Pharmaceutical importance of zinc and metallothionein in cell signalling

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ABSTRACT: Zinc as an essential element plays the crucial role in many physiological, but also pathological processes - zinc controls cell proliferation, differentiation, and viability including the apoptosis. These facts are especially based on the structural role of zinc ions in many proteins including the transcription factors. Due to the role of zinc in many crucial physiological processes, its homeostasis must be intensively controlled. The controlling mechanisms are based especially on the zinc transporters and low-molecular proteins metallothioneins (MTs) of the molecular weight between 6 and 10 kDa, which were firstly discovered in a horse kidneys as the cadmium-binding protein. High affinity of metallothioneins to the zinc ions indicates their significant participation in the physiological, but also pathological processes, which are controlled by zinc. This paper summarizes the connection between metallothioneins and zinc and its significance on the molecular basis.

THE ROLE OF ZINC IN ORGANISMS

Zinc is the essentials for the prenatal as well as postnatal normal development of the cells, tissues and organs for the higher organisms because it controls the cell proliferation, differentiation and viability (1). This fact is connected with the role of the zinc ions as the structural components of many proteins in modulation of the cell genesis, differentiation, proliferation and apoptosis (2), respectively with the regulation of zinc-dependent enzymes, such as caspase-3 (3) mediated by p38/MAPK involving DNA fragmentation (4, 5), and signals – transcriptional factors – participating in the direction of cell cycle (ERK1/2, p53, NF-kappaB) (6). Where is the molecular basis of the regulation of transcription by zinc ions? It was demonstrated that significant number of the transcriptional factors contains evolutionary conserved the zinc finger motifs; the main type of the eukaryotic zinc finger motif is Cys_2His_2, which employs two cysteine and two histidine residues coordinated by one zinc(II) ion, which establishes $\alpha\beta\beta$ framework (7).

This zinc finger is able to form complex with DNA based on interactions between α-helix of zinc finger and DNA specific bases (8-10). Number of these domains in proteins varies from 1-37, but one domain itself is insufficient for the binding to the specific DNA sequence (11-13). The function of the zinc fingers consists in the DNA recognition and transcriptional activation, but also in RNA packaging (14), protein folding (15, 16) and apoptosis (17), which regulation is important not only in the development of the "normal" tissues, but also in neoplastic transformation and proliferation (18). In these processes, the zinc finger transcriptional factors, such as ZnF652 (proliferation and differentiation of prostate cells, breast and vulvar carcinomas, chronic lymphocytic leukemia) (19-21), ZFP637 (cell proliferation and differentiation, progression of cell cycle) (2), ZnF424 (repressor suppressing NFAt and p21) (22), ZnF418 (negative regulator in MAPK signalling pathway, fetal and adult tissues) (23), ZnF268 (blood cells differentiation, pathogenesis of leukemia) (24), ZPF423 (control of proliferation and differentiation of neural precursors), THAP1 (endothelial cells proliferation) (25), or HZF1 (erythroid and megakaryocytic differentiation) (26) play the essential role in the regulation of the gene expression of the proteins and enzymes regulating the cell cycle and proliferation.

The zinc finger nuclease plays the role in cleavage of pro-apoptic Bax and Bak proteins, which cause permeabilization of mitochondrial membranes (27). The inhibition of Bax and Bak and all subsequent steps of apoptosis activation including cytochrome c release was also recorded (28). The cell lacking above mentioned pro-apoptic proteins grow normally, but activation of caspases as response to apoptic stimuli is missing (27). The zinc modulates cellular signals recognition, metabolism of the second messengers, activities of protein kinases and phosphatases, metabolism of cGMP, and activities of protein kinase C (1). The regulation of *Bcl-2* by zinc ions is intensively investigated (29). Down-regulation of *Bcl-2* by zinc ions with the subsequent apoptosis in case of prostate epithelial cells was demonstrated (30).

The important zinc role consists in the interaction with other ions; competition between zinc and iron ions was suggested (31). This fact is important in the inhibition of iron-induced oxidative damage (32).

ZINC TRANSPORTERS

Our knowledge are still limited in the field of the small-molecule zinc(II) complexes in the regulation of gene expression and apoptosis. Some studies indicate effect of these small zinc complexes on the regulation of cell cycle and anti-apoptic genes *Bcl-2* and *Bcl-xL* (33).

It is obvious that each cell must have mechanisms, which are able to regulate levels of the zinc ions. These mechanisms include sequestration of the zinc in the zinc storing vesicles, which are called zincosomes, nucleoplasmic distribution, redistribution, and elimination. The zinc ions regulate also metal transcription factor MTF-1, which controls transcription of the genes for metallothioneins – MTs, and zinc transporters ZnT-1 (1) or ZIP6 connected with influx of free zinc ions and increased cytoplasmatic free zinc ions level (34). Human ZnT transporters include family of nine transporters – ZnT-1-9. Expression of different ZnT types is connected with zinc homeostasis in various types of cells and tissues (35). ZnT-1 is the first described mammalian zinc transporter with the ubiquitous expression. Other ZnT types, especially ZnT-4 and ZnT-6 are intensely studied in connection with nervous tissue and neurodegenerative diseases (36); ZnT-8 is recently associated with regulation of the insulin and glucagon secretion in some pancreatic cell line types (37). Fourteen members of human ZIP transporters have been identified (38).

Cell and tissue specificity of ZIP transporters has been demonstrated in many works (39-40-41). Some zinc transporters are associated only with tumour cells – LIV1 is the zinc transporter gene induced by the treatment of the histone deacetylase inhibitors in the tumour cells, but not in normal cells (42). LIV1 is connected with the zinc homeostasis and activation of caspase-3 and expression of some *Bcl-2* family genes (43).

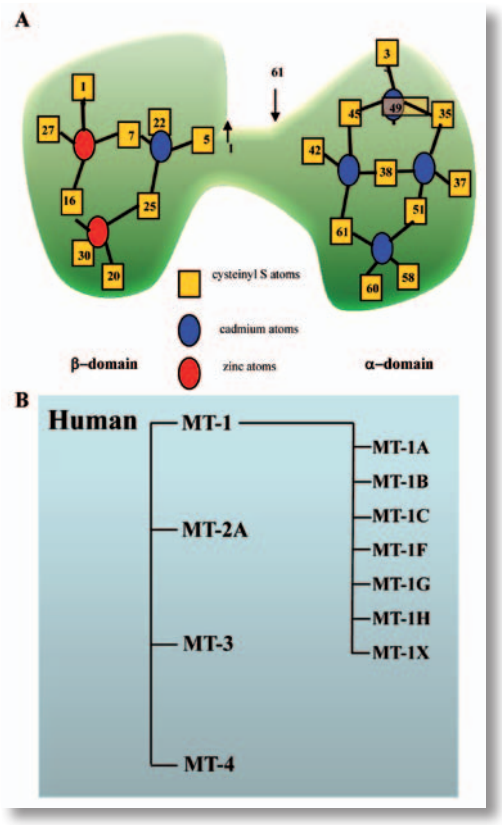

Figure 1. The structure of metallothionein, its two domains, and binding sites. Cysteine residues with –SH groups are marked in yellow; contribution of these groups in metal binding is evident (A). The biologically important isoforms of metallothionein gene in human (B).

METALLOTHIONEINS

The crucial role in the zinc homeostasis play major zinc binding proteins metallothioneins (44), which participate in the regulation of free zinc ions and their nuclear translocation during the cell cycle and processes of cell differentiation (Figure 1A) (1). Metallothioneins (MTs) belong to the group of the ubiquitous intracellular low-molecular proteins of molecular weight between 6 and 10 kDa. They were discovered as the cadmium-binding protein isolated from the horse kidneys. The human MT gene family consists of 18 isoforms, containing pseudogenes as well as the genes encoding the functional proteins (Figure 1B). MTs have high affinity to the divalent ions, such as the toxicologically important cadmium and mercury, but also to the essential copper and zinc ions. Their main function consists in the maintenance of the homeostasis of these ions. MT expression significantly increases after exposition to many exogenous as well as endogenous factors, such as UV radiation, heavy metals, stress hormones, reactive oxygen species, cytokines liberated from the injured or damaged tissue and xenobiotics (45). MT is thermodynamically stable, which makes MT ideal zinc reservoir (Figure 2). Release of zinc from Zn-MT complex makes zinc available for function of other molecules (46). MT is localized in the cytoplasm, but enhancement of MT level in nuclei was recorded as the effect of several physiological and pathological stimuli, such as hydrogen peroxide and nitric oxide. MT translocation into nucleus is connected with the control of the cell differentiation.

The connection between the transition from G0/G1 phase to S phase of the cell cycle and zinc and MT levels in nucleus was demonstrated. This fact indicates the role of MT in the translocation of zinc ions to the nuclear factors in the mitogenic processes (47). MT is connected with the cell differentiation and direction of the cell cycle, as it was demonstrated on the chronic myeloid leukemia derived cell line K562 (48). In addition, other studies indicates role of MT in the direction of the cell cycle. It was demonstrated that down-regulation of MT expression in the endothelial cells inhibits proliferation, cell migration, and angiogenesis. MT down-regulation results in the cell cycle arrest in G1 phase. The connection between vascular endothelial zinc finger 1 (VEZF1) transcriptional factor, which plays important role in angiogenesis, and MT expression regulation was proved (49). MT is the important transporter of the metals´ ions in the organisms. As the transporter of zinc ions, MT can serve as reservoir of these ions for the important transcriptional factors; nuclear and cytoplasmic distribution of zinc ions is important in the controlling of transcription of many genes. High MT levels in the nucleus, may be connected with the increasing requirements of the zinc for many metalloenzymes and transcription factors during the rapid growth of malignant tumour cells (50).

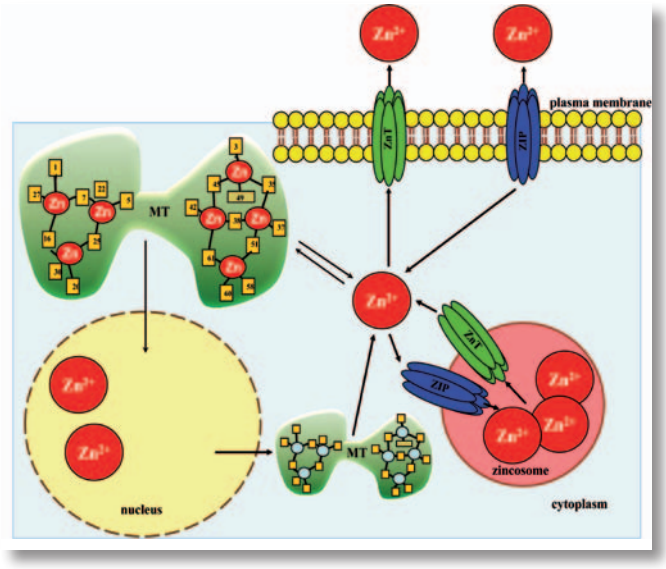

Figure 2. The zinc homeostasis and its control by the different mechanisms: the transport of the zinc ions through the plasma membrane by the zinc transporters ZnT and ZIP families, transport of tree zinc ions to the zincosomes, which represent pool of zinc ions, through ZnT and ZIP transporters, and role of metallothionein in the zinc ions homeostasis and controlling of tree zinc ions and their transport to the nucleus.

In addition, anti-apoptic effects of MT are connected with its antioxidant activity, which includes zinc ions homeostasis – zinc ions, which are able to cause oxidative damage of cell structures and induce apoptosis (51). Contrariwise, the protective MT properties against the oxidative damage of the cells and tissues is connected with the protection of the tissues during the treatment by some drugs, such as anthracyclines, and during diseases, which are connected with the action of the reactive oxygen species (52). The antioxidant effect of MT is also connected with the inflammatory stress, but the explanation of these mechanisms is still missing (53).

MOLECULAR BASIS OF THE EXPRESSION OF METALLOTHIONEIN

The molecular mechanism of MT expression is still almost unknown. In this mechanism participates the metal itself by the binding to the specific transcription factor – MTF-1. MTF-1 has well-conserved protein structure from insects to the human; it contains six tandem repeats of Cys_2His_2 zinc finger motif in its N-terminal part and three transcriptional activation domains in its C-terminal part (54). Phosphorylation of MTF-1 plays crucial role in its activation by zinc, but also cadmium ions. Complex metal-MTF-1 subsequently binds in the nucleus to metal-responsive element (MRE) in the promoter area of MT gene and activates its transcription. In MT synthesis, next regulation proteins participate through the responsive elements, such as glucocorticoid response element (GRE), interferon response element (IRE), signal transducers and activator protein (STAT) or antioxidant response element (ARE), the binding receptors connected with the activity of the second messengers or activation of "common" transcription factors.

Induction of MT synthesis was investigated in nervous tissues. MT synthesis is induced in the central nervous system by the pathogens and disorders, such as radiation that indicates the neuroprotective role of MT during the neuropathological conditions (55). MT decreases the inflammation and oxidative tissue damage, in which zinc ions plays the crucial role, and promote the reparation processes, such as angiogenesis, neurogenesis and the tissue remodelling connected with the signal transduction of the density lipoprotein family of the receptors on the cell surface involving lipoprotein receptor-1 (LRP1) and megalin (LRP2) (56). Accumulation of zinc ions was observed in case of the neuronal death following the acute brain injury as well as during the ontogenic development. This fact may be used as the marker of apoptosis of the neurons undergoing processes of apoptosis (57).

METALLOTHIONEIN IN PATHOGENESIS

The oxidative damage of the tissues is connected with many diseases, such as diabetes, but also Crohn's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Menke's disease or Wilson's disease (53, 58, 59); in the case of these diseases, the increasing MT expression was demonstrated. Menke's and Wilson's diseases are two hereditary disorders of copper metabolism. Menke's gene in localized on the chromosome X13.3 and encodes copper-transporting ATPase. Mutation of this gene leads to significant accumulation of the metallothionein-bound copper in the cytosol of affected cells and copper transport is disturbed (60). Mutation of ATP7B – type of ATPase transporting copper ions expressed in liver, kidney and placenta that transports copper into bile and incorporates it into ceruloplasmin – leads to Wilson's disease that is connected with accumulation of copper in brain and liver (61). There were published first studies, which investigate the role of MT in diabetes (62). As it was indicated above, the oxidative

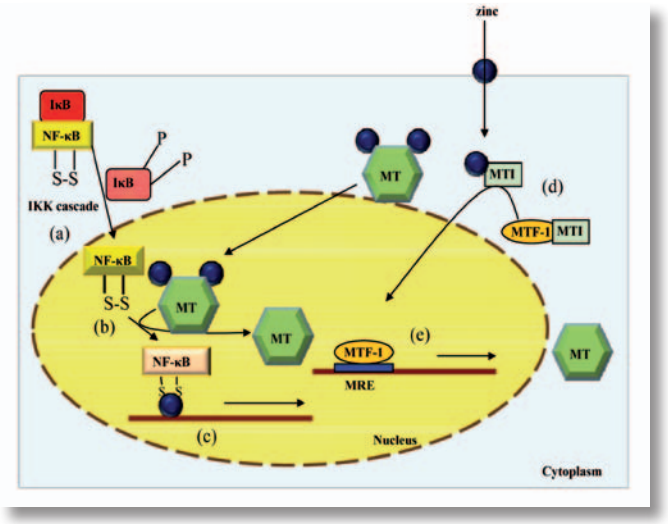

Figure 3. The possible relation between metallothionein and transcription factor NF-κB. The transcription factor NF-κB is activated through the signal from IKK cascade (a). The activated transcription factor passes to nucleus, where reacts with MT molecule binding zinc (b). Zinc is subsequently transferred to transcription factor NF-κB, which binds on regulatory sequences of DNA (c). Level of zinc is regulated by the transcription factor MTF-1 (d), which binds to MRE of DNA (e).

stress is connected with the excess of free zinc ions – increasing of the inflammation by zinc was demonstrated (63). On the other hand, in the case of inflammatory lung disease, Zn protects lung epithelium against the oxyradicals and other nonoxious agents with the significant implications for asthma and other inflammatory diseases (64, 65). In the case of Parkinson's disease, zinc-induced apoptosis of the experimental MES23.5 dopaminergic cells may due to the enhancement of TEA-sensitive potassium channel activity (66, 67).

METALLOTHIONEIN AND CANCER

MT is connected with the malignant tumour diseases and their prognosis, especially due to its antiapoptic, antioxidant, proliferative and angiogenic effects (68). The negative MT role in apoptosis is still investigated (69). MT is associated with the higher cell proliferation rates and fewer positive apoptotic cells (5). Most recently was studied the role of the over-expression of MT in the different types of tumour tissues. MT overexpression was demonstrated in the various tumour tissues, such as breast, kidney, nasopharynx, lungs, prostate, testes, urinary bladder, cervix, endometrium, salivary glands, pancreas, acute lymphoblastic leukaemia or melanoma, where MT level is directly connected with the grade and prognosis of disease (70-81). Some studies indicate that MT expression is connected with the zinc homeostasis and correlates with the level of free zinc ions and with the cell proliferation (82). The antioxidant action of metallothionein was highlighted by the summarizing of the most important findings that confirm the role of zinc in the cellular protection in the relation to metallothionein expression and apoptotic processes (83) with the direct connection to the resistance to the apoptosis through zinc-dependent antiapoptic transcription factor nuclear factor kappaB (NF-kappaB, NF-κB) and its regulation, which plays important role in the inflammatory responses (Figure 3) (84, 85). Recently, MTs are connected with resistance of tumour cells to cisplatin. It was demonstrated that this resistance is connected with overexpression of MT-1H in non-small cell lung cancer cell lines (86), ovarian carcinoma cell lines (87, 88), tongue squamous cell carcinoma cell lines (89), or gastric tumour cell lines (90).

CONCLUSION

Due to the significant affinity of MT to zinc ions, it can be supposed that MT represents the most important and effective mechanism in the regulation of the intracellular levels of zinc ions, thereby plenty of the cell processes, including the cell division, proliferation, differentiation and apoptosis are regulated. Zinc modulates in the organisms the function of the second messengers, participates in the regulation of cell signalling, and influences functions of the protein kinases and phosphatases. Zinc ions form the stable chemical bond with MT. Metalloproteins associated with zinc have both structural functions and regulation functions participating in many metabolic pathways as the cofactors. The zinc ions demonstrate also the important signalling function of the regulating the cell proliferation, especially through MAPK kinases.

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