# Up-Regulation of Peroxiredoxin 1 in Lung Cancer and Its Implication as a Prognostic and Therapeutic Target

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**Abstract**

**Purpose:** Peroxiredoxin 1 and 2 are highly homologous members of the Prx (or Prdx) protein family. Prx1 and Prx2 are elevated in several human cancers, and this seems to confer increased treatment resistance and aggressive phenotypes. This study was undertaken to examine the expression profiles of Prx1 and Prx2 in non – small cell lung cancer (NSCLC), and to test their prognostic value in predicting patient survival.

**Experimental Design:** To gain insight into the regulatory mechanisms of Prx1 and Prx2 expression in NSCLC, their respective transcript profiles were examined in NSCLC cell lines from the NCI-60 panel Affymetrix database sets, and the promoter compositions of the two genes were investigated using computer-based multiple sequence alignment analyses. Immunohistochemical analyses of Prx1 and Prx2 were done on a total of 235 NSCLC specimens with stage I through IV disease. The expression profiles of Prx1 and Prx2 in tumor specimens, and their associations with survival, were investigated.

**Results and Conclusion:** The levels of *prx1* transcript were higher than those of *prx2* in NSCLC cell lines, and the upstream regulatory sequences of the two genes display striking differences. The relative risk of death increased as Prx1 expression levels increased ($P$ = 0.036) in a multivariate Cox model, independent of other clinicopathologic variables associated with survival. No statistically significant correlation was observed between Prx2 and survival. These results suggest that Prx1 may possess unique functions and regulatory mechanisms in NSCLC which are not shared with Prx2, and that Prx1 may serve as a new prognostic biomarker and therapeutic target in NSCLC.

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Lung cancer continues to be the leading cause of cancer-related mortality in the United States and worldwide, with an estimated 213,380 new cases in 2007, lung cancer is expected to kill more than 160,000 Americans this year (1). Non – small cell lung cancer (NSCLC) accounts for up to 80% of all lung cancer cases and includes adenocarcinoma, squamous carcinoma, large-cell carcinoma, and mixed types. The prognosis of patients with lung cancer is poor, and the 5-year survival rate of patients with NSCLC remains among the lowest of all major human cancers at less than 15%. Studies suggest that conventional therapies may have reached a therapeutic plateau (2). The current challenge is to identify new therapeutic targets and strategies, and to incorporate them into recent treatment regimens with the goal of improving therapeutic gain. Identifying reliable markers predictive of clinical outcome would also be desirable in order to guide clinicians in selecting new treatment options and monitoring the treatment response of patients.

Peroxiredoxin 1 and 2 are highly homologous members of the peroxiredoxin (also known as Prx or Prdx) protein family (3). Both Prx1 and Prx2 have been found to be elevated in several human cancer cells and tissues, and to influence diverse cellular processes including cell survival, proliferation, and apoptosis. Elevation of Prx1 has been shown in oral, esophageal, pancreatic, follicular thyroid, and lung cancers (4 – 12). Prx1 and Prx2 were found to be elevated in mesothelioma, breast, and head and neck cancers (13 – 15). Elevated Prx1 was implicated in the chemotherapy resistance of breast cancer (16) and in radiotherapy resistance of lung cancer cells (17). On the other hand, down-regulation of Prx1 was shown to sensitize lung cancer cells to radiation and reduce metastasis (18, 19), and to increase the sensitivity of prostate cells to androgen ablation treatment (20). Similarly, overexpression of Prx2 rendered leukemia and stomach cancer cells resistant to various chemotherapeutic agents (21, 22), whereas down-regulation of Prx2 sensitized head and neck cancer cells to radiation and gastric carcinoma to cisplatin (23, 24). These studies suggest that both Prx1 and Prx2 enhance the aggressive survival phenotype of cancer cells, and confer an increased

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Received 9/26/07; revised 11/21/07; accepted 11/25/07.
**Grant support:** NIH grants CA109480, CA111846, and CA126804; Department of Defense grant PC050127; and Cancer Center Support grant CA16056.
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doi:10.1158/1078-0432.CCR-07-4457

resistance to treatment. Although the cell survival–enhancing function of Prx1 and Prx2 has traditionally been attributed to their antioxidant activity, a molecular chaperone–like function of Prx1, in particular, is gaining increased attention in recent years (17, 20, 25–27).

Human Prx1 and Prx2 share >90% homology in their amino acid sequences. Are they duplicative proteins and functionally redundant in NSCLC, or do they play unique roles in influencing tumor progression and treatment response? In this study, we show that levels of *prx1* transcript are consistently higher than those of *prx2* in all nine NSCLC cell lines included in the well-characterized Affymetrix NCI-60 panel database sets, indicating that regulation of their respective expression may differ in NSCLC. Our results, obtained from computer-based multiple sequence alignment analyses, support this notion, showing dramatic differences in the promoter compositions of the human *prx1* and *prx2* genes. Upstream sequence analyses also revealed that the characteristic regulatory elements and transcription factor binding sites (TFBS) present in the human *prx1* and *prx2* promoters are markedly conserved across species. Furthermore, the expression profiles of Prx1 and Prx2 and their respective associations with clinicopathologic variables were clearly distinguishable in our retrospective immunohistochemical study of 235 NSCLC patients with stage I through IV disease. Our results also show that Prx1 possesses prognostic utility for assessing patient survival in NSCLC. The results obtained from the current study corroborate our previous finding that Prx1 is an independent prognostic factor for poor survival outcome in stage I NSCLC (28). No statistically significant correlation was observed between Prx2 and survival.

## Materials and Methods

***Patients and clinicopathologic data collection.*** Institutional review board approval was obtained to investigate the immunohistochemical expression of Prx1 and Prx2 in stages I to IV NSCLC. A total of 1,528 patients were diagnosed and received their first line treatment at Roswell Park Cancer Institute between January 1993 and March 2002. Of these, staging and clinicopathologic information was complete for 633 patients. Either specimen blocks or follow-up records were not available for 398 out of the 633 patients. For the remaining 235 patients, we were able to retrieve suitable specimen blocks as well as clinicopathologic information and follow-up data which were used for the current study. Information regarding patient demographics, smoking history, tumor stage, histopathologic diagnosis, grade of differentiation, date of diagnosis, and date of death or last follow-up was collected as previously described (28). Performance status was based on the Eastern Cooperative Oncology Group scale (0, no symptoms; 1, minor symptoms; 2, symptoms present, out of bed or chair for more than 50% of waking hours; 3, symptoms present, in bed or chair for more than 50% of waking hours). The presence or absence of weight loss was based on weight change reported at initial presentation (±5% in 3 months). Tumors were staged pathologically according to the revised international system (29), and histologically classified according to the WHO criteria for lung and pleural tumors (30). The cutoff date for vital status analysis was December 2006.

***Specimen preparation and immunohistochemistry.*** Tissue specimens obtained from diagnostic or therapeutic procedures were fixed in neutral buffered formalin (10% vol/vol formalin in water; pH 7.4) and embedded in paraffin wax. Serial sections of 4-μm thickness were cut and mounted on charged glass slides (Superfrost Plus, Fisher Scientific). Immunohistochemistry conditions for Prx1 and Prx2 were optimized and evaluated by two independent pathologists (J-H. Kim and P.N. Bogner). In brief, for both Prx1 and Prx2, sections were microwaved twice for 10 min in citrate buffer (pH 6.0) for antigen retrieval. The sections were then treated with 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block nonspecific binding. Rabbit polyclonal antibodies against Prx1 and Prx2 (both from Lab Frontier, Korea) were used at a dilution of 1:2,000. The avidin-biotin detection method was used on a Ventana Automated System (Ventana Medical Systems). An irrelevant rabbit antiserum served as a negative control.

***Assessment of immunostaining and statistical analysis.*** Each slide was evaluated for Prx1 or Prx2 immunoreactivity using a semiquantitative scoring system for both the intensity of the stain and the percentage of positive malignant cells. Prx1 immunoreactivity was observed primarily in the cytosol, although nuclear expression was also noted in some malignant cells. Prx2 immunoreactivity was predominantly in the cytosol. The intensity of cytosolic Prx1 and Prx2 staining was coded as: 0, lower than the adjacent normal-appearing bronchial epithelium; 1, similar to the adjacent bronchial epithelium; or 2, stronger than the adjacent bronchial epithelium. The percentage of cancer cells displaying a stronger staining intensity compared with the adjacent bronchial epithelium was categorized as 1 (0-24% cancer cells stained), 2 (25-49% cancer cells stained), 3 (50-74% cancer cells stained), or 4 (75-100% cancer cells stained). For Prx1 staining, 9 cases were grade 0, 57 cases were grade 1, and 169 cases were grade 2. Out of 169 cases of grade 2 specimens, 20 cases fall into category 1, 51 cases to category 2, 67 cases to category 3, and 31 cases to category 4. Sixty-six cases displaying either grade 0 (9 cases) or 1 (57 cases) were categorized as "1". For Prx2 staining, 28 cases were grade 0, 58 cases were grade 1, and 148 cases were grade 2. Out of 148 grade 2 specimens, 43 cases were category 1, 57 cases were category 2, 40 cases were category 3, and 8 cases were category 4. Eighty-six cases belonging to either grade 0 (28 cases) or 1 (58 cases) were categorized as 1. The association between Prx1 and Prx2 staining categories and ordinal measures was analyzed using Kendall's $\tau$. The association between Prx1 and Prx2 staining categories and nominal variables was analyzed using Kruskal-Wallis's rank sum test. Cox proportional hazards models were used for multivariate analyses of patient survival to adjust for potential confounding factors including tumor stage, performance status, and age. Survival curves for the patients based on the Prx1 and Prx2 staining categories were generated using the Efron method for handling ties. To confirm and further validate goodness-of-fit, additional stratified and unstratified analyses were done. A one-sided log-rank trend test was used to evaluate the statistical significance of differences in survival distribution among Prx1 and Prx2 staining categories and other covariates. Statistical analyses were conducted using the R software package (31).[6] Results were considered statistically significant if $P < 0.05$.

## Results

***The levels of* prx1 *transcript are higher than those of* prx2 *in human NSCLC cell lines.*** In order to understand the regulation of human *prx1* and *prx2* gene expression in NSCLC, we examined the transcript profiles of *prx1* and *prx2* genes in NSCLC cell lines. Several Affymetrix database sets are available for the 60 human cancer cell lines (the NCI-60) used by the National Cancer Institute's Developmental Therapeutics Program to screen chemically defined compounds and natural product extracts (32–35). The NCI-60 lines have been characterized more extensively than any other set of cells in existence, and their molecular databases are publicly available (36). A total of nine NSCLC cell lines are included in the NCI-60 panel. We extracted three Affymetrix transcript data

[6] http://www.R-project.org

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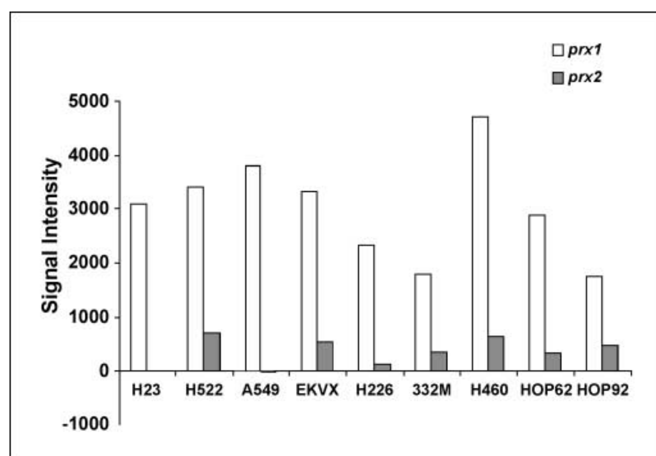


**Fig. 1.** Comparison of *prx1* and *prx2* transcripts in the NCI-60 panel of human NSCLC cell lines. Columns, levels of *prx1* and *prx2* transcript in the nine NSCLC cell lines included in the NCI-60 panel. *Y*-axis, intensity values obtained from the Affymetrix 6,800-feature set arrays.

sets available online[7] in the CellMiner program package: the original transcript profile data set on the HU-6800 chip and the two new NCI-60 transcript profile data sets on the HG-U95 and the HG-133A chips. Our results showed that levels of *prx1* transcript were consistently higher than those of *prx2* in all nine NSCLC cell lines in these data sets, although the levels of *prx1* transcript varied among cell lines. Figure 1 displays the levels of *prx1* and *prx2* transcripts obtained from the HU-6800 data set.

***Promoter structure and composition of* prx1 *and* prx2 *genes display striking differences.*** The analysis of *prx1* and *prx2* transcript levels indicated that the regulatory mechanisms of *prx1* and *prx2* gene expression may differ in NSCLC. When we examined the chromosome ideograms and the genomic location of *prx1* and *prx2* genes using the UCSC Genome Browser,[8] it was apparent that *prx1* has a much longer upstream region compared with *prx2*. The human *prx1* gene is localized to chromosome 1p34 with ~30 kb of upstream regions shared with the *Akr1a1* gene in a head to head orientation. The human *prx2* gene is localized to chromosome 13q12 with a much shorter upstream region (~5 kb) shared with its neighboring gene, *Rnaseh2a*, also in a head to head orientation. The genomic regions containing *prx1* and *prx2* seemed to be conserved syntenic (i.e., same genes and orders in the corresponding chromosomal loci), at least across mammalian species.

To further explore the molecular basis of *prx1* and *prx2* gene expression, we carried out computer-based upstream sequence analyses for each gene. The genomic DNA sequences upstream of the first exon of *prx1* (15 kb) and *prx2* (5 kb) genes were retrieved from the UCSC genome browser[9] in Fasta format. Considering that critical regulatory sequences are likely to be conserved across species, multiple sequence alignment analyses were done using upstream noncoding sequences from human, mouse, and rat. First, in order to identify conserved noncoding sequences (CNS), four data sources and methods were used, which were DIALIGN (37),[10] mVISTA (38),[11] Vertebrate Multiz Alignment and PhastCons Conservation (39), and ESPERR Regulatory Potential (40). The results of these four analyses for the upstream regions of each gene were compared, and the regions of CNS supported by more than two methods were chosen as the reliable CNS (Fig. 2, sequences shown with gray highlight). The selected CNS regions for *prx1* or *prx2* gene were then subjected to the TFBS analysis using a locally installed Match program of TRANSFAC Professional. In order to minimize false positives and negatives, TFBS were selected based on the stringent criteria fulfilling both the core match score of 1 and a matrix match score >0.9.

As shown on the right hand side of the corresponding sequences in Fig. 2, the composition of TFBS from *prx1* (panel *A*) and *prx2* (panel *B*) in the selected CNS regions showed marked differences. The characteristic TFBS identified for each gene were also found to be highly conserved across species. The *prx1* gene seems to possess more TFBS in its CNS regions, and among them, Oct-1, CREB, ATF, AR, GR, C/EBP, SOX, LEF1, TBP, MZF-1, v-Myb, YY1, p53, and AP-1 were featured repeatedly across species. The presence of the Nrf2 site in the *prx1* CNS was also noted, the significance of which on the transcriptional activation of the *prx1* gene was reported previously (41). In contrast, the TFBS detected in *prx2* CNS regions seemed to be mainly AP-2, AP-4, TATA, E2F-1, E2F, and Nkx2-5. Promoter composition analyses support the hypothesis that the transcriptional regulatory mechanisms of *prx1* and *prx2* expression may differ significantly. A possible epigenetic mechanism affecting either genes' expression was explored by analyzing the presence of CpG islands (42). As shown in Fig. 2, the *prx1* and *prx2* genes possess CpG islands (sequences shown in boldface) within their core promoter regions, indicating that both genes may be subjected to epigenetic regulation.

***Immunohistochemical analysis of Prx1 and Prx2 expression profiles in NSCLC tumor specimens.*** There is little information regarding the expression profile of Prx1 and Prx2 in NSCLC. We carried out immunohistochemical analyses and comparatively evaluated the expression patterns of Prx1 and Prx2 in tumor tissues obtained from 235 NSCLC patients representing the spectrum of pathologic stages I to IV disease. The distribution of histopathologic diagnoses by pathologic disease stage of the 235 NSCLC patients is shown in Table 1. There was no association between stage and histopathologic subtype of NSCLC ($P = 0.46$). Although elevated levels of Prx1 and Prx2 in cancer cells were detectable in nearly all cases, trends in Prx1 and Prx2 staining in tumors seemed to differ when they were grouped by the percentage of positively stained tumor cells (Table 2). Whereas a large number of tumor tissues (129 cases) contained <25% of Prx2-stained cancer cells, only eight cases contained ≥75% of Prx2 positive cancer cells. On the other hand, tumors contained a higher proportion of Prx1 positive cancer cells with a total of 98 cases displaying ≥50% of Prx1 staining, of which 31 cases contained ≥75% of Prx1-positive cancer cells. The distributions of Prx1 and Prx2 staining categories in tumors were significantly different ($P < 0.001$) based on the Wilcoxon signed rank test.

[7] http://discover.nci.nih.gov
[8] http://genome.ucsc.edu
[9] http://genome.ucsc.edu
[10] http://bioweb.pasteur.fr/seqanal/interfaces/dialign2-simple.html
[11] http://genome.lbl.gov/vista

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***Association of Prx1 and Prx2 expression levels with clinicopathologic characteristics.*** The clinical and pathologic characteristics of the 235 patients investigated in the current study are detailed in Table 3. The median age of the patients at the time of diagnosis was 64.4 years (range, 35.2-88.2 years). A total of 103 patients had stage I, 46 had stage II, and 86 had stages III or IV disease. Approximately 56% of the patients (132) were men, and 44% (103) were women. Ethnically, the majority of patients were white (222 cases), whereas 12 were African American and 1 was Hispanic. Forty of the 235 patients never smoked, 195 were active or past smokers. The Eastern Cooperative Oncology Group performance status score was 0 in 155 patients, 1 in 69 patients, 2 or 3 in 10 patients, and information was not available for 1 patient. Thirty-one patients had lost >5% of their body weight in the 3 months before diagnosis, 203 had stable weight, and data was unavailable for 1 patient. The histopathologic distribution of the 235 patients included 125 adenocarcinomas, 85 squamous carcinomas, 15 large cell carcinomas, and 10 mixed NSCLC. In terms of tumor grade, 19 cases were well-differentiated, 78 were moderately differentiated, and 129 cases were poorly differentiated.

When we tested for correlations between Prx1 expression levels and the clinicopathologic variables potentially predictive of prognosis, Prx1 expression levels were found to correlate

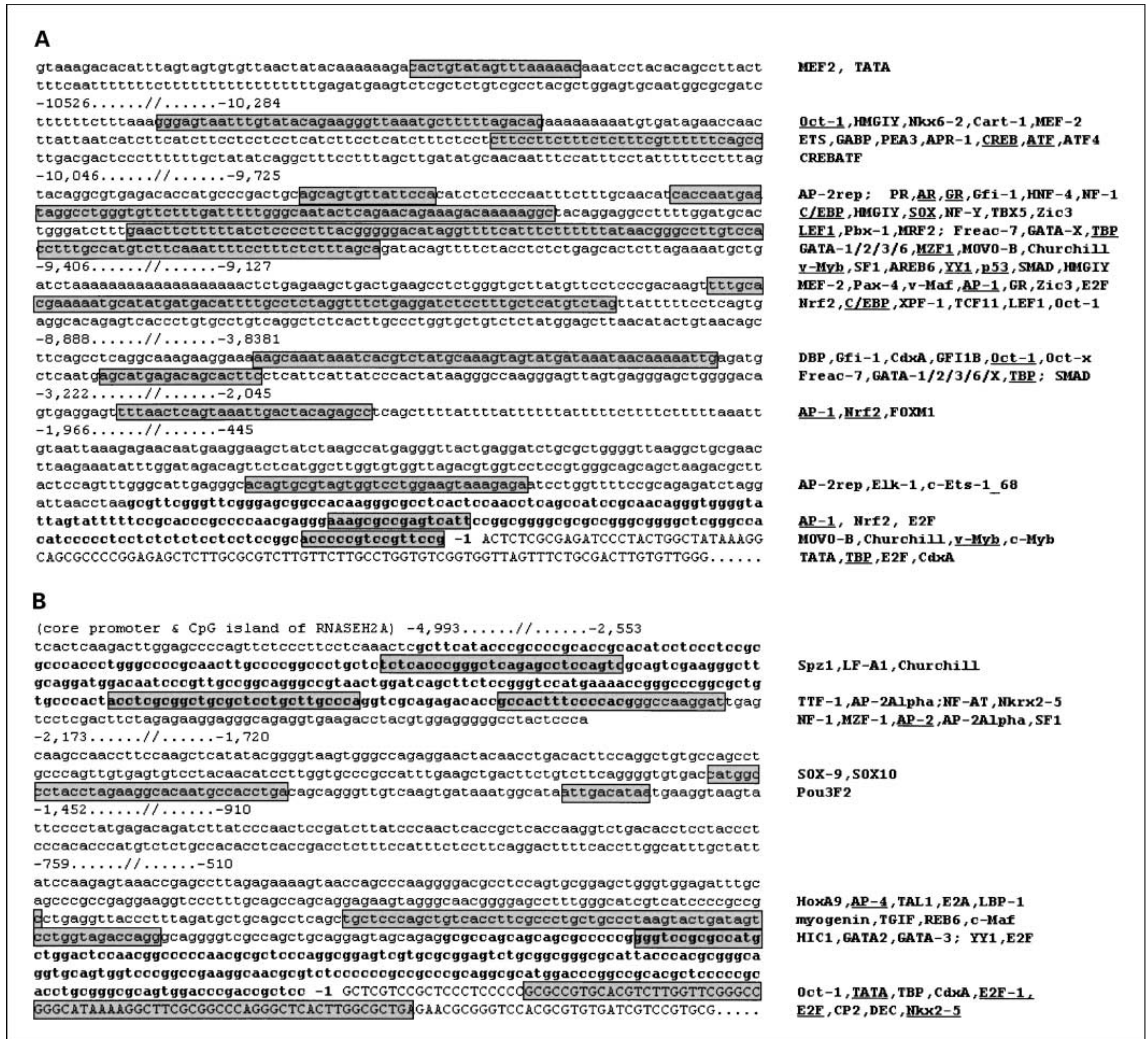


**Fig. 2.** Multiple sequence alignment analyses of 5′ upstream sequences of *prx1* and *prx2* genes. The CNS in the 15 kb upstream of *prx1* gene (*A*) or the 5 kb upstream of *prx2* gene (*B*) were analyzed to identify TFBS. Sequences with gray highlights, CNS regions supported by more than two types of multiple sequence alignment analyses. The TFBS identified in the highlighted regions are listed on the right side of the corresponding sequences. TFBS with multiple hits in the region are underlined. Sequences intervening the CNS regions were deleted ("......//......"). Numbers, positions of each CNS region in relation to the transcription initiation site; boldface, conserved CpG islands; sequences in uppercase, regions of the first exon.

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significantly with the grade of tumor differentiation. Poorly differentiated tumors had a higher percentage of Prx1-stained cells ($P$ = 0.016). No other variables showed statistically significant associations with Prx1 expression levels. Consistent with our previous findings (28), no correlation was observed between Prx1 expression levels and smoking history ($P$ = 0.36), suggesting that the elevated expression of Prx1 in NSCLC is unlikely to result from a general stress response to tobacco smoke exposure. On the other hand, when the correlations between Prx2 expression levels and the clinicopathologic factors were tested, the presence of smoking history and gender were found to correlate significantly with Prx2 expression levels. Higher levels of Prx2 expression correlated significantly with the absence of a smoking history ($P$ = 0.03) and also with female gender ($P$ = 0.05) in this patient cohort. These results are congruent with the results of the NSCLC cell lines and promoter composition analyses, and suggest that the mechanisms of *prx1* and *prx2* gene expression in NSCLC differ significantly between the two genes.

***Prx1 expression level is an independent prognostic factor for overall survival.*** A multivariate Cox analysis was carried out to assess the prognostic value of Prx1 and Prx2 levels on patients' survival. Survival was defined as the time from the date of diagnosis to the date of last follow-up or death from any cause. All other potentially prognostic factors including age, tumor stage, and grade were adjusted for in this analysis. Our results showed that levels of Prx1 expression significantly correlate with overall survival; the hazards ratio increased progressively as the percentage of Prx1-stained cancer cells increased ($P$ = 0.036; Table 4). Using patients with tumors containing <25% stained cancer cells as the reference group (risk = 1), the relative risk of death was 1.18 for patients with tumors containing 25% to 49% of Prx1-stained cancer cells, 1.27 with tumors containing 50% to 74% of Prx1-stained cancer cells, and 1.52 with tumors containing ≥75% of Prx1-stained cancer cells. Conversely, the relative risk of death seemed to decrease progressively as the levels of Prx2 expression increased. Although it did not reach statistical significance ($P$ = 0.099), the relative risk of death was 0.88 in patients with tumors containing 25% to 49% of Prx2-stained cancer cells, 0.77 with tumors containing 50% to 74% of Prx2-stained cancer cells, and 0.75 with tumors containing ≥75% of Prx2-stained cancer cells compared with those containing <25% of Prx2-stained cancer cells. $P$ values for all other clinicopathologic variables except for age, tumor stage, and performance status were >0.05 in correlation with overall survival. Survival curves for the patients were generated based on the Prx1 staining categories, and are displayed in Fig. 3. Cumulative overall survival curves of patients were significantly split by Prx1 staining categories ($P$ = 0.036).

## Discussion

Lung cancer continues to be the number one cause of cancer-related mortality. In order to improve the outcome for patients, advances in the understanding of the pathophysiology of lung cancer, and identification of proteins and molecular pathways that affect key proliferation and survival mechanisms are needed. Accumulated evidence suggests that Prx1 and Prx2 may be important components in these mechanisms. No previous study, however, has undertaken an investigation of the prognostic value of Prx1 and Prx2 in NSCLC. In this study, we showed that a high level of Prx1 expression is an independent and clinically significant prognostic factor for poor survival outcome in patients with NSCLC. The relative risk of death assessed using a multivariate Cox proportional hazards model increased in NSCLC patients as Prx1 expression levels increased ($P$ = 0.036), independent of clinicopathologic variables potentially predictive of patient survival. No significant correlation was observed between Prx2 levels and survival. Our results on NSCLC cell lines and tumor tissues also indicated that the regulation of *prx1* and *prx2* gene expression might differ significantly. Computer-based promoter composition analyses further support the existence of separate and unique regulatory mechanisms distinguishing the expression profiles of Prx1 and Prx2 in NSCLC. The current findings, together with the previous results reported by us and others, suggest that Prx1 may be a new player in mediating the malignant progression of NSCLC.

Although the cell survival–enhancing function of Prx1 has traditionally been attributed to its antioxidant activity, the physiologic significance of this function is unclear because it is highly susceptible to inactivation by oxidative stress. Studies suggest that Prx1 may act as a molecular chaperone, and modulate the activities of growth regulatory proteins with an outcome favoring cell survival (17, 20, 25, 26). Based on the results from a combination of mutagenesis, biochemical, and X-ray crystallographic studies, we recently reported that despite >90% homology in their amino acid sequences, human Prx1 and Prx2 proteins are structurally and functionally distinguishable (27). Furthermore, results obtained from purified human Prx1 and Prx2 proteins showed that Prx1 is highly efficient as a molecular chaperone, whereas Prx2 is not (27). These findings are consistent with the behavior of Prx1 in interacting physically with various cellular proteins. The interaction of Prx1 with c-Abl, c-Myc, and the macrophage inhibiting factor, MIF, has been shown using a yeast two-hybrid system (43–45). The wide range of effects attributed to Prx1 in various cells and organisms may in part be explained by the physical interaction

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**Table 1.** Distribution of pathologic stage by tumor histopathology

| Stage | Adenocarcinoma ($n$ = 125) | Squamous cell carcinoma ($n$ = 85) | Large cell carcinoma ($n$ = 15) | Others ($n$ = 10) |
|---|---|---|---|---|
| I | 51 (21.7) | 41 (17.4) | 6 (2.6) | 5 (2.1) |
| II | 25 (10.6) | 19 (8.1) | 2 (0.9) | 0 (0.0) |
| III | 32 (13.6) | 18 (7.7) | 4 (1.7) | 4 (1.7) |
| IV | 17 (7.2) | 7 (3.0) | 3 (1.3) | 1 (0.4) |
| Total | 125 (53.2) | 85 (36.2) | 15 (6.4) | 10 (4.3) |

NOTE: Value in table expressed as $n$ (%).

**Table 2.** Number of tumor specimens by Prx1 and Prx2 staining categories

| Tumor cell staining category | Prx1 specimens (*n* = 235) *n* (%) | Prx2 specimens (*n* = 234) *n* (%) |
|---|---|---|
| (1) 0-24% | 86 (36.6) | 129 (55.1) |
| (2) 25-49% | 51 (21.7) | 57 (24.4) |
| (3) 50-74% | 67 (28.5) | 40 (17.1) |
| (4) 75-100% | 31 (13.2) | 8 (03.4) |

of Prx1 with various growth regulatory proteins (43–47). As studies of Prx1 advance, the spectrum of molecules interacting with Prx1 is likely to expand. Although the functional consequences of these interactions are likely to manifest in a cell type–, tissue context–, and/or disease site–dependent manner, and thus need to be interpreted with caution, inhibiting Prx1 may provide an approach for the simultaneous targeting of multiple proteins and molecular pathways that contribute to tumor progression and treatment response.

The NF-E2–related factor 2, Nrf2, belongs to the Cap'n' Collar family of basic region-leucine zipper (bZip) transcription factors. The human *prx1* gene was recently reported to be a target of Nrf2 (41). It is noteworthy that studies by Padmanabhan et al. (48) and Singh et al. (49) suggest that occurrence of genetic abnormalities in the Nrf2 repressor, Keap1, may be a frequent event in lung cancer. Based on the results from several cell lines and tumor tissues, these authors suggested that the genetic defects of Keap1 might lead to the constitutive nuclear accumulation and activation of Nrf2 in lung cancer. Further research is necessary to determine whether loss of heterozygosity and/or mutation in the Keap1 genomic loci contributed to the elevation of Prx1 in the NSCLC tissues investigated in the current study. As illustrated in our promoter composition analysis of the *prx1* and *prx2* genes, however, it is highly likely that in addition to Nrf2, various other transcription factors may be involved in coordinating and relaying different cellular signals to turn on/off the transcriptional machinery of the *prx1* (or *prx2* gene), as is true for other genes involved in cell growth and survival regulation. The results of our promoter composition analyses may serve as a basis upon which the molecular regulatory mechanisms of *prx1* and *prx2* expression in NSCLC and other human malignancies can be unraveled.

Aberrant cytosine methylation of promoter CpG islands and regulatory elements of genes, and/or changes in histone acetylation, are often involved in silencing gene expression in cancer cells. The possible involvement of aberrant methylation

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**Table 3.** Clinicopathologic variables and the expression status of Prx1 and Prx2

| Characteristics | Total | Prx1 staining category | | | | *P* | Total | Prx2 staining category | | | | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | | 1 | 2 | 3 | 4 | |
| Age | | | | | | 0.76 | | | | | | 0.22 |
| <Median | 117 | 42 | 25 | 34 | 16 | | 116 | 59 | 31 | 22 | 4 | |
| ≥Median | 118 | 44 | 26 | 33 | 15 | | 118 | 70 | 26 | 18 | 4 | |
| Tumor stage | | | | | | 0.16 | | | | | | 0.38 |
| I | 103 | 32 | 22 | 36 | 13 | | 103 | 60 | 22 | 16 | 5 | |
| II | 46 | 20 | 9 | 8 | 9 | | 46 | 26 | 13 | 7 | 0 | |
| III | 58 | 23 | 12 | 17 | 6 | | 57 | 29 | 14 | 12 | 2 | |
| IV | 28 | 11 | 8 | 6 | 3 | | 28 | 14 | 8 | 5 | 1 | |
| Gender | | | | | | 0.34 | | | | | | 0.05 |
| Female | 103 | 41 | 21 | 30 | 11 | | 103 | 47 | 35 | 17 | 4 | |
| Male | 132 | 45 | 30 | 37 | 20 | | 131 | 82 | 22 | 23 | 4 | |
| Race | | | | | | 0.15 | | | | | | 0.75 |
| White | 222 | 79 | 48 | 65 | 30 | | 221 | 122 | 55 | 36 | 8 | |
| Others | 13 | 7 | 3 | 2 | 1 | | 13 | 7 | 2 | 4 | 0 | |
| Smoking history | | | | | | 0.36 | | | | | | 0.03 |
| Never | 40 | 10 | 11 | 16 | 3 | | 39 | 16 | 10 | 12 | 1 | |
| Ever | 195 | 76 | 40 | 51 | 28 | | 195 | 113 | 47 | 28 | 7 | |
| Performance status | | | | | | 0.38 | | | | | | 0.42 |
| 0 | 155 | 59 | 35 | 43 | 18 | | 154 | 82 | 37 | 30 | 5 | |
| 1 | 69 | 21 | 14 | 23 | 11 | | 69 | 41 | 17 | 9 | 2 | |
| 2/3 | 10 | 6 | 1 | 1 | 2 | | 10 | 5 | 3 | 1 | 1 | |
| Weight loss | | | | | | 0.35 | | | | | | 0.74 |
| Absent | 203 | 76 | 45 | 56 | 26 | | 203 | 113 | 48 | 34 | 8 | |
| Present | 31 | 10 | 5 | 11 | 5 | | 30 | 15 | 9 | 6 | 0 | |
| Histopathology | | | | | | 0.52 | | | | | | 0.96 |
| Adenocarcinoma | 125 | 54 | 23 | 30 | 18 | | 125 | 70 | 31 | 19 | 5 | |
| Squamous cell | 85 | 24 | 22 | 29 | 10 | | 84 | 45 | 23 | 15 | 1 | |
| Large cell | 15 | 5 | 3 | 5 | 2 | | 15 | 9 | 0 | 5 | 1 | |
| Others | 10 | 3 | 3 | 3 | 1 | | 10 | 5 | 3 | 1 | 1 | |
| Differentiation | | | | | | 0.02 | | | | | | 0.70 |
| Well | 19 | 10 | 4 | 3 | 2 | | 19 | 10 | 4 | 3 | 2 | |
| Moderate | 78 | 31 | 20 | 21 | 6 | | 78 | 45 | 20 | 12 | 1 | |
| Poor | 129 | 42 | 25 | 40 | 22 | | 128 | 68 | 33 | 23 | 4 | |

NOTE: When the sum of subset numbers does not match patient totals, data were missing or unavailable.

**Table 4.** Cox proportional hazards model analysis with Prx1 and Prx2 for overall survival

| | Relative risk (95% confidence interval) | *P* |
|---|---|---|
| Prx1 staining category | | 0.036 |
| (1) 0-24% | 1.00 | |
| (2) 25-49% | 1.18 (0.77-1.80) | |
| (3) 50-74% | 1.27 (0.88-1.86) | |
| (4) 75-100% | 1.52 (0.90-2.27) | |
| Prx2 staining category | | 0.099 |
| (1) 0-24% | 1.00 | |
| (2) 25-49% | 0.88 (0.60-1.29) | |
| (3) 50-74% | 0.77 (0.49-1.20) | |
| (4) 75-100% | 0.75 (0.30-1.84) | |
| Age | | 0.001 |
| <Median | 1.00 | |
| ≥Median | 1.03 (1.28-2.48) | |
| Tumor stage | | <0.001 |
| I | 1.00 | |
| II | 1.40 (0.89-2.21) | |
| III | 3.28 (2.19-4.91) | |
| IV | 3.23 (1.97-5.30) | |
| Gender | | 0.160 |
| Male | 1.00 | |
| Female | 0.78 (0.56-1.10) | |
| Smoking history | | 0.790 |
| Never | 1.00 | |
| Ever | 1.06 (0.70-1.60) | |
| Performance status | | 0.001 |
| 0 | 1.00 | |
| 1 | 1.44 (1.01-2.05) | |
| 2/3 | 4.18 (1.81-9.65) | |
| Weight loss | | 0.820 |
| Absent | 1.00 | |
| Present | 1.06 (0.64-1.74) | |
| Histopathology | | 0.998 |
| Adenocarcinoma | 1.00 | |
| Squamous cell | 0.96 (0.66-1.40) | |
| Large cell | 0.95 (0.38-2.40) | |
| Others | 0.98 (0.47-2.07) | |
| Differentiation | | 0.061 |
| Well | 1.00 | |
| Moderate | 0.89 (0.45-1.75) | |
| Poor | 1.22 (0.65-2.30) | |

in *prx2* expression was reported in malignant melanoma (50). Based on the methylation-sensitive representational difference analysis, it was shown that Prx2 is expressed in normal melanocytes, and its expression is lost in melanomas with methylation. The loss of expression was restored by treatment of melanomas with a demethylating agent (5-aza-2′-deoxycytidine). These findings led the authors to suggest that silencing of Prx2 might be involved in melanoma progression.

Our results also indicate that low levels of Prx2 might be associated with poor survival outcome in patients with NSCLC. Although it did not reach statistical significance at the level of 0.05, the relative risk of death decreased as the Prx2 levels increased with $P$ = 0.099. The median survival of patients with <25% Prx2 staining category was shorter by 13 months than those in the combined categories with ≥25% positively stained cancer cells (40 versus 53 months), whereas the median survival of patients with <25% Prx1 staining category was longer by 8.1 months than those with ≥25% positively stained cancer cells (48.3 versus 40.2 months). Whether the down-regulation of Prx2 contributes to the pathophysiology of lung cancer, and whether the expression of Prx2 is under epigenetic control in NSCLC remains to be determined. Given the presence of CpG islands in the core promoter regions of *prx1*, a possible involvement of epigenetic reactivation of *prx1* in NSCLC cannot be ruled out. It also remains to be established in which cell types and tissues, and under what pathophysiologic conditions, these genetic and epigenetic mechanisms influence *prx1* and/or *prx2* gene expression.

To the best of our knowledge, this current study is the first comprehensive analyses of Prx1 and Prx2 expression profiles and their prognostic value in NSCLC. Our results suggest that Prx1 may possess unique functions and regulatory mechanisms in NSCLC which are not shared with Prx2. That a high Prx1 expression level correlates significantly with worse survival in NSCLC, together with the cell survival–enhancing functions of Prx1 previously reported by us and others, indicates a possible role for Prx1 in influencing tumor progression and treatment response. We suggest that Prx1, in addition to serving as a prognostic marker, may also serve as a therapeutic target in NSCLC. To explore this possibility, elucidation of the full range of *cis* elements and transcriptional factors involved in the regulation of *prx1* gene expression in NSCLC will be necessary. Future investigation into the potential of Prx1 inhibition as an effective means of enhancing tumor response to treatment and/or delaying tumor progression is clearly warranted.

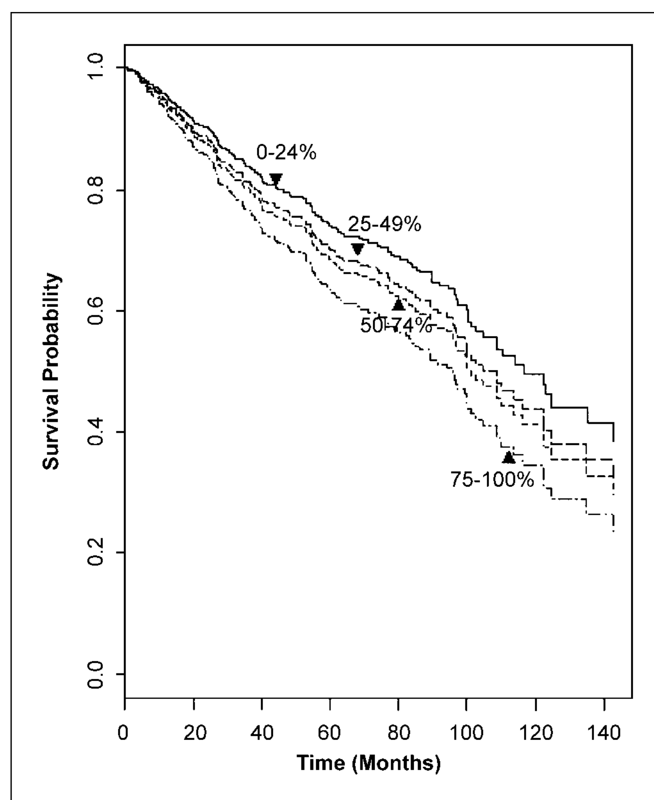


**Fig. 3.** Adjusted Cox regression survival curves for Prx1 expression levels. Survival curves were adjusted for tumor stage, performance status, weight loss, gender, histopathology, and smoking history, and plotted for the Prx1 staining categories. Overall survival curves for patients whose Prx1 expression levels belonging to the staining category <25%, 25% to 49%, 50% to 74%, or ≥75% were estimated by maximizing the Cox partial likelihood. A one-sided log-rank trend test with equidistant spacing of categories was used to evaluate the statistical significance of differences in survival distribution among Prx1 staining categories.

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