

NUCLEAR ZINC IN METASTATIC TUMORS OF PROSTATIC ADENOCARCINOMA

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ABSTRACT

The zinc-binding proteins regulate genes, which play an important role in cell differentiation and proliferation. Moreover, zinc is involved in DNA and RNA transcription and replication. Also, zinc was elevated and decreased in benign prostatic hyperplasia and adenocarcinoma of the prostate, respectively. The hypothesis that the behaviours of metastatic tumors of prostate might affect the nuclear zinc concentration was postulated, since zinc have been demonstrated in the nuclei of normal rat, and dog prostate gland. The X-ray microanalysis technique was used to detect and quantitate zinc in nuclei of metastatic tumor cells of prostatic adenocarcinoma R-3327H cell line, in skin, liver and lung. S.C. implants, or I.V. infusion of about 106 cells were used to establish metastatic tumors. It was found that zinc concentration in euchromatin and heterochromatin was higher in metastatic tumors of I.V. treated group. However, there was a variation in zinc concentration across the treatment and across the organs. From these results, it was suggested that the route of metastasis could affect nuclear sequestration of zinc. The euchromatin/heterochromatin zinc concentration ration and zinc concentration could be used as an estimated factor in diagnosis of prostatic tumor metastasis.

Keywords: X-ray micro-analysis, protein expression, proliferation, RNA, DNA, Zn.

INTRODUCTION

Prostate cancer is the most leading cause of death in the United States after age of 70, and is second to the Lung cancer after the age of 50 (Gyorkey, 1973). The number and nature of events leading to neoplastic transformation remain ill defined. It is generally believed that more than one event is required for the transformation of a normal cell to malignant one (Bishop, 1995). In previous studies we have reported that several proteins were differentially expressed in human leukemia, in solid tumor and in normal cell proliferation and these proteins were associated with N-myc gene amplification (Hanash *et al.*, 1988; Hailat *et al.*, 1990; Keim *et al.*, 1990). Others have reported that zinc binding proteins were involved in the regulation of genes, which play an important role in cell differentiation and proliferation (Duncan, 1984; Valee, 1988, Vallee, 1993).

Early diagnosis and staging of cancer and the metastatic behaviour of these cancers have been a challenge to the physicians and the scientists as well. A strong association was reported between reduced expression of the nm23-H1 gene and acquisition of metastatic behaviour. In a model system of rodent metastasis, nm-23 RNA levels were lower in highly metastatic potential (Steeg *et al.*, 1988). On the other hand, tumors from patients with infiltrating ductal breast carcinomas with lymph node metastasis had low nm23 RNA content (Bevilacqua *et al.*, 1989).

Variations in metal content were also reported in different pathological conditions (Poukkula *et al.*, 1987; Diplock, 1990; Takeda *et al.*, 1992). Zinc levels were decreased and increased in adenocarcinoma of the prostate and benign prostatic hyperplasia, respectively (Brys *et al.*, 1997; Ogunlewe and Osegbe, 1989). The intracellular localization of zinc in normal, hyperplastic, and neoplastic human prostate (Tvedt *et al.*, 1989), and in normal rat (Chandler *et al.*, 1977 a) and dog prostate (Chandeler *et al.*, 1977 b) was reported, using X-ray microanalysis.

The X-ray microanalysis (Tvedt *et al.*, 1989) and atomic absorption spectrophotometry (Brys *et al.*, 1997) techniques have been used to detect the intracellular distribution of zinc in normal, hyperplastic and neoplastic human prostate gland. Neither of these studies has demonstrated the zinc concentration in the nuclei. In this study, X-ray microanalysis technique determined the nuclear zinc concentration in the metastatic foci of the prostatic adenocarcinoma R-3327H cell line of the Copenhagen rat. It is anticipated that this investigation will promote a better understanding of the role of zinc in the aetiology and diagnosis of prostatic cancer.

MATERIALS AND METHODS

Animals

Twenty Copenhagen rates were used in this experiment. For establishing tumors in vivo, ten rates

were injected with 10^6 tumor cells via tail vein. The same amount of tumor cells was inoculated subcutaneously into the flanks of other ten rats. These procedure was done under aseptic conditions. The tumor cell line was the Dunning R-3327H prostatic adenocarcinoma of the Copenhagen rat. The rats were killed after 4 weeks by ether anesthesia. Metastatic foci were encountered in liver, lung, and subcutaneous layer of the skin.

Tissues

Tissues were prepared for X-ray microanalysis using a modification of the potassium pyroantimonate technique (Chandler, 1977). The tissues were diced into 1 mm^3 pieces in small amount of 2% potassium pyroantimonate in 1% aqueous solution of osmium tetroxide. Tissues then were transferred to fresh fixative and left at room temperature for 90 minutes. Rapid dehydration in absolute alcohol (3×10 minutes) preceded emersion in 50/50 absolute alcohol and propylene oxide for 10 minutes each, followed by 50/50 propylene oxide and Spurr's resin for 10 minutes. Two changes of resin 30 minutes each preceded final resin embedding of tissues in Beem plastic capsules, and polymerization at 70°C overnight. Sections of approximately 100-120 nm were cut using a diamond knife in a Reichert ultramicrotome, collected on 150 mesh aluminum grids, left unstained by coated with a thin (10 nm) layer of carbon in vacuo to stabilize the sections against the electron beam.

X-ray microanalysis

The microanalysis technique used in the present study has employed extensively in both biological and non-biological samples. The principles, techniques, instrumentation and calculations have been discussed in details by many investigators (Chandler, 1977; Marshall, 1980). At least twenty randomly selected areas, for euchromatin and heterochromatin regions in the nuclei of the metastatic cells in the liver, lung, and skin, in both groups were analyzed. Grids with standard zinc concentration of 10, 25 and $50 \mu\text{g}\%$ were used to calculate the final concentration of zinc in euchromatin and heterochromatin. X-ray microanalysis for zinc was performed using Hitachi H-600 analytical microscope, coupled with a Kevex multichannel analyzer.

Statistics

The T-test was used to demonstrate the statistical differences between zinc concentrations in euchromatin and heterochromatin among the tissues and across the treatments.

RESULTS

The nuclei of cells in the metastatic foci in skin, lung, and liver showed a variation in zinc concentration. The variation was noted across the organs and across

the treatments, both in euchromatin and heterochromatin (Fig. 1, 2).

Fig. 1 showed zinc concentration in the euchromatin of the nuclei of metastatic foci in the skin, lung and liver. In I.V. treated group, zinc concentration was the highest in the skin and lowest in the lung. There were statistical differences between zinc concentration in the skin and lung, and between lung and liver. Although the zinc concentration in the skin was higher than that in the liver, but it was not statistically significant. In S.C. treated group, zinc showed a variation in zinc concentration across the organs, being the highest in the skin and lowest in the lung. Statistical differences were noted between skin and lung, and between skin and liver, but not between lung and liver. Zinc concentration in the euchromatin was higher in the I.V. treated group than that in the S.C. treated group in the three organs. The statistical differences were noted in the lung and liver but not in the skin.

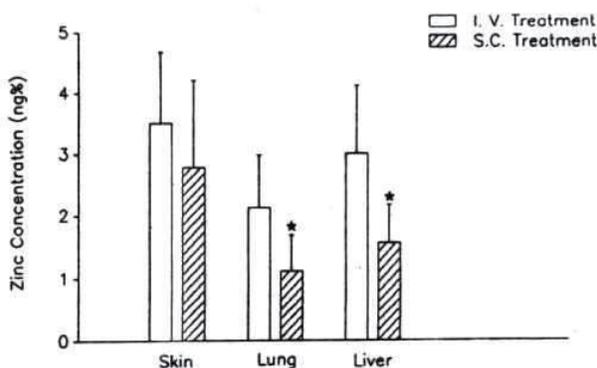


Fig. 1: A comparison of zinc concentration in euchromatin of the metastatic foci in the skin, lung, and liver as determined by X-ray microanalysis. Values are expressed in ng%. Vertical lines indicate mean \pm SE; white bars I.V. treated; Oblique striped, S.C. treated. The statistically different pairs ($P < 0.05$) are indicated by stars.

Fig. 2 showed zinc concentration in the heterochromatin of the nuclei of the metastatic foci in skin, lung and liver. In I.V. treated group, there was a variation in zinc concentration, being the highest in liver and lowest in the lung, but there was no statistical difference between the three organs. Also, there was a variation in zinc concentration in the S.C. treated group, being the highest in the skin and lowest in lung, but there was no statistical difference between the three organs. Again, as in the euchromatin, zinc concentration was higher in the I.V. treated group than

that in the S.C. treated group. Statistical difference was noted in the liver, but not in the skin and lung. The ratio of zinc concentration in euchromatin to that of heterochromatin in the skin of both treatments was > 1 and whereas, the ratio was < 1 in the lung and liver of both types of treatments. In I.V. and S.C. treated groups, if the zinc concentration values of euchromatin and heterochromatin, in each organ, were pooled together, the total zinc concentration in chromatin was higher in the metastatic group compared to the S.C. treated group.

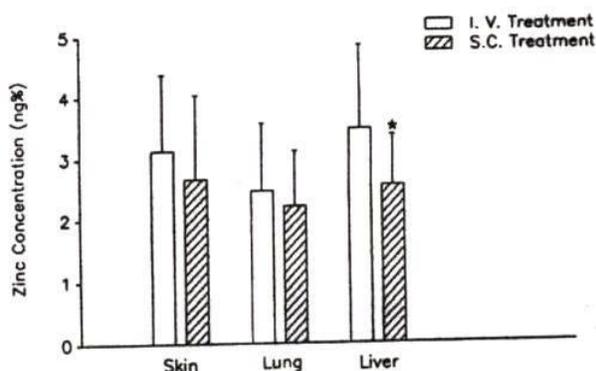


Fig. 2: A comparison of zinc concentration in heterochromatin of the metastatic foci in the skin, lung, and liver as determined by X-ray microanalysis. Values are expressed in ng%. Vertical lines indicate mean \pm SE; white bars I.V. treated; Oblique striped, S.C. treated. The statistically different pairs ($P < 0.05$) are indicated by stars.

DISCUSSION

The present study represents the first study in demonstrating zinc concentration in euchromatin and heterochromatin of metastatic tumors of prostatic adenocarcinoma R-3327H cell line of Copenhagen rats utilizing X-ray microanalysis. In previous study, it was demonstrated by histopathological means, that the metastatic tumors in the skin, liver and skin, in both types of treatment, did not show any significant difference, in the histological profile of all nuclei. It was shown that all metastatic foci exhibited essentially the same fine histopathological structural features (Heidger *et al.*, 1986). These differences in nuclear zinc content may give additional criteria in describing neoplastic changes, and may serve to distinguish certain metastatic tumor cells exhibiting a similar morphology.

The consistent finding of higher zinc concentration in euchromatin and heterochromatin in I.V. treated group compared to the S.C. treated group, led us to suggest that the route of metastasis could

affect nuclear sequestration of zinc. The differences in nuclear zinc concentration in different sites of metastasis in both groups could be due to the interaction of extracellular matrix components, which provide a favourable interface for seeding, and growth of metastatic lesions within host tissues.

Nuclear zinc concentration per se was used as an estimating factor for the chromatin stability. It was found that low chromatin stability was associated with low zinc content in human sperm (Kvist *et al.*, 1988). Moreover, zinc has a role in structural integrity of DNA and RNA (Eichhorn *et al.*, 1971; Mooser and Gunderson, 1983). In colorectal cancers, an increase in zinc levels has been shown with advanced pathological conditions (Kollmeier *et al.*, 1992). It was found that all hepatic cirrhosis had a very low zinc concentration, whereas, zinc concentration was increased in liver tumors and invasive metastases.

There is a consistent ratio of zinc concentration in euchromatin to zinc concentration in heterochromatin of metastatic tumors in lung and liver (< 1) and in skin (> 1), in both groups, could be used as an index for the nuclear status. In this regard, copper/zinc ratio in lung cancer was suggested as a diagnostic test in lung cancer patients, and a correlation between increasing copper/zinc ratio and tumor extension and postoperative survival was suggested (Diz *et al.*, 1989). On the other hand, quantitative microscopic nuclear features have been used as a new method in grading the malignancy of prostate carcinoma (Robutti *et al.*, 1989).

It is concluded that our findings could be used as working hypothesis to establish a correlation between zinc status of human prostate and the development of pathological conditions. A work is in progress including correlation of the zinc concentration in euchromatin and heterochromatin with various stages of prostatic adenocarcinoma.

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