Expression of Antioxidant Enzymes (Catalase, Superoxide Dismutase, and Glutathione Peroxidase) in Human Bladder Cancer

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Purpose: Intrinsic antioxidant enzymes (AOE) are essential for protection against potential cellular damage by reactive oxygen species (ROS), which affect many biological processes including carcinogenesis. The aim of the present study was to characterize the expression of antioxidant enzymes in human bladder cancer tissue and to evaluate the relationship with histopathological characteristics.

Materials and Methods: Immunohistochemical stainingfor catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) was performed on formalin-fixed, paraffin-embedded tissues obtained from 75 bladder cancersand 30 normal bladders. The degree of AOE expression was compared with cancer invasiveness or the cell grade.

Results: The expression of catalase and SOD were significantly lower in cancer tissue than in normal bladder tissue (44% vs 73.3%, p=0.012; 49.3% vs 80%, p=0.007, respectively) but GPx expression was not significantly different (45.3% vs 63.3%, p=0.146). Catalase and SOD expression were significantly lower in invasive transitional cell carcinomas than in superficial transitional cell carcinomas (32.4% vs 53.7%, p=0.034; 32.4% vs 63.4%, p=0.014, respectively), but again GPx expression was not significantly different (38.2% vs 51.2%, p=0.26). Moreover, no significant difference was observed between the expression of all three enzymes and the cancer cell grade.

Conclusions: Down-regulation of the antioxidant enzyme system, as indicated by the expression of catalase and SOD, appears to be related with carcinogenesis and progression in bladder cancer. (**Korean J Urol 2007; 48:921-926)**

Key Words: Antioxidants, Immunohistochemistry, Urinary bladder neoplasms

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PURPOSE

Reactive oxygen species (ROS) are aerobic metabolites that are physiologically active at low concentration, but they may provoke several types of injury, including DNA, cell membrane, protein and lipid damage by oxidative injury when present at high concentration.[1] However, cells possess effective antioxidant systems that either prevent or allow recovery from oxidative injury. These systems consist of low molecular weight antioxidants like glutathione, and vitamins E, C and A, and antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx).[2-6] An adequate balance between ROS generation and the antioxidant defense systems is indispensable for the maintenance of cellular or organic health, and the disruption of this balance induces oxidative injury, which may become pathologic.

ROS are known to be involved in DNA mutation-induced tumorigenesis and in tumor promotion and progression, whereby they induce changes in signal transfer processes or in the activities of proteins or genes involved in cellular proliferation, differentiation, and apoptosis.[7] In particular, the antioxidant

enzyme system, is one of more important defense modalities against oxidative injury, and is believed to have an important role in cellular proliferation, tumor infiltration, and in the development of chemo-resistance by malignant cells.[8-10] Recently studies have reported that the transfection of Mn-SOD cDNA inhibits the malignant characteristics of human malignant melanomas, and induces fibrosarcoma susceptibility to radiation therapy in mice.[11,12] However, much remains to be resolved, and considerable disagreement exist concerning the effects and roles of antioxidant enzymes in different tumor types and some have been reported elevated antioxidant enzyme activities in tumor tissues.[13-15]

To clarify the roles of the antioxidant enzymes catalase, SOD, and GPx in bladder cancer, we investigated and compared the expressions of these enzymes immunohistochemically in superficial and invasive bladder cancer and in normal bladder tissues.

MATERIALS AND METHODS

1. Tissue selection

One hundred and five surgical specimens were obtained from 75 bladder cancer patients between 1981 and 2003. There were 41 cases of superficial bladder cancer, 34 cases of invasive bladder cancer, and 30 normal bladder tissues as controls. Cancer tissues were obtained from transurethral resection or radical cystectomy specimen. Normal bladder tissues were obtained from biopsy specimens during 23 TURP, 5 URS, and 2 internal urethrotomy. All of the specimens used were formalin fixed and routinely processed as paraffin- embedded tissue blocks.

2. Immunohistochemical staining

Immunohistochemical staining was performed using a streptavidin-biotin immunoperoxidase kit, as described by the manufacturer (Dako LSAB kit, Carpinteria, U.S.A.). In brief, paraffin-embedded sections were deparaffinized in xylene and rehydrated in graded ethanol. After quenching endogenous peroxidase activity in 0.3% hydrogen peroxide for 30 minutes and treating section with blocking reagents for 30 minutes, primary antibodies to catalase, SOD, and GPx (Abcam, England) were applied at a dilution of 1:200 and incubated in a moist chamber for 2 hours at room temperature. After rinsing with PBS, sections were incubated with biotinylated goat antibody to rabbit-mouse IgG (Dako LSAB kit, Carpinteria, U.S.A.) for 10 minutes, and after washing out excess complex, antibody localizations were visualized by incubating sections for 10 minutes in 3,3'- diamino benzidine tetrahydrochloride (Research Genetics, Huntsville, Ala). Sections stained in this manner were analyzed by one pathologist at a magnification of x200. The presence of staining and the percentages of cells stained were evaluated for each enzyme in both tumor cells and normal bladder epithelium. The results obtained were divided into three groups: no staining; weak staining ($\leq$10% of cells showed positive cytoplasmic staining); or strong staining ($\geq$ 10% of cells showed positive cytoplasmic staining).

3. Statistical analysis

The enrolled data were analyzed using SPSS statistical software, release 12.0 (SPSS, Inc., Chicago, USA). To estimate the impact of antioxidant enzymes according to tumor stage and nuclear grade, we constructed contingency tables and analysed using Pearson's chi-squared test. Statistical test was evaluated at 0.05 level.

RESULTS

1. Patients characteristics

Cancer specimens were obtained from 61 male and 14 female bladder cancer patients of average age 66 years. Superficial caners were present in 41 cases and invasive cancers in 34 cases. Twenty-four (32%) cases had a grade 1 cancer, 24 (32%) grade 2, and 27 (36%) grade 3. The normal bladder tissues were obtained from 25 men and 5 women of average

Table 1. Clinical characteristics of the tumors and control group

	Tumor	Control group
No. of patients	75	30
Mean age (range)	66.6±8.25 (43-85)	62.6±6.67 (50-76)
Sex		
Male	61 (81.3%)	25 (83.3%)
Female	14 (18.7%)	5 (16.7%)
Stage		
Superficial	41 (54%)	
Invasive	34 (46%)	
Grade		
1	24 (32%)	
2	24 (32%)	
3	27 (36%)	

age 62 years. No significant difference in age was found between the cancer patients and the normal control (Table 1).

2. Immunohistochemical staining of antioxidant enzymes

Patterns of immunostaining are illustrated in Fig. 1. For example, when stained for catalase, various intensity of staining were seen in epithelial cells throughout the specimen removed for bladder cancer, which shows catalase amounts as a representative case.

We investigated expression rates according to sex and age, but found no significant difference between male and female in expression of each antioxidant enzyme (catalase; p=0.59,

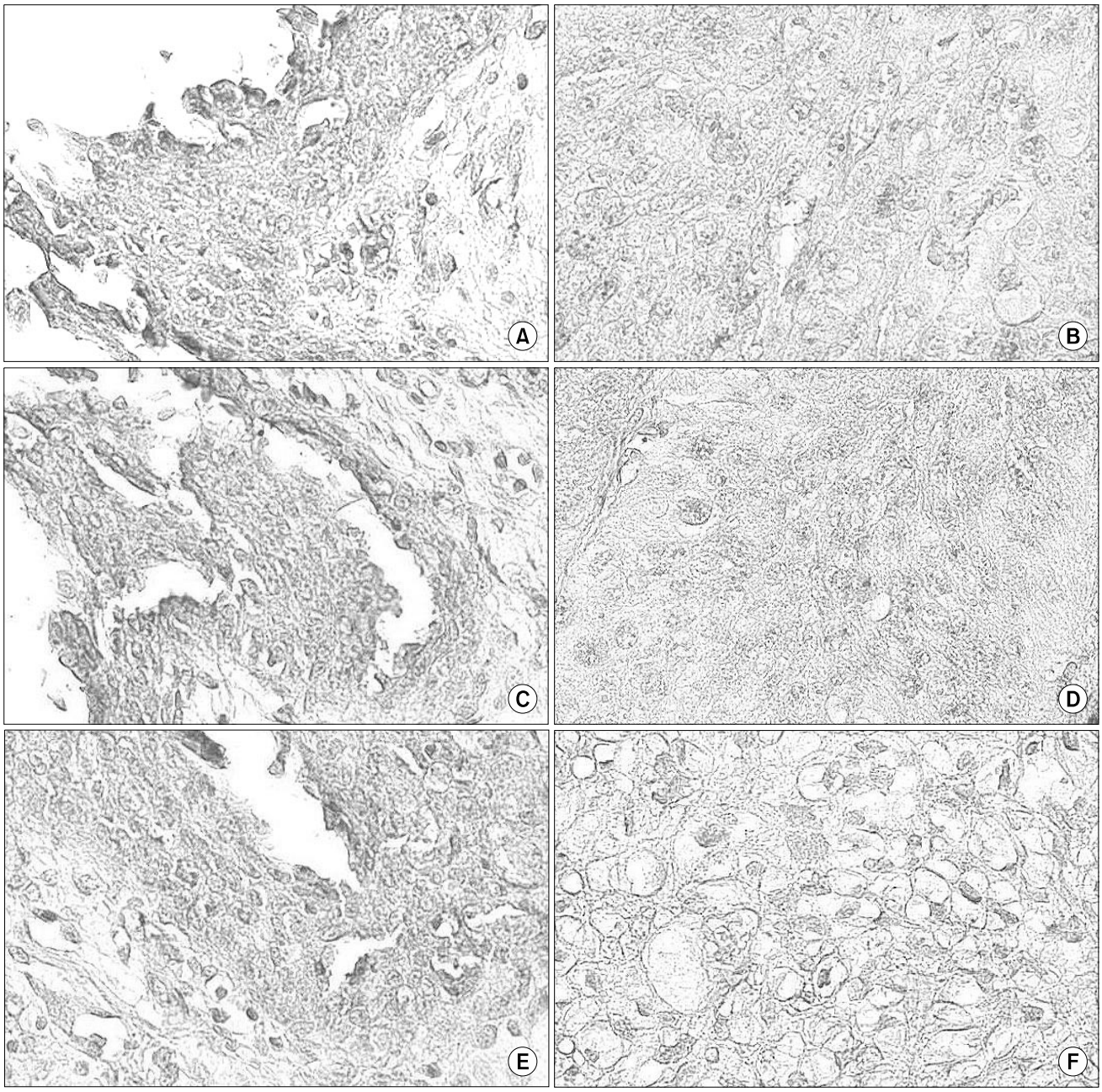

Fig. 1. Immunohistochemical analysis of the antioxidant enzymes in transitional cell carcinoma of the bladder and adjacent normal transitional epithelium. (A) Strong staining for catalase is seen in normal transitional epithelial cells. (B) Staining for catalase is nearlyun-detectable in transitional cell carcinoma. (C) Strong staining for Cu, Zn-superoxide dismutase is seen in normal transitional epithelial cells. (D) Light staining for Cu, Zn-superoxide dismutase is seen in transitional cell carcinoma. (E) Light staining for glutathione peroxidase is seen in normal transitional epithelial cells. (F) Staining for glutathione peroxidase is nearly undetectable in transitional cell carcinoma.

Table 2. Antioxidant enzyme expression in the bladder tumors and control group

	No. of patients (%)		
	Catalase	SOD	GPx
Bladder tumor group			
Positive	33 (44)†	37 (49.3)‡	34 (45.3)
Weak	30	27	29
Strong	3	10	5
Sex*			
Male (n=61)	26 (42.6)	30 (49.2)	28 (45.9)
Female (n=14)	7 (50)	7 (50)	6 (42.9)
Age§			
<60 (n=12)	5 (41.7)	5 (41.7)	5 (41.7)
60-69 (n=41)	19 (46.3)	22 (53.7)	19 (46.3)
≥70 (n=22)	9 (40.9)	10 (45.5)	10 (45.5)
Control group			
Positive	22 (73.3)†	24 (80)‡	19 (63.3)
Weak	12	15	13
Strong	10	9	6
Sex*			
Male (n=25)	19 (76)	20 (80)	16 (64)
Female (n=5)	3 (60)	4 (80)	4 (80)
Age§			
<60 (n=8)	6 (75)	6 (75)	5 (62.5)
60-69 (n=19)	14 (73.7)	16 (84.2)	12 (63.2)
≥70 (n=3)	2 (66.7)	2 (66.7)	2 (66.7)

SOD: superoxide dismutase, GPx: glutathione peroxidase, *,§: tested by chi-square test within each group, $p>0.05$, †,‡: tested by chi-square test between two groups, $p<0.05$.

SOD; p=1.0, GPx; p=0.64 in normal tissue, catalase; p=0.77, SOD; p=1.0, GPx; p=1.0 in cancer tissue) (Table 2). Moreover, the chi-square test showed no significant difference in the expressions of each enzyme by patient divided into three age, i.e., <60, 60-69, and ≥70 years old, (catalase; p=1.0, SOD; p=0.84, GPx; p=1.0 in normal tissue, catalase; p=0.85, SOD; p=0.7, GPx; p=0.89 in cancer tissue) (Table 2). 33 (45%) of 75 cancer tissues and 22 of 30 normal tissues (73.3%) showed catalase expression, with significance (p=0.0123). In addition, a significant difference was found between SOD expressions in cancer and normal bladder tissue, i.e., 37 (49.3%) cancer tissues and 24 (80%) normal tissues were stained for SOD (p=0.0079). GPx was expressed in 34 cancer tissues (45.3%) and in 19 normal tissues (63.3%), but without significance (p=0.1469) (Table 2).

Table 3. Comparison of expression of the antioxidant enzymes according to the disease extension

	Superficial	Invasive	p-value
Catalase			
Positive	22/41 (53.7)	11/34 (32.4)	0.03
Negative	19/41 (46.3)	23/34 (67.6)	
SOD			
Positive	26/41 (63.4)	11/34 (32.4)	0.01
Negative	15/41 (36.6)	23/34 (67.6)	
GPx			
Positive	21/41 (51.2)	13/34 (38.2)	0.26
Negative	20/41 (48.8)	21/34 (61.8)	

Tested by chi-square test, SOD: superoxide dismutase, GPx: glutathione peroxidase

Table 4. Comparison of expression of the antioxidant enzymes according to the nuclear grade

	Grade 1	Grade 2	Grade 3	p-value
Catalase				
Positive	11/24 (45.8)	10/24 (41.7)	12/27 (44.4)	0.95
Negative	13/24 (54.2)	14/24 (58.3)	15/27 (55.6)	
SOD				
Positive	13/24 (54.2)	10/24 (41.7)	14/27 (51.9)	0.65
Negative	11/24 (45.8)	14/24 (58.3)	13/27 (48.1)	
GPx				
Positive	12/24 (50)	10/24 (41.7)	11/27 (40.7)	0.77
Negative	13/24 (50)	14/24 (58.3)	16/27 (59.3)	

Tested by chi-square test, SOD: superoxide dismutase, GPx: glutathione peroxidase

3. The correlation between cancer stage and grade with the expressions of antioxidant enzymes

The frequencies of catalase and SOD expression were significantly lower in the invasive cancer group than in the superficial group (catalase, 32.4% vs 53.7%, p=0.03; SOD, 32.4% vs 63.4%, p=0.01). However, no significant difference was found between these two groups in terms of GPx expression (38.2% vs 51.2%, p=0.26) (Table 3). As shown in Table 4, cancer grade was not related to the expression of any of the three enzymes.

DISCUSSION

Reactive oxygen species (ROS) are a product of normal aerobic metabolism and are generally produced in mitochondria. This process has a decisive role in many various important

biological processes, such as, in inflammation, cell division, differentiation, and apoptosis.[1,16,17] Cellular exposure to certain types of stresses induces the generation of ROS, and excessive ROS generation induces DNA injury, which may induce cell death or carcinogenesis. The intrinsic antioxidant enzyme system is a defense mechanism that protects cells against oxidative injury. This system is composed of 3 types of protein, SOD, which converts superoxide to hydrogen peroxide, and catalase and GPx, which convert hydrogen peroxide to water.[4-6] There are two types of SOD, namely, manganese (Mn)-SOD, which exist mainly in mitochondria, and Cu, Zn-SOD, which exists mainly in the cytoplasm. This system converts two toxic radicals, namely, superoxide and hydrogen peroxide into water.

Several recent studies have shown that the expression of antioxidant enzymes are organ, tissue, and cell dependent, although generally their expressions are reduced in cancer tissues. In particular, in renal cell carcinoma antioxidant enzymes expression were found to be cancer cell dependent. For example, Mn-SOD and catalase expressions were elevated in granular cancer cells versus the granular cells in normal tissues, whereas all antioxidant enzyme expressions were attenuated in clear cell type cancer cells.[14] Urothelial cancer in renal pelvis is easily compared with adjacent normal urothelium. Oberley et al[14] showed that all antioxidant enzyme expressions were attenuated in urothelial cancer cells versus adjacent normal urothelial cells. Our data on urothelial cancer of the bladder showed a significant reduction in catalase and Cu, Zn-SOD expressions in cancer tissue versus normal urothelium, except for GPx. In particular, the expression rate of GPx in normal urothelium was relatively low at 63.3%, which we consider an organ-specific phenomenon; however, the issue requires further clarification. Actually, most cancer tissues in the literature have been found to show low SOD and catalase expression. However, results on GPx expression in cancer are more diverse.[13,18]

Based on the information available to date, antioxidant enzyme levels appear imbalanced in cancer cells versus normal tissues. However, the implications of an increase in the ratio ROS to antioxidant enzymes are not well understood. It is questionable whether an antioxidant enzyme imbalance causes oxidative injury or whether excessive ROS generation modulates the antioxidant enzyme system. However, our preliminary data demonstrate that low levels of oxidative stress do not affect the antioxidant enzyme system, but that high levels of oxidative stress due to excessive ROS generation can suppress antioxidant enzyme activity (data not shown). Cao et al[19] also reported that excessive oxidative stress suppresses the activity and expression of catalase. These findings suggest that imbalance in the antioxidant enzyme system occurs in parallel with oxidative injury rather than a causative event. However, further studies are required to elucidate the relationship between the antioxidant enzymes and oxidative injury.

The lower expressions of catalase and SOD in invasive cancers than superficial cancers, suggests that oxidative injury might be involved in cancer progression (Table 3). Although little data is available on the role of oxidative injury in cancer progression, Kondo et al[20] reported a lower level of oxidative injury in adenoma than in adenocarcinoma of the human colon. Another study demonstrated a step-wise reduction in the level of antioxidant enzymes in normal tissue, chronic pancreatitis, and pancreatic cancer, which suggests the involvement of oxidative injury in cancer progression.[21]

Moreover, recent studies on human malignant melanoma, breast cancer, and neuroglioma cell lines have demonstrated that the augmentation of antioxidant enzyme activity via Mn-SOD cDNA transcription can suppress cancer cell growth.[9,11,22] This suggest the possibility of cancer treatment by altering cellular redox states using antioxidant enzymes. Further studies on the antioxidant enzymes should clarify the roles of oxidative injury and antioxidant enzymes in terms of pathophysiology and with respects to the preventive and therapeutic aspects of bladder cancer.

CONCLUSIONS

Our results showed that bladder cancer tissues expressed catalase and SOD less frequently than normal tissues, and that invasive cancers expressed these species less frequently than superficial bladder cancer. Moreover, these findings suggest that oxidative injury might be involved in the pathogenesis and progression of bladder cancer. If future studies including large clinical series, can clarify the role of oxidative injury in bladder cancer, we believe that the pharmacological or molecular biological control of antioxidant enzymes will become an important mean of preventing or treating cancer.

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