

The Significance of the Nuclear and Cytoplasmic Localization of Metallothionein in Human Liver and Tumor Cells

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Metallothioneins are a group of low-molecular-weight intracellular proteins present in high levels in fetal mammalian livers, bound to zinc and copper. They are also present in two major isoforms in low basal levels in various organs of adults in several species. Although a number of functions have been proposed for metallothioneins, their major biological roles may be in the storage of zinc and copper during rapid growth and development, and also in the detoxification of certain toxic metals. In adult liver, metallothionein is mainly localized in the cytoplasm, it is localized also in the hepatocyte nuclei in human fetal liver and fetal and neonatal rat liver, as determined by immunohistochemical staining with a specific metallothionein antibody. Because of its high expression in fetal development, the potential role of metallothioneins in human tumors was investigated. The cellular localization of metallothionein was demonstrated in various human tumors such as thyroid tumors, testicular germ cell carcinoma, bladder transitional cell carcinomas, and salivary gland tumors. In most of these tumor tissues, metallothioneins were found in high levels in nucleus and cytoplasm in both benign and malignant tumors, although the proliferating edge of the malignant tumors showed most intense metallothionein staining. The expression of metallothionein is not universal to all tumor growth; its presence may depend on various factors, such as the type of tumor, cellular origin, morphological heterogeneity, or stage of growth. Human testicular seminomas, which are well differentiated, showed little expression of metallothionein irrespective of the staging, as compared to less well-differentiated embryonal carcinomas. Preliminary studies with DNA flow cytometry on adenocarcinoma of breast and colon, using a fluorescent antibody to metallothionein, showed that metallothionein was present in the nucleus of the epithelial cells only in the S-phase of the cell cycle. Thus the nuclear presence of metallothionein in tumor cells is indicative of their proliferative activity, and metallothionein staining may be used as a marker of the proliferation index of certain tumors. — Environ Health Perspect 102(Suppl 3):131-135 (1994).

Key words: metallothionein, cell cycle, differentiation, cell proliferation, immunohistochemistry, cancer, DNA flow cytometry, epithelial malignancies

Introduction

The cellular requirement of essential metals, their homeostatic control, and biological and toxicological effects are controlled by a number of specific metal-binding proteins. These metalloproteins are involved in the storage, transport, and biological properties of many metals. Therefore it is important to study the type of binding of metals to proteins and their intracellular localization to understand the biological functions and mechanisms of action of metals. The role of proteins such as ceruloplasmin, transferrin, and ferritin in the metabolism of copper and iron is extensively studied in various laboratories (1-5). Because of their high affinity for metals, metallothioneins may play a physiologic role in the absorption, storage, and metabolism of important trace metals such

as zinc and copper, as well as in detoxification of certain toxic metals such as cadmium and mercury. This brief review will focus on some of the recent work in our laboratory on the changes in intracellular localization of metallothioneins in the liver during mammalian development and on its expression in certain human tumors.

Although metallothionein was first isolated and characterized as a cadmium-binding protein from horse kidney (6), several studies (7-9) showed its presence in many

tissues as a zinc-and copper-binding protein. Most adult mammalian tissues contain low basal levels of metallothioneins which may vary with age and type of tissues. The presence of high levels of intracellular metallothionein during gestation and the early postnatal period, bound to zinc and copper in mammalian liver, is well documented (7-11). The estimation of metallothionein and metals in human liver and kidney for different age groups showed (12) high levels of zinc and copper bound

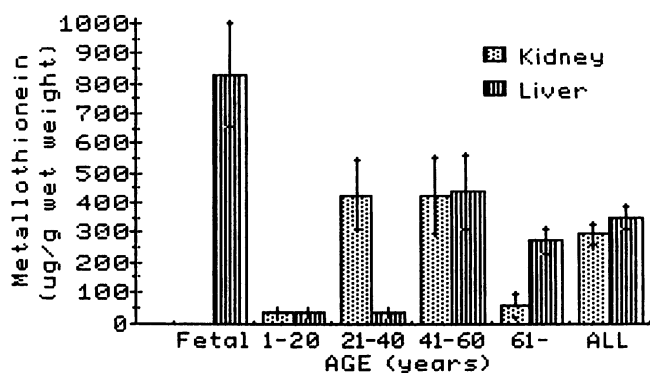


Figure 1. Metallothionein levels in human liver and kidney for different age groups. Metallothionein was estimated by Cd-saturation method; details of the data are presented in Chung et al. (12).

This paper was presented at the Second International Meeting on Molecular Mechanisms of Metal Toxicity and Carcinogenicity held 10-17 January 1993 in Madonna di Campiglio, Italy.

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Table 1. Localization of metallothionein in human tumors.^a

Organ	Tumor	Reference
Thyroid	Adenoma Follicular adenocarcinoma Papillary adenocarcinoma	Nartey et al. (17)
Testes	Seminoma Embryonal carcinoma Teratocarcinoma Choriocarcinoma	Chin et al. (20) Kontazoglou et al. (19)
Urinary bladder	Transitional cell carcinoma	Bahnon et al. (18)
Salivary gland	Pleomorphic adenoma Adenoid cystic carcinoma	Chauvin et al. (21)
Breast	Adenocarcinoma	Haile-Miskel et al. (22)
Colorectal	Adenocarcinoma	Haile-Miskel et al. (22)

^aArchival paraffin-embedded tissue sections fixed in formalin were stained for metallothionein using a specific polyclonal antibody and peroxidase-antiperoxidase method as described in detail by Nartey et al. (17).

to metallothionein in human fetal livers (14 to 16 gestational weeks). There is no detectable cadmium in any human tissue at birth. In contrast to high hepatic metallothionein levels in the fetus, the concentration of metallothionein in the kidney is low (Figure 1). The few samples analyzed from newborn children and those under the age of 20 showed low metallothionein levels in both liver and kidney. The binding of zinc and copper to metallothionein and its high expression in human fetal liver suggests that metallothionein may serve as a temporary reservoir for these metals during fetal development. The requirement for zinc is universal during mammalian growth and development. Since DNA and RNA polymerases and a number of other enzymes may require zinc, the homeostatic control of this metal is essential for catalysis of metabolic processes, nucleic acid metabolism, and protein synthesis.

Similar to human fetal liver, a high level of metallothionein was detected in fetal and neonatal rat liver. In Sprague-

Dawley rats, while metallothionein was barely detectable in liver on day 15 of gestation, a dramatic increase in hepatic metallothionein concentration was observed on day 18 and it was continued until birth (Figure 2). Both zinc and metallothionein concentrations in liver were highest soon after birth and they remained high until about 14 days of age. As metallothionein content in the liver increased on day 18 of gestation, the Zn/metallothionein molar ratio in liver decreased and then remained constant at about 11 in late gestation and neonatal periods. The results suggest that

about 65 to 70% of total zinc pool in the rat liver during this period is bound to metallothionein. The high hepatic concentration of both zinc and metallothionein in newborn rats decreased at a linear logarithmic rate as the pups grew older and attained near adult levels on weaning (9,11,13). Developmentally regulated expression of metallothionein also has been studied in cell culture using embryonal carcinoma cells induced to differentiate to primitive visceral endoderm by retinoic acid treatment (14). These results suggest that the increased synthesis of metallothionein in hepatocytes during fetal life may be due to the high requirement for essential metals such as zinc, which is necessary for various metabolic processes in embryonic development, cellular differentiation, and cell proliferation.

In addition to the high expression of metallothionein in mammalian liver during early development, there is a marked difference in the cellular localization of metallothionein as determined by immunohistochemical staining with a specific metallothionein antibody. We have demonstrated that although metallothionein is localized mainly in the cytoplasm in adult liver (Figure 3), it can also be detected in hepatocyte nuclei (Figure 4) in human fetal liver and in fetal and neonatal rat liver (9,11,13,15,16). The high expression of metallothionein and its mRNA in fetal rat liver is dependent on the maternal zinc sta-

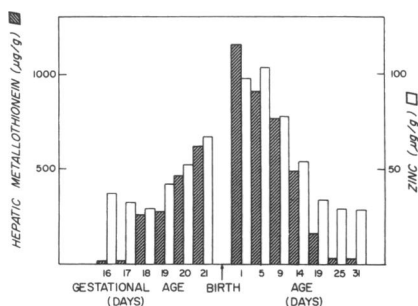


Figure 2. Hepatic zinc and metallothionein levels in rats during perinatal and postnatal development. The data are adapted from Panemangalore et al. (9) and Templeton et al. (13).

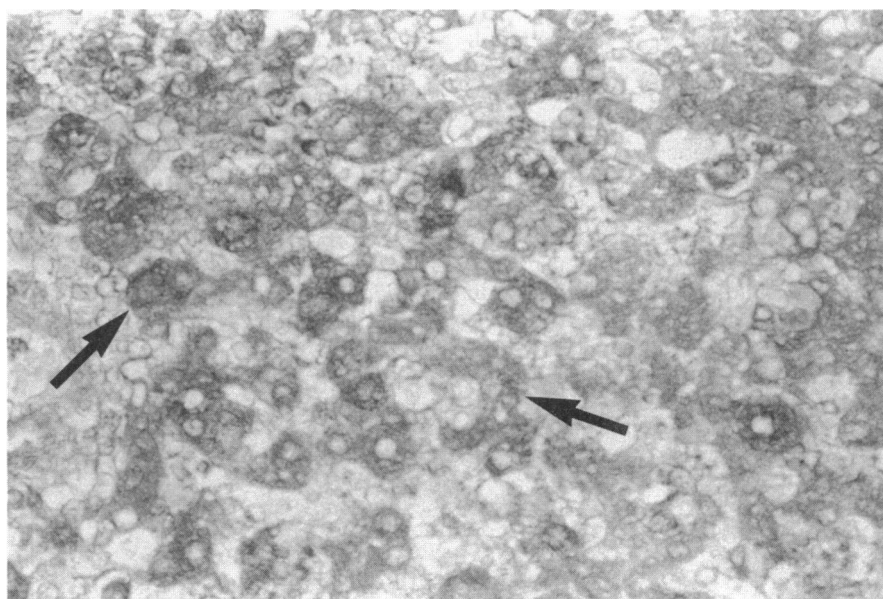


Figure 3. Cellular localization of metallothionein in adult human liver. Metallothionein was localized by immunohistochemistry using a specific polyclonal antibody and peroxidase-antiperoxidase method as described in Nartey et al. (15). The staining for metallothionein was mainly in the cytoplasm and most of the nuclei did not stain. The cytoplasmic staining is indicated by no counter stain.

Table 2. Distribution of metallothionein staining and clinical staging of germ cell tumors.

Tumor	Stage			
Seminoma (n = 10)				
Metallothionein staining	I	II	III	
0 ^a	1	6	1	
+	2	0	0	
++	0	0	0	
+++	0	0	0	
Nonseminoma (n = 22)				
Metallothionein staining	I	IIA	IIIB	III
0	1	2	0	0
+	3	0	0	0
++	0	6	0	0
+++	2	2	3	3

0^a no metallothionein staining; +, weak; ++, moderate; +++, strong staining. Nonseminomas are embryonal carcinoma, teratocarcinoma, and choriocarcinoma. Adapted from Chin et. al. (20).

tus (16). The hepatic metallothionein level in newborn rats is markedly decreased when the pregnant rats are fed a zinc-deficient diet at late gestation period (11,16). The neonates born from zinc-deficient mothers were able to induce metallothionein synthesis following injection of zinc salts, suggesting that the accumulation of zinc in the fetal liver during late gestation plays an important role in the induction of metallothionein. Although there are distinct differences in the nuclear-cytoplasmic presence of endogenous metallothionein in hepatocytes depending on the developmental stage, the significance of these changes in intracellular localization of metallothionein is not yet understood. These changes in developmental and intracellular localization profiles and gene expression of metallothionein suggest that metallothionein could be considered an oncodevelopmental gene product.

There is increasing evidence that certain proteins such as α -fetoproteins whose normal expressions are restricted to the embryonal and fetal periods, are frequently reexpressed in certain tumors. The re-expression of the proteins in tumors may be closely related to cellular differentiation and some of the proteins are used as tumor markers in certain neoplasia. In a report published in 1987, we used immunohistochemical localization to demonstrate the distinct presence of metallothionein in 31 of 34 human thyroid tumors in archival paraffin-embedded tissue samples; less than 20% of normal thyroid glands showed a diffused distribution of metallothionein (17). The positive staining for metallothionein was present in both nucleus and cytoplasm of thyroid adenomas and carcinomas. In subsequent studies (Table

1), we have demonstrated the presence of metallothionein in various human tumors including transitional cell carcinoma of urinary bladder (18), testicular embryonal carcinoma (19), other types of testicular tumors (20), pleomorphic adenoma and adenoid cystic carcinoma of salivary gland (21), and adenocarcinoma of breast and colorectum (22). These studies show that metallothionein is expressed in both benign adenomas and malignant carcinomas and can be detected in nucleus and cytoplasm of tumor cells. However, the staining for metallothionein is not universal to all tumor growth; but it may depend on various factors that may be related to the type

of tumor, its cellular origin, morphological heterogeneity, or stage of growth. The development of subpopulations of microregions of heterogeneous cells with a variety of growth and functional properties within tumors (23) creates additional obstacles to study the role of expression of certain specific proteins such as metallothionein.

The immunohistochemical staining of metallothionein in various tumors showed its presence mainly in epithelial cells, especially on the proliferating edge of the tumor (19). There was a distinct difference between metallothionein staining in seminomas and nonseminomas in human testicular tumors (Table 2). Pure seminomas showed little or no staining for metallothionein, irrespective of clinical stage, while nonseminomas stained heavily for metallothionein, especially in the more advanced stages (20). In general, seminomas are well-differentiated tumors and are very sensitive to both radiation and chemotherapy (24). However, it is unknown whether there is any direct relationship between the radiosensitivity or chemosensitivity of seminomas and the absence of metallothionein or differences in subcellular localization. The staining of metallothionein in pleomorphic adenomas and adenoid cystic carcinomas of human salivary gland showed its presence mainly in myoepithelial cells with intense staining in the proliferating edge (21). These tumors are known to be relatively radioresistant. The differences in metallothionein localization in

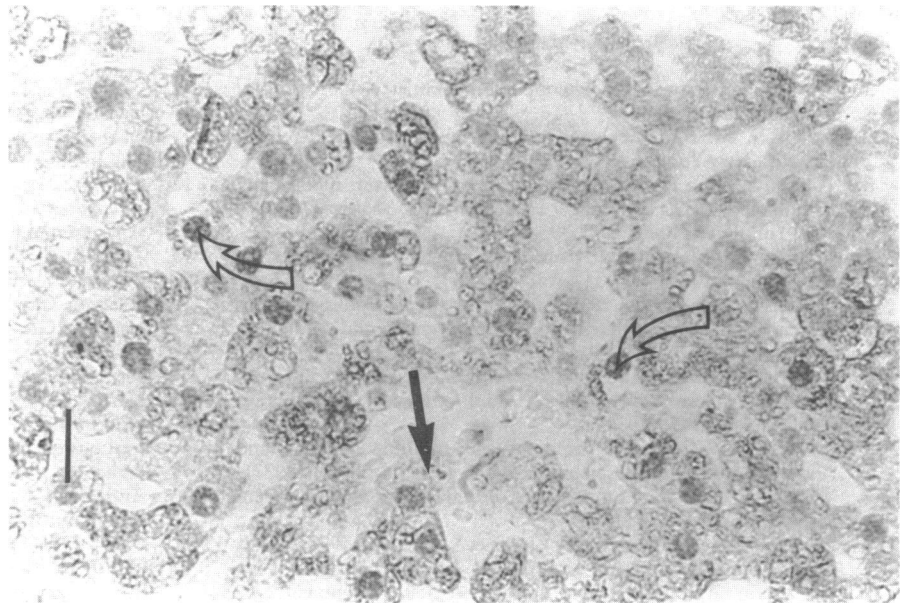


Figure 4. Cellular localization of metallothionein in human fetal liver. Fourteen-week-old fetal liver was stained with a specific antibody to metallothionein as described by Nartey et. al. (15). Cytoplasmic (closed arrows) and nuclear (open arrow) staining for metallothionein are shown. No counter stain.

embryonal carcinoma cells are closely related to their well-defined histomorphological patterns (19). This study showed that tumors with a typical glandular-papillary pattern, corresponding to the epiblastic or ectodermal pattern were strongly positive for metallothionein, while tumors growing in solid masses, designated embryoblastic, were negative or weakly positive for metallothionein at the periphery. These results suggest that the variability of metallothionein staining in human tumors may reflect their morphological heterogeneity, which may also correlate with the degree of differentiation and maturation of the tumor cells. Thus, the tumors with solid pattern, which resembles the better-differentiated seminomas, may have less cellular metabolic activity and less requirement for zinc than other morphological patterns of embryonal carcinomas, which therefore may have little metallothionein. These differences in growth patterns within the tumors may explain the different expression of metallothionein in various tumors.

The significance of the presence of metallothionein in the nucleus of hepatocytes during development (9,15) or partial hepatectomy (25) and also in certain tumor cells (17-22) is not yet understood but may be related to the interactions of metal ions like zinc with various nuclear constituents during the cell cycle. The metals that bind to metallothionein can also bind to histones, nucleolar RNA, and nuclear acidic proteins, and may modify gene

expression and phosphorylation of regulatory proteins. The presence of metallothionein in the cell nucleus can effectively donate zinc to several enzymes or chelate zinc from transcription factors such as zinc finger proteins (26). These protein-metal interactions in the nucleus can modify various cellular processes, including gene transcription and enzymatic activity.

The subcellular localization of metallothionein in primary cultured adult rat hepatocytes stimulated by epidermal growth factor and insulin showed that there was a transient presence of metallothionein in the nucleus during the early S-phase of cell cycle (27). Metallothionein also has been localized in hair follicles in normal skin and in the basal layer of hyperplastic epidermis, indicating its association with proliferation of epidermal keratinocytes (28). In a recent DNA flow cytometry study (22), we demonstrated the localization of metallothionein in the nucleus of both diploid and aneuploid cells during the S-phase and not during G-1 phase of cell cycle of adenocarcinomas of breast and colon. In a previous study (9), the attempts to demonstrate the presence of metallothionein in cell nuclei isolated by biochemical techniques from newborn rats were not successful because of the leaching out of low-molecular-weight metallothionein through the nuclear pores. In the DNA flow cytometry study, the cell nuclei were isolated from archival tumor tissue, and fixed in formalin by the method of

Hedley et al. (29). The polymerized form of metallothionein in the nucleus of formalin-fixed tissues cannot leach out, and therefore intact nuclei containing metallothionein can be isolated. Thus the physiological role of metallothionein is closely related to its nuclear presence in the proliferating phase of the cell cycle, where metallothionein may have a significant role to supply zinc to enzymes involved in the synthesis of nucleic acid and protein or to chelate zinc from transcriptional factors.

It is well established that the cell kinetic status of malignant tumors has prognostic implications (30,31). An active tumor growth implies cell proliferation and loss of differentiation, which may depend on the growth fraction, cell cycle time, and cell loss. The abnormal ploidy and elevated proliferative activity are associated with poor clinical outcome in locoregional breast, non-small cell lung, ovarian, and colorectal cancers. Therefore, for diagnostic and therapeutic purposes, it is of interest to characterize tumors with expression of different antigens related to the cell cycle. The results of preliminary DNA flow cytometry studies of breast and colon carcinoma to localize metallothionein in cellular nucleus suggest that it may provide a good indication of the proliferating fraction of cell cycle in tumors. Further studies are required to demonstrate whether metallothionein labeling index in flow cytometry can be used for prognosis in histopathological evaluation of epithelial malignancies.

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