ORIGINAL ARTICLE

Metallothionein isoform II expression in hyperplastic, dysplastic and neoplastic prostatic lesions

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Background: Metallothionein is a low-molecular-weight cysteine-rich protein that has the ability to bind and sequester heavy metal ions. It is associated with metalloregulatory functions such as cell proliferation, growth and differentiation.

Aims: To investigate the expression of metallothionein in hyperplastic, dysplastic and neoplastic prostatic lesions and to correlate its expression with histological grade of prostatic carcinoma.

Method: The study was carried out on formalin-fixed and paraffin-wax-embedded tissue blocks from 8 patients with benign prostatic hyperplasia, 6 patients with prostatic intraepithelial neoplasia (PIN) and 30 patients with prostatic carcinoma, using the streptavidin-biotin technique. The histological grade was defined and the carcinomas were divided into low-grade (Gleason Score 2–4), 12 moderate grade (Gleason Score 5–6) and 10 high-grade (Gleason Score 7–10) carcinomas.

Results: Patchy metallothionein staining of epithelial cells was observed in normal and benign prostatic tissues. All cases of PIN and 20 of 30 patients with prostatic carcinoma showed positive staining for metallothionein. Metallothionein expression considerably increased from low-grade to high-grade tumours. The proportion of cells staining positively for metallothionein was directly correlated with histological grade of prostatic carcinoma. The epithelial cells lack uniformity in staining intensity, but the percentage of strongly positive cells increased with the histological grade of prostatic carcinoma.

Conclusions: The high incidence of metallothionein expression in PIN in our study suggests that it is associated with early prostate tumorigenesis. Also, metallothionein expression was directly correlated with the histological grade of prostatic carcinoma, suggesting that metallothionein may be a useful marker for predicting the prognosis of prostate cancer.

Metallothioneins constitute a family of several intracellular, low-molecular-weight proteins with a high affinity to various heavy metals such as zinc, cadmium, copper, mercury and platinum.¹ Different isoforms (0–IV) of metallothionein have been identified. Metallothionein I and II are major isoforms in mammalian tissues. They are believed to serve an important role in the homeostasis of essential metals such as zinc and copper during growth and development, as well as in the detoxification of heavy metals such as cadmium and mercury, rendering the metallothioneins important mediators and attenuators of toxicity induced by heavy metals.^{2–4}

Metallothioneins are associated with metalloregulatory functions such as cell proliferation, growth and differentiation. In recent years, metallothionein expression has been linked with carcinogenesis, resistance to cancer treatment and tumour progression.^{5–7} Immunohistochemically detected metallothionein is not usually found in normal tissues, except in myoepithelial cells of the breast and epithelial cells of the kidney and thyroid.⁴

Overexpression of metallothionein occurs frequently in human malignant tumours, but the underlying mechanism is unknown.⁸ Several lines of evidence suggest that metallothionein may be associated with cell proliferation and differentiation in carcinogenesis.^{8–10} Many studies have shown an association between metallothionein overexpression and type and grade of tumour.¹¹ Metallothionein has been expressed in various types of human cancers such as colorectal,^{8, 12} bladder¹³ and breast cancers.^{6, 14}

Prostatic carcinoma is the most prevalent cancer among men in the Western world. Preneoplastic lesions, known as prostatic intraepithelial neoplasia (PIN), have been found in men as early as 20 years of age and are commonly observed

in men >50 years of age. PIN lesions are thought to be precursors of invasive prostate cancer in which incidence considerably increases in the sixth decade of life.^{15–17} Ageing, genetic factors, environmental carcinogens and steroid hormone levels are factors that have been associated with the development of prostatic cancer.¹⁸

Our study investigated the expression of metallothionein in hyperplastic, dysplastic and neoplastic prostatic lesions and correlated its expression with the histological grade of prostatic carcinoma.

MATERIALS AND METHODS

Formalin-fixed and paraffin-wax-embedded tissue blocks from 8 patients with benign prostatic hyperplasia, 6 patients with PIN and 30 patients with prostatic carcinoma were collected from the National Institute of Urology and Nephrology, Cairo, Egypt. They were obtained by radical prostatectomy, transurethral resection or prostatic needle biopsy. Five specimens composed of normal prostate adjacent to tumours were included in this study. The Gleason system was used for histological grading of malignant tumours.¹⁹ According to Xiang-Hua *et al.*,²⁰ the tumours were classified as 8 low grade (Gleason Score 2–4), 12 moderate grade (Gleason Score 5–6) and 10 high grade (Gleason Score 7–10).

Immunohistochemical staining of metallothionein

The streptavidin-biotin technique was used to investigate metallothionein expression. Tissue samples were fixed in 10% buffered formalin for 24 h and cut into 4-µm-thick sections. After deparaffinisation and hydration, the tissue sections were incubated for 30 min in 0.3% hydrogen peroxide to

Abbreviation: PIN, prostatic intraepithelial neoplasia

Figure 1 Benign prostatic hyperplasia showing patchy cytoplasmic staining for metallothionein (immunoperoxidase $\times 150$).

quench endogenous peroxidase activity. Antigen retrieval was carried out by microwave pretreatment for 10 min in 0.01 M citrate buffer. The sections were incubated for 20 min with normal blocking serum to suppress non-specific binding of immunoglobulin. The tissue sections were incubated at 4°C overnight with anti-metallothionein monoclonal antibody (Dako- MT, E9; from Dako Corporation, Glostrup, Denmark), which reacts with metallothionein isoforms I and II at a dilution of 1:50. These steps were followed by 30 min of incubation with biotinylated horse anti-mouse antibody at room temperature, avidin–biotin peroxidase complex for 50 min at room temperature and finally diaminobenzidine for 3–5 min. The slides were counterstained with haematoxylin, dehydrated and mounted. In each metallothionein staining patch, normal kidney tissue was included as a positive control. Cytoplasmic or nuclear staining and cytoplasmic brown coloration indicated positive staining. A negative control was obtained by omitting the primary antibody.

Results from metallothionein immunostaining were scored as follows: 0, no; 1, <25%; 2, 25–50%; and 3, >50% staining cells.²⁰ The results were judged positive if >25% of the tissue section stained. Staining intensity was recorded semiquantitatively as mild (+), moderate (++) or strong (+++), following Somji *et al.*¹³

Statistical analysis

Difference in distribution of variables between groups was tested using the χ^2 test. Values of $p < 0.01$ were considered to be significant.

RESULTS

A total of 44 prostatic lesions were evaluated for metallothionein immunoreactivity. Normal and benign prostatic tissues showed patchy metallothionein staining of epithelial cells (fig 1). All cases of PIN and 20 (66.7%) of 30 cases with prostatic carcinoma showed positive staining for metallothionein (>25% of cells). Although some cells showed only cytoplasmic staining, most cancer cells showed cytoplasmic and nuclear staining. Only one case of PIN and five cases of prostatic carcinoma showed positive staining of the basement membrane surrounding the cancer cells (figs 2 and 3).

Table 1 shows the correlation of metallothionein expression with histological grade of prostatic carcinoma. Metallothionein expression increased significantly from low-grade (37.5%), to moderate-grade (66.7%) to high-grade (90%) tumours ($p < 0.01$).

Table 2 shows the correlation between positively stained cells and histological grades of prostatic carcinoma. Between 76 and 100% of cells were positively stained in 8 (88.8%)

Figure 2 Prostatic intraepithelial neoplasia with diffuse cytoplasmic and focal membranous staining for metallothionein (immunoperoxidase $\times 300$).

high-grade and 5 (62.5%) moderate-grade tumours. On the other hand, 2 (66.7%) cases of low-grade and 1 (12.5%) case of moderate-grade tumours showed positive staining in 26–50% of cells. These results showed that the proportion of cells stained positively for metallothionein was directly correlated with the histological grade of prostatic carcinoma ($p < 0.01$).

Regarding metallothionein staining intensity, the epithelial cells did not immunoreact uniformly: some stained strongly whereas others stained weakly, and the percentage of strongly positive cells increased from low-grade to high-grade prostatic carcinomas (figs 3–5).

DISCUSSION

Prostatic glands contain heavy metals such as zinc and cadmium, and epidemiological studies have shown that both metals are associated with the development of prostate cancer.²¹ Metallothionein is a low-molecular-weight cysteine-rich protein that has the ability to bind and sequester heavy metal ions. Metallothionein synthesis is induced in a variety of tissues by these metal ions, as well as by endogenous factors such as glucocorticoids, interferon, interleukin 1 and vitamin D.²² Several lines of evidence have indicated that metallothionein may have a role in carcinogenesis and in drug resistance of tumours.^{7–23}

Few studies previously evaluated expression of metallothionein in prostatic lesions with conflicting results. We studied the expression of metallothionein in benign prostatic hyperplasia, PIN and prostatic carcinoma to evaluate its role

Figure 3 Moderate-grade prostatic carcinoma showing cytoplasmic, membranous and focal nuclear staining for metallothionein (immunoperoxidase $\times 300$).

Table 1 Correlation of MT expression and histological grade of PC

MT expression	Low grade, n (%)	Middle grade, n (%)	High grade, n (%)	Total, n (%)	p Value
+	3 (37.5)	8 (66.7)	9 (90.0)	20 (66.7)	<0.01
–	5 (62.5)	4 (33.3)	1 (10.0)	10 (33.3)	
Total	8 (100)	12 (100)	10 (100)	30 (100)	

MT, metallothionein; PC, prostatic carcinoma; +, positive; –, negative.

Table 2 Correlation between positively stained cells and histological grade of positively stained cells with prostatic cancer

Positive cells (%)	Low grade, n (%)	Middle grade, n (%)	High grade, n (%)	Total, n (%)	p Value
26–50	2 (66.7)	1 (12.5)	0 (0.0)	3 (15.0)	<0.01
51–75	1 (33.3)	2 (25.0)	1 (11.1)	4 (20.0)	
76–100	0 (0.0)	5 (62.5)	8 (88.9)	13 (65.5)	
Total	3 (100)	8 (100)	9 (100)	20 (100)	

in prostatic carcinogenesis. Metallothionein expression was also correlated with the histological grade of prostatic carcinoma to study its usefulness as an indicator of aggressive behaviour of the tumours.

In this study, normal and benign prostatic tissue showed patchy metallothionein staining of epithelial cells. These results were in agreement with previous observations by Moussa *et al.*²³ and Zhang *et al.*²⁴ Also, metallothionein expression was increased in PIN and further increased in a highly variable fashion in prostatic carcinoma. These results were in accordance with the study of Garrett *et al.*²⁵

We observed metallothionein expression in 66.7% of patients with prostatic carcinoma. This result is higher than that in the study of Zhang *et al.*²⁴ in which metallothionein was expressed in only 33.3% of patients with prostatic carcinoma. In contrast, Moussa *et al.*'s²³ study showed that all tumour foci were positively stained for metallothionein. These conflicting results may be explained by the differences in antibodies, methods and interpretation of immunostaining results.

Most cancer cells showed cytoplasmic and nuclear staining, similar to the previous findings of Moussa *et al.*²³ and Tekur and Ho.²⁶ Although metallothionein is thought to be a cytoplasmic protein, many studies^{1, 27–28} suggest that metallothionein is present in the cytoplasm and nucleus of normal and malignant cells. Subcellular localisation of metallothionein is regulated in cells undergoing proliferation, developmental progression and tumorigenesis, and during the cell

cycle.²⁸ In our study, only 1 of 6 patients with PIN and 5 of 20 patients with prostatic carcinoma showed positive staining of the basement membrane surrounding the cancer cells, which is in agreement with the study by Zhang *et al.*²⁴ in which 2 of 15 cases of prostatic carcinoma showed the same finding. It is well known that the glandular epithelial cells (normal cells, particularly the basal cells or malignant cells) may participate in biosynthesis of the basement membrane. Consequently, this phenomenon may be regarded as an actively secretory result of the cancer cells.²⁴

A close correlation of metallothionein expression and invasive breast carcinoma with a poor prognosis has been reported,^{7–29} as well as a close correlation with tumour grade in breast carcinoma²⁴ and bladder carcinoma.⁴ In this study, metallothionein expression was markedly correlated with the histological grading of prostatic carcinoma, with its expression increasing from low-grade to moderate-grade to high-grade tumours. These results are in accordance with those of Zhang *et al.*²⁴ Garrett *et al.*¹¹ and Dutta *et al.*³⁰ Also, the proportion of positively stained cells correlated considerably with the histological grade of prostatic carcinoma. As regards metallothionein staining intensity, the epithelial cells did not immunoreact uniformly (some stained strongly whereas others stained weakly), and the percentage of strongly stained cells increased from low-grade to high-grade prostatic carcinoma. This was in accordance with the study of Suzuki *et al.*³¹ Generally, it is believed that anaplastic and

Figure 4 Low-grade prostatic carcinoma with cytoplasmic staining, which lacks uniformity in staining intensity (immunoperoxidase ×300).

Figure 5 High-grade prostatic carcinoma showing cytoplasmic staining for metallothionein, with a high proportion of strongly stained cells (immunoperoxidase ×300).

aggressive tumours with more cellular metabolic activity may have greater zinc and metallothionein requirements.³² Therefore, this wide range of non-uniformity of metallothionein expression may have resulted from changes in zinc or cadmium metallothionein in prostate cancer.²⁰

CONCLUSION

The high incidence of metallothionein expression in PIN in this study suggests that it is associated with early prostate tumorigenesis. In addition, the direct correlation of metallothionein expression with histological grade of prostatic carcinoma suggests that metallothionein may be a useful marker for predicting the prognosis of prostate cancer.

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