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Up-regulation of defense enzymes is responsible for low reactive oxygen species in malignant prostate cancer cells

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Abbreviations: BSO, L-buthionine-(S,R)-sulfoximine; CDNB, 1-chloro-2,4-dinitrobenzene; GPx, glutathione peroxidase; GR, glutaredoxin reductase; Grx, glutaredoxin; GST, glutathione-S-transferase; MnSOD, Mn superoxide dismutase; NO, nitric oxide; ROS, reactive oxygen species; SNP, sodium nitroprusside; TR, thioredoxin reductase; Trx, thioredoxin

Abstract

Reactive oxygen species (ROS) are involved in a diversity of important phenomena in the process of tumor development. To investigate the alterations of oxidative stress and their related systems in tumor progression, a variety of components in the antioxidative stress defense system were examined in prostate cancer cell lines, PC3 and LNCaP. Cell surface molecules involved in metastasis were expressed highly in PC3 cells compared with LNCaP cells, and strong invasion ability was shown in PC3 cells only. ROS level in LNCaP cells was twice higher than that in PC3 cells, although nitric oxide (NO) level was similar between the two cell lines. The content of GSH increased up to about 2-fold in PC3 compared with LNCaP. Activities of glutathione reductase, thioredoxin reductase, and glutathione S-transferase except catalase are significantly higher in PC3 cells than in LNCaP cells. Furthermore, oxidative stress-inducing agents caused down-regulation of GSH and glutathione S-transferase much more significantly in LNCaP cells than in PC3 cells. These results imply that malignant tumor cells may maintain low ROS content by preserving relatively high anti-oxidative capacity, even in the presence of stressful agents.

Keywords: catalase; glutathione; glutathione reductase; glutathione S-transferase; metastasis; oxidative stress; prostatic neoplasms; thioredoxin

Introduction

Reactive oxygen species (ROS) and free radicals are generated *in vivo* as a result of aerobic metabolism or host defense mechanisms, including superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and nitrogen oxide (NO_x) (Cleveland and Kastan, 2000). They can influence carcinogenesis and cancer progression by causing oxidative damage to macromolecules such as protein, lipid, and DNA, and by inducing expression of a variety of transcriptional factors involved in neoplastic transformation, such as c-fos and c-jun (Das, 2002). It is known that cancer cells are oxidatively stressed due to overproduced ROS and suppressed intracellular antioxidants. Large amounts of H_2O_2 are produced *in vitro* without exogenous stimulation in several human carcinoma cell lines, including malignant melanoma, colon carcinoma, pancreatic carcinoma, neuroblastoma, breast carcinoma and ovarian carcinoma (Szatrowski and Nathan, 1991). Antioxidant enzymes such as catalase and Mn superoxide dismutase (Mn SOD) are down-regulated in tumor cells (Sato *et al*., 1992). It is postulated that such persistent oxidative stresses present in cancer cells lead to cell proliferation rather than apoptosis, which is usually caused by severe oxidative stresses. Sublethal oxidative stress promotes cell proliferation *in vitro*, with both superoxide- and H_2O_2-stimulating growth (Burdon, 1995). Besides, although several antioxidant enzymes such as catalase and SOD are negatively regulated in various cancer cell lines, other redox enzymes or non-enzymatic antioxidant molecules (e.g., GSH) participate in antioxi-

dant defense of cancer cells (Sun, 1992).

Thiol group-containing molecules such as thioredoxins (Trx) and GSH are known as efficient antioxidant. Trx is a small redox protein that undergoes reversible NADPH-dependent reduction by a selenocyteine-containing flavoprotein Trx reductase (TR) (Nordberg and Arner, 2001). Trx, through its redox activity, regulates activities of enzymes such as apoptosis signal-regulating kinase 1 and protein kinase C, and increases the DNA binding and transactivating activity of transcription factors such as nuclear factor-κB (NF-κB), the glucocorticoid receptor, and p53, suggesting that Trx would be a potent cell growth and survival factor (Makino *et al*., 1999; Ueda *et al*., 2002; Djavaheri-Mergny *et al*., 2004). GSH scavenges oxidative stress-inducing molecules. Within cytoplasm, micromolar concentrations of reduced GSH efficiently clear hydrogen peroxide. This clearance results in the accumulation of oxidized GSH (GSSG), which is converted back to GSH by GR in an NADPH-dependent redox cycle, thereby maintaining adequate levels of GSH inside the cells (Perry *et al*., 1993). Higher concentration of GSH is maintained in malignant cell lines. It is possible that up-regulated activities of these antioxidative enzymes or high content of GSH may facilitate the emergent survival of cancer cells under persistent oxidative stress (Batist *et al*., 1986; Tsuchida *et al*., 1989).

Although most studies have mainly focused on the involvement of oxidative stresses in starting cancer, oxygen radicals are also known to act on tumor metastasis. ROS can specifically damage certain protease inhibitors, including α_1-proteinase inhibitor and α_2-macroglobulin, plasminogen activator inhibitor and α_2-plasmin inhibitor by oxidizing methionine residues in the active sites (Swaim and Pizzo, 1988). Resultant inactivation of these protein inhibitors could enhance actions of proteases, such as elastase, plasminogen activator and plasmin (Szatrowski and Nathan, 1991), which may facilitate tumor invasion and metastasis. Oxygen radicals also augment tumor cells' migration by affecting activation of p38 MAPK, resulting in phosphorylation of heat shock protein-27 (HSP-27) and changes in actin dynamics (Huot *et al*., 1997; Wang *et al*., 1998; Rust *et al*., 1999).

Prostate cancer, a leading cause of cancer-related deaths in men, progresses slowly and can be treated effectively when detected early; however, the metastatic disease presents a major obstacle to improve survival rate and treatment efficacy (Landis *et al*., 1999). To overcome this problem, it is critical to understand the mechanism in which prostate cancers will progress and metastasize. Metastatic prostate cancer proceeds through a series of distinct states, such as transformation of normal prostatic epithelial cell to preinvasive primary tumor, androgen-dependent invasive cancer, and androgen-independent metastatic disease (Chakrabarti *et al*., 2002). These stages of prostate cancer involve multiple molecular changes some of which can be implicated in metastatic process (Dong *et al*., 1995; Sheng *et al*., 1996). An increasing body of evidence points to a prominent role for oxidative stresses, such as ROS and reactive nitrogen species (RNS), in the pathogenesis of prostate cancer development, and most of them have focused on their roles in the initial development of prostate cancer (Sikka, 2003). Though many forms of prostate cancer initially are androgen-dependent, the response to androgen ablation therapy is transient. After certain periods, the majority of prostate cancer relapses into the status of androgen independence, resulting in more malignant and metastatic cancer (Oh *et al*., 1998). It is necessary to investigate whether changes in oxidative stresses and their defense mechanisms occur in androgen-dependent or independent and noninvasive or invasive prostate cancer cells. In this preliminary work, PC3 and LNCaP cells were chosen since PC3 is invasive and androgen-independent while LNCaP is androgen-dependent and noninvasive (Witkowski *et al*., 1993; Liu, 2000).

Materials and Methods

Cell lines

Human prostate cancer cell lines, LnCAP and PC3, were kindly provided by Dr. B. C. Kim (Kangwon National University, Chuncheon, Korea). These cell lines were grown in RPMI medium supplemented with 10% (v/v) heat-inactivated FBS (Biowhittaker Inc., Walkerville, MD), 100 U/ml penicillin and 100 μg/ml streptomycin. The both cell lines were grown at 37°C in a humidified air/CO_2 (19:1) atmosphere.

Treatment and cell harvest

To examine the effects of various stress-inducing agents on stress-related enzymes, PC3 and LNCaP cells were cultured upto 90% confluence. The cells were then treated with H_2O_2, superoxide-generating sodium nitroprusside (SNP), 1-chloro-2,4-dinitrobenzene (CDNB) and L-buthionine-(S,R)-sulfoximine (BSO) at the given concentration for 5 h, followed by centrifugation at 1,000 $\times$ *g* for 5 min.

Preparation of cytosolic extracts

Cytosolic extracts from the human cell lines were prepared as described previously (Lee *et al*., 2002).

Briefly, grown cells were disrupted in animal cell lysis buffer (50 mM HEPES, 10% sucrose, 0.1% Triton X-100, and 1 mM PMSF, pH 7.5) and centrifuged at 12,000 *g* for 15 min. The protein concentration was determined according to the method of Bradford, using bovine serum albumin as a standard (Bradford, 1976).

Determination of total intracellular GSH content

About 2×10^5 cells were obtained by scraping off the bottom of the dish with a cell scraper. Cell pellets were once washed with ice-cold PBS and resuspended in lysis buffer for 30 min on ice. Supernatants were taken by centrifugation at 22,000 *g* for 15 min. Total GSH content in the supernatants was determined by the method of Nakagawa *et al*. (1990), using an enzymatic recycling assay based on GR.

Enzyme assays

TR was measured as the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich Co., St. Louis, MI) in the presence of NADPH. The assay mixture contained 0.2 M phosphate buffer (pH 7.6), 1 mM EDTA, 0.25 mM NADPH, 1 mM DTNB and cell extract. The increase in the absorbance at 412 nm was monitored over 5 min at 25°C. GR activity was spectrometically assayed following the oxidation of NADPH at 340 nm (Carlberg and Kaplowitz, 1985). The reaction mixture (0.2 ml) contained 0.1 M phosphate buffer (pH 7.0), 1 mM GSSG, and 0.1 mM NADPH. The reaction was initiated by the addition of cell extract. GST activity was assayed spectrophotometrically as described (Habig *et al.*, 1974; Kimura *et al.,* 2004) with minor modifications. The reaction mixture contained 100 mM phosphate buffer (pH 6.5), 5.0 mM GSH, 2.0 mM CDNB, and crude extract in a vol of 1.0 ml. The reaction, conducted at 25°C, was initiated by the addition of CDNB, and the change in the absorbance at 340 nm was monitored with a spectrophotometer. All initial rates were corrected for corresponding background non-enzymatic reaction. Specific activities were calculated as the changes in absorbance per min per mg protein .

In vitro invasion assay

Cell invasiveness assay was conducted with the QCM Cell Invasion Assay Kit (Chemicon International, Tumecula, CA) according to the manufacturer's instruction. The 2×10^5 cells were seeded in the upper chamber in RPMI containing 10% (v/v) FBS. RPMI supplemented with 10% (v/v) FBS was placed in the lower chamber. After 5 h of incubation at 37°C in 5% CO_2/95% air (v/v), the media in the upper chamber was changed with serum-free RPMI containing 0.25 μM [Glu]plasminogen. After 18 h of incubation, invaded cells in the lower chamber were detached, lysed, and detected by CyQuant GR dye.

Immunoblot analysis

Whole cell extracts were obtained in 1% Triton X-100 lysis buffer (50 mM Tris-Cl, pH 8.0, 150 mM sodium chloride, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 10 μg/ml apoprotinin, and 250 μM phenylmethylsulfonyl fluoride). Protein samples were heated at 95°C for 5 min and analyzed by sodium dodecyl sulfate (SDS)-12% polyacrylamide gel electrophoresis (PAGE). Western blotting was performed using anti-α-tubulin, anti-PECAM-1, anti-ICAM1, anti-uPAR, anti-cathepsin B and anti-integrin-a antibodies (Santa Cruz Biotechnology, San Diego, CA).

Results and Discussion

Properties of PC3 and LNCaP cell lines

The invasion and metastasis of cancer cells require several proteins responsible for such cell-cell or cell-matrix interaction and proteolytic degradation. Recently, the expression pattern of cell surface molecules in prostate cancer cells has been reported to be different. Expression screening of 119 cell surface molecules showed that the profiles of strongly invasive prostate cancer cell lines, PC3 and DU145, are more similar than that of the noninvasive LNCaP (Liu, 2000). Integrin molecules mediating cellular adhesion to extracellular matirx are highly expressed in PC3 cell lines, whereas LNCaP prostate cancer cell line did not express them (Zheng *et al*., 1998). Thus, the expression levels of various invasion-related proteins such as cellular adhesion molecules, cysteine protease, and plasminogen activator were investigated to identify differential invasion ability in PC3 and LNCaP before comparing the changes in oxidative stresses and their related systems between the two cell lines. As shown in Western blotting analysis (Figure 1A), integrin, PECAM and ICAM, best known cellular adhesion molecules, were strongly expressed in PC3 but not in LNCaP cell lines. Furthermore, the action of proteases, known to facilitate tumor invasion, was compared between the two cell lines. Human prostate cancer cells produce and secrete the serine protease, urokinase-type plasminogen activator (uPA), which is found bound to receptors (uPAR) at the leading edge of some migrating cells. The receptor-bound uPA, whose proteolytic activity degrades the extracelluar matrix, may help PC3 cells metastasize.

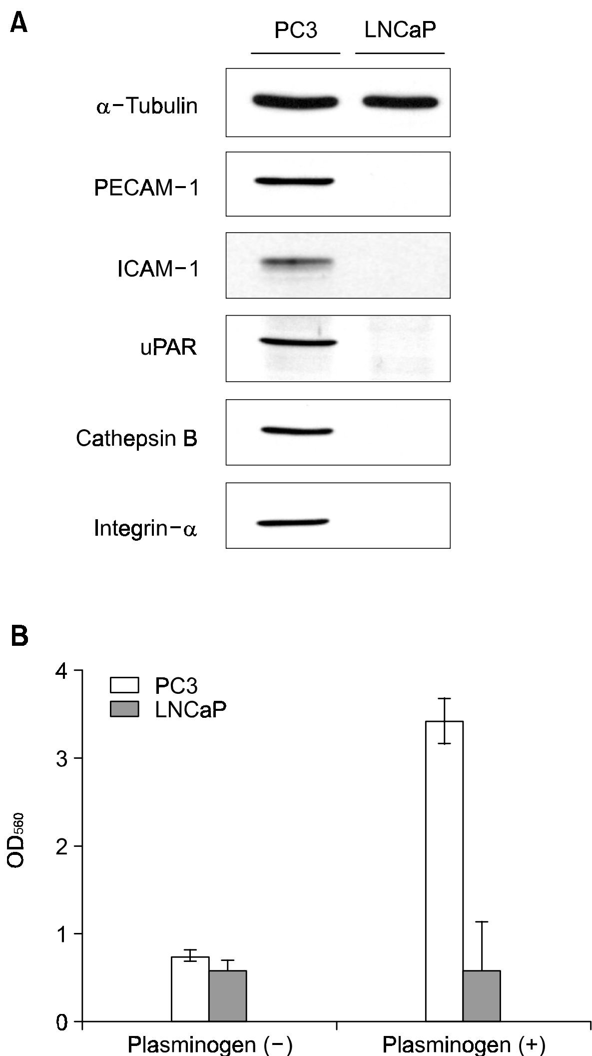


Figure 1. The expression of cell surface molecules (A) and invasion ability (B) in PC3 and LNCaP cells. A few surface proteins were determined by immunoblot analysis. α-Tubulin was determined as a control. Invasiveness of the two cell lines was assessed by using QCM Cell Invasion Assay Kit. Data represent the mean ± SD.

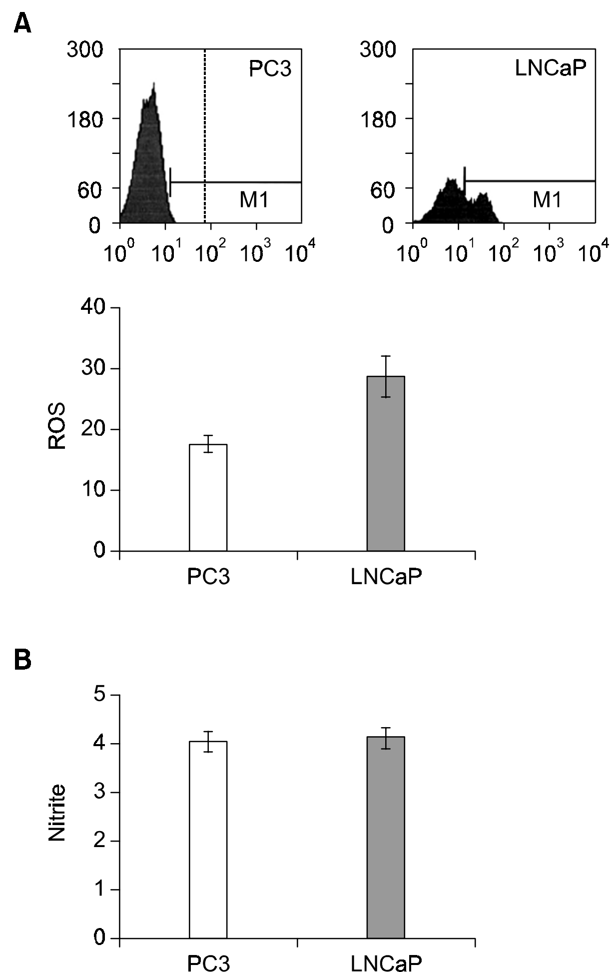


Figure 2. The intracellular level of ROS (A) and nitrite (B) in PC3 and LNCaP cells. ROS production was determined by FACS analysis of the cells loaded with carboxy-dichlorodihydrofluorescein diacetate. Nitrite content was determined with fresh cytosolic extract using Griess reagent. Data represent the mean ± SD.

The expression of uPAR was elevated only in the PC3 cell line but not in the LNCaP cell line (Figure 1A). The expression of a cysteine protease cathepsin B was measured since it is up-regulated in a variety of tumors, particularly capable of invasion, leading to tumor progression (Mai *et al.*, 2000). Like other molecules, cathepsin B expression in PC3 cells was much higher than that in the LNCaP cells. Finally, as being postulated from the expression pattern of invasion-related proteins, strong invasion ability was observed in PC3 cells compared with LNCaP cells (Figure 1B). Thus, these results revealed that the selected two cell lines are suitable to study alterations in oxidative stresses and intracellular antioxidative system in accordance with tumor invasive ability.

Oxidative stress levels in PC3 and LNCaP cell lines

A series of epidemiologic studies and laboratory observations suggest that oxidative stress may be linked to the effects of androgen, antioxidant system, the pre-malignant condition, and high-grade prostatic intraepithelial neoplasia (Pathak *et al.*, 2005). Prostate cancer cells produce substantial amounts of ROS, in part from stimulation of a nonphagocytic Ca^{2+}-regulated NADPH oxidase (Sauer *et al.*, 2001; Lim *et al.*, 2005). ROS produced by prostate cancer

Table 1. The levels of oxidative stress-defense systems in PC3 and LNCaP cell lines.

	Intracellular antioxidants[a,b]				
Cells	Catalase[c]	GSH[d]	GR[e]	GST[f]	TR[g]
PC3	1.97 ± 0.29	1.57 ± 0.15	0.34 ± 0.03	0.46 ± 0.02	0.91 ± 0.05
LNCaP	2.47 ± 0.21	0.80 ± 0.14	0.08 ± 0.01	0.04 ± 0.01	0.35 ± 0.05

[a]Content and activities of intracellular antioxidants were measured in the cell extracts prepared from PC3 and LNCaP cells. [b]Values represent mean ± SD. [c]Catalase activity was represented as ΔA_{240}/min/mg protein. [d]Glutathione level was represented as μg/mg protein. [e,f]Activities of GR and GST are represented as ΔA_{340}/min/mg protein. [g]TR activity was represented as ΔA_{412}/min/mg protein.

cells can activate the p90 ribosomal S6 kinase (p90 RSK) and the redox-regulated NF-κB and activator protein-1 (AP-1), leading to cell proliferation (Sauer *et al*., 2001). Selenite was found to induce apoptosis of non-malignant Chang liver cells *via* ROS generation and JNK1 activation (Kim *et al*., 2004). The modulation of ROS can affect the growth of prostate cancer cells (Arbiser *et al*., 2001; Venkataraman *et al*., 2004). It is likely that ROS accumulation may give rise to up-regulation of hypoxia-inducible factor-1 (HIF-1)-induced VEGF expression and angiogenesis, leading eventually to invasion (Dachs and Tozer, 2000). Thus, oxidative stress levels were compared between PC3 and LNCaP cells. To determine whether growing PC3 and LNCaP cells spontaneously generate ROS, the cells were treated with DCFH-DA for flow cytometry and determined whether it was oxidized to DCF, indicating the intracellular production of H_2O_2 and other oxidants (Chung *et al*., 2001). ROS level was markedly lower in PC3 than in LNCaP cell lines as shown in Figure 2A, implying that the increased cellular level of ROS in LNCaP cells may be related to susceptibility to androgen rather. Probably, this hypothesis might be supported by the fact that stimulation with physiological level of androgen resulted in enhanced levels of ROS only in LNCaP cells but not in PC3 (Ripple *et al*., 1997; Sun *et al*., 2001). However, the cellular level of nitrite is similar between the two cell lines (Figure 2B), indicating modulation of ROS level seems to be more critical for prostate cancer pathogenesis. Although ROS production may accelerate cell proliferation and invasion, the control or detoxification of ROS is required for cell survival because the high content of ROS can induce cell injury accompanied by damage to DNA, proteins and lipid. Thus, next studies were focused on changes in the intracellular control system of oxidative stresses between two cell lines.

Oxidative stress defense systems in PC3 and LNCaP cell lines

Multiple antioxidant defense mechanisms affect ROS production and cycle signaling, leading to the decreased proliferation of cancer cells. Thus, not only are oxidants important in cancer progression, but the defense systems might also be crucial in understanding the mechanism of malignant cancer generation. The first line of defense includes two SODs, the cytosolic SOD1 and mitochondrial SOD2, which convert superoxide to H_2O_2. The produced H_2O_2 is removed by other enzymatic pathways involving GPx, catalase and peroxiredoxin. GST is directly responsible for the elimination of electrophilic oxidants at the expense of GSH (Habig *et al*., 1974). The second line of cellular defense against oxidative stress involves generation of intracellular reducing power mediated by GSH/GR and Trx/TR, leading to detoxification of ROS. Previously, we compared TR activity, GSH content and GSH-related antioxidant enzymes such as γ-glutamylcysteine synthetase, glutathione synthetase, GR, and γ-glutamyltranspeptidase between normal hepatic cell line Chang and hepatoma cell line HepG2 (Lee *et al*., 2002; Jung *et al*., 2004). Although there was no big difference in TR activity in two cells, GSH content and activities of all relative enzymes were much higher in HepG2 cells than in Chang cells. Oxidized ascorbate, known to retain pro-oxidant properties, is reduced by GSH back to ascorbate, which results in loss of cellular GSH and oxidative stress in liver cells (Song *et al*., 2003). Recently, many studies have shown that antioxidant enzymes are involved not only in prostate carcinogenesis but also in resistance to chemotherapy (Jung *et al*., 1997; Suzuki *et al*., 2000; Li *et al*., 2005). Thus, alterations in the antioxidant systems was examined in this study. Although catalase activity was similar between in PC3 and LNCaP cells, not only are GSH content and GSH- related enzymes, GR and GST, but also TR activity was enhanced in PC3 (Table 1), implying that changes of overall antioxidative systems may be related with malignant processes, leading to adaptation to oxidative stresses. Elevated antioxidant systems may be partly responsible for lower level of ROS in PC3 as shown in Figure 2A. A recent

article reported that maspin, a novel serine protease inhibitor, suppressed tumor progression in prostate cancer bone metastasis although the exact molecular mechanism remains unknown (Cher *et al*., 2003). An inhibitory effect of maspin on cell surface-associated uPA/uPAR complex is thought to be charged in such invasion suppressive potential (McGowen *et al*., 2000). More recently, the yeast two-hybrid method has shown that GST directly interacted with maspin and the interaction correlated with an elevated GST activity, leading to decreased basal level of ROS in prostate cancer cells (Yin *et al*., 2005). The maspin suppression by siRNA decreased GST activity followed by increasing the basal ROS generation as well as H_2O_2-treated ROS generation in PC3 cells (Yin *et al*., 2005). This implies that activation of oxidative stress defense system may be needed for maintenance of low ROS level in invasive cancer cells although the effect of siRNA-mediated maspin suppression on PC3 invasion ability is not defined yet.

Effects of stressful agents on antioxidative defense systems in PC3 and LNCaP cell lines

Accumulated studies have elicited that tumor growth seems to be controlled by modulating intracellular ROS level. Animal experiments have shown that the addition of SOD and catalase lead to a clearly reduced antitumor effect of oxygen radicals produced by hypoxanthine (Yoshikawa *et al*., 1995). A reduced level of GSH intensifies the antitumor effect of chemotherapeutic agents (Lutzky *et al*., 1989). Likewise, PC3 cell lines containing significantly higher content of GSH (Table 1) are expected to be less susceptible to chemotherapeutic agents compared to LNCaP cell lines. However, BSO-induced GSH depletion increases the cytotoxicity of melphalan to PC3 cells (Canada *et al*., 1993). These facts imply the possibility of cancer treatments based on the modulation of oxidative stresses. Based on these findings, it seems useful to investigate the relationship between oxidative stresses and their defense systems in accordance with malignancy level, and also helpful to provide a reasonable target for anti-cancer therapy. A few oxidative stress-inducing agents were used to treat PC3 and LNCaP cells for 5 h. The agents at given concentrations did not seriously affect cell viability (Table 2). In the first experiment, TR activities were measured in treated PC3 and LNCaP cells, and compared between the two cell lines. As shown in Figure 3, the treatment of 0.02 mM CDNB, an irreversible TR inhibitor, markedly abolished TR activity in both cells but did not reduce mRNA levels for cytoplasmic TR1 and mitochondrial TR2 based on RT-PCR (data not shown). Although 3 mM NO-generating SNP reduced around 50% of total TR activity in PC3, it is still higher than that in untreated LNCaP cells (Figure 3). However, TR activity in SNP-treated LNCaP cells is similar to that in the CDNB-treated cells (Figure 3). It indicates that NO might have comparable down-regulating activity towards TR. Treatments with 1.0 mM BSO and 0.5 mM H_2O_2 could not largely influence TR activities in both the cells (Figure 3). Previously, we reported that TR activity was differentially regulated in human normal hepatic and hepatoma cell lines in response to various oxidative stress-inducing reagents lines (Jung *et al*., 2004). For example, aluminum and zinc

Table 2. Cytotoxicity of various agents used in this study on the survival of the prostate cancer cell lines, PC3 and LNCaP.

Agents	Relative survival[a]	
	PC3	LNCaP
Control	100.00 ± 0.21	100.00 ± 0.35
0.5 mM H_2O_2	68.32 ± 0.07	53.62 ± 0.07
3.0 mM SNP	73.27 ± 0.14	72.46 ± 0.71
20 μM CDNB	63.37 ± 0.85	68.12 ± 0.35
0.5 mM BSO	92.08 ± 0.21	81.16 ± 0.00

[a]Relative survival values were calculated by considering the survival of the corresponding untreated cells (Control) as 100. Viable cells were counted using a trypan blue exclusion assay. Data represent the mean ± SD.

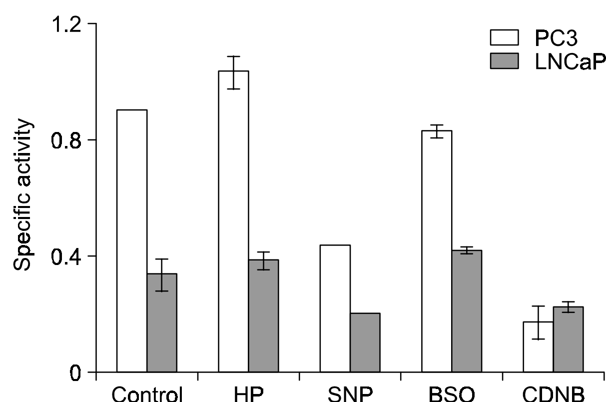
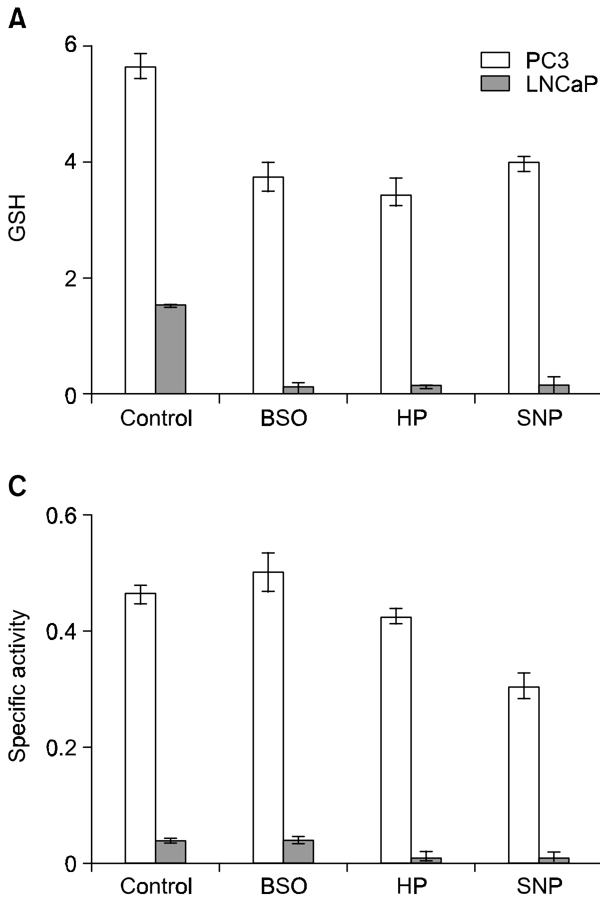
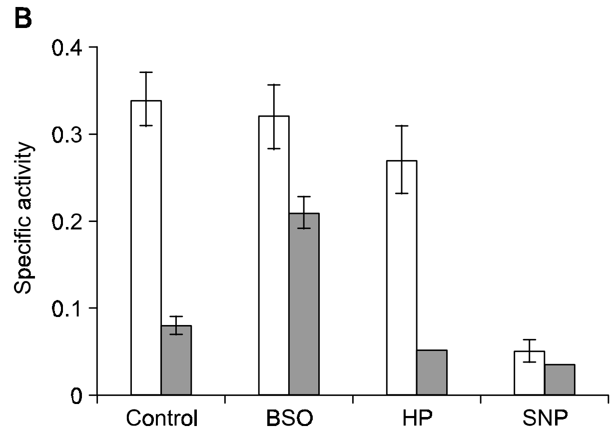


Figure 3. Effects of stress-inducing agents on thioredoxin reductase (TR) in PC3 and LNCaP cells. TR activity was measured in the cell extracts prepared from the cells treated with 0.5 mM H_2O_2 (HP), 3 mM SNP, 0.02 mM CDNB, and 0.5 mM BSO. The specific activity is represented as ΔA_{412}/min/mg protein. Data represent the mean ± SD.





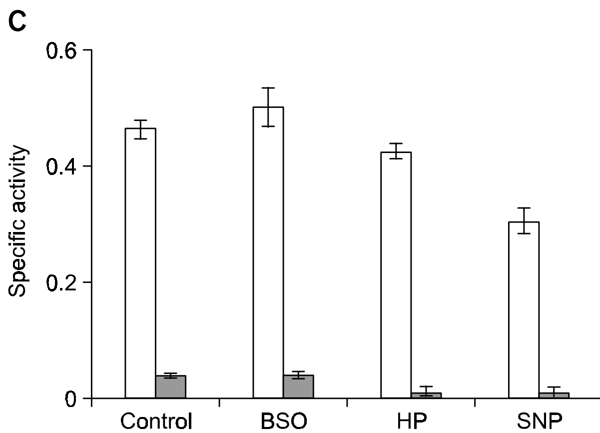


Figure 4. Effects of stress-inducing agents on GSH (A), glutathione reductase (GR, B), and glutathione S-transferase (GST, C) in PC3 and LNCaP cells. Cell extracts were prepared after treatments with 0.5 mM H_2O_2 (HP), 3 mM SNP, and 0.5 mM BSO. GSH content, and GR and GST activities were represented as μg/mg protein and ΔA_{340}/min/mg protein, respectively. Data represent the mean ± SD.

enhanced TR activity in the Chang cell line but not in the HepG2 cell line. In contrast, copper, cadmium, and H_2O_2 markedly induced TR activity only in the HepG2 cells. Therefore, we can not rule out the possibility that the regulation of TR activities from PC3 and LNCaP cells may be affected differentially by various stress-inducing reagents. Secondly, changes in GSH content and GSH-related antioxidant enzymes were measured in the two cell lines after the treatments with 1.0 mM BSO, 3.0 mM SNP and 0.3 mM H_2O_2. Intracellular GSH content was completely reduced in LNCaP cells by all three agents whereas the agents slightly reduced GSH content in PC3 cells (Figure 4A). It might support the higher proliferative activity of PC3 cells under these agents. GSH, as one of primary antioxidants, may play a regulatory role in proliferation of cancer cells at least in the presence of stressful agents. Recently, up-regulation of GSH biosynthesis has been reported in NIH3T3 cells transformed with the ETV6-NTRK3 gene fusion (Kim *et al.*, 2005). GR activity in PC3 cells was clearly reduced by 3.0 mM SNP but not by BSO and H_2O_2 (Figure 4B). Interestingly, GR activity in LNCaP cells was increased by BSO, and

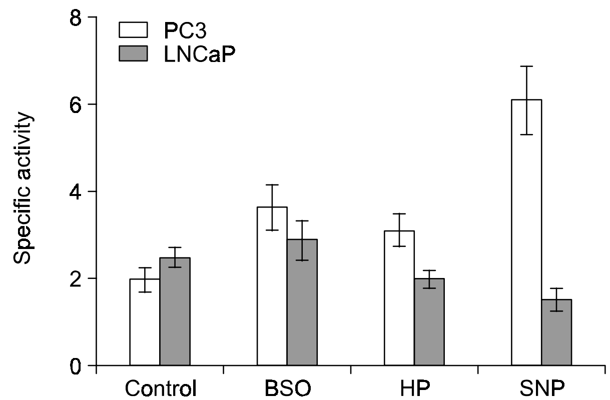


Figure 5. Effects of stress-inducing agents on catalase in PC3 and LNCaP cells. Catalase activity was measured in the cell extracts prepared from the cells treated with 0.5 mM H_2O_2, 3 mM SNP, and 0.5 mM BSO. Catalase activity was determined by monitoring consumption of H_2O_2 (HP) at 240 nm. The specific activity is represented as ΔA_{240}/min/mg protein. Data represent the mean ± SD.

was reduced by SNP (Figure 4B). From these results, changes in GR activity is not assumed to contribute to differential proliferation of the two cells

under stressful conditions. However, higher GR activity itself in PC3 cells is believed to have some kinds of regulatory roles in the apoptotic process of cancer cells. GST activity was not largely changed in the treated PC3 cells while it was completely inhibited in the treated LNCaP cells with the low GST activity (Figure 4C). Finally, effects of the stress-inducing agents on catalase activity was studied in PC3 and LNCaP cells (Figure 5). All the agents used did not induce catalase activation in LNCaP cells. In contrast, all of them enhanced catalase activity in PC3 cells. In particular, the catalase activity was enhanced more than 3-fold in 3.0 mM SNP-treated PC3 cells. Though nitrite levels are similar in the PC3 and LNCaP cells (Figure 2B), exogenous NO could act as a strong catalase inducer in PC3 cells (Figure 5). Different regulatory mechanism of catalase in cancer cells remains to be explained. Taken together, metastatic PC3 cells have more efficient antioxidant systems and are better suited to oxidative stress than LNCaP cells. Consistently, such oxidative stress-inducible agents were unable to affect invasion ability of PC3 cells probably due to efficient antioxidant systems (data not shown). To better understand the roles of ROS in cancer progression including invasion and metastasis, the effects of changes in ROS level, derived from the suppression of antioxidative proteins by siRNAs or antisense RNAs, need to be examined.

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