Metallothioneins in neoplastic cells – distribution in subfractions

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Abstract: Metallothioneins are intracellular macromolecules with a high potential for binding metallic ions. They are proteins which bind metals indispensable for the organism, as well as proteins which have found their way into the body by chance as a result of environmental pollution (e.g. cadmium, lead). Further studies have shown that the content of metallothioneins rises in neoplastic cells. Apart from metals entering a cell, metallothionein synthesis induction can be triggered by some inflammatory agents or reactive forms of oxygen. It follows from the studies of metallothionein content in a cell that these proteins are present in all cellular subfractions, and the highest content of metallotioneins is present in the cytosol subfraction. It has been found that the cellular subfractions of a neoplasm are characterized by significant changes in the distribution of metallothioneins.

Key words: metallothionein, neoplastic cell, cell subfractions, astrocytoma

Abbreviations: ADP – adenosine diphosphate; Cd-MT – cadmium bound metallothionein; EGF – epidermal growth factor; HGF – hepatocyte growth factor; MT – metallothionein; NO – nitrogen oxide; ROS – reactive oxygen species; SH – thiolic groups; TNF – tumour necrosis factor; Zn-MT – zinc bound metallothionein.

INTRODUCTION

Metallothioneins (MT's) are low molecular weight proteins of 6-7 kDA. MT molecules contain 20 residues of cystein, which approximately amounts to 30% of the amino acid content [1]. Large amounts of cystein with sulphydril groups determine protein activity. Metallothioneins help maintain the homeostasis of metallic ions crucial for undisturbed metabolism (zinc, copper); they regulate the functioning and biosynthesis of zincprotein (such as zinc dependent transcription factors), participate in detoxication processes, protect the cell against reactive oxygen species (ROS), ionizing radiation and electrophilic pharmaceuticals used in the treatment of cancer, and from other mutagens [2-5]. Metallothionein synthesis is triggered by many different factors: heavy metals, inflammatory agents, free radicals, glycocorticoids and pharmacological agents [6-11].

METALLOTHIONEINS IN THE NEOPLASTIC CELL

An increased intracellular content of metallothioneins has been demonstrated in numerous neoplasms in humans and animals [12-16]. Studies on animals have shown that there was an increase of MT content not only in the neoplastic tissue but also in the healthy liver [17]. Also, the concentration of MT in the plasma rose along with the increase of MT concentration in the liver.

The mechanism triggering the induction of MT synthesis in neoplastic diseases is little known. Numerous studies have shown that elements such as cytokins, interleukin 1, interleukin 6, tumour necrosis factor (TNF) and interferons could be the culprits [18, 19]. It is possible that neoplastic cells can release cytokins into the blood stream which trigger MT synthesis, both in the neoplastic lesion as well as in the healthy liver. Some authors insist that increased expression of metallothionein synthesis may mean increased cell metabolism [20], faster cell proliferation [21] or onkogen activation [22].

Tashiro-Itoch et al. [23] suggest that MT expression may depend on the degree of cancer differentiation. The above-mentioned authors used immunohistochemical methods and observed a correlation between the amount of metallothioneins and the proliferative activity of tumour cells. MT count decreased with the degree of histological differentiation: the highest level of MT was seen in highly differentiated cells, a lower level characterized low differentiated cells, and no MT was present in poorly differentiated cells.

Fresno et al. [24], Hearhev et al. [25], Shmid [26] and Zhang et al. [27] are of the opinion that MT expression seems to correspond with the progression of the disease and poor prognosis of cancer.

In further studies, MT content was assayed in cell subfractions. From the studies it follows that metallothioneins appear in all examined cell subfractions. The highest content of MT is present in the cytosol subfraction. In a neoplastic cell signifiant changes were observed in the distribution of metallothioneins [7-11, 28, 29].

NUCLEAR SUBFRACTION

A higher MT content in the nuclear subfraction has been reported by numerous authors [7, 10, 11]. Nartev et al. [7,8] and Tsijikawa et al. [10, 11] in their experiments on rats observed that during the embryonic development and at

infancy metallothioneins are mostly located in the nuclear fraction. MT content in the nuclear subfraction of rat liver was highest immediately after birth and gradually decreased until it reached the level typical for adults.

The significance of these changes in MT distribution was unclear. Initially, it was thought that such a location protects proteins from degradation [7, 8]. Later, it was found that the presence of large quantities of MT in the nucleus was connected with a higher demand for zinc during increased synthesis of nucleic acids. This has been confirmed by subsequent studies [30]. Moreover, it was shown that MT concentration in a hepatocytic nucleus is higher also during the regeneration of hepatic tissue.

In our studies [28] concerning breast cancer (*carcinoma ductale invasivum*, $T_2N_1M_0$) we noted a higher content of MT in the nuclear subfraction compared with that in the mastopathic lesion (*mastopathia fibrosa cystica*) treated as a reference group. Also, in the studies of gliomas (*astrocytoma*) at various degrees of histological differentiation, we noted a higher content of MT for the nuclear subfraction in poorly differentiated cancers G-4 [29].

The literature offers little elucidation on the factors causing metallothionein translocation to the nuclar subfraction. Tsuikawa et al. [11] suggest that one of many such factors is the *hepatocyte growth factor* (HGF). Due to the fact that the pores in the nuclear membrane can allow permeation of the molecules of 60 kDa molecular mass, it seems puzzling which factors - given the small molecular mass of metallothioneins - maintain a high degree of the protein concentration between the nucleus and the cytoplasm. Tsuilawa et al. [10, 11] offer two theories. According to one, metallothioneins could be bound with some unspecified macromolecules in the extranuclear space, and only factors such as HGF (*hepatoma growth factor*), EGF (*epidermal growth factor*) or insulin could cause dissociation and allow their transmission through the pores of the nuclear membrane. According to the other theory, MT-macromolecule coupling is supposed to be located in the nucleus. The dissociation of the macromolecule-metallothionein coupling might depend on the cell cycle as it was shown that there was an MT increase in the nucleus in the S and G_2 phases. The dissociation might also take place as a result of the activity of the growth factors.

During the study of MT functioning in the nucleus it was stated that the loci binding zinc with metallothionein is very similar to GL4 protein domain binding with DNA, which is a transcription factor in *Saccharomyces cerevisiae*. This discovery suggests that MT might be the kind of proteins that enable transcription by forming so-called zinc clusters. In mammalian cell cultures they can also remove zinc from transcription factors which contain in their make-up "zinc digits", such as Sp1, Xenopus TFIIIA [31, 32].

MITOCHONDRIAL SUBFRACTION

The literature does not explain which conditions are required to trigger MT relocation to the mitochondrial subfraction. It is known that the permeability of the outer mitochondrial membrane is 10,000 which, given the MT molecular mass as 6-7 kDa, enables the protein translocation to the intramembrane space. In our studies, we found evidence of noticeable translocation of MT to the mitochondrial subfraction, both in breast cancer and in brain carcinoma [28, 29].

There is evidence of MT influence on the metabolism of the mitochondria. Simpkins et al. [9] *in vitro* examined the effect of MT on mitochondrial functioning. They demonstrated that MT-1 (resulting from stress) modulates oxygen consumption in the mitochondria isolated from rat liver. Metallothionein then increases oxygen consumption in the presence of succinate and suppresses oxygen consumption in the presence of ADP.

The above-mentioned authors [9] suggest that a primary effect of MT activity is the depolarization of the inner mitochondrial membrane, followed by increased permeability, which results in the swelling of the mitochondria.

The influence of MT on the electron transportation in the respiratory chain is possible thanks to the thiolic groups present in the MT - the SH, which can be electron donors for cytochrome C or other electron acceptors. The authors also demonstrated that the effects resulting from MT activity on the mitochondria are suppressed by cyclosporin – a compound that seals the outer mitochondrial membrane.

Furthermore, the cited authors [9] chose to use spermin in their studies, having shown previously that this compound reverses the effect of MT activity such as, among other things, the swelling of the mitochondria.

The disturbing of the balance between the compounds may result in a disturbance of the mitochondria functioning. A decrease in the level of spermin can therefore lead to depolarization, swelling and abnormal oxygen utilization. A decrease in spermin can take place during the suppression of ornithine decarboxylaze, a decrease in the level of substrate for ornithine decarboxylaze, or a decrease in the level of arginin. Arginin is also a substrate for the syntase of nitrogen oxide (NO), an enzyme limiting NO synthesis in stress [9].

MICROSOMAL SUBFRACTION

The essential part in MT removal is played by lysosomal subfraction. Using different inhibitors for cathepsins, it was discovered that cathepsin B is crucial for the process. The rate of MT breakdown was also examined depending on whether it was a free protein (apometallothionein) or one containing metals. The protein that did not contain metals broke down faster compared with zinc and cadmium bound metallothionein (with Cd-MT breakdown slower than Zn-MT). The studies indicate the protective role that metals play in MT breakdown. For example, the metal content of at least 5 eq Zn/mol of protein suppresses metallothionein breakdown in a dramatic way [33].

Metal release from the protein is also found in lysosomes since the process needs an acidic environment. Klaassen et al. [33] studied the process of metal release depending on pH. With pH at 4.5, about 70% of the zinc dissociated from MT, and with pH at 4.0 all the zinc was released. The content of metals significantly affects the duration of biological functioning of metallothioneins.

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