Metallothionein expression in human prostate gland

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ABSTRACT

Objective: This study is conducted to detect metallothionein (MT) distribution in the epithelial cells of prostate gland from patients with benign prostatic hypertrophy and adenocarcinoma.

Methods: Prostatic tissues from patients with benign prostatic hypertrophy and adenocarcinoma were processed for immunocytochemistry using indirect peroxidase antiperoxidase procedure and primary antibody against MT. The samples were collected over a period of 2-3 years and were processed at Jordan University of Science and Technology, Irbid, Jordan in the year 2002.

Results: All prostatic tissues showed a positive reaction for MT. In benign prostatic hypertrophy, MT was mainly localized in the nuclei of epithelial cells while in the adenocarcinoma; MT was mainly localized in the cytoplasm of the epithelial cells.

Conclusion: Metallothionein expression may be affected by the pathological status of the prostate. In addition, these findings could be used in diagnosing and evaluating the prognosis of different pathological conditions of the prostate.

Saudi Med J 2003; Vol. 24 (11): 1246-1249

Metallothionein (MT) is a low molecular weight, cytosine rich metal-binding protein.[1] It has been isolated and characterized in many tissues and organs.[2,3] Metallothionein can be induced by metals[4] and anticancer drugs.[5] Induction of MT is associated with high tolerance to the toxic effect of cadmium[6] and cisplatin.[7] Metallothionein has been localized in normal rat[8] and human[9] prostate gland. Metallothionein has been detected in normal human prostatic tissue and benign prostatic hyperplasia.[9] It was found that zinc within the cytosol of cultured (human prostatic adenocarcinoma) PC-3 cells[10] and in the prostatic fluid[11] was bound to MT. Metallothionein may be involved in zinc homeostasis, which may be required for tumor growth and progression.[12,13] Immunocytochemical studies at light and electron microscopic levels have demonstrated the localization of MT in the rat prostate gland[14] in sites corresponded closely with sites of subcellular distribution of zinc.[15] This suggests the involvement of MT in storage and transport of zinc in the prostate gland. Metallothionein may also be involved in cell proliferation and differentiation in carcinogenesis.[16] Expression of MT is associated with various types of tumors such as thyroid,[17] testicular germ cell[18] and urinary bladder tumors.[19] The induction of tumor growth in the ventral lobe of the rat prostate was attributed to the deficiency of MT.[20] In previous study, it has been shown that zinc concentrations were low and high in the nuclei of prostatic epithelial cells of adenocarcinoma and benign prostatic hypertrophy.[12] The aim of this study is to localize MT in the epithelial cells of human prostate in adenocarcinoma and benign prostatic hyperplasia and to correlate this with the nuclear zinc localization. We anticipate that the finding will through the light to a better understanding of the etiology and the

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Received 21st April 2003. Accepted for publication in final form 15th July 2003.

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pathogenesis of different pathological states of the prostate.

Methods. Prostatic tissues were taken from patients with benign prostatic hyperplasia (21 cases) and prostatic adenocarcinoma. Gleason stage IV (7 cases) through transurethral or supra pubic resection. All tissues were fixed in SUSA fluid consisting of formalin, mercuric chloride and glacial acetic acid. Tissues were processed routinely and embedded in paraffin. Immunocytochemical staining was performed on 5 μm thick sections. Sequentially, sections were treated with rabbit anti-MT polyclonal antibody, goat anti-rabbit and peroxidase anti-peroxidase (PAP). Visualization of the reaction was carried out using 3,'3 diaminobenzidine. The detailed procedure was described earlier.[14] Specificity of the reaction was tested by incubating sections from malignant prostatic tissues (Gleason IV) with either pre-absorbed primary antibody with excess antigen for 48 hours or incubating sections in a buffer from which the primary antibody has been deleted, followed by incubation, otherwise, as usual.

Results. All prostatic tissues from patients with benign prostatic hyperplasia and adenocarcinoma (Gleason stage IV) showed a positive reaction for MT, but with variation in the distribution and intensity of the reaction product. **Figure 1** showed a positive reaction for MT in prostatic tissues from patients with benign prostatic hyperplasia. The reaction was confined to the epithelium and connective tissue as well. The reaction was most intense in the nuclei, while the cytoplasm showed a mild reaction. In contrast, the connective tissue had the least reaction product. Both cytoplasm and nuclei of epithelial cells lacked uniformity in reaction; namely some nuclei stained deeply; others stained lightly. Also, the cytoplasm of some cells stained lightly and others stained faintly. **Figure 2** showed a positive reaction for MT in prostatic tissues from patients with prostatic adenocarcinoma (Gleason stage IV). Both the epithelial cells and the connective tissue were stained. The cytoplasm showed the most intense reaction, whereas, the nuclei showed a negative reaction for MT in most of the epithelial cells. Some nuclei showed a faint reaction. On the other hand, the secretory products in the acinar lumina showed a positive reaction. In addition, the connective tissue was stained for MT. **Figure 3** showed malignant prostatic tissue (Gleason IV), which has been used as a control to validate the technique. No reaction product for MT, neither in the epithelium nor in the connective tissue, has been detected.

Discussion. All prostatic tissues have shown a positive reaction for MT with variation in the distribution and intensity of the reaction product. The variation in MT distribution and intensity may reflects

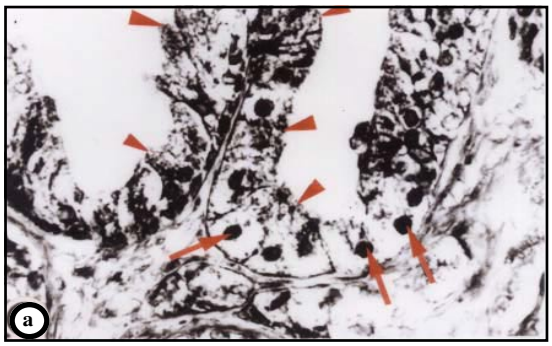

Figure 1 - Photographs of benign prostatic hyperplasia of human prostate after peroxidase anti-peroxidase treatment. Portions of prostatic acini are shown at higher magnification. The nuclei of epithelial cells showed intense reaction (arrows) and the cytoplasm shows a mild reaction (arrowheads) (x 2800).

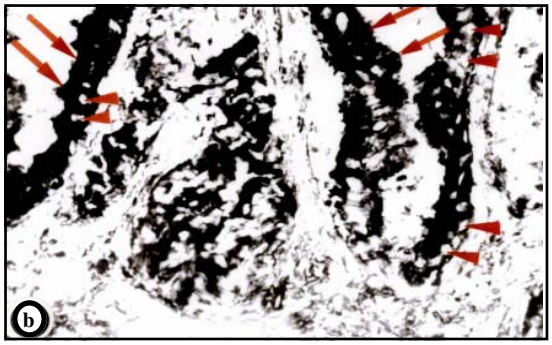

Figure 2 - Photographs of malignant prostatic tissue (Gleason stage IV) from human prostate after peroxidase anti-peroxidase treatment. Portions of prostatic acini at higher magnification are shown. The cytoplasm of the epithelial cells shows intense reaction (arrows), while the nuclei show no reaction (arrow heads). The secretory products in the lumen of the acini, as well as, the connective tissue between the acini shows a mild to moderate reaction. (x 2600).

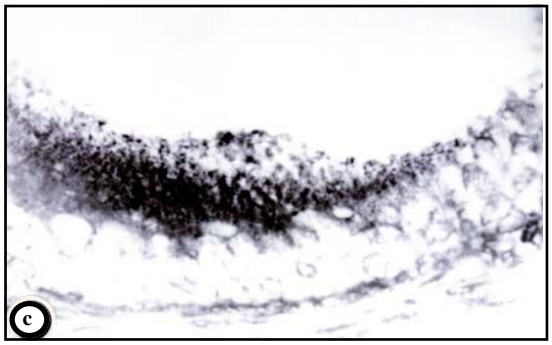

Figure 3 - Photographs of the malignant prostate (Gleason stage IV) of human prostate gland. Control sections that have been treated with a preabsorbed primary antibody. The epithelium and connective tissue show a negative reaction A (x 2600), B (x 2800).

differences in specific functional response with different pathological conditions. It was found that different lobes of adult[14] and very young[9] prostate gland showed variation in distribution and intensity of MT. These lobe-specific differences of MT localization may suggest functional significance of MT. Metallothionein was localized in the nuclei of epithelial cells of prostate from benign prostatic hyperplasia. Other study has supported this finding[10] due to MT binds zinc avidly, it is tempting to speculate that MT donates its zinc to many zinc enzymes to modify their catalytical, structural and regulatory effect.[21,22] Also, zinc proteins are involved in transcription and translation of genetic material, accounts for its essentiality to all forms of life.[23] In a previous study, it has been shown that the nuclei of epithelial cells of benign prostatic hyperplasia contain the highest zinc concentration compared to normal and adenocarcinoma of the prostate.[21] The cytoplasm of the epithelial cells of prostatic adenocarcinoma demonstrated the presence of high amount of MT. The tumor cells are rapidly proliferating the cells. In this regard MT was found in proliferating and differentiated cells of female reproductive organs and mammary gland of rat and guinea pig[3] and human neuroblastoma IMR-32 cells.[24] The lack of MT expression in the ventral prostate of the rat may explain the development of cadmium-induced tumors in this lobe,[25] whereas a strong immunoreactivity for MT was observed in the peripheral zone of the human prostate where the adenocarcinoma usually develops.[10] On the other hand, the central zone showed a weak staining and adenocarcinoma rarely develops in this zone.[10] Positive staining of the cytoplasm may show active protein synthesis in rapidly proliferating cells of prostatic adenocarcinoma. On the other hand, positive nuclear staining in the prostatic hypertrophy may reflect an active transcriptional process involving MT, the zinc carrying protein, in prostate gland. The positive reaction for MT in the secretory products in the adenocarcinoma of the prostate suggests that MT regulates zinc secretion from the prostatic epithelium since ample amount of zinc is bound to MT[26] and MT is secreted by the prostatic epithelium and secreted into the prostatic fluid.[11] The differences found in MT distribution could be used as a cytological parameter in the diagnosis of disease states of the prostate. In this regard, the intensity of prostatic acid phosphatase was used to differentiate between normal and benign prostatic hyperplasia from adenocarcinoma of the prostate.[27] Other studies have used the prostatic specific antigen in localization and staging[28] and prognosis of prostatic adenocarcinoma. Although this study did not determine that MT expression is an independent predictor of the pathological states of the prostate, MT expression is of significant interest and warrants further investigations as a potential marker for cell proliferation in prostate. This marker could be used for diagnosis and prognosis of prostatic diseases. The results also may show a potential role of MT in cell proliferation and differentiation in human prostatic diseases.

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