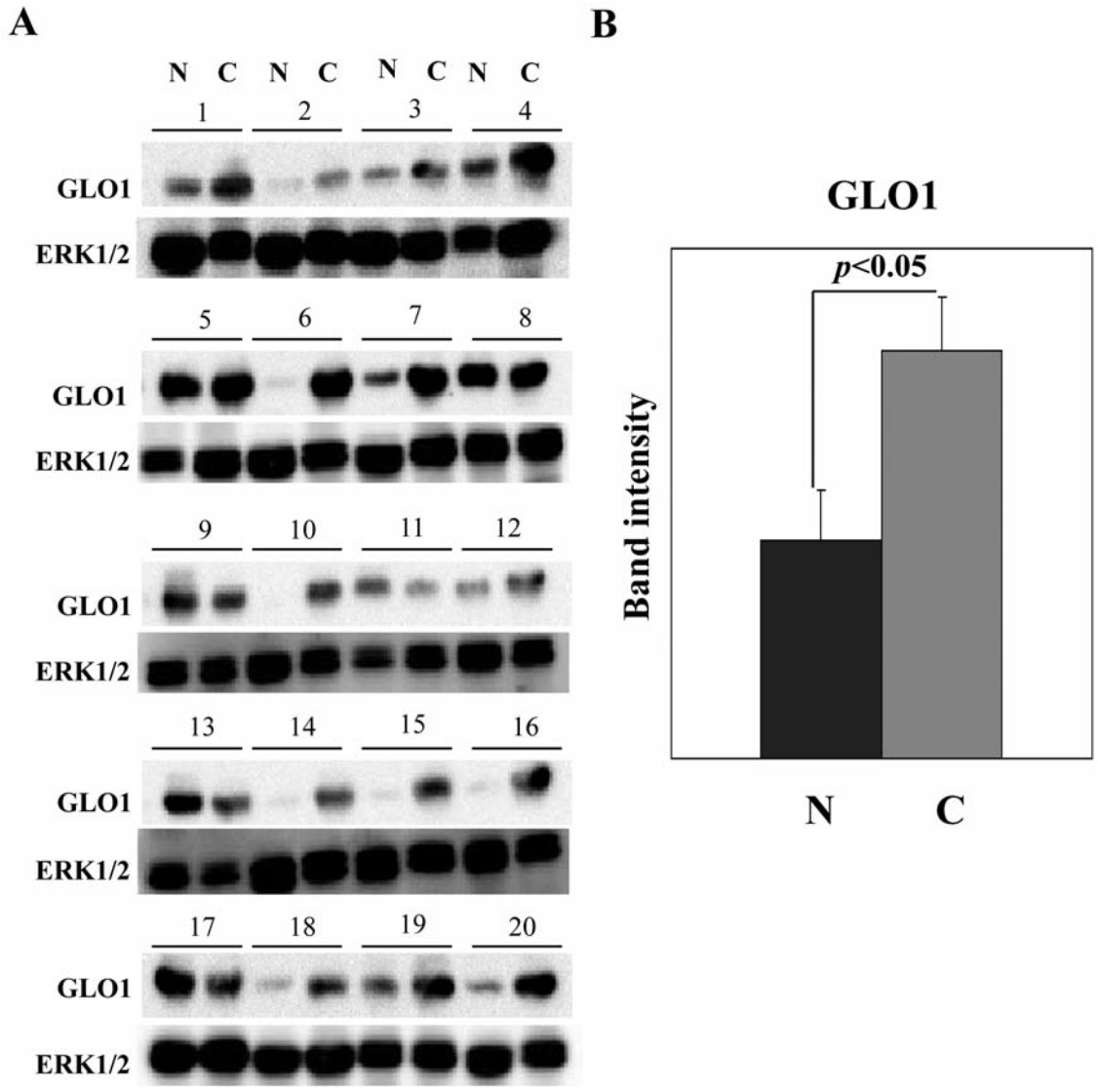

Figure 1. *Western blot analysis of glyoxalase I (GLO1) in PC. A: Tissues from 20 patients with pancreatic cancer (C) and paired non-cancerous tissues (N) were used for western blotting with anti-GLO1 and anti-extracellular regulated protein kinases (ERK1/2) antibody. The expression of GLO1 was confirmed 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 the Student's 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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