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Copper and metallothioneins in cancer cells

Metallothioneins (MTs) are a widespread protein in the animal world. These proteins are characterized by great invariability of their structure. While isolated from various animals they only slightly differ from one another in the amino acid composition. The number of amino acids is constant in every animal group, and that is 61 amino acids, 20 of which are the cysteins radicals, which makes over 30% of the amino acids composition (Fig. 1.). Such a high amount of cysteins which include the reactive sulphydryl groups – SH determines the metallothionein's functions (2,3).

Metallothioneins take part in the homeostasis of the ions of the metals which are necessary for the proper metabolism of the organism (zinc, copper), biosynthesis regulation and zincprotein activity (for example the activity of the zinc-dependent transcription factors) and they also take part in the detoxication of the tissue from toxic metals. Apart from this, they also protect the tissue from oxygen-free radicals, radiation, electrophilic pharmacological agents used in the cancer therapy and the mutagens (4,6,9). The induction of metallothionein synthesis is influenced by many factors: heavy metals, inflammatory factors, free radicals, glycocortycoids and pharmacological agents (7).

ESSENTIAL ROLE OF COPPER

The essential role of copper (Cu) in the animal world ought to be seen in the context of its appearance in numerous oxidation-reduction enzymes. Copper is an important component of cytochrome-c oxidase (which is one of the elements of the respiratory chain), lysine oxidase (an enzyme taking part in the processes of collagen fibre maturing), Cu/Zn superoxide dismutase (an enzyme assisting in free radicals elimination), dopamine β-hydroxylase (the enzyme of the biosynthesis path of the catechol amines – adrenaline and noradrenaline), ceruloplasmin (which is a double-function protein fulfilling an enzymatic and transporting role), tyrosinase – catechol oxidase (which is an enzyme protein participating in melanin synthesis) and a protein that probably does not perform any enzymatic function, called metallothionein (15).

Copper, one of the essential metals, is required for the activity of enzymes associated with ferrum (Fe) metabolism, elastin and collagen formation, melanin production and integrity of the central nervous system. On the other hand, Cu ions are known to be toxicant, to both eukaryotic and prokaryotic cells. Copper ions can bind to proteins and nucleic acids and can cause the oxidation of lipids and proteins. The formation of deleterious free radicals is also enhanced by copper ions (13).

The concentration of protein in tissues is highly variable and is induced by many nutritional, physiological and developmental factors. For example, concentrations are greatly decreased in tissues of zinc-deficient animals and are increased after imposition of many types

of stress or metal administration. They are generally elevated during fetal development and vary dramatically among species (2). For cell viability, regulation of intracellular copper activity is thus crucially important and mechanisms must exist for the homeostasis of copper (12).

COPPER AND METALLOTHIONEINS IN CANCER CELLS

Metallothioneins have been isolated from a wide range of tissues, including liver, kidney, pancreas, and intestine. The immunologic techniques for their detection have improved, metallothionein have been found in most other tissues, including brain, thymus, bone marrow, and reproductive organs. Detection by subcellula fractionation indicates that metallothioneins occur principally in the cytosol, but immuhistochemical studies have also consistently revealed its presence in nuclei. Although metallothioneins are mainly of intracellular origin, they also occur in small amounts in extracellular fluids such as plasma, bile and urine (2).

The characteristic features of metallothioneins as are their low molecular weight and their unusual amino acid composition: cysteine accounts for 30% of the residues and aromatic acids absence (Fig. 1). Sequence studies showed that the distribution of the cysteine residues along the polypeptide chain is fixed, regardless of the source or isoform of the protein. Another main feature of metallothioneins is their high metal content, with 7 gram atoms of cadmium or zinc per mole or up to 12 gram atoms of copper per mole. This content is equivalent to one metal atom per three or two cysteine residues, respectively. The cadmium- and copper-induced metallothioneins usually contain also zinc as a secondary metal (2).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cys	Ala	Met	Asp	Pro	Asn	Cys	Ser	Cys	Ala	Ala	Gly	Asp	Ser	Cys
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Thr	Gly	Ser	Cys	Lys	Cys	Lys	Glu	Cys	Lys	Cys	Thr	Ser	Cys	Lys
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Lys	Ser	Cys	Cys	Ser	Cys	Cys	Pro	Val	Gly	Cys	Ala	Lys	Cys	Ala
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
Gln	Gly	Cys	Ile	Cys	Lys	Gly	Ala	Ser	Asp	Lys	Cys	Ser	Cys	Cys	Ala

Fig. 1. Metallothionein – amino acid composition

The responsibility for the homeostasis of copper inside the cell is held by metallothioneins –SH sulphydryl groups frequently occuring in these proteins permit the binding of metallic ions. Although metallothioneins bind Cu with considerable affinity, copper exchange is possible both between particular MT molecules as well as other proteins and micro-molecular ligands. This exchange helps to supply proteins with copper, which they need in order to function properly (3).

In the conducted experiments an increase of copper content was found in the neoplastic tissue. The increase was perhaps caused by more rigorous demands of the neoplastic cell for copper imposed by its more dynamic metabolism. An elevated copper level is noted in many types of neoplastic tissue (8,9,10,11).

Brem et al. (1), while experimenting on rats with implanted gliosarcoma and showing copper deficiency, noticed the inhibition of neoplastic growth. In the histological images of those rats no neoplastic processes were present on the margins of the neoplasm. These

observations, which suggest defective migration of neoplastic cells, drew the researchers' attention to the angiogenetic function of copper.

Copper is considered to be an important co-factor of proteins participating in and controlling angiogenesis. The growth of neoplasm is known to be dependent on its ability to produce new blood vessels. The induction of the blood vessels is required for the expansion of a neoplasm and is stimulated by specific compounds like fibronectine or fibroblastic growth factor (FGF), which are compounds produced by cancer cells.

The examined neoplastic tissue also exhibited an elevated metallothionein level. An increased intracellular metallothionein expression was found in many human and animal neoplasms (5,14). MT synthesis induction is stimulated by such factors as metallic ions, free radicals, cytokines, lymphokines and stress. Experiments conducted on animals show an MT increase in the neoplastic tissue. However, MT synthesis induction was also present in a liver free from the neoplastic process. Also, the concentration of MT's in the plasma of the blood grew together with the MT concentration increase in the liver.

The mechanism that governs the induction of MT synthesis is not well known. Research shows that cytokines like interleukin 1, interleukin 6, tumour necrosis factor (TNF) or interferon may induce MT synthesis. It is not impossible that neoplastic cells can release cytokines into the bloodstream that induce MT synthesis both in a tumour and in a neoplasm-free liver. The existence of the interdependence between the neoplasm and MT induction in the liver is supported by the fact that after surgical removal of tumour proper values return. Comparing the correlation coefficients of the levels of MT's and copper it may be supposed that there is an interdependence between the MT level and that of Cu, especially in the neoplastic tissue (8).

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SUMMARY

Metallothioneins (Mts) are intracellular proteins whose biological funtion is zinc and copper regulation as well as detoxification of toxic metals. Another function of these proteins is sweeping away free radicals. MT synthesis induction is stimulated by such factors as metallic ions, free radicals, cytokines, lymphokines and stress. An increased intracellular metallothionein expression was found in many human and animal neoplasms. Copper functions as cofactor in various redox enzymes. At the same time, copper is very toxic to both eukaryotic and prokaryotic cells. Copper ions can bind to proteins and nucleic acids and cause the oxidation of lipids and proteins. The formation of deleterious free radicals is also enhanced by copper ions. For cell viability, regulation of intracellular copper activity is thus crucially important and mechanisms must exist for the homeostasis of copper. An elevated copper level is noted in many types of neoplastic tissue.

Metalotioneiny oraz miedź w komórkach nowotworowych

Metalotioneiny są białkami wewnątrzkomórkowymi, których biologiczna funkcja polega na regulacji zawartości cynku i miedzi oraz detoksykacji komórki z metali toksycznych. Ponadto białka te biorą udział w usuwaniu wolnych rodników tlenowych. Syntezę metalotionein indukują jony metali, cytokiny oraz hormony. Wzrost poziomu metalotionein stwierdzono w nowotworach u ludzi i zwierząt. Miedź funkcjonuje jako kofaktor wielu enzymów oksydoredukcyjnych. Jednocześnie pierwiastek ten jest toksyczny zarówno dla organizmów eukariotycznych, jak i prokariotycznych. Jony miedzi mogą wiązać się z kwasami nukleinowymi oraz białkami i powodować utlenienie lipidów i białek. Pierwiastek ten bierze udział również w wytwarzaniu aktywnych form tlenu. Zatem w organizmach muszą występować mechanizmy regulujące ilość miedzi oraz jej aktywność. Podwyższoną zawartość miedzi stwierdzono w wielu typach nowotworów.