

Distinct prognostic values of mRNA expression of glutathione peroxidases in non-small cell lung cancer

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Introduction: Glutathione peroxidases (GPxs) constitutes an enzyme family which has the ability to reduce free hydrogen peroxide to water and lipid hydroperoxides to their corresponding alcohols, and its main biological roles are to protect organisms from oxidative stress-induced damage. GPxs include eight members in different tissues of the body, and they play essential roles in carcinogenesis. However, the prognostic value of individual GPx in non-small cell lung cancer (NSCLC) remains elusive.

Materials and methods: In the current study, we investigated the prognostic value of GPxs in NSCLC patients through the “Kaplan–Meier plotter” database, wherein updated gene expression data and survival information from a total of 1,926 NSCLC patients are included.

Results: High expression of *GPx1* mRNA was correlated with worse overall survival (OS) in adenocarcinoma patients. High expression of *GPx2* mRNA was correlated with worse OS for all NSCLC patients. In contrast, high expression of *GPx3* mRNA was associated with better OS for all NSCLC patients. High expression of *GPx4* mRNA was significantly correlated with worsening adenocarcinoma in these patients. *GPx5* mRNA high expression correlated with worsening OS for all NSCLC patients.

Discussion: The current findings of prognostic values of individual mRNA expression of GPxs in NSCLC patients indicate some GPxs may have prognostic value in NSCLC patients, and this needs further study.

Keywords: NSCLC, glutathione peroxidases (GPxs), cancer stem cell, prognosis, KM plotter, hazard ratio

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide in both men and women.^{1,2} Non–small cell lung cancer (NSCLC) includes adenocarcinoma (ADE) and squamous cell carcinoma (SCC), and accounts for 75–80% of cases of lung cancer. A multidisciplinary approach combining the application of chemotherapy with alternative treatment modalities as well as targeted therapy have aided improvement in survival outcomes. However, success with the above treatment modalities has continually been constrained by various limitations.^{3,4} Therefore, further investigation on the mechanism of progression, as well as identification of prognostic markers, will help select patients with a higher chance of lung cancer recurrence and provide better prognosis and individualized treatment.

Glutathione peroxidases (GPxs) belong to an enzyme family which has the ability to reduce free hydrogen peroxide to water and lipid hydroperoxides to their

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corresponding alcohols; their main biological roles are to protect organisms from oxidative stress-induced damage.^{5–8} GPxs include eight members in different tissues of the body with different substrate specificity, and they play essential roles in the protection of the organism from oxidative damage and carcinogenesis.^{8–10} Reactive oxygen species (ROS)-induced oxidative stress from mitochondrial dysfunction or NADPH oxidase (NOX) overactivation and ectopic expression of antioxidative enzymes were involved in EGFR-mediated tumor progression and drug resistance in malignancies including NSCLC.^{11,12} However, the prognostic values of individual GPxs in NSCLC remain elusive. The “Kaplan–Meier plotter” (KM plotter) generated data from the Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/)¹³ database. The KM plotter database was initially established using data on expression manifested by 22,277 genes in a group of 1,809 patients with breast cancer.^{14,15} Later, this database also included gene expression data and survival information from a total of 1,715 NSCLC patients.¹⁶ Recently, this database has included gene expression data and survival information from patients with ovarian cancer,¹⁷ gastric cancer, as well as liver cancer.¹⁸ Thus, KM plotter can be utilized for the analysis of individual genes with clinical results to relapse-free survival and total survival of the patients. So far, a number of genes have been identified and/or validated by KM plotter in breast cancer^{19–27} as well as in NSCLC.²⁸ In this study, we determined the prognostic value of individual GPx in human NSCLC patients using the KM plotter database.

Materials and methods

We used an online database¹⁴ to determine the relevance of mRNA expression of individual GPx members to relapse-free survival. The database was established using gene expression data and survival information of 1,926 NSCLC patients downloaded from Gene Expression Omnibus (GEO). Briefly, eight GPx sub-members (*GPx1*, *GPx2*, *GPx3*, *GPx4*, *GPx5*, *GPx6*, *GPx7*, and *GPx8*) were entered into the database (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>) to obtain Kaplan–Meier survival plots in which the number-at-risk is indicated below the main plot. The certain gene mRNA expression above or below the median separates the cases into high and low expression, respectively. HR (95% CIs) and log rank *p* were calculated and displayed on the webpage.

Results

The GPxs family comprises a total of eight sub-members. Among all the eight GPxs isoenzymes, only *GPx8* is not

included in the database; *GPx6* and *GPx7* share the same mRNA in www.kmplot.com.

We first examined the prognostic value of *GPx1* mRNA expression in www.kmplot.com. The desired Affymetrix IDs is valid: 200736_s_at (*GPx1*). Survival curves were plotted for all patients (*n*=1,926; Figure 1A), for ADE (*n*=720; Figure 1B), and for SCC (*n*=524; Figure 1C). High expression of *GPx1* mRNA was not found to be correlated with overall survival (OS) for all NSCLC patients followed for 20 years (HR 1.03 [95% CI 0.9–1.16]; *p*=0.7). However, high expression of *GPx1* mRNA was found to be correlated to worsen OS in ADE patients (HR 1.49 [95% CI 1.18–1.89]; *p*=0.00083), but not in SCC patients (HR 0.81 [95% CI 0.64–1.03]; *p*=0.082).

We then examined the prognostic value of *GPx2* mRNA expression in www.kmplot.com. The desired Affymetrix ID is valid: 239595_at (*GPx2*). High expression of *GPx2* mRNA correlated with worse OS for all NSCLC patients (HR 1.63 [95% CI 1.38–1.93]; *p*=5.1e-09; Figure 2A). However, high expression of *GPx2* mRNA was not significantly correlated with worse OS in ADE patients (HR 1.21 [95% CI 0.95–1.54]; *p*=0.12; Figure 2B) and SCC patients (HR 1.37 [95% CI 1–1.87]; *p*=0.051; Figure 2C).

Figure 3 shows the prognostic value of *GPx3* mRNA expression in www.kmplot.com. The desired Affymetrix ID is valid: 201348_at (*GPx3*). High expression of *GPx3* mRNA correlated with better OS for all NSCLC patients (HR 0.76 [95% CI 0.67–0.86]; *p*=2e-05; Figure 3A). However, high expression of *GPx3* mRNA was not significantly correlated with better OS in ADE patients (HR 0.85 [95% CI 0.67–1.07]; *p*=0.16; Figure 3B) and SCC patients (HR 0.87 [95% CI 0.69–1.11]; *p*=0.27; Figure 3C).

Figure 4 shows the prognostic value of *GPx4* mRNA expression in www.kmplot.com. The desired Affymetrix ID is valid: 201106_at (*GPx4*). The curves show that high expression of *GPx4* mRNA above or below the median do not separate the cases into significantly different prognostic groups among all NSCLC patients (HR 0.97 [95% CI 0.85–1.1]; *p*=0.63; Figure 4A). However, high expression of *GPx4* mRNA was significantly correlated with worse OS in ADE patients (HR 1.3 [95% CI 1.03–1.65]; *p*=0.029; Figure 2B), but not in SCC patients (HR 0.98 [95% CI 0.78–1.25]; *p*=0.9; Figure 4C).

Figure 5 shows the prognostic value of *GPx5* mRNA expression in www.kmplot.com. The desired Affymetrix ID is valid: 214648_at (*GPx5*). *GPx5* mRNA high expression was found to be correlated with worse OS for all NSCLC patients (HR 1.19 [95% CI 1.05–1.35]; *p*=0.0064; Figure 5A). In addition, high expression of *GPx5* mRNA was found to be correlated with worse OS in ADE patients (HR 1.57

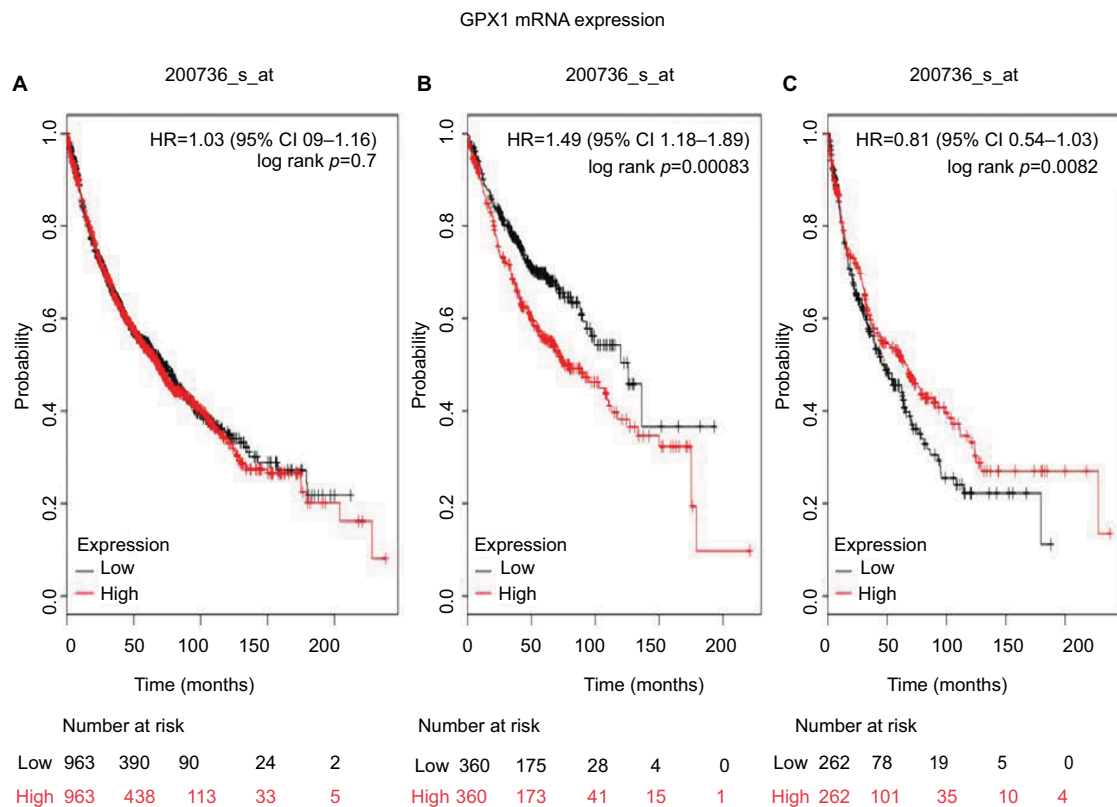


Figure 1 The prognostic value of *GPx1* expression according to the database of Kaplan–Meier plotter.

Notes: The desired Affymetrix ID is valid: 200736_s_at (*GPx1*). (A) Survival curves are plotted for all patients ($n=1,926$). (B) Survival curves are plotted for adenocarcinoma ($n=720$). (C) Survival curves are plotted for squamous cell carcinoma ($n=524$). Probability: overall survival.

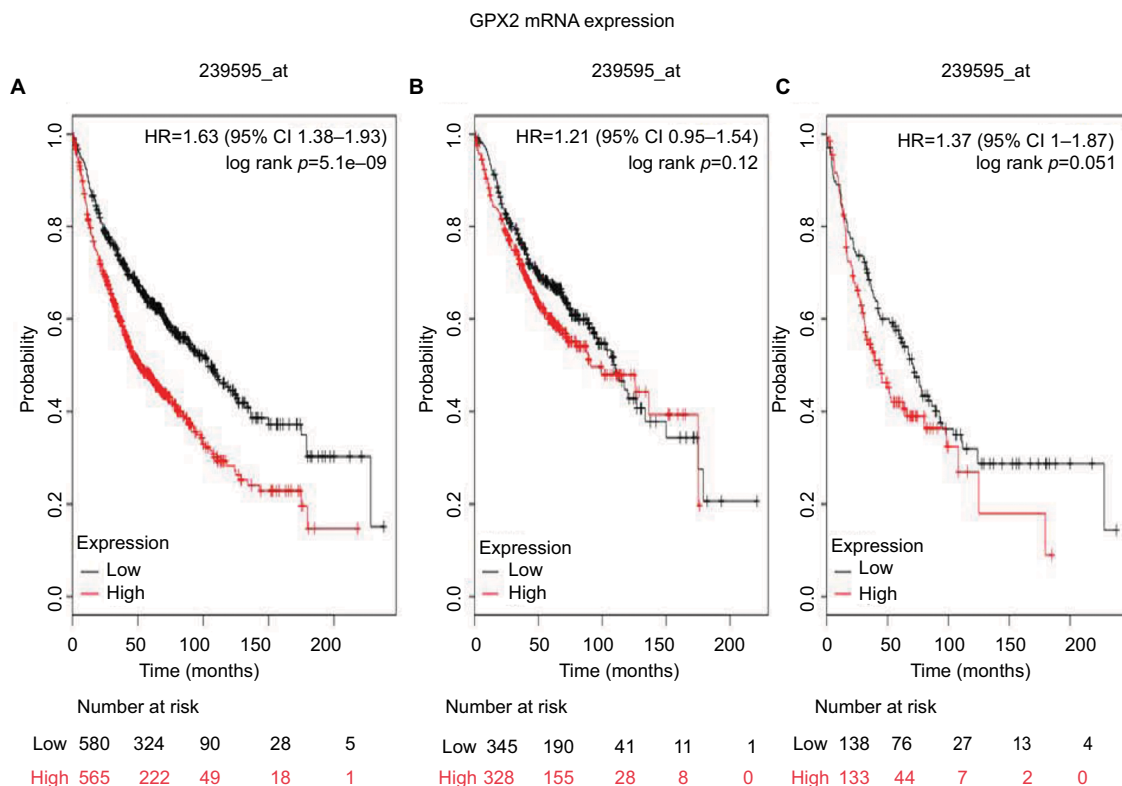


Figure 2 The prognostic value of *GPx2* expression according to the database of Kaplan–Meier plotter.

Notes: The desired Affymetrix ID is valid: 239595_at (*GPx2*). (A) Survival curves are plotted for all patients ($n=1,145$). (B) Survival curves are plotted for adenocarcinoma ($n=673$). (C) Survival curves are plotted for squamous cell carcinoma ($n=271$). Probability: overall survival.

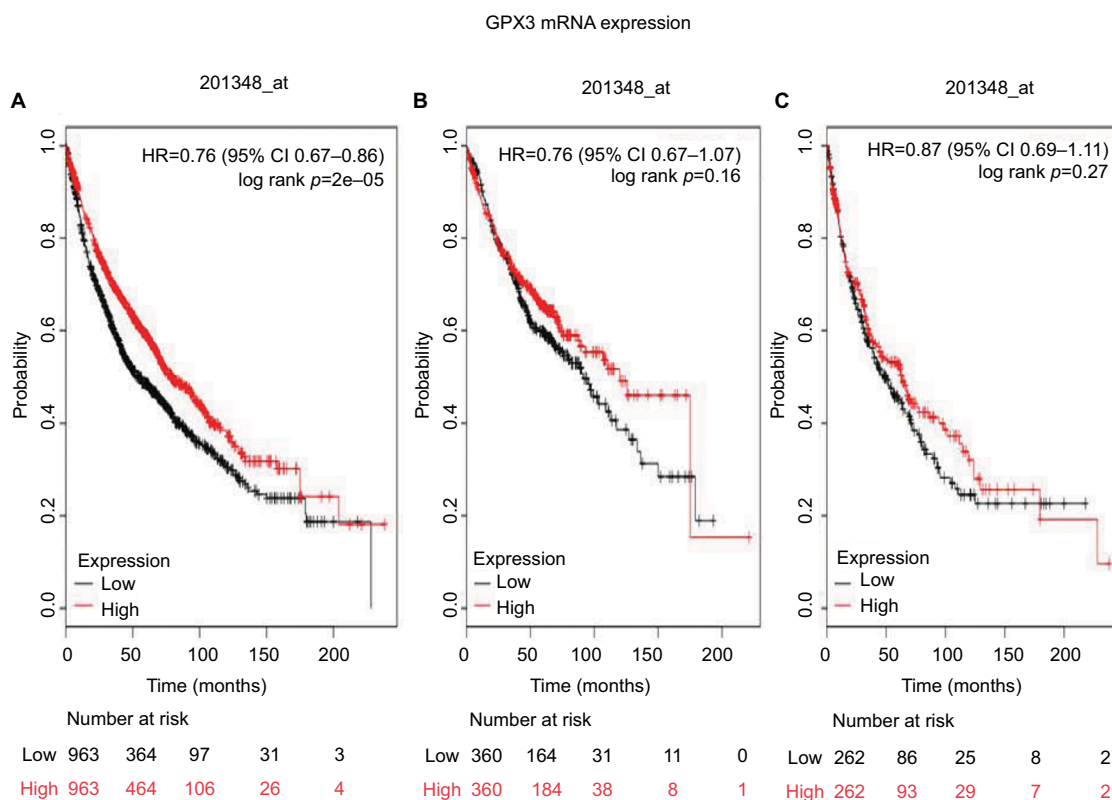


Figure 3 The prognostic value of GPX3 expression according to the database of Kaplan–Meier plotter.

Notes: The desired Affymetrix IDs is valid: 201348_at (GPX3). (A) Survival curves are plotted for all patients ($n=1,926$). (B) Survival curves are plotted for adenocarcinoma ($n=720$). (C) Survival curves are plotted for squamous cell carcinoma ($n=524$). Probability: overall survival.

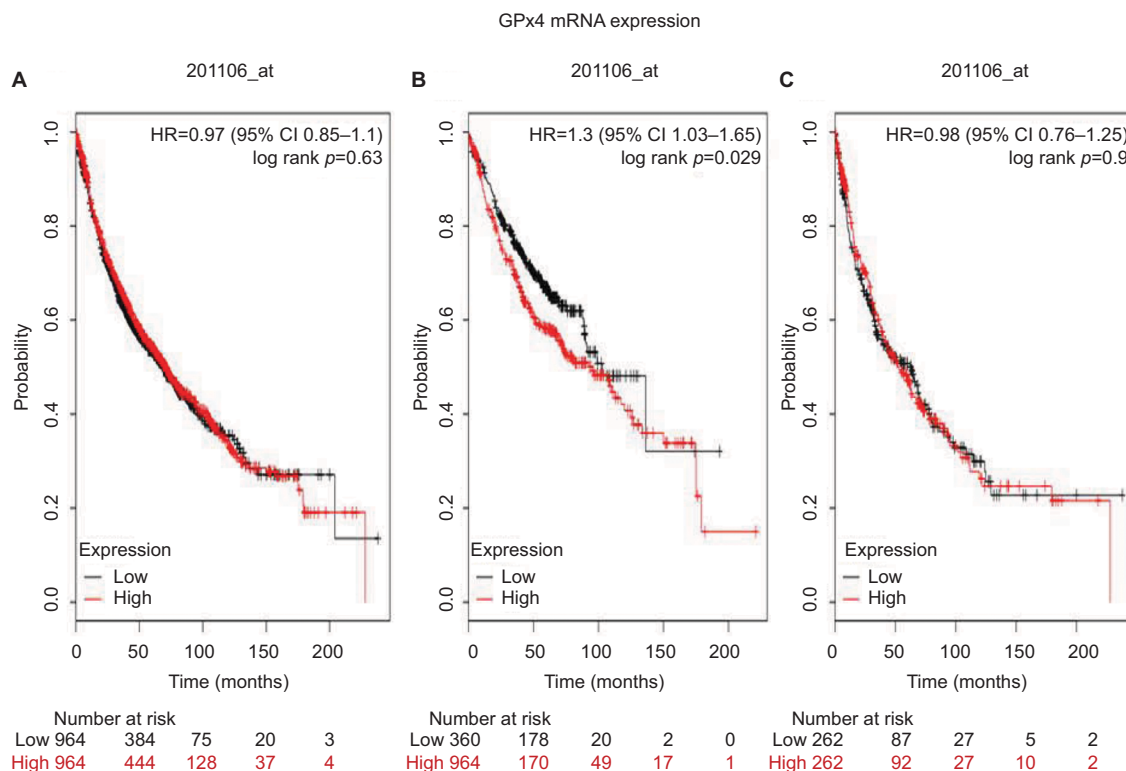


Figure 4 The prognostic value of GPX4 expression according to the database from Kaplan Meier plotter.

Notes: The desired Affymetrix ID is valid: 201106_at (GPX4). (A) Survival curves are plotted for all patients ($n=1,926$). (B) Survival curves are plotted for adenocarcinoma ($n=720$). (C) Survival curves are plotted for squamous cell carcinoma ($n=524$). Probability: overall survival.

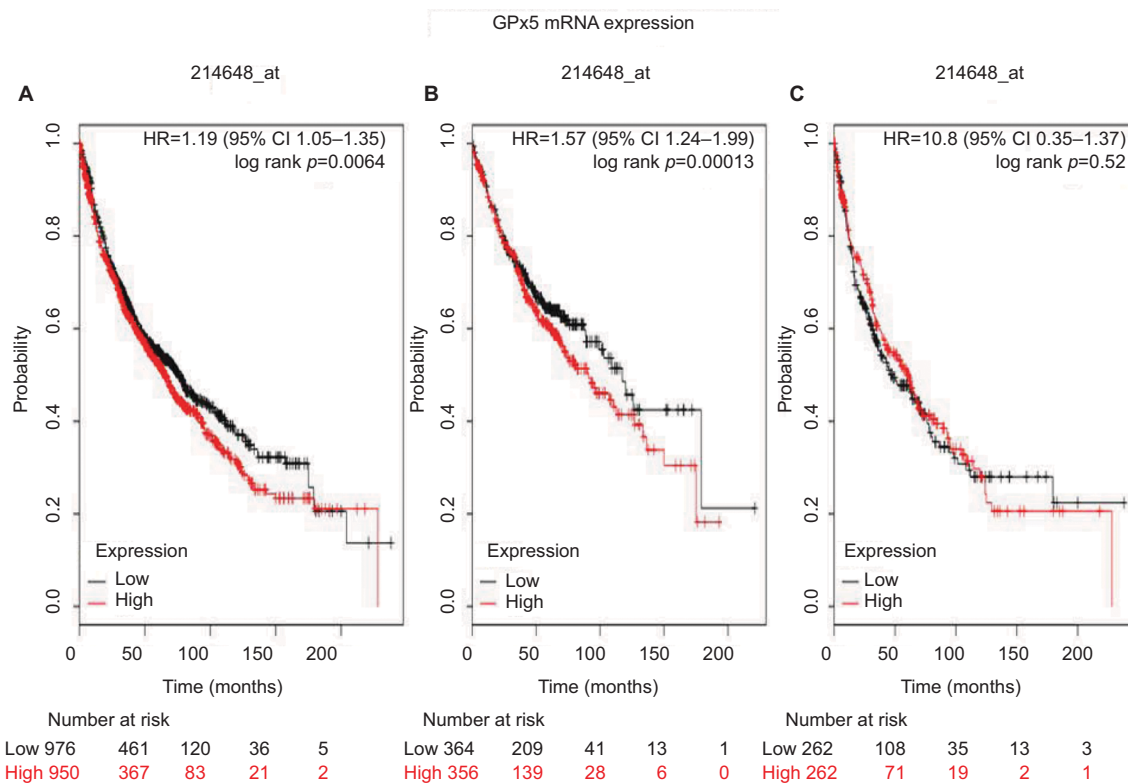


Figure 5 The prognostic value of GPx5 expression according to the database of Kaplan–Meier plotter.

Notes: The desired Affymetrix ID is valid: 214648_at (GPx5). (A) Survival curves are plotted for all patients ($n=1,926$). (B) Survival curves are plotted for adenocarcinoma ($n=720$). (C) Survival curves are plotted for squamous cell carcinoma ($n=524$). Probability: overall survival.

[95% CI 1.24–1.99]; $p=0.00013$; Figure 5B), but not in SCC patients (HR 1.08 [95% CI 0.85–1.37]; $p=0.52$; Figure 5C).

Figure 6 shows the prognostic value of GPx6 or GPx7 mRNA expression in www.kmplot.com. The desired Affymetrix ID is valid: 213170_at (GPx6 or 7). The curves show that high expression of GPx6/7 mRNA above or below the median do not separate the cases into significantly different prognostic groups in all NSCLC patients (HR 1.13 [95% CI 1–1.28]; $p=0.056$; Figure 6A), in ADE patients (HR 1.19 [95% CI 0.94–1.5]; $p=0.15$; Figure 6B), as well as in SCC patients (HR 0.95 [95% CI 0.75–1.2]; $p=0.67$; Figure 6C).

To further determine the correlation of individual GPx with other clinicopathological features, we determined their correlation with the smoking status (Table 1), clinical stages (Table 2), and chemotherapy (Table 3) of NSCLC patients. As shown in Table 1, high expression of GPx5, GPx6, or GPx7 mRNA was correlated with worse OS in never-smoked NSCLC patients. From Table 2, it is apparent that GPx2, GPx3, GPx6, or GPx7 are significantly associated with clinical stages of NSCLC patients. From the data in Table 3, it is

evident that none of the GPxs are significantly associated with NSCLC patients, with or without chemotherapy, probably due to the relatively limited number of patients.

Discussion

Changes in GPx levels in several types of tumor have been reported. GPx1 was reported to prevent oxidative DNA mutations and, thus, GPx1 may prevent tumorigenesis.²⁹ Overexpressed GPx1 reduced tumor growth, which indicates that it has a role in protecting against tumorigenesis.³⁰ The dual role of GPx2 in tumorigenesis has been reviewed recently.^{9,31} Overexpression of GPx2 was observed in several tumors,^{32,33} including lung cancer,³⁴ indicating that GPx2 may be an oncogene. GPx3 is considered to be a novel tumor suppressor, as hypermethylation of GPx3 was detected in tumor samples from patients with Barrett's esophagus^{35–37} as well as in endometrial³⁸ and prostate cancers,³⁹ and downregulation of GPx3 was generally correlated with worse prognosis. Moreover, GPx4 is considered to be a tumor suppressor, because it is downregulated in pancreatic³⁰ and breast cancers.⁴⁰ In

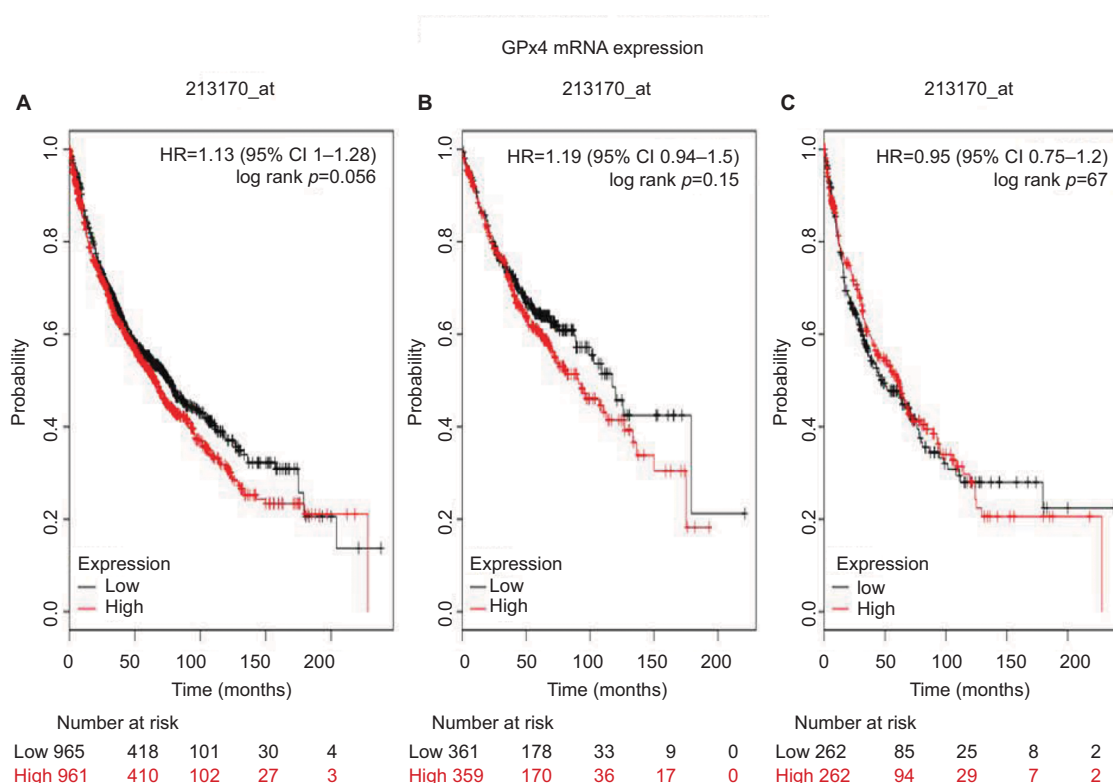


Figure 6 The prognostic value of GPx6/7 expression according to the database of Kaplan-Meier plotter.

Notes: The desired Affymetrix ID is valid: 213170_at (GPx6/7). **(A)** Survival curves are plotted for all patients ($n=1,926$). **(B)** Survival curves are plotted for adenocarcinoma ($n=720$). **(C)** Survival curves are plotted for squamous cell carcinoma ($n=524$). Probability: overall survival.

Table 1 Correlation of GPx mRNA expression with smoking status of NSCLC patients

GPxs	Smoking status	Cases	HR (95% CI)	p-value
GPx1	Never smoked	205	1.45 (0.83-2.54)	0.19
	Smoked	820	1.01 (0.82-1.24)	0.92
GPx2	Never smoked	141	0.74 (0.33-1.67)	0.47
	Smoked	300	1.18 (0.78-1.76)	0.43
GPx3	Never smoked	205	1.05 (0.6-1.82)	0.87
	Smoked	820	0.86 (0.7-1.06)	0.16
GPx4	Never smoked	205	1.55 (0.88-2.73)	0.13
	Smoked	820	0.93 (0.76-1.15)	0.51
GPx5	Never smoked	205	2.39 (1.33-4.29)	0.0027
	Smoked	820	1.15 (0.94-1.42)	0.17
GPx6/7	Never smoked	205	2.35 (1.31-4.22)	0.0032
	Smoked	820	1.11 (0.91-1.37)	0.3

Abbreviations: GPxs, glutathione peroxidases; NSCLC, non-small cell lung cancer.

addition, GPx4 overexpression reduced fibrosarcoma cell growth.⁴¹ So far, there are no reports on the role of GPx5, -6, -7, and -8 in tumorigenesis. With the exception of GPx3, all GPx members were reported to be associated with the

Table 2 Correlation of GPx mRNA expression with clinical stages of NSCLC patients

GPxs	Clinical stages	Cases	HR (95% CI)	p-value
GPx1	I	577	1.29 (0.98-1.29)	0.066
	II	244	1.17 (0.81-1.69)	0.4
	III	70	1.09 (0.63-1.88)	0.77
GPx2	I	449	1.52 (1.11-2.08)	0.0086
	II	161	1.08 (0.69-1.7)	0.74
	III	44	1.29 (0.64-2.59)	0.47
GPx3	I	577	0.63 (0.48-0.83)	0.00083
	II	244	1.28 (0.89-1.85)	0.19
	III	70	1.08 (0.62-1.87)	0.78
GPx4	I	577	1.25 (0.95-1.64)	0.11
	II	244	1.26 (0.87-1.82)	0.22
	III	70	1.36 (0.79-2.36)	0.27
GPx5	I	577	1.28 (0.98-1.68)	0.072
	II	244	1.57 (1.09-2.27)	0.015
	III	70	0.7 (0.4-1.21)	0.19
GPx6/7	I	577	1.81 (1.37-2.38)	1.9e-05
	II	244	1.28 (0.89-1.85)	0.18
	III	70	0.81 (0.47-1.4)	0.45

Abbreviations: GPxs, glutathione peroxidases; NSCLC, non-small cell lung cancer.

Table 3 Correlation of GPx mRNA expression with chemotherapy of NSCLC patients

GPxs	Chemotherapy	Cases	HR (95% CI)	p-value
GPx1	No	310	1.12 (0.8–1.57)	0.51
	Yes	176	1.11 (0.73–1.67)	0.63
GPx2	No	21	0.35 (0.06–1.93)	0.21
	Yes	34	1.89 (0.56–6.35)	0.3
GPx3	No	310	0.79 (0.56–1.1)	0.16
	Yes	176	1.31 (0.87–1.98)	0.2
GPx4	No	310	0.76 (0.54–1.06)	0.11
	Yes	176	0.96 (0.64–1.45)	0.85
GPx5	No	310	0.85 (0.61–1.19)	0.33
	Yes	176	0.79 (0.53–1.19)	0.26
GPx6/7	No	310	1.01 (0.73–1.42)	0.93
	Yes	176	1.19 (0.79–1.79)	0.4

Abbreviations: GPxs, glutathione peroxidases; NSCLC, non-small cell lung cancer.

prognosis of tumor patients. For example, increased GPx1 expression was significantly associated with poor prognosis in oral SCC⁴² and laryngeal SCC.⁴³ In contrast, loss of GPx1 expression was associated with aggressiveness and poor survival in patients with gastric cancer.⁴³ Furthermore, GPx2 overexpression was correlated with poor prognosis in patients with hepatocellular carcinoma⁴⁵ and gastric cancer.⁴⁶ In contrast, loss of GPx2 expression was associated with patients who had esophageal SCC.⁴⁷ The distinct different prognostic values of GPx1 and GPx2 in different types of cancer patients have not been well explained so far.⁴⁸

Several reports exist about the liver changes associated with mRNA expression of GPxs in NSCLC cell lines and/or tumor tissues;^{49–51} however, the prognostic value of mRNA expression of GPxs in NSCLC patients has not been reported. KM plotter is a widely used database containing gene expression data and survival information.¹⁷ Until now, a number of genes, such as *ALDH1*, *GLI1*, *ITIH5*, *CK2*, *RAI14*, and *GREB1*, have been identified and validated by KM plotter in lung,^{16,28,52,53} breast,^{19–28,54} gastric,^{55,56} and ovarian cancers.^{28,57,58} Using KM plotter, we determined the prognostic value of individual GPx mRNA in NSCLC patients. High expression of *GPx1* mRNA was not correlated with OS for all NSCLC patients followed for 20 years. However, high expression of *GPx1* mRNA was correlated with worse OS in ADE patients (HR 1.49 [95% CI 1.18–1.89]; $p=0.00083$). High expression of *GPx2* mRNA correlated with worse OS for all NSCLC patients (HR 1.63 [95% CI 1.38–1.93]; $p=5.1e-09$). In contrast, high expression of *GPx3* mRNA correlated with better OS for all NSCLC patients (HR 0.76 [95% CI 0.67–0.86]; $p=2e-05$). High expression of *GPx4* mRNA was significantly correlated with worse OS in ADE patients (HR 1.3 [95% CI

1.03–1.65]; $p=0.029$). High expression of *GPx5* mRNA correlated with worse OS for all NSCLC patients (HR 1.19 [95% CI 1.05–1.35]; $p=0.0064$). High expression of *GPx6/7* mRNA was not correlated with OS for all NSCLC patients. GPx8, as a novel member belonging to the GPx family, has been identified in a phylogenetic analysis in amphibia and mammalia.⁵⁹ GPx8 is a membrane protein, lung-abundant enzyme, and is detected in the endoplasmic reticulum.^{60,61} However, little is known about its role. In addition, individual GPx may interact among themselves and finally affect the prognosis of NSCLC patients. However, the KM plotter cannot be used to analysis the impact between the various isoforms of GPx. Moreover, the KM plotter cannot be used to analyze the correlation between isoenzyme expression.

Nicotine can regulate a number of biological functions such as cell proliferation, invasion, inflammation, apoptosis, and angiogenesis.⁶² Through inducing the secretion of stem cell factors, nicotine is able to regulate the growth and metastasis of NSCLC.⁶³ There is no evidence about the direct correlation between nicotine and GPx expression in NSCLC. However, nicotine treatment may significantly impact the expression of GPx1 in peripheral blood lymphocytes,⁶⁴ rat kidneys,⁶⁵ as well as in a novel cell line – Danio rerio gill (DrG) – derived from the gill tissue of zebrafish.⁶⁶ Furthermore, nicotine treatment was reported to significantly impact the expression of GPx4, GPx6, and GPx7 in rat brain.⁶⁷ In this report, we observed that high expression of *GPx5*, *GPx6*, or *GPx7* mRNA correlated with worse OS in never-smoked NSCLC patients.

There are a number of reports about the prognostic values of GPxs in tumor patients. For example, loss of GPx1 expression was associated with tumor aggressiveness and poor survival in patients with gastric cancer.⁴³ GPx2 overexpression indicates poor prognosis in patients with hepatocellular carcinoma,⁴⁵ gastric cancer,⁴⁶ and urothelial carcinomas of the upper urinary tract and urinary bladder.⁶⁸ However, patients with prostate cancer with high GPx2 expression on the biopsy specimen had significantly lower prostate-specific antigen (PSA), recurrence-free survival, and OS than those without any GPx2 expression.⁶⁹ Downregulation of GPx3 significantly correlated with poor prognosis in hepatocellular carcinoma,⁷⁰ cervical cancer,⁷¹ and gallbladder cancer.⁷² GPx3 is considered to be a novel tumor suppressor, because hypermethylation of the GPx3 promoter was detected in tumor samples from patients with Barrett's esophagus^{35–37} as well as endometrial³⁸ and prostate cancers,³⁹ and downregulation of GPx3 was generally associated with a poor prognosis. However, so far, no prognostic significance of GPxs protein

expression in NSCLC patients has been reported. The current findings of prognostic values of individual mRNA expression of GPxs in NSCLC patients indicate some members of the GPx family may also have prognostic values in NSCLC patients, and this needs further study.

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Disclosure

The authors report no conflicts of interest in this work.

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