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Immunohistochemical Localization of Metallothionein in Human Thyroid Tumors

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High levels of metallothionein (MT) are present in the developing mammalian liver; however, a remarkable decrease is observed during postnatal life after weaning. This developmental profile is similar to that of certain oncofetal gene products such as α-fetoprotein, which is used as a tumor marker. This study deals with the reexpression of MT genes in thyroid tumors. With an immunohistochemical method, the presence of MT was investigated in tissue sections of normal and neoplastic human thyroid glands. Tissue sections of 34 thyroid tumors and 10 normal human thyroid glands were studied by means of the peroxidase-antiperoxidase method. MT was localized in 31 of the thyroid gland tumors. MT was also present in two of the normal thyroid glands. These findings indicate that although high levels of MT are mainly found in the fetal liver, it may also be expressed actively in certain human thyroid neoplastic tissues, and occasionally in normal thyroid tissue. (Am J Pathol 1987, 129:177–182)

METALLOTHIONEINS (MTs) are a group of structurally similar low-molecular-weight intracellular proteins with high content of cysteinyl residues (30%) and complete absence of aromatic amino acids and histidine.[1] MTs have a high affinity for essential metals such as zinc (Zn) and copper (Cu) and nonessential metals like cadmium (Cd) and mercury (Hg).[2,3]

High levels of endogenous MT bound to Zn and Cu have been observed in mammalian liver during gestation or the early postnatal period in various species.[4–8] The role of MT during development is not yet understood. However, most studies point to an intracellular storage function of essential metals, Zn and Cu.[9,10] Similarities between the gene expression of MT and α-fetoprotein have been reported.[11] It was also observed that MT mRNA was abundant in the developing liver as well as the visceral and parietal yolk sacs in mice, similar to α-fetoprotein.[11] Thus, the metallothionein gene is expressed very early in mammalian development.

The observation of high levels of MT in fetal and neonatal livers but very low concentration in the adult mammalian liver[7,8,12] prompted this study to localize MT in normal and neoplastic tissues. We report the localization of MT in various types of thyroid tumors by immunohistochemical methods using a specific rabbit antibody to rat liver MT. This polyclonal antibody readily cross-reacted with human MT.[13–15]

Materials and Methods

Paraffin-embedded blocks of all the different thyroid tumors resected surgically between the years 1980 and 1985 were obtained from St. Joseph's Hospital, London, Ontario. Ten paraffin-embedded blocks of normal thyroid gland from autopsy specimen were also obtained at random from the same hospital. The surgical specimens were fixed in 10% buffered formalin solution for a minimum of 6 hours and a maximum of 12 hours, while the autopsy specimens were fixed for a minimum of 48 hours and a maximum of 72 hours. Five-micron sections were deparaffinized and incubated with 20% normal swine serum for 30 minutes to block nonspecific binding sites. The excess normal swine serum was tapped off, and the tissue sections were layered with rabbit anti-MT serum (100 times diluted) or control serum (nor-

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mal rabbit serum or rabbit anti-MT serum absorbed with rat liver MT).[12] The tissue sections were stored at 4 C in a humid chamber for 16–18 hours, then washed in Tris-HCl buffer (0.1 M, pH 7.4) and incubated with the following reagents in sequence at room temperature[16]: swine anti-rabbit IgG (linking antibody), peroxidase–antiperoxidase complex (PAP), and 6 mg 3,3-diaminobenzidine tetrahydrochloride (DAB) in 10 ml Tris-HCl buffer (0.1M, pH 7.4) containing 3 drops of 3% H_2O_2.

Antibody to rat liver MT was prepared in rabbits, and its cross-reactivity was tested in our previous study.[10,12] The rabbit anti-MT readily reacted with human MT.[13–15] All the reagents for immunohistochemistry were obtained from Cedarlane Laboratories Ltd., Hornby, Ontario, and Sigma Company Ltd., St. Louis, Missouri

The specificity of the antibody was checked in three different control experiments: 1) prior absorption of MT antibody with purified rat liver MT; 2) substitution of the antiserum with normal rabbit serum (100 times diluted); 3) omission of primary antiserum (anti-MT) from the procedure.

Results

The immunohistochemical reaction using rabbit anti-MT serum showed specific staining for MT, because none of the negative control procedures gave any staining. The absorption of rabbit anti-MT with rat liver MT abolished all staining (Figure 1), and the use of normal rabbit serum did not produce any staining. There was no staining observed when the primary staining antiserum (anti-MT) was omitted from the procedure.

Fifty-four different types of thyroid tumors were obtained from the files of St. Joseph's Hospital, London, Ontario. Twenty thyroid tumors were rejected from this study either because the tissue sample was too small to allow any interpretation of the results or there was inconsistency in staining on three different analyses due to extensive necrosis or poor fixation of the tissue.

A detailed immunohistochemical localization of MT was performed on 34 surgically resected thyroid tumors and 10 normal human thyroid glands obtained from autopsy cases. The ages of the patients with thyroid neoplasia range from 18 to 87 years, with an average age of 47 years; those of the autopsy cases range from 35 to 80 years, with an average age of 52 years. There is a female predilection in the population studied: 82% of the patients were female, and 18% were male.

The thyroid tumors consist of 19 follicular adenomas, 16 normofollicular (simple type), 3 microfollicular (fetal type), 6 follicular adenocarcinomas, and 9 papillary adenocarcinomas. MT was localized in 31 of the 34 thyroid tumors studied. The distribution of staining for MT in the individual tumors is shown in Table 1. The presence of MT was observed only in 2 of the 10 normal human thyroid glands. On the other hand, MT was localized in 17 follicular adenomas, 10 of which showed both nuclear and cytoplasmic staining. Fourteen normofollicular adenoma (simple type) stained positive with anti-MT serum. Nuclear cytoplasmic staining was also observed in 10 of these adenomas (Figure 2), while nuclear staining alone was observed in 3 of the normofollicular adenomas (Figure 3) and cytoplasmic staining alone in 2 tumors (not shown). There was positive nuclear staining for MT

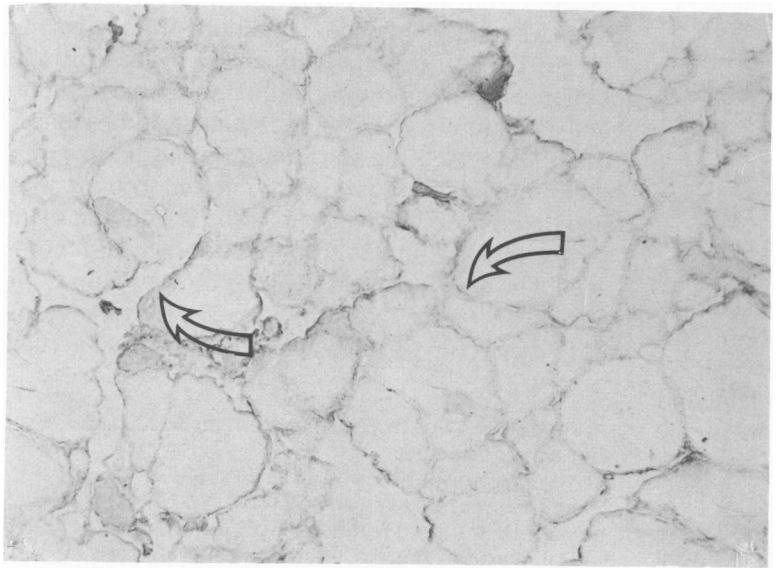

Figure 1—A control section of normofollicular adenoma that had been treated with preabsorbed primary antibody. There was no staining of the follicular epithelium (*open arrows*). (Original magnification, ×150)

Table 1—Immunohistochemical Localization of Metallothionein in Thyroid Tumors

Type of thyroid tumor	No. of tumors	Positive	Nucleus only	Staining cytoplasm only	Nucleus & cytoplasm	Negative
Follicular adenoma	19	17	6	1	10	2
Normofollicular (simple type)	16	14	3	1	10	2
Microfollicular (fetal pattern)	3	3	3	—	—	—
Follicular adenocarcinoma	6	5	—	—	5	1
Papillary adenocarcinoma	9	9	—	—	9	—
Total	34	31	6	1	24	3
Control	10	2	2	—	—	8

The different types of staining were assigned to each case after tissue sections of a particular tumor gave consistent staining in three consecutive procedures.

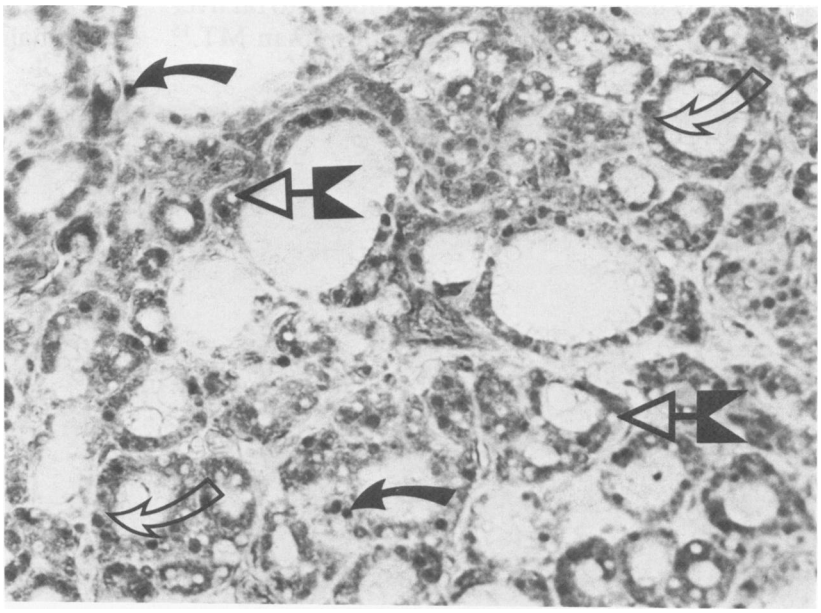

Figure 2—Normofollicular adenoma (simple type). *Closed arrows* indicate the presence of nuclei staining for MT. *Open arrowheads* indicate negative nuclear staining. *Open arrows* show cytoplasmic staining. (Unlabeled PAP, no counterstain, original magnification, ×200)

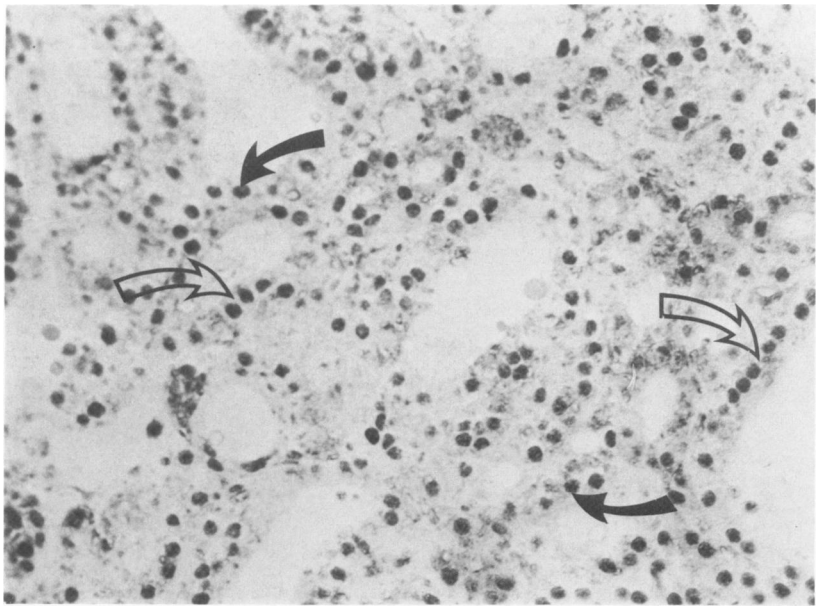

Figure 3—Normofollicular adenoma (simple type). *Closed arrows* show positive nuclear staining for MT. Cytoplasm (*open arrows*) are unstained. (Unlabeled PAP, no counterstain, original magnification, ×200)

and a weak cytoplasmic staining in all the three microfollicular adenomas (fetal pattern) (Figure 4). All the follicular adenocarcinomas, with the exception of 1, stained positively with the anti-MT serum. MT staining was observed in both the nucleus and the cytoplasm of the neoplastic cells (Figure 5). Nine of 9 papillary adenocarcinomas showed positive nuclear cytoplasmic staining for MT (Figure 6).

Discussion

The result of the present study demonstrates the presence of MT in various thyroid tumors. MT was localized in 91% of various thyroid tumors and only in 20% of normal thyroid glands. The immunohistochemical localization of MT in thyroid tumors was achieved by using a specific rabbit antisera to rat liver MT which readily cross-reacted with human MT.[15] MT has a unique amino acid composition and structure.[3] Therefore, the polyclonal rabbit antibody to rat liver MT-II will cross-react with both the isoforms of MT from various species and organs,[13,15] but it is unlikely that it will cross-react with other proteins in human thyroid, because no staining was observed in any of the control tissues. In this study, MT staining was observed in both the nucleus and the cytoplasm in most of the thyroid tumors. The biologic significance of the presence of MT in the cellular nucleus is not yet clearly understood. However, it may be classified as a morphologic feto-neonatal pattern for MT and may be related to increased synthesis of MT in these cells.[7,12] Similar changes in localization of heat shock proteins have been reported after induction of their synthesis by various experimental conditions.[17]

Previous reports suggest that the synthesis of MT in mammalian liver depends on the stage of develop-

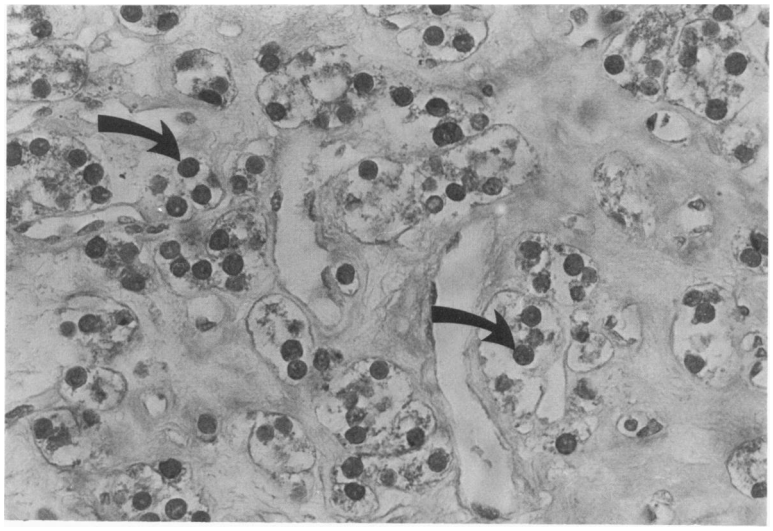

Figure 4—Microfollicular adenoma (fetal type). MT localization in the nucleus of fetal adenoma (*closed arrows*). (PAP, no counterstain, ×450)

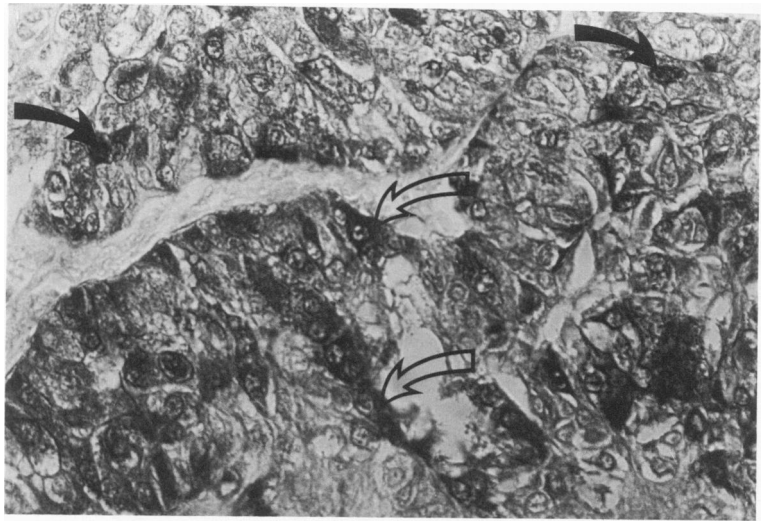

Figure 5—Follicular adenocarcinoma. Nuclear (*closed arrows*) and cytoplasmic (*open arrows*) staining for metallothionein. (PAP, no counterstain, original magnification, ×450)

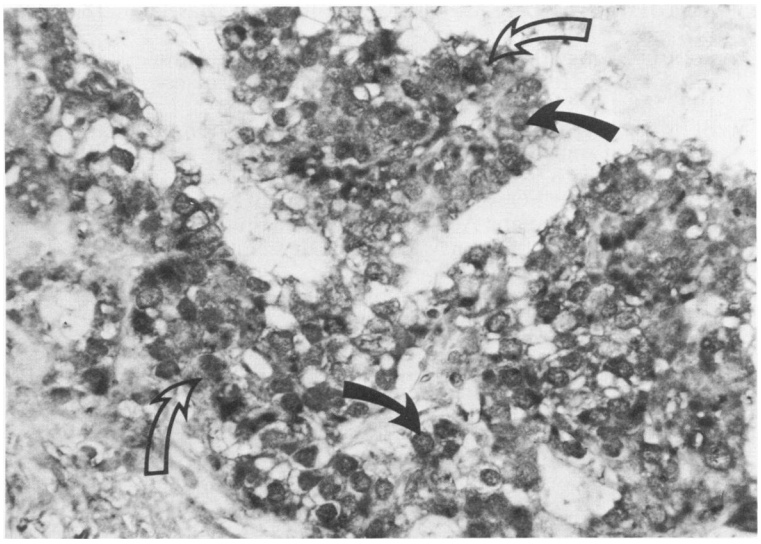

Figure 6—Papillary adenocarcinoma. MT localization in the nucleus (*closed arrows*) and cytoplasm (*open arrows*) of the neoplastic cells. (PAP, no counterstain, original magnification, ×450)

ment[7] and the physiologic conditions.[18] The levels of both hepatic zinc and MT in mammals are high during late fetal and early neonatal life, and they decrease dramatically by weaning in rats.[7,19] Although there is very little MT synthesis in the liver of adult animals, its synthesis can be induced by injection of certain metals[20–22] and stress conditions.[18,23] MT has been localized in the cell nucleus and cytoplasm of rat liver and kidney after injection of cadmium chloride for about 2 weeks.[12] In the same study,[12] it was observed that MT localization in the cell nuclei occurred at the time of increased concentrations of MT in the liver and kidney cells.

The present study is the first attempt on immunohistochemical localization of MT in various thyroid tumor cells. The biologic significance of the presence of MT in the nucleus of thyroid neoplastic cells is not yet understood, but it may indicate increased synthesis of MT in these tumors during growth of the tumor cells. The different types of staining patterns (only nuclear, only cytoplasmic, and combined nuclear and cytoplasmic) for MT in various thyroid tumors cannot be explained by the type of tumor. Nuclear localization of MT was observed in both adenomas and carcinomas, and there was no distinct staining pattern specific to a particular type of tumor. However, in most of the carcinomas both nuclear and cytoplasmic staining for MT was observed (Table 1).

There is increased requirement for zinc during the synthesis and metabolism of proteins and nucleic acids during rapid growth and increased cellular metabolic activity.[24] MT has been suggested to serve as an intracellular zinc storage protein in the fetal liver.[7]

A report shows that MT can be detected in cells containing thymulin, a zinc-bound hormone in both human and mouse thymuses.[25] High levels of MT and α-fetoprotein have been observed in the visceral and parietal yolk sacs in mice during early fetal development and in the liver during late gestation.[11] Similar to α-fetoprotein, high levels of MT mRNA are present in certain hepatomas and differentiated teratocarcinoma cells.[11] These studies suggested that MT may have a developmental profile similar to that of α-fetoprotein. A recent study on the estimation of MT suggested that MT levels are decreased in hepatocarcinoma tissues as compared with those of normal liver samples.[26] However, it is possible that in that study the tumor tissues were obtained from terminal cases where the rapid growth rate has subsided. It is also known that various enzymatic activities and glycolytic pathways are increased to a maximum during rapid tumor growth and then markedly decreased.[27,28]

We have demonstrated the presence of MT in detectable amounts in 31 of 34 thyroid tumors analyzed. However, the significance of the various types of staining for MT in tumors is not understood clearly. More studies are needed to delineate the factors involved in the reexpression of fetal genes such as that of MT in tumors and also to evaluate the potential use of MT as a tumor marker.

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