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World J Gastroenterol 2007 February 21; 13(7): 993-996
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Metallothionein: An overview

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Received: 2006-07-27 Accepted: 2007-01-10

Abstract

Metallothioneins (MTs) were discovered in 1957 by Margoshes and Vallee and identified as low-molecular weight and sulphydryl rich proteins. It is not surprising that most mammalian tissues contain age related basal levels of MTs since they are involved in metalloregulatory processes that include cell growth and multiplication. In an effort to understand the biology of this intriguing tumor, various biomarkers such as oncogenes, p53 tumor suppressor gene, waf 1 protein, proliferating cell nuclear antigen, telomerase, microsatellite markers and cytogenetic changes have been examined. One biomarker which has recently shown to be expressed in various human tumors but still less reported in carcinoma is MT. Immunohistochemical detection of MT proteins in cold acetone-fixed paraffin embedded liver sections was performed by the streptavidin-avidin-biotin immuno-peroxidase complex method.

Key words: Metallothioneins; Protective function; Immuno-histochemical detection; Anti-oxidant character; Metal regulatory gene; Oncogene; Apoptosis; Genotoxic; Non-genotoxic environment; Detoxification

Thirumoorthy N, Manisenthil Kumar KT, Shyam Sundar A, Panayappan L, Chatterjee M. Metallothionein: An overview. *World J Gastroenterol* 2007; 13(7): 993-996

http://www.wjgnet.com/1007-9327/13/993.asp

INTRODUCTION

Metallothioneins (MTs) were discovered in 1957 by Margoshes and Vallee and identified as low-molecular weight sulphydryl rich proteins. Due to their high metal content and unusual bioinorganic structure, they are distinguished as metalloproteins. It is not surprising that most mammalian tissues contain age related basal levels of MTs since they are involved in metalloregulatory processes that include cell growth and multiplication. The presence of high levels of MTs in developing mammalian cells is well documented and it has been suggested that the MT-expressed protein is similar to onco-developmental gene products such as alpha-fetoprotein[1].

Classification of MTs

MT is a small protein with a high affinity for divalent heavy metal ions. MTs are a family of Mr 6000 proteins comprised of MT-Ⅰ, MT-Ⅱ, MT-Ⅲ and MT-Ⅳ classes with multiple isoforms within each class. MT-Ⅰ and MT-Ⅱ are ubiquitously expressed and are stress inducible. MT-Ⅰ isoform inducibility is reported to depend on the embryonic germ layer from which a tumor is derived[2].

MTs are a group of ubiquitous low molecular mass cysteine-rich intracellular metal binding proteins. In human, MTs are encoded by a family of genes consisting of 10 functional MT isoforms and the encoded proteins are conventionally subdivided into four groups: MT-1, MT-2, MT-3 and MT-4 proteins. While a single MT-2A gene encodes MT-2 protein, MT-1 protein comprises many subtypes encoded by a set of MT-1 genes (MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H and MT-1X), accounting for the micro-heterogeneity of the MT-1 protein. Different MT genes in humans possibly play different functional roles during development or under various physiological conditions[3,4].

The known functions of MTs include metalloregulatory roles in cell growth and differentiation, and enhanced synthesis of MTs in rapidly proliferating tissues suggests its crucial role in normal and neoplastic cell growth[5].

Characteristics of MTs

These intracellular proteins are characterized by their unusual high cysteine content (30%) and lack of aromatic amino acids. Because of their rich thiol content, MTs bind a number of trace metals including cadmium, mercury, platinum and silver, and also protect cells and tissues against heavy metal toxicity. Additionally MTs are among the most abundant intracellular aspects for biologically essential metals, zinc and copper. MT metal-thiolate fractions being dynamic and of high affinity also facilitate metal exchange in tissues[6].

They are present in a great variety of eukaryotes[3], functioning as anti-oxidants; they also play a protective role against hydroxyl free radicals. This is relevant in tumors (nasopharyngeal carcinoma) which are known to be

markedly radio-sensitive, where radiotherapy (which kills cells *via* free-radical-induced apoptosis) is the treatment of choice[7].

MT AS TUMOR MARKER

Alteration in MT function due to carcinogenesis

Development of carcinogenesis is a dynamic process in which irregulation of gene function occurs. Time is crucial in this process. During a latency period of several years or even decades, the genetic material of cells is subject to multiple 'hits' by damaged substances, carcinogens, etc. Accumulation of damage leads to altered gene function and colonal expansion of mutated cells. However, most of these early lesions never develop into more aggressive and invasive states, but disappear spontaneously. This feature of carcinogenesis does not solely influence the initial phase of this process but actively intervenes and changes the course of the disease[5].

Protective functions of MT

MTs have a high binding affinity to bivalent metal ions. A number of studies have demonstrated the presence or enhanced synthesis of MT in rapidly proliferating normal cells, regenerating cells and cancer cells. The studies have suggested a relationship between high expression of MT protein and aggressive neoplastic cell growth. Due to their nucleophilicity, MTs have been shown to protect cells against the cytotoxic effects of electrophilic anticancer drugs. However, protection of cells by MT from the cytotoxic effect of agents such as 1-β-D-arabinofuranosyl cytosine and nuclear factor DF-α must involve mechanisms other than simple covalent binding[8].

Relation between MT and apoptosis

Recent reports of various studies have shown that the enhanced expression of MT in cells induces the anti-apoptotic effects and a lack of MT in MT null cells increases the susceptibility to apoptic cell death after exposure to certain anticancer drugs. The down-regulation of MT in MCF-7 cells within 18-mer antisense has not only shown inhibition in growth but also initiated apoptosis[8].

MTs INVOLVED IN PATHOPHYSIOLOGICAL PROCESSES IN HUMAN

Role of MT in detoxification

MTs play a homeostatic role in the control and detoxification of the heavy metals; several evidences indicate that MT has the capacity to scavenge reactive oxygen metabolite (ROM), particularly the hydroxyl radical. These substances which are produced continuously during normal aerobic metabolism may become noxious in situations of imbalance with endogenous antioxidants and then can induce DNA damage, lipid peroxidation, enzyme oxidation, *etc.*, leading to cellular destruction, chromosomal aberrations and finally to cancer. Paradoxically, by anticancer treatment such as radiotherapy, chemotherapy and photodynamic therapy, tumor cells are killed by generating toxic amounts of ROM[2].

MT was considered as a potential prognostic marker in invasive ductal carcinoma of the breast[9], skin[10], cervix[11] and pancreas[12]. Irregular cell growth, due to increased cell proliferation or failure of cells to undergo apoptosis is recognized as a major contributory factor to the malignant process.

Dual functions of MT

MTs appear to perform dual functions of influencing the growth and survival of tumor cells as they are known to have metallo-regulatory functions in cellular repair processes, growth and differentiation and to play a protective role in oxidative stress by scavenging free radicals, thus protecting the cell against apoptosis induced by oxidative stress. MT can be induced by a number of endogenous and exogenous stimuli including glucocorticoids, interferon, interleukin-1, progesterone, vitamin D_3 endotoxins, serum factors, and heavy metals, storage of metal ions and regulation of cellular zinc[1].

MT and its response to anti-cancer drugs

The association of MT expression with spontaneous mutagenesis response to anti-cancer drugs and tumor progression has emerged in recent years. This has been more significant in the later stage, with reliable methods for detecting the two isoforms of MT-Ⅰ and MT-Ⅱ by immunohistochemistry in archival tissues. MT over expression has been associated with more malignant and higher grade tumors in some cancers and with more differentiated lower grade tumors in others[6].

Over expression of MT

The *in vitro* studies suggest that MT over expression in ovarian cancer may induce chemo-resistance. It has been proposed that the sequestration of drugs or their metabolites may prevent the reaction of these compounds with the respective intracellular target, thus decreasing the efficacy of certain anti-cancer drugs.

Recurrence of drug resistant ovarian tumors that initially appear to respond well to chemotherapy may be explained by over expression of drug resistance proteins such as MT.

Another opinion of the same investigators is that growth factor signals might activate transcription factors that control the expression of drug-resistant enzymes and proteins. However, two independent groups of investigators were unable to find a direct causal relationship between MT expression and chemo-resistance[1].

Genotoxic and non-genotoxic effects

Humans are exposed to mixtures of genotoxic and non-genotoxic environmental chemicals that may be linked to cancer. Robust biomarkers of somatic stem cell mutation and mutant clonal expansion may provide cancer surrogates that are useful for risk assessment. Acquired mutation of a selectable endogenous reporter gene like glucose-6-phosphate dehydrogenase (G6PD) within a colonic crypt stem cell induces a crypt-restricted phenotype change.

Stem cell mutation

Stable crypt-restricted immunopositivity for MT is a recently described stem cell mutation marker for mouse colon that can be assayed in paraffin-fixed tissue sections and has been validated against the G6PD assay. MT-immunopositive crypt frequency has shown a dose response to three different chemical mutagens that further confirmed the evidence that it is a somatic mutation marker[13].

Low levels of MTs and their susceptibility

Accumulating evidence indicates that cells with low levels of intracellular MT are more susceptible to DNA damage and apoptotic death after exposure to stress stimuli including oxidative stress, whereas prior induction of MT appears to confer protection. The findings of changes in unicellular localization of MT from cytoplasm to the nucleus during early differention of myoblasts coincide with increased apoptosis of newly formed myotubes[14].

Markers of carcinogenesis

The basal expression of MT in normal cells has generally been associated with heavy metal detoxification, intracellular trace elements storage and scavenging of free radicals. Authors Tridip and Thirumoorthy studied the roles of alterations of hepatic levels of trace elements and significance of the expression of MT and Ki-67 proteins as important markers of carcinogenesis in the development of pre-malignant phenotype and the reported dose (0.5 ppm) of vanadium in suppressing 2-AAF-induced carcinogenicity. Several reports are available to show that, over expression and up-regulation of MT and Ki-67 proteins are associated with the carcinogenic processes. But the reports documenting the anti-neoplastic potential of chemo-preventive agents in modulating these indicts are meager.

It was reported that the chemo-preventive potential of vanadium is suppressing in MT and Ki-67 expression in preneoplastic rat liver. The role of vanadium on p53 expression and induction of apoptosis in a defined rat model of experimental hepato-carcinogenesis has also been brought into focus[15].

MT as anti-oxidants

MTs can function as antioxidants. It has been suggested that intracellular oxidants may play a role in anticancer drug mediated programmed cell death and it was observed that MT expression can be regulated by ambient oxygen levels. This has led to speculation that MT may be an inducible anti-apoptotic gene product[6].

Proteins like MT may play an important role in carcinogenesis, including tumor cell pathology and drug resistance. It was shown that colorectal and gastric carcinogenesis was associated with a significant increase in the level of manganese (Mn-SOD) containing superoxide dismutase, an antioxidant enzyme that detoxifies superoxide to hydrogen peroxide[2].

Factors influencing synthesis of MT

The synthesis of MT is regulated by polymorphic genes and induced by many factors such as metals, hormones, cytokinen, drugs and physical and oxidative stresses. Tumor MT levels have also been reported to correlate with resistance to anticancer reagent[16].

MT regulates intracellular concentration of zinc and other metal ions. MT over expression can influence transcription, replication and protein synthesis, and might explain why MT over expression is associated with high-grade tumors, including carcinomas of the head and neck[17].

IMMUNOSTAINING OF MT

Positive control

Immunohistochemical detection of MT protein in cold acetone-fixed paraffin embedded liver sections was performed by the streptavidin-avidin-biotin-immuno peroxidase complex method (Jin *et al*, 2002). Briefly, 5 μm thin sections on poly-L-lysine coated slides were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 1% H_2O_2 in 0.1 mol/L Tris-NaCl (pH 7.6) for 30 min. After incubation with 5% normal goat serum for 1 h at 37℃, sections were incubated overnight at 40C with the primary antibody rabbit anti-rat MT-1 in 1% BSA using 1:50 dilution. Sections were then incubated with a bio-tinylated secondary antibody goat anti-rabbit IgG (Sigma) for 30 min at 37℃ with 1:200 dilutions.

This was followed by incubation with streptavidin peroxidase (1:100) for 1 h and subsequent chromogen development with 0.5% of 3, 3'-diamino benzedrine tetra hydrochloride (DAB) and 0.33% H_2O_2 in 0.5 mol/L Tris-NaCl as substrate. The sections were then counterstained with Harris haematoxylin (H&E), then dehydrated, mounted and served as positive control.

Negative control

Negative control was prepared following the above steps but by omitting the primary antibody. MT immuno-staining was considered positive when the nuclei and cytoplasm of the hepatocytes were stained prominently (purplish brown/reddish brown). MT immunoreactivity was expressed as percentage of immuno-positive cells. A total of 10 high power fields were randomly chosen. The numbers of positive cells were determined in relation to the total number of cells in the field[15].

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S- Editor Liu Y **L- Editor** Ma JY **E- Editor** Lu W