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REVIEW

Metallothionein – a promising tool for cancer diagnostics

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Abstract: The latest research outcomes indicate that metallothionein (MT) levels in peripheral blood and serum from cancer patients can provide many interesting information about type or clinical stage of the disease, or response to therapy.
MT plays a key role in transport of essential heavy metals, detoxification of toxic metals and protection of cells against oxidation stress. Serum MT levels of cancer patients are three times higher than control patients (0.5 μM). The elevated MT levels in cancer cells are probably related to their increased proliferation and protection against apoptosis.
Automated electrochemical detection of MT allows its serial analysis in a very small volume with excellent sensitivity, reliability and reproducibility and therefore it can be considered as a new tool for cancer diagnosis *(Fig. 4, Ref. 55).* Full Text (Free, PDF) www.bmj.sk.
Key words: cancer, tumour, metallothionein, marker, blood serum.

It is a general knowledge that the sooner a cancer is detected, the better are the chances to treat it successfully. For this purpose new diagnostic tools and approaches are in development. The latest research indicate that metallothionein level in peripheral blood and serum of cancer patients can provide a lot of interesting information about the type or clinical stage of the disease or response to therapy.

What is metallothionein?

Since 19th century it has been known that various organs such as liver, kidney, gonads or spleen contained higher level of heavy metals. In 20th century an elevated content of heavy metals were discovered also in tumour tissues (1–4). In 1957, protein rich in cysteine and able to bind cadmium was isolated from the horse kidney and named as metallothionein according to its structural properties (5). Further, this protein and metallothionein-like proteins were found in tissues of other animal species, yeasts, fungi and plants (6–8). The highest metallothionein (MT) content has been found in the same tissues, where formerly the highest level of heavy metal was determined. An elevated metallothionein level was determined in various tumour tissues (9–11). It was also shown that expression of MT is inducible by the presence of heavy metals (6, 8, 12, 13).

Four major isoforms (MT-1 through MT-4) have been identified in mammals (14). Genes for MT consist of eleven MT-1 genes and one gene for every other MT isoform (the MT-2 A gene, MT-3 gene, and MT-4 gene) (15). MT-1 and 2 have ubiquitous tissue distribution particularly in liver, pancreas, intestine, and kidney (16), whereas MT-3 is found in brain (17). MT-4, which was found in epithelial cells, is less known (18).

Is there any relation between metallothionein and cancer?

The first discovered and still not fully understood roles of MT are transporting essential heavy metals, detoxification of toxic ones and protection of cell compartments against oxidation stress (19). Thanks to high cysteine content it is able to bind up to seven divalent and up to twelve monovalent heavy metal ions (Fig. 1). Although MT exhibits the highest affinity for Cu^{2+}, under physiological conditions Zn^{2+} ions are bound most frequently what contributes to its homeostasis. Toxic heavy metal ions (e.g. Cd^{2+}, Pb^{2+}, Hg^{2+}) are capable to displace Zn^{2+} from MT structure (19, 20). MT molecules with boud toxic heavy metals are transported to kidney, where supposedly their elimination from the organism occurs.

Thanks to thiol moieties from cysteine residues, MT has important antioxidant properties. In couple with glutathione (GSH), it is able to decrease the level of free radicals (ROS) and thus to protect easily oxidizable molecules against their action

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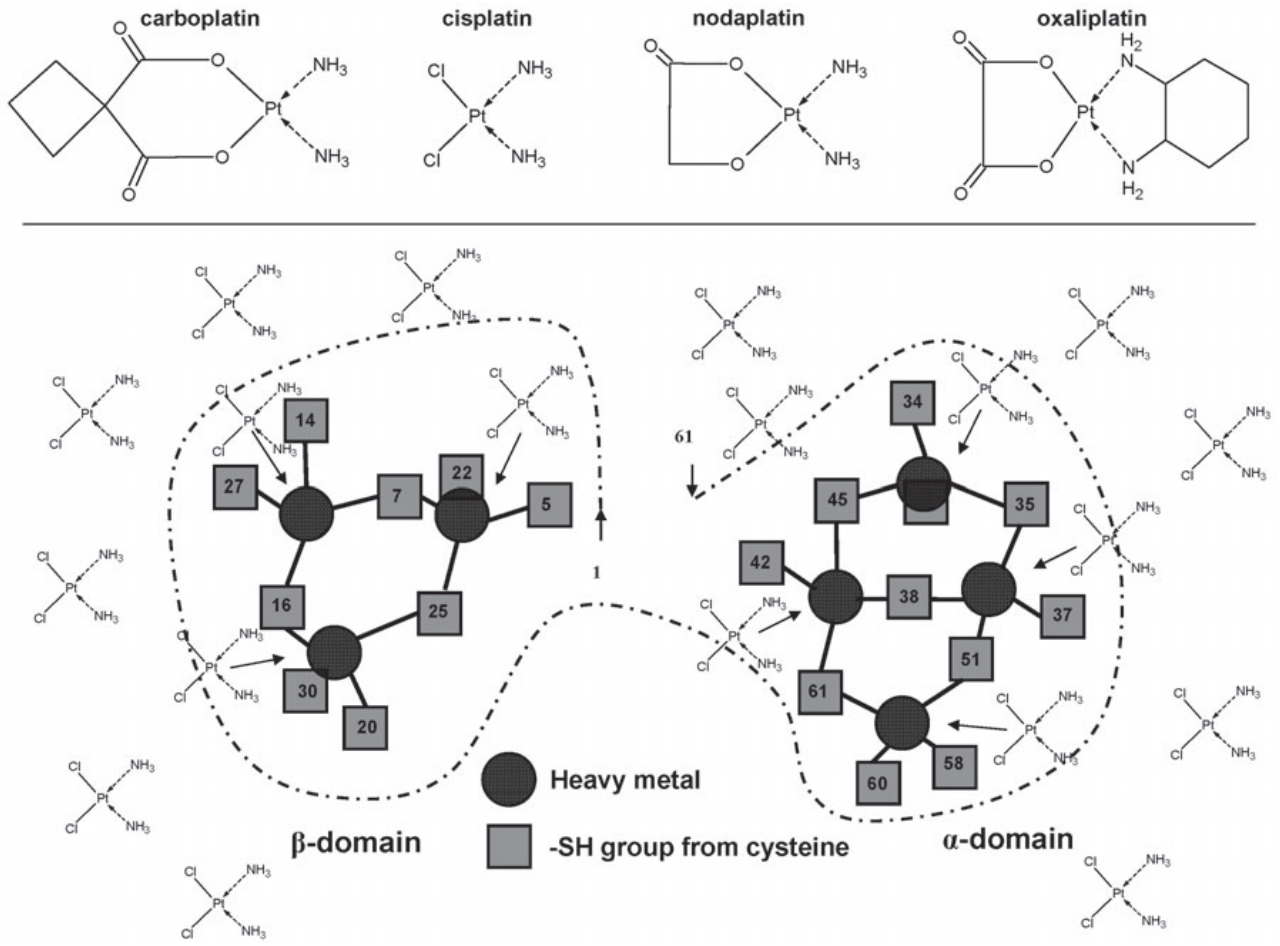

Fig. 1. Key roles of MT are transporting essential heavy metals, detoxification of toxic ones and protection of cell compartments against oxidation stress . Thanks to a high cysteine content it is able to bind up to seven divalent and up to twelve monovalent heavy metal ions. It is not surprised that MT can bind heavy metal based cytostatics e.g. cisplatin and its derivatives, and thus decrease the therapeutic concentration of these drugs.

(19). This protein plays a role in many intracellular physiological processes, where an increased occurrence of molecules causing oxidative stress can be expected (e.g. liver, digestive tract and kidney) and/or where preservation of structures susceptible to free radicals (nucleic acids, phospholipids membranes, proteins etc.) is needed such as proliferation or embryo development (17, 21).

Another role of MT consists in regulation of programmed cell death (22). An increased level of MT was found to prevent apoptosis in cell cultures. Two possible MT roles are regulation of intracellular zinc concentration and interaction of MT with some proteins involved in apoptosis. Zinc is an intracellular mediator of apoptosis, which can interfere with action of calcium. Zinc is able to inhibit many proteins connected to apoptosis, e.g. caspase-3 and some proteases. Zinc addition prevents DNA fragmentation and inhibits calcium-magnesium dependent proteases. MT interacts with the p50 subunits of NF-κB, with kinase domain of PKCl, and with GTPase Rab3A. Those interactions are important for growth of some tumours e.g. activation of NF-κB may mediate anti-apoptotic effect of MT. MT can also modulate the biological activity of p53 via zinc exchange. MT-1 and MT-2 regulate the level, activity and cellular location of the transcription factor NF-B (23–26). NF-κB is necessary to ensure cells protection from the apoptotic cascade induced by TNF and other stimuli through activation of anti-apoptotic genes and proto-oncogenes such as Bcl-2, c-myc and TRAF-1. In addition, apo-MT-1 (metal-free form of MT-1) but not MT-1 (MT-1 with metal ion) forms a complex with p53 (25, 27, 28). Activation of p53 is an important factor that increases metal-dependent expression of metal-responsive-elements (29).

The ability of MT to regulate protein expression by activation of zinc-dependent transcription factors and consequent transcription initiation was discussed (30, 31). Several authors report the evidence about metalloenzymes (de)activation by zinc supply from MT molecule (21, 32, 33). Another role of MT is the immunomodulation. It was shown that MT can interact with

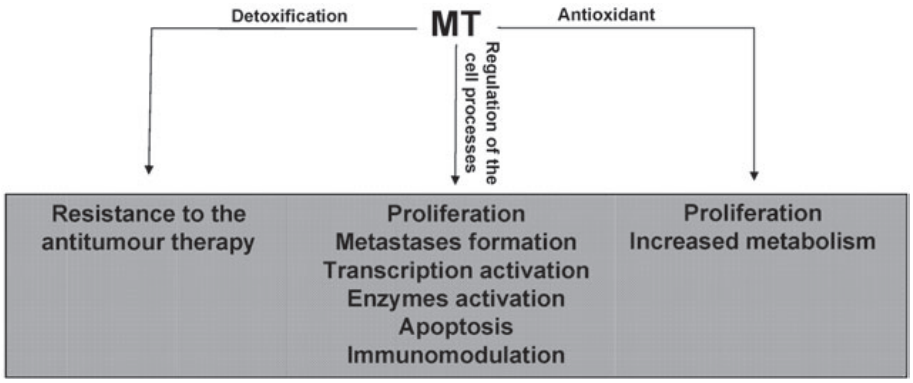

Fig. 2. Main roles of MT in healthy and tumour cells and tissues.

the cellular membrane of lymphocytes and modulate functions of cellular immunity (22). MT can be induced by acute phase cytokines (IL-1, IL-6, TNF-alpha, and IFN-gamma). It is possible to consider MT as a regulator of the immune response suppressing the autoimmune attack on self-tissues.

The role of metallothionein in tumour tissue remains still unclear. Nevertheless the main roles of these proteins in a tumour tissue are similar to those in healthy ones. Its increased expression in tumour cells is probably related to an increased proliferation, protection against apoptosis and others (10, 11). Scheme of these functions is shown on Figure 2. The connection between an increased MT expression and tumour invasiveness and activation of MMP (matrix metalloproteinases) degrading the extracellular matrix, which allows the tumour to grow and to form metastases, is still discussed. One of the possible explanations is the activation of the metalloproteinases by metallothioneins (34–36). It was published that MT can suppress the proper immune response in the tumour (37, 38).

An increased level of MT in tumour cells can be also related to formation of multidrug resistance, first of all on heavy metals based cytostatics e.g. cisplatin, its derivatives, arsenic and ruthenium compounds. It is related to radiation resistance of the tumour too (39, 41). Mechanisms of the tumour cell protection against cytostatics can be described as follows: i) cytostatics transport out of the cell or ii) detoxification of these compounds by MT (Fig. 3). MT molecules are able to bind platinum-based cytostatics and thus to decrease their therapeutic concentration (42).

Determination of MT level

Analytical and molecularly-biological instruments are used for detection of MT, but they differ in instrumentation, character of the information obtained and usability for particular purpose (42–44). Immunochemical methods like ELISA, immunocytochemistry, imunohistochemistry and western-blotting are probably the most utilized for metallothionein determination in clinical practice. For the research purpose, a chromatography with various detectors or spectrometric instruments are used (44). Detection of mRNA of MT isoforms using RT PCR or Northern blotting is also used in many studies (45).

Electroanalytical methods are very sensitive with a big potential for routine laboratory analysis thanks to their rapidity,

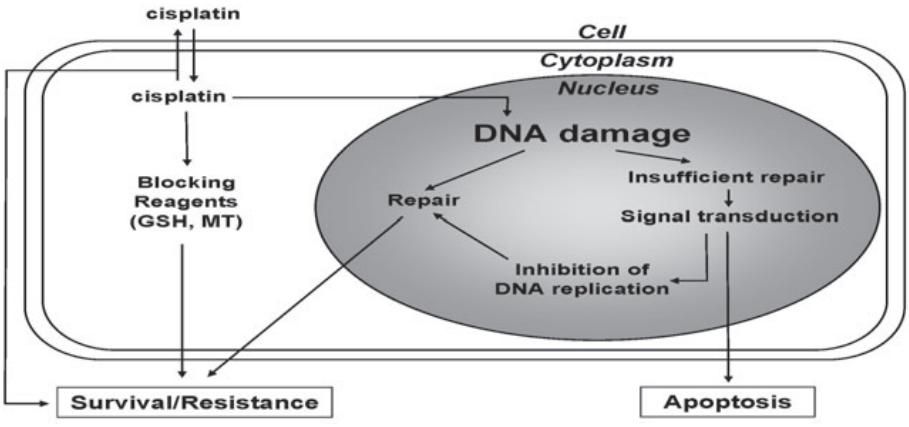

Fig. 3. Mechanisms of the tumour cell protection against cytostatics via synthesis of metallothioneins.

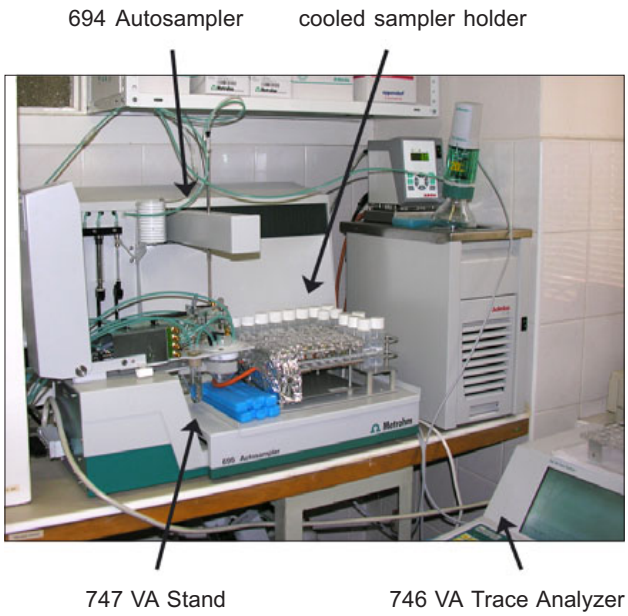

Fig. 4. Photo of stationary electrochemical analyser coupled with autosampler: 695 Autosampler with cooled sample holder, 747 VA Stand instrument with potentiostat/galvanostat using a standard cell with three electrodes and 746 VA Trace Analyzer for data processing. Vessels with washing water and the supporting electrolyte are other parts of instrument.

low-cost and miniaturization possibility (46). Its using in clinical practice is limited by high demands on laboratory stuff due to difficult automation. In 1933, Brdicka reaction was firstly used for detection of proteins. This method is based on electrochemical detection of the catalytic hydrogen evolution from the supporting electrolyte. The hydrogen evolution is catalyzed by complex of proteins thiol groups with Co^{3+} present in supporting electrolyte (47, 48). This method was successfully used for cancer diagnosis (49, 50). Thanks to a high content of SH moieties, MT exhibits a considerable electrochemical activity. The coupling of adsorptive-transfer technique with Brdicka method lowered detection limit for MT at 100 pM, which means a possibility to detect 3.5 pg of the target protein. In spite of the fact that automation of this method was nearly impossible for many years, recently an unique instrument was suggested, which enable us to carry out the automated analysis of samples (51). The photography of the instrument used for automated MT determination is shown on Figure 4.

What kind of information can the MT analysis provide for clinician?

It is known that MT is involved in many processes linked to carcinogenesis but using of this protein for prognostic purposes or as a tumour marker is controversial due to the interpretation of information. It was shown that MT level depends on tumour grading, clinical stage and additional tumour cells characteristics (42, 52). However, the relation of MT with tumour diseases needs deeper investigations.

In serum of control patients, the average determined MT level was 0.5 μM. A slightly increased MT level was found in smokers and in case of exposition to heavy metals and oxidation stress. Nevertheless, the level of MT in patients with tumour disease is three times higher compared to control samples (9, 52–54). MT level depends on clinical stage, tumour grade, its histological type and the therapy (53, 55). MT level in the field of theoretical research shows to be a very promising indicator, which can help us to answer questions connected to carcinogenesis and response to therapy.

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