# Expression and prognostic significance of human peroxiredoxin isoforms in endometrial cancer

SEULHEE HAN[1], HAIYING SHEN[1], MINHYUNG JUNG[2], BUM-SOO HAHN[3,4], BYUNG KWAN JIN[5], INSUG KANG[1], JOOHUN HA[1] and WONCHAE CHOE[1]

[1]Department of Biochemistry and Molecular Biology, Medical Science and Engineering Research Center for Bioreaction to Reactive Oxygen Species, Biomedical Science Institute; [2]Department of Obstetrics and Gynecology, Kyung Hee University School of Medicine, Seoul 130-701; [3]National Academy of Agricultural Science, Suwon 441-707; [4]Department of Genetic Engineering; [5]Neurodegeneration Control Research Center, Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, Seoul 134-727, Republic of Korea

Received December 17, 2011; Accepted February 28, 2012

DOI: 10.3892/ol.2012.648

**Abstract.** Endometrial cancer is a common type of malignant tumor of the human female genital tract, which typically occurs after menopause. Asian nations, including Korea, Japan and China, have a 4-5 times lower incidence than Western industrialized nations. However, in recent years, there has been a marked increase in the incidence of the disease. Peroxiredoxin (Prx) is an antioxidant enzyme that consists of six isoforms in mammals. These enzymes share a common reactive Cys residue in the N-terminal region, and are capable of breaking down $H_2O_2$ as a peroxidase and involving thioredoxin or glutathione as the electron donor. In the present study, we evaluated the expression of Prx isoforms in normal endometrium, endometrial hyperplasia and endometrial cancer. A total of 240 patients, diagnosed with endometrial cancer by immunohistochemistry, were enrolled in this study. Results showed that Prx I, III, IV and V were negative or weakly expressed in normal endometrium, whereas levels of Prx II and VI were strongly expressed. Notably, the expression levels of Prx III and V were upregulated in endometrial cancer, compared with normal endometrium and endometrial hyperplasia. However, no differences in the staining intensities according to the grade of lesion were observed in the other Prx isoforms. The Kaplan-Meier survival analysis demonstrated that Prx V expression in endometrial cancer is significantly associated with survival rate. Therefore, we suggest that Prx V is a clinically significant prognostic marker for the development of endometrial cancer.

*Correspondence to:* Professor Wonchae Choe, Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemoon-Gu, Seoul 130-701, Republic of Korea
E-mail: wchoe@khu.ac.kr

*Key words:* peroxiredoxin, reactive oxidative species, endometrial cancer

## Introduction

Endometrial cancer is the most frequent gynecological malignancy in the world (1). Endometrial adenocarcinoma is the most common type of diagnosed endometrial cancer, which originates in glandular tissue and is characterized via a sequence of hyperplastic changes in the endometrium; each with increasing malignant potential. Endometrial adenocarcinoma is graded histologically according to the International Federation of Gynecology and Obstetrics. In this system, grade 1 designates a well-differentiated tumor with a <5% solid growth pattern, grade 2 designates a moderately differentiated tumor of 5-50% solid growth, and grade 3 designates a poorly differentiated tumor with >50% solid tumor. Peroxiredoxins (Prxs), synonymous with thioredoxin peroxidases, are a family of proteins, whose members were initially identified as thiol-specific antioxidant enzymes (2-4). Prxs are involved in the enzymatic degradation of hydrogen peroxide, organic hydroperoxides and peroxynitrite (5-7). They are found in a wide range of organisms, including bacteria, plants and mammals, and are classified into three major subclasses: typical 2-cysteine Prxs (Prx I-IV), atypical 2-cysteine Prxs (Prx V) and 1-cysteine Prxs (Prx VI). A number of studies have reported Prx overexpression in various types of malignant cancer cells (8,9). Therefore, we investigated the Prx isoforms (Prx I-IV) to determine whether their expression is associated with cancer progression in endometrial cancer.

## Patients and methods

*Patient follow-up and tissue array material*. Patient samples for tissue array were retrieved from the files of the Department of Gynecologic Oncology at the Kyung-Hee Medical Center. A total of 240 patients, who were diagnosed with endometrial cancer by immunohistochemistry and underwent surgery at the Kyung-Hee Medical Center for preinvasive and invasive

cervical cancer, were enrolled in this study. Each sample was classified into 3 groups: normal endometrium (61 patients), endometrial hyperplasia (56 patients) and endometrial cancer (123 patients) (Table I).

The study was performed with the informed consent of the patients and with the approval of the local Ethics Committee of the Kyung-Hee Medical Center in Korea.

*Immunohistochemistry*. The expression levels of the Prx isoforms were determined by immunohistochemistry. The paraffin-embedded specimens were fixed in 4% buffered formalin. The tissues were cut into 4 $\mu$m sections, and attached on a glass slide. They were then deparaffinized in xylene and rehydrated through ethanol and distilled water, and the endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 10 min. The tissues were then immersed in 10 mM citric acid monohydrate (pH 6.0) for 8 min, and boiled in a microwave oven at 850 W. The specimens were chilled on ice for 20 min. The samples were blocked by protein block, serum-free media for 20 min. The specimens were incubated overnight at 4˚C with a monoclonal antibody against Prx I, II, III, IV, V or VI (Ab Frontier, Korea) at a dilution of 1:500. The immunostained sections were visualized using an EnVision Detection kit (Dako, Denmark).

*Statistical analysis*. Statistical analysis was performed with SPSS for Windows 11.5. The associations were determined using a two-tailed t-test, Fisher's exact probability test and Pearson's correlation test. Agreement of the double evaluation was calculated by Cohen's K-correlation analysis. Cumulative survival curves were plotted according to the Kaplan-Meier method and the generalized log-rank test was applied to compare the survival curves. $P \leq 0.05$ was considered to indicate a statistically significant difference.

## Results

*Prx I*. To determine whether the expression level of Prx I is associated with endometrial cancer development, we examined the immunohistochemical expression of Prx I in endometrial cancer tissue. For this assay, we used 42 tissue samples from patients (normal sample, 11; endometrial hyperplasia, 11; endometrial cancer, 20). The normal tissues were expressed as negative (45.5%) or weakly positive (36.4%) for Prx I (Figs. 1 and 2 and Table II). In the endometrial hyperplasia samples, Prx I expression levels were moderately positive (36.4%) or strongly positive (27.3%). In the cancer samples, Prx I expression was strongly positive (25%) or moderately positive (20%). However, 20 and 30% of the endometrial cancer samples were negative and weakly positive for Prx I, respectively (Figs. 1 and 2, Table II). Therefore, we did not find any direct association between Prx I and endometrial cancer progression.

*Prx II*. Immunohistochemisty with Prx II antibody (normal sample, 9; endometrial hyperplasia, 8; endometrial cancer, 19) was performed. The endometrial cancer samples were strongly positive (57.9%) or moderately positive (42.1%) for Prx II (Figs. 1 and 2 and Table II). However, 33.3 and 22.2% of the normal samples also demonstrated strongly positive and weakly positive expression levels of Prx II, respectively (Figs. 1 and 2, Table II). Therefore, we concluded that Prx II expression levels are not directly associated with endometrial cancer development.

Table I. Patient samples analyzed by immunohistochemistry.

| Histopathological diagnosis | No. of cases |
|---|---|
| Normal[a] | 61 |
| Hyper[b] | 56 |
| EC[c] | 123 |
| Total | 240 |

[a]Normal endometrium, [b]endometrial hyperplasia, [c]endometrial cancer.

*Prx III*. We performed immunohistochemistry using the Prx III antibody (normal sample, 9; endometrial hyperplasia, 8; endometrial cancer, 18). In the normal tissues, 55.6 (p=0.004) and 44.4% (p=0.001) of the samples demonstrated a negative and weak expression of Prx III, respectively (Figs. 1 and 2 and Table II). However, 62.5 (p=0.004) and 44.4% (p=0.0005) of the samples were moderately positive for Prx III in endometrial hyperplasia and endometrial cancer samples, respectively. Additionally, 33% (p=0.001) of the endometrial cancer samples demonstrated a strong Prx III expression (Figs. 1 and 2, Table II). These results suggest that Prx III is at least associated with endometrial cancer development.

*Prx IV*. Immunohistochemistry using the Prx IV antibody (normal sample, 11; endometrial hyperplasia, 10; endometrial cancer, 20) was performed. A total of 55% (p=0.001) of the endometrial cancer samples were strongly positive, and 50% (p=0.003) of the endometrial hyperplasia samples were weakly positive for Prx IV (Figs. 1 and 2 and Table II). However, a moderately positive (45.5%) and weakly positive (45.5%) expression of Prx IV was also observed in the normal samples, suggesting that Prx IV is not directly associated with endometrial cancer development.

*Prx V*. We performed immunohistochemistry using the Prx V antibody (normal sample, 11; endometrial hyperplasia, 10; endometrial cancer, 21). A total of 65.3% (p=0.002) of the endometrial cancer samples were strongly positive for Prx V, whereas 72.7% of the normal tissues were weakly positive (Figs. 1 and 2 and Table II). In the endometrial hyperplasia samples, 40.0% were strongly positive for Prx V (p=0.001) (Figs. 1 and 2, Table II). These results suggest that Prx V is upregulated during endometrial cancer development.

*Prx VI*. We performed immunohistochemistry using the Prx VI antibody (normal sample, 10; endometrial hyperplasia, 10; endometrial cancer, 20). The majority of samples, including normal and endometrial cancer, were moderately or strongly positive for Prx VI, indicating that there is no notable difference in Prx VI expression between the normal and endometrial cancer samples (Fig. 1 and Table I).

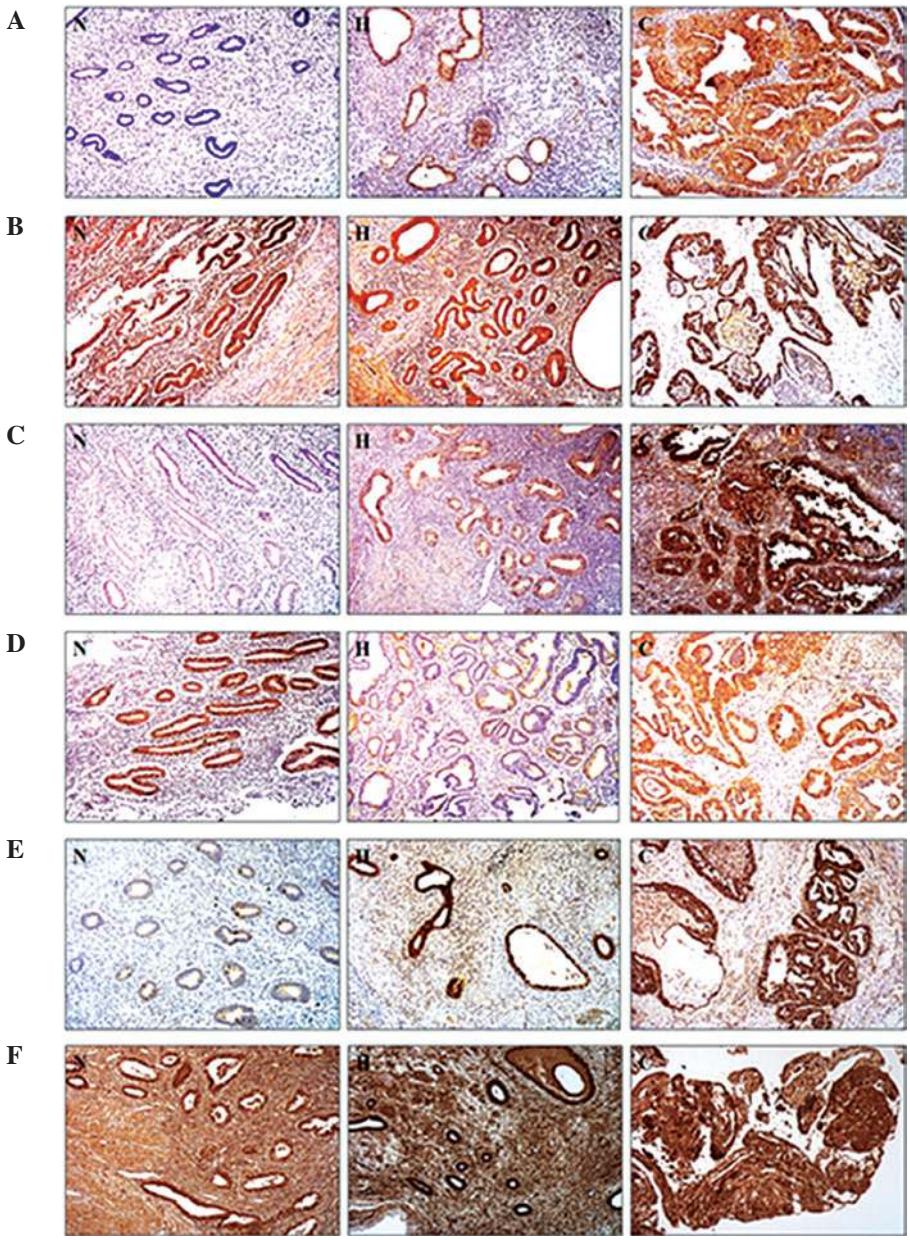


Figure 1. Immunohistochemical staining of Prx isoforms in human endometrial tissues. (A) Prx I, (B) Prx II, (C) Prx III, (D) Prx IV, (E) Prx V and (F) Prx VI. Each Prx isoform (A, B, C, D, E and F) was immunostained in (N) normal endometrium, (H) endometrial hyperplasia and (C) endometrial cancer samples. Prx, peroxiredoxin.

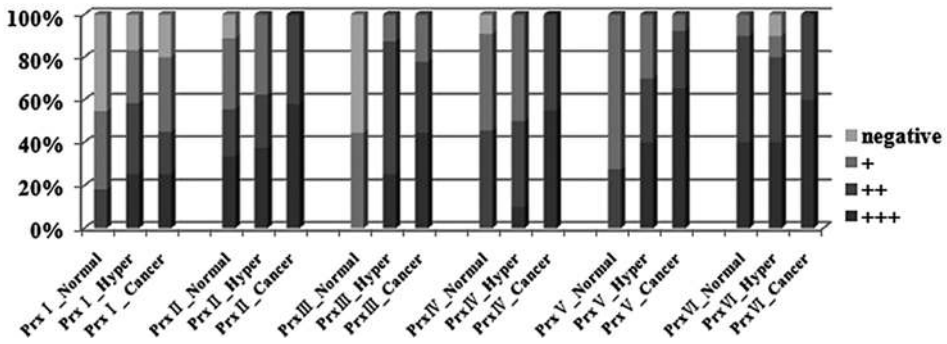


Figure 2. Immunohistochemical staining results of the peroxiredoxin isoforms in human endometrial tissues. Distribution of the Prx I, II, III, IV, V and IV expression in each histopathological group of normal endometrium (normal), endometrial hyperplasia (hyper) and endometrial cancer (cancer) tissues are shown as: intensity +++, intensity ++, intensity + and negative. The immunostaining of Prx III and Prx V were significantly increased during endometrial cancer progression. ($p \leq 0.05$). Prx, peroxiredoxin.

Table II. Intensity of Prx immunostaining in normal, hyperplasia and endometrial cancer samples (%).

| Prx isoform | Sample | - | + | ++ | +++ |
|---|---|---|---|---|---|
| I | Normal[a] | 5/11 (45.5) | 4/11 (36.4) | 2/11 (18.2) | 0/11 (0.0) |
| | Hyper[b] | 2/11 (18.2) | 3/11 (27.3) | 4/11 (36.4) | 3/11 (27.3) |
| | Cancer[c] | 4/20 (20.0) | 7/20 (35.0) | 4/20 (20.0) | 5/20 (25.0) |
| II | Normal[a] | 1/9 (11.1) | 3/9 (33.3) | 2/9 (22.2) | 3/9 (33.3) |
| | Hyper[b] | 0/8 (0.0) | 3/8 (37.5) | 2/8 (25.0) | 3/8 (37.5) |
| | Cancer[c] | 0/19 (0.0) | 0/19 (0.0) | 8/19 (42.1) | 11/19 (57.9) |
| III | Normal[a] | 5/9 (55.6) | 4/9 (44.4) | 0/9 (0.0) | 0/9 (0.0) |
| | Hyper[b] | 0/8 (0.0) | 1/8 (12.5) | 5/8 (62.5) | 2/8 (25.0) |
| | Cancer[c] | 0/18 (0.0) | 4/18 (22.2) | 6/18 (33.3) | 8/18 (44.4) |
| IV | Normal[a] | 1/11 (9.1) | 5/11 (45.5) | 5/11 (45.5) | 0/11 (0.0) |
| | Hyper[b] | 0/10 (0.0) | 5/10 (50.0) | 4/10 (40.0) | 1/10 (10.0) |
| | Cancer[c] | 0/20 (0.0) | 0/20 (0.0) | 9/20 (45.0) | 11/20 (55.0) |
| V | Normal[a] | 0/11 (0.0) | 8/11 (72.7) | 3/11 (27.3) | 0/11 (0.0) |
| | Hyper[b] | 0/10 (0.0) | 3/10 (30.0) | 3/10 (30.0) | 4/10 (40.0) |
| | Cancer[c] | 0/26 (0.0) | 2/26 (7.7) | 7/26 (27) | 12/21 (65.3) |
| VI | Normal[a] | 0/10 (0.0) | 1/10 (10.0) | 5/10 (50.0) | 4/10 (40.0) |
| | Hyper[b] | 1/10 (10.0) | 1/10 (10.0) | 4/10 (40.0) | 4/10 (40.0) |
| | Cancer[c] | 0/20 (0.0) | 0/20 (0.0) | 8/20 (40.0) | 12/20 (60.0) |

[a]normal endometrium, [b]endometrial hyperplasia, [c]endometrial cancer.

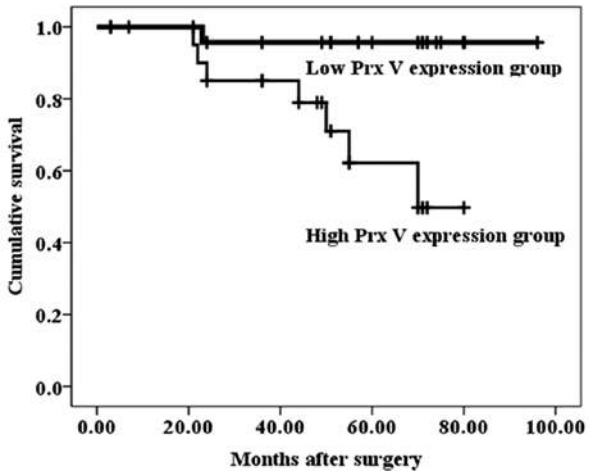


Figure 3. Kaplan-Meier survival curve for endometrial cancer patients based on the expression of Prx V. Patients were followed-up for a period of 96 months after surgery. High Prx V expression is significantly associated with lower cumulative survival rates (P=0.006). The log-rank test was used to compare the difference in survival times between the high and low Prx V expression groups.

*Survival analysis*. We investigated whether the expression of the Prx isoforms served as a prognostic biomarker for endometrial cancer by using the follow-up data of surgically treated endometrial cancer patients. The patients were followed up for a period of 96 months. The Prx V expression group was divided into the low Prx V expression group, which included the patients with a weak or moderate Prx V expression, and the high Prx V expression group, which included the patients with a strong Prx V expression, in endometrial cancer samples. The survival rate and mean survival length for the low Prx V expression group were 96.2% and 92.8 months, respectively. However, the survival rate and mean survival length for the high Prx V expression group were 66.7% and 63.3 months, respectively. The Kaplan-Meier survival analysis demonstrated that Prx V expression in endometrial cancer is significantly associated with the survival rate (Fig. 3). When evaluated with the log-rank test, the other Prx isoforms did not demonstrate a significant association.

## Discussion

To the best of our knowledge, few studies have been conducted on all six Prx isoforms in various types of human cancer (9,10). Prx III, IV and V are known to be upregulated in breast malignancy, while in colorectal neoplasms, Prx I, II, III and V are elevated (11), and recently, it was reported that Prx III and IV are consistently upregulated in prostate cancer (12). These studies suggest the induction of Prx isoforms in response to increased free radicals in cancer tissue.

For the first time, we assessed the expression levels of Prx isoform members in endometrial cancer samples. Our data have shown that Prx III and V were clearly elevated in the majority of endometrial cancer cells as assessed by immunohistochemistry (Figs. 1 and 2, Table II). Notably, Prx III and V were highly expressed during endometrial cancer development, suggesting that they may be used as tumor markers to predict the progression of endometrial cancer. In accordance with our data, Prx III has been known to be associated with the formation and development of hepatocellular carcinoma (13). Additionally, the preferential overexpression of Prx III was reported in breast,

colorectal and prostate cancer. Previously, we reported the upregulated expression of Prx III in cervical cancer development (14). In the present study, we report that Prx V is upregulated in response to endometrial cancer development, and that Prx V expression in endometrial cancer is significantly associated with the patient survival rate. Few studies on Prx V and cancer development are currently available. There is, however, indirect evidence that Prx V protects cells from oxidative stress. Prx V is known to prevent the p53-dependent generation of reactive oxidative species (ROS) and p53-induced apoptosis in the mouse cell line (15). Prx V was recently reported to be highly expressed in immunostimulated macrophages (16). Although the protective role of Prx V against ROS in endometrial cancer cells is assumed, more studies are required to understand the pathophysiological meaning of preferentially expressed Prx V over other types of Prx in endometrial cancer, and its association with the survival rate.

In conclusion, we have demonstrated that Prx III and V are preferentially overexpressed in human endometrial carcinoma, and the expression levels are associated with tumor grade. In addition, a high expression of Prx V correlates with a worse survival rate. Based on these observations, we suggest that Prx V is a potential prognosis marker for endometrial cancer.

## Acknowledgements

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (Nos. 2009-0072431 and 20090063274), and a grant from the Next-Generation BioGreen 21 Program (No. PJ008086), Rural Development Administration, Republic of Korea.

## References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J and Thun MJ: Cancer statistics. CA Cancer J Clin 57: 43-66, 2007.
2. Chae HZ, Kim IH, Kim K and Rhee SG: Cloning, sequencing, and mutation of thiol-specific antioxidant gene of *Saccharomyces cerevisiae*. J Biol Chem 268: 16815-16821, 1993.
3. Chae HZ, Kim IH, Kim K and Rhee SG: Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. Proc Natl Acad Sci USA 91: 7017-7021, 1994.
4. Chae HZ, Uhm TB and Rhee SG: Dimerization of thiol-specific antioxidant and the essential role of cysteine 47. Proc Natl Acad Sci USA 91: 7022-7026, 1994.
5. Hofmann B, Hecht HJ and Flohe L: Peroxiredoxins. Biol Chem 383: 347-364, 2002.
6. Rhee SG, Kang SW, Netto LE, Seo MS and Stadtman ER: A family of novel peroxidases, peroxiredoxins. Biofactors 10: 207-209, 1999.
7. Wood ZA, Schröder E, Robin HJ and Poole LB: Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 28: 32-40, 2003.
8. Chang JW, Lee SH, Jeong JY, Chae HZ, Kim YC, Park ZY and Yoo YJ: Peroxiredoxin-I is an autoimmunogenic tumor antigen in non-small cell lung cancer. FEBS Lett 579: 2873-2877, 2005.
9. Karihtala P, Mäntyniemi A, Kang SW, Kinnula VL and Soini Y: Peroxiredoxins in breast carcinoma. Clin Cancer Res 9: 3418-3424, 2003.
10. Noh DY, Ahn SJ, Lee RA, Kim SW, Park IA and Chae HZ: Overexpression of peroxiredoxin in human breast cancer. Anticancer Res 21: 2085-2090, 2001.
11. Wu XY, Fu ZX and Wang XH: Peroxiredoxins in colorectal neoplasms. Histol Histopathol 25: 1297-1303, 2010.
12. Basu A, Banerjee H, Rojas H, Martinez SR, Roy S, Jia Z, Lilly MB, De León M and Casiano CA: Differential expression of peroxiredoxins in prostate cancer: consistent upregulation of PRDX3 and PRDX4. Prostate 71: 755-765, 2010.
13. Choi JH, Kim TN, Kim S, Baek SH, Kim JH, Lee SR and Kim JR: Overexpression of mitochondrial thioredoxin reductase and peroxiredoxin III in hepatocellular carcinomas. Anticancer Res 22: 3331-3335, 2002.
14. Kim KY, Yu MR, Han SH, Oh IK, Choi YJ, Kim SS, Yoon KS, Jung MH and Choe W: Expression of human peroxiredoxin isoforms in response to cervical carcinogenesis. Oncol Rep 21: 1391-1396, 2009.
15. Zhou Y, Kok KH, Chun AC, Wong CM, Wu HW, Lin MC, Fung PC, Kung H and Jin DY: Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. Biochem Biophys Res Commun 268: 921-927, 2000.
16. Abbas K, Breton J, Picot CR, Quesniaux V, Bouton C and Drapier JC: Signaling events leading to peroxiredoxin 5 up-regulation in immunostimulated macrophages. Free Radic Biol Med 47: 794-802, 2009.