ORIGINAL ARTICLE

Cadmium Concentration and Metallothionein Expression in Prostate Cancer and Benign Prostatic Hyperplasia of Humans

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Background/Purpose: Cadmium (Cd) causes various genitourinary disorders and is a carcinogen for prostate cancer. Metallothionein (MT) is a protein that detoxifies heavy metals. We evaluated changes in Cd concentration and MT expression in human prostate cancer (CaP) and benign prostatic hyperplasia (BPH). Our goal was to clarify the relationship between Cd concentration and MT expression in prostatic diseases.
Methods: The experimental group consisted of 18 patients who underwent radical prostatectomy for CaP. The control group consisted of 35 patients who underwent transurethral resection of the prostate for BPH. Tissue samples were acquired from the gross tumor site and from resected chips. We determined Cd concentration by atomic absorption, MT expression by immunoblotting, and immunohistochemical staining. The significance of between-group differences for these outcomes was analyzed using Student's *t* tests.
Results: There was no statistically significant difference in Cd concentration between the CaP and BPH groups. Immunoblots from both groups revealed a single band. The relative intensity of the MT band was 0.58 ± 0.09 in the BPH group and 0.17 ± 0.03 in the CaP group. MT expression in patients with BPH was 3.4-fold higher than in those with CaP.
Conclusion: MT may bind heavy metals and protect patients from CaP. Additional studies are needed to reveal the factors that influence the expression of MT in prostate epithelial cells, and to analyze the free and compound forms of Cd at the same time. [*J Formos Med Assoc* 2009;108(7):554–559]

Key Words: benign prostatic hyperplasia, cadmium, metallothionein, prostate cancer

Cadmium (Cd) is a metallic toxin of major environmental and occupational concern. It is a suspected human prostatic carcinogen and has been shown to induce prostatic tumors and proliferative lesions in rats.[1,2] Some studies have indicated that tissue levels of Cd in the human prostate correlate with malignant disease.[3] Therefore, Cd has a possible role as an etiological agent in human prostate cancer (CaP).[1–4] Cd, like other metallothionein (MT)-bound metals, is very toxic in a variety of tissues.[5]

MT is a low-molecular-weight protein that is rich in cysteine and is a heavy-metal-binding protein. MT is rich in thionein moieties, which play a role in detoxification of metals such as Cd and Hg, in homeostasis of essential metals such as Zn and Cu, and in the protection of cells against damage induced by alkylating agents, oxygen radicals, and ionizing radiation.[6–11]

In the present study, we investigated Cd concentration and MT expression in patients with benign prostatic hyperplasia (BPH) or CaP.

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Our goal was to clarify the relationship between Cd concentration and MT expression in prostatic diseases.

Material and Methods

Patients

We enrolled 53 patients between 1 January 2005 and 30 June 2007. Their ages ranged from 65 to 75 years. None were smokers. The experimental group included 18 patients who had radical prostatectomy for primary CaP. Specimens were obtained from resected tumor tissue (the tumor area was defined by our pathologist). The control group consisted of 35 patients who had a prostate-specific antigen level < 4 ng/mL, who underwent transurethral resection of the prostate for BPH (determined by the same pathologist). All prostatic tissues were resected and some stored at −80°C until Cd measurement and MT determination with atomic absorption spectrophotometry and western blotting, respectively. The other tissues were fixed with formalin and embedded in paraffin blocks for subsequent immunohistochemical staining. All specimens were removed only after obtaining informed written consent from the patients.

Measurement of Cd

Cd was measured in tissue specimens using a modification of our previously described method.[12] Fifty-three prostatic specimens were washed three times in tap water and briefly rinsed in double-deionized water within 1 minute to remove blood contaminants and ions from the tissue surface; water left on the prostate surface was blotted dry with filter paper. Prostatic tissues were dried at 65°C overnight in order to nitrate tissues completely—then each was nitrated overnight with 200 μL of 13.1 N HNO_3 at 40°C. The digested solutions, as well as aqueous samples from the incubation media, were diluted with double-deionized water and subjected to atomic absorption spectrophotometry (Z-8000; Hitachi, Japan), using a graphite furnace for Cd analysis according to standard methods modified from Chang et al.[13] Cd standard solutions (Merck, Germany) were used for establishing standard curves. The standard addition method was used for background correction to eliminate the matrix effect.

MT immunoblotting

MT extraction and immunoblotting were carried out as described in our previous study.[14] Prostatic tissues were prepared by homogenization in 10 mM Tris–HCl with 5 mM 2-mercaptoethanol, pH 7.0. The homogenate was centrifuged at 12,000*g* for 40 minutes at 4°C. The supernatant was inactivated at 80°C for 10 minutes, and was centrifuged again at 12,000*g* for 40 minutes at 4°C. The final supernatants were subjected to MT western blotting as described below.

Samples (20 μg) of total prostate protein were loaded in triplicate and subjected to polyacrylamide gel electrophoresis on 15% polyacrylamide gels for 1 hour at 125 V. The separated proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA) by electroblotting. After preincubation for 2 hours in PBST buffer, which contained 1–2% (w/v) nonfat dried milk to minimize nonspecific binding, the blots were trimmed into two pieces. One of the two pieces was incubated for 1 hour in primary anti-MT antibody (rabbit monoclonal antibody; United States Biological, Swampscott, MA, USA) and diluted in 5% BSA (1:8000). The other piece was incubated with β-actin antibody (Sigma, St Louis, MO, USA) as a positive control. Both were washed in PBST and allowed to react for 2 hours with alkaline-phosphatase-conjugated secondary antibody (KPL, Gaithersburg, MD, USA; 1:4000). Blots were developed after incubation with 0.015% nitro-blue tetrazolium and 0.007% bromochloroindolyl phosphate in a reaction buffer that contained 100 mM Tris, 100 mM NaCl and 5 mM $MgCl_2$ (pH 9.5). Immunoblots were scanned and imported as JPG files into a commercial software package (Photo-Capt; Vilber Lourmat, Marne-la-Vallée, France). The results were converted to numerical values and a ratio of MT to

β-actin was calculated using the same lane, in order to compare the relative intensities of the immunoreactive bands.

Immunohistochemistry for MT

Sections were dewaxed with xylene and ethanol, and rinsed in phosphate buffered saline (PBS). Endogenous peroxidase was inactivated by incubating the sections with 3% H_2O_2. Sections were stained immunohistochemically with rabbit polyclonal antibody IgG (sc-11377; Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:200) for all MT isoforms, followed by analysis for MT using a commercial kit (PicTure; Zymed, South San Francisco, CA, USA). The negative control was PBS instead of primary antibody. Finally, sections were counterstained with hematoxylin (Merck) and rinsed with tap water. Sections were observed using a light microscope (Olympus BX50; Tokyo, Japan) and micrographs were taken with a digital camera (Nikon Coolpix 5000; Tokyo, Japan).

Statistical analysis

Data were analyzed with Student's *t* tests; $p < 0.05$ was considered statistically significant.

Results

MT immunoblots from both groups revealed a single band (Figure 1). There were no significant differences in Cd concentration between the BPH (1.11 ± 0.08 μg/kg) and CaP (1.09 ± 0.12 μg/kg) groups (Figure 2). However, the relative intensity of the MT band was 0.58 ± 0.09 in the BPH group and 0.17 ± 0.03 in the CaP group. Hence, MT expression in BPH patients was about 3.4-fold higher than in patients with CaP (Figure 2). Immunohistochemical staining for MT showed stronger immunoreactivity in the cytoplasm of prostatic epithelium in the BPH group (Figures 3A and 3B) than in the CaP group (Figures 3C and 3D).

Discussion

Cd is a toxic metal and has been reported to increase the risk of CaP.[1,2] The pathogenesis of Cd

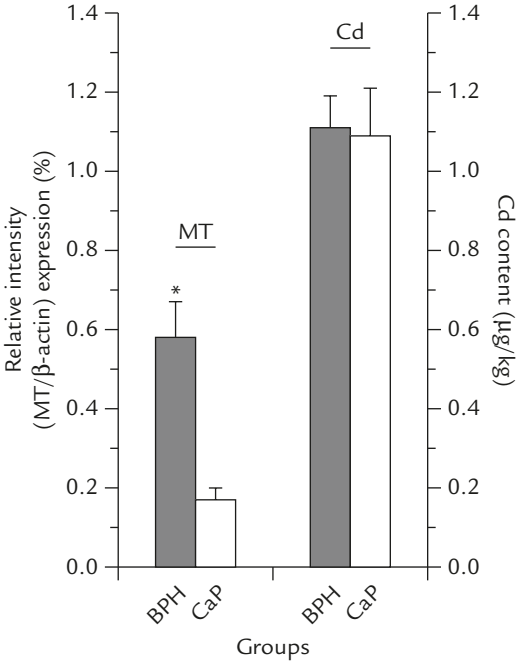

Figure 2. Mean metallothionein protein (MT) levels in the benign prostatic hyperplasia (BPH) group were 3.4-fold higher than in the human prostate cancer (CaP) group. However, there was no significant difference in cadmium (Cd) concentrations.

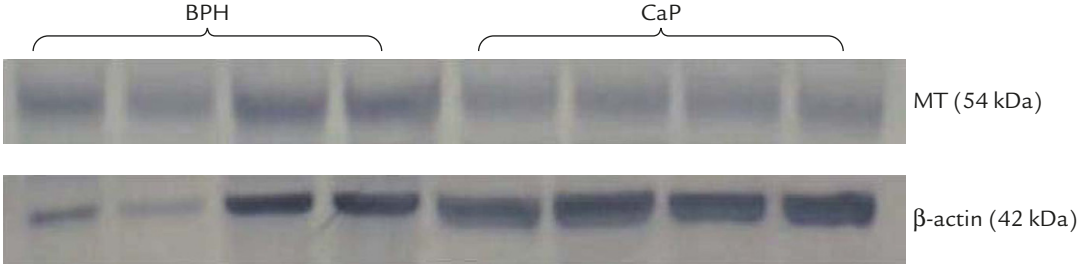

Figure 1. Representative immunoblots and relative intensity of metallothionein protein in human prostate cancer and benign prostatic hyperplasia groups. β-actin was used as a loading control.

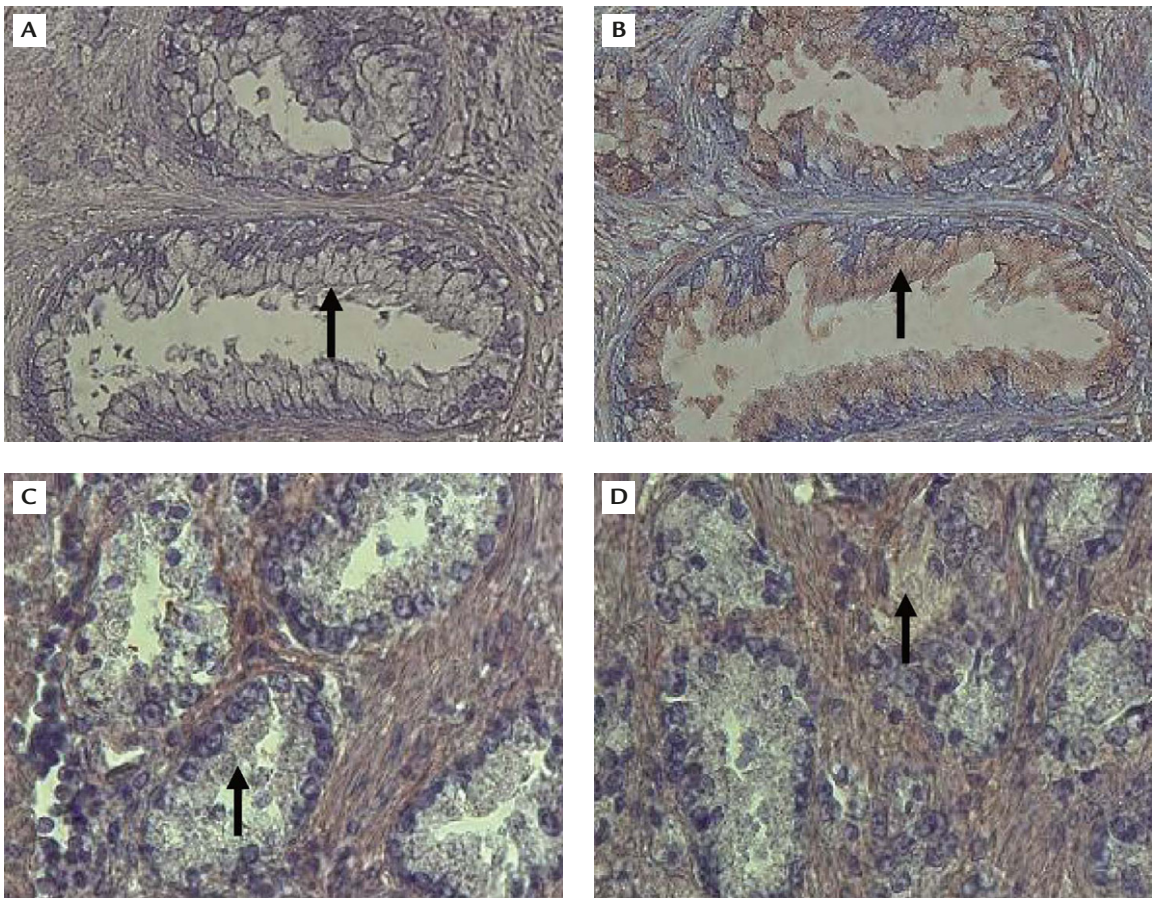

Figure 3. Metallothionein immunohistochemical staining pattern in prostatic sections from the benign prostatic hyperplasia groups (A and B) and human prostate cancer groups (C and D). (A) Photomicrograph of a representative prostatic section after hematoxylin staining. This is a negative control (400×). (B) Intense immunohistochemical staining for metallothionein, particularly in the cytoplasm (arrow) of prostatic epithelium (400×). (C) Photomicrograph of a representative human prostate cancer section after hematoxylin staining. This is a negative control (400×). (D) Weak immunohistochemical staining of prostatic epithelium (arrow) for metallothionein (400×).

prostatic carcinogenesis might include aberrant gene expression that results in stimulation of cell proliferation or inhibition of apoptosis.[4] However, the Cd–MT complex formed is nontoxic. It has been proposed that MT is a chelator that binds to harmful heavy metals and excessive amounts of essential metals.[15] In the present study, MT expression in the BPH group was 3.4-fold higher than the CaP group. We suggest that the greater level of Cd–MT complexes and lower level of free Cd^{2+} in BPH patients lead to less Cd-induced cell toxicity and carcinogenesis than the CaP group. Previous studies have reported that adenoma appears to contain a higher Cd content than normal prostate.[16] Ogunlewe and Osegbe[17] have also reported that Cd concentrations are higher in CaP patients compared with normal prostates or BPH patients. However, these studies have not researched the relationship between CaP, Cd concentration, and MT expression in the same specimens. In addition, they have focused only on total Cd content and ignored free Cd level.

MT is a low-molecular-weight, cysteine-rich protein. It is inducible during acute stress and is a free radical scavenger that protects cells from oxidative damage during inflammation.[18] The antioxidant properties of MT may participate independently or in conjunction with glutathione to protect cells against injurious agents and stress stimuli.[19] Each of these responses and intermediates, including metals, glucocorticoids and cytokines, are able to induce MT protein synthesis.[18]

Recent studies have demonstrated that MT mRNA and protein are correlated highly with Cd levels at low doses, but MT expression is reduced at high doses.[15] These results imply that the dose response for MT expression is relevant only when heavy metals do not exert detrimental effects on physiological functions.[15,20] The present study showed that MT synthesis was lower in the CaP than the BPH group, but there was no statistically significant difference in total Cd concentration (free+bound). Combining the previous and present data, there are two possibilities: (1) cancer cells cannot carry out normal MT synthesis; and (2) MT chelates more Cd in patients with BPH compared with CaP. However, both candidate hypotheses need to be confirmed in future studies.

A number of studies have shown an increase in MT expression in human tumors of the breast, colon, kidney, lung, nasopharynx, ovary, thyroid, and urinary bladder.[21,22] However, MT is downregulated in other tumors such as hepatocellular carcinoma and liver adenocarcinoma.[20] Hence, the expression of MT is not universal to all human tumors, but may depend on their differentiation status and proliferative index.[21] Most previous studies have ignored a number of factors in their analysis, including stimulators such as free radicals, smoking, foods, toxic heavy metals, and some genes—all of these can interfere with MT synthesis.[23–28]

To the authors' knowledge, this is the first study to analyze the relationship between Cd content and MT expression in prostatic diseases simultaneously. Previous studies have not researched the relationship between prostate tissues, Cd concentration and MT expression in the same specimens. Our results showed that the BPH group had greater MT expression than the CaP group, but the Cd concentration did not appear to differ significantly between the groups. We suggest that MT may have protective effects against CaP. However, additional studies are needed to reveal the factors that influence the expression of MT in prostate epithelial cells and to analyze the free and compound forms of Cd at the same time.

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