Immunohistochemical Study of Metallothionein in Human Seminal Vesicles

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Metallothionein (MT) in human seminal vesicles was examined by use of the avidin-biotin-peroxidase complex method. Tissues were obtained from six patients with prostate cancer who underwent luteinizing hormone-releasing hormone agonist or estrogen therapy before radical prostatectomy (group 1) and from 18 patients without hormone therapy (three with prostate cancer, three with urinary bladder cancer, and twelve free of urogenital diseases at autopsy) (group 2). MT was localized in the cytoplasm and nuclei of epithelial cells and also in secretory products in the lumen. The epithelial cells lacked uniformity in immunoreaction; for instance, some stained strongly while others stained weakly. Smooth muscle cells were found to have positive immunoreaction, but other connective tissues had no immunoreaction. The number of strongly positive cells in group 1 was fewer than that in group 2 (not significant), and the secretory products in group 1 had no immunoreaction. These results suggest that MT is synthesized in the epithelial cells of the seminal vesicles and secreted into the fluids, and that the synthesis of MT is suppressed by the hormone therapy.

— metallothionein; seminal vesicle; human; immunohistochemistry

Metallothionein (MT) is a metal-binding protein with a low molecular weight of approximately 6,000 (61 amino acid). It is able to bind class I b and II b metals such as zinc and cadmium (Margoshes and Vallee 1957). It was identified in the
male reproductive tracts such as those of the testis (Nishimura et al. 1990; De et al. 1991) and prostate (Bataineh et al. 1986; Umeyama et al. 1987; Suzuki et al. 1991). In general, the biological role of MT is considered to be the detoxification of harmful metals and the metabolism of essential metals (Kojima 1990), but its function has not been clarified in the male reproductive tract. Recently, MT genes were reported to be actively regulated in the process of spermatogenesis in the seminiferous tubules (De et al. 1991); furthermore, MT was synthesized in the prostatic cells and secreted into the prostatic fluids (Umeyama et al. 1987; Suzuki et al. 1991). There has been little investigation of MT in the reproductive organs except for the testis and prostate (Nishimura et al. 1990), and MT has not been previously reported in human seminal vesicles. This study investigated the detection of MT in human seminal vesicles, and the changes occurring after hormone therapy for prostate cancer by immunohistochemistry.

**MATERIALS AND METHODS**

Tissues of the seminal vesicles were obtained from patients with prostate or urinary bladder cancers as well as at autopsy. There were nine patients with prostate cancer in clinical stage B (aged 61-71 years, average 64.2 years) who received radical prostatectomy; of the nine patients, six were treated with luteinizing hormone-releasing hormone (LH-RH) agonist or estrogen therapy for an average period of 5.1 months before surgery and three had no hormone therapy. Three patients with urinary bladder cancer (aged 62-76 years, average 70.2 years) underwent cystoprostatectomy. Twelve autopsied patients had diseases other than urogenital cancers and were from 44 to 85 years old (average 66.7 years). The patients were divided into two groups. Group 1 was composed of the six patients treated with hormone therapy before receiving prostatectomy, and group 2 was of the 18 patients without hormone therapy; group 2 included the patients with urinary bladder cancer and the autopsied patients. Pathologically, there was not any tumor invasion into the seminal vesicles in all cases of prostate cancer.

The seminal vesicles were fixed in a buffered solution of 10% formalin for about 48 hr and embedded in paraffin. The sections were cut at a thickness of 3 μm and mounted on a poly-L-lysine coated slide glasses. They were deparaffinized and incubated in 0.1% trypsin solution for 30 min at 37°C to improve immunoreaction for primary anti-MT antibody (Kemeny et al. 1983; Nakajima et al. 1991). They were then immersed in 0.5% (5 mM) periodic acid solution for 10 min in order to inhibit intrinsic peroxidase, and incubated with normal serum for 20 min to block nonspecific binding sites. The primary antibody was mixed with anti-MT-1 antibody and ascaris antigen (Suzuki et al. 1991). The slides were layered with the primary antibody for 2 hr at room temperature and washed with 0.01 M phosphate-buffered saline, pH 7.2 (PBS). The secondary antibody was applied for 1 hr and sections were again washed with PBS. They were treated with avidin-biotin complex for 30 min and washed with PBS. The sections were submersed in 0.05%, dianinobenzidine tetrahydrochloride in 0.05 M Tris buffer (pH 7.6) to which H₂O₂ (0.01%) had been added just before use. These reagents were prepared using a Vectastain ABC Kit (Vector Laboratories, Inc., Burlingame, CA, USA). Subsequent counterstaining was performed with Mayer's hematoxylin. The specificity of the staining reaction was confirmed in a prior absorption of MT antibody with pure rabbit liver MT (MT-1, MT-2; Sigma Chem. Co., St. Louis, MO, USA) and omission of primary antibody from the procedure, and hematoxylin and eosin staining was also performed.

The intensity of the immunoreaction for MT was divided into none, weakly positive, and strongly positive, and the areas observed to have positive immunoreaction were calcu-
lated. In each case, 200-500 epithelial cells were counted in the areas of strongly positive immunoreaction, and the percent of strongly positive cells was calculated (percent of strongly positive areas x percent of strongly positive cells). Statistical analysis was performed using the Student's t-test.

RESULTS

Immunohistochemical control

No positive immunoreaction for MT was found in sections treated with primary antibody preabsorbed with MT antigen (Fig. 1). In addition, no positive immunoreaction for MT was found in sections treated with PBS.

Immunohistochemical findings of patients without hormone therapy

The immunoreaction for MT was observed in the epithelial cells of the seminal vesicles, and the localization of MT was found to be in the cytoplasm and nuclei. Basal cells had mostly negative immunoreaction. Both the cytoplasm and nuclei of epithelial cells lacked uniformity in immunoreaction, with some being stained strongly and others weakly (Fig. 2). The strongly positive cells for MT immunoreaction were mostly localized in the epithelia projecting into the lumen or facing the large cavity, and the weakly positive cells were mostly observed in the epithelia forming shallow or deep folds (Fig. 3). The secretory products in the lumen had a strongly positive immunoreaction for MT (Fig. 3). Smooth muscle cells in the muscular wall layer had a positive immunoreaction for MT which was localized in the cytoplasm and nuclei (Fig. 4). No other connective tissues had an immunoreaction. In the portion near the ejaculatory duct, the epithelial cells facing the large lumen were shown to have a positive immunoreac-

Fig. 1. Photomicrograph of a control section treated with preabsorbed primary antibody by adding MT-2 antigen. The epithelium, secretory products, and connective tissues have a negative immunoreaction (×112).
Changes of immunoreaction for MT between groups with and without hormone therapy

In group 1 (with hormone therapy), the strongly positive area averaged 6.7%, the weakly reactive area 26.7%, and the nonreactive area 66.7%; in group 2
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(without hormone therapy), the results were 10.6%, 26.1%, and 63.3%, respectively. A negative immunoreaction for MT in the epithelial cells was observed in one case of group 1 and in three of group 2. The area of positive immunoreaction had almost the same ratio in groups 1 and 2; the strongly positive cells averaged 0.55% in group 1 and 4.19% in group 2 (Fig. 6). The strongly positive cells in group 1 decreased compared with group 2 (Fig. 7). The secretory products had no

Fig. 4. Photomicrograph of a negative area for MT immunoreaction. The epithelial cells have no immunoreaction. The smooth muscle cells of the muscular layer show a positive immunoreaction for MT, which is localized in the cytoplasm and nuclei (×112).

Fig. 5. Photomicrograph of the portion near the ejaculatory duct. Strong immunoreaction for MT is found in the cytoplasm and nuclei of epithelial cells facing the large lumen, while the epithelia forming acini have a weak immunoreaction (×280).
Fig. 6. Percentages of strongly positive cells for MT immunoreaction in groups 1 and 2. Horizontal bars indicate the mean values for individual determinations plotted in the figure (0.55% in group 1 and 4.19% in group 2), but the difference is not significant.

Fig. 7. Photomicrograph of a case with hormone therapy. Strongly positive cells are fewer than those in cases without hormone therapy (cf. Fig. 2) (×280).
immunoreaction for MT in any case of group 1.

**DISCUSSION**

There has been little previous investigation of MT in the seminal vesicles. Nishimura et al. (1990) reported that MT was localized in the basal but not in the epithelial cells in Wistar rats. It was suggested that the basal cells play a role as nurse cells (Aumüller et al. 1981) and supply zinc ions to the epithelial cells, because MT has been thought to act as a storehouse for and a donor of zinc, and, furthermore, zinc ions are required for various enzymes involved in cell division (Vallee 1959). In this study of human seminal vesicles, MT was identified in the epithelial cells and secretory products, not in the basal cells. Therefore, these findings suggested that MT was synthesized in the epithelial cells and secreted into the fluids of the seminal vesicles. In the rat and human prostates, MT was demonstrated by immunohistochemistry and radioimmunoassay to be synthesized in the epithelial cells and secreted into the prostatic fluids (Bataineh et al. 1986; Umeyama et al. 1987; Suzuki et al. 1991, 1992). The number of immunoreactive cells in the seminal vesicles was fewer than that in the prostate reported previously (Suzuki et al. 1991). It was thought that MT plays the role of a secretory protein in the male genital organs, and that the secretory function of MT in the seminal vesicles is weaker than that in the prostate.

The physiological function of the seminal vesicles is regulated by androgen (Mathieson and Hays 1945; Higgins et al. 1976), and the function of androgen is suppressed by the estrogenic and LH-RH agonistic therapies for prostate cancer. In patients treated with hormone therapy, the strongly positive cells for MT decreased, and the secretory products had no immunoreaction. This result suggested that the synthesis of MT was suppressed by the hormone therapy.

In conclusion, MT was speculated as being synthesized in the epithelial cells of the seminal vesicles and secreted into the fluids, but the function of MT was suggested to be limited compared with that in the prostate glands. Furthermore, the synthesis of MT was suppressed by hormone therapy.

**References**


