INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **Metalothionein**

Školitel: Dr. Ing. Branislav Ruttkay-Nedecký

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NanoBioMetalNet

Laboratoř Metalomiky a Nanotechnologií
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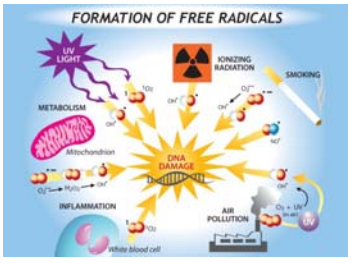
METALLOTHIONEIN AND ITS RELATION TO REDOX METABOLISM

Branislav Ruttkay-Nedecky, Lukas Nejdl

INVESTICE DO ROZV

Free Radicals



Free radicals are chemical particles containing one or more unpaired electrones and they are highly reactive. Free radicals:

a) can be generated during UV radiation, X-ray or gamma radiation
b) are the products of reactions catalysed by metals
c) are present in the air as pollutants
d) are produced by neuthrophils and macrophages during inflammation
e) are by-products of the mitochondrial respiratory chain

The most important free radicals in aerobic organisms are reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Source: http://best-skincare.net/rooibos/rooibos-nutrition/

Overview of Reactive Oxygen and Reactive Nitrogen Species

Reactive Oxygen Species				Reactive Nitrogen Species			
Free Radicals		Other Substances		Free Radicals		Other Substances	
Superoxide anion radical	$O_2^{\bullet-}$	Hydrogen peroxide	H_2O_2	Nitric oxide radical	$NO^{\bullet}$	Peroxynitrite	$ONOO^-$
Hydroxyl radical	$HO^{\bullet}$	Hypochlorous acid	HOCl	Nitric dioxide radical	$NO_2^{\bullet}$	Nitrites	NO_2^-
Alkoxyl radical	$RO^{\bullet}$	Ozone	O_3			Nitrates	NO_3^-
Peroxyl radical	$ROO^{\bullet}$	Singlet oxygen	$^1O_2$			Nitrosyl	NO^+

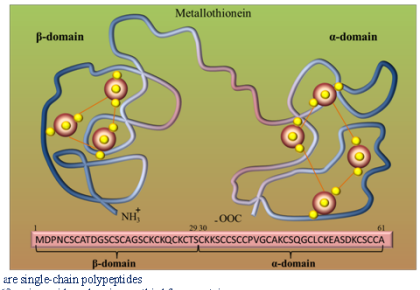
Metallothioneins (MTs)



- metallothioneins (MTs) were discovered by Margoshes and Vallee in 1957
- they were isolated from horse kidney as Cd-binding proteins
- discoverers called the protein metallothionein, which means a metal-binding sulfuric protein
- MTs occur in humans and the whole animal kingdom with a large degree of homology
- MTs are also known in eukaryotic microorganisms, many prokaryotes and higher plants
- they are small proteins of the size (6 – 10 kDa)
- 4 mammalian MT isoforms (MT1, MT2, MT3 and MT4) are known
- MT1 and MT2 are present primarily in liver, kidney, pancreas and intestine
- MT3 is expressed in brain, also in heart, kidney and reproductive organs
- MT4 was detected in stratified squamous epithelial cells (oral epithelia, esophagus, upper stomach and neonatal skin)
- MTs are involved in the detoxification of cells and mantaining metal ion homeostasis
- MTs participate in the protection of body against free radicals

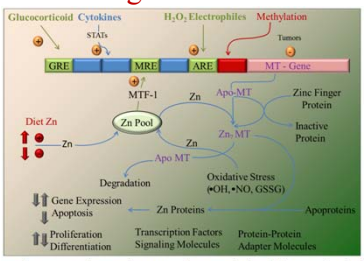
Source:https://www.google.cz/search?q=hearth+anatomic+pictures&rlz=1C1GIGM_enCZ560CZ560&espv=210&es_sm=93&tbm=isch&tbo=u&source=univ&sa=X&ei=MdToUq2cHMaK7AaDxYDABA&ved=0CCoQsAQ&biw=1920

The Structure of Metallothionein



- mammalian MTs are single-chain polypeptides
- they contain 61 - 68 amino acids , wherein one third form cysteines
- to the SH groups of cysteines metal ions are bound
- MTs has the greatest affinity to Cu^+ , then Cd^{2+} and Zn^{2+}
- Its tertiary structure is divided into two metal binding domains, α and β – cysteine clusters
- α (C-terminal part) is more stable and has four binding sites for divalent metal ions, β (N-terminal part) has the ability to bind 3 divalent metal ions.
- Adopted and modified according to Petrlova, Potesil et al. 2006

MT Gene Regulation and Function



The MT promoter has several response elements that upregulate transcription. MT expression is induced by metal ions through MRE (metal response element), cytokines through STAT (signal transducers and activators of transcription), stress hormones glucocorticoids through GRE (glucocorticoid response element) and free radicals (electrophiles) through ARE (antioxidant response element). ARE is activated in response to oxidative stress of a cell. Metals which induces the MT gene expression are mainly Zn, Cd, Cu, however at physiological processes acts mainly Zn. Methylation of the MT promoter decreases the expression of MT in some tumor cells. Expression of MT is initiated upon binding of the metal regulatory transcription factor 1 (MTF-1) to the MT gene regulatory part called MRE (metal responsive element). Zn cell stocks are influenced by food intake of Zn. Zn together with MT plays an important role in the regulation of key processes such as apoptosis, cell proliferation and differentiation. Adopted and modifed according to Davis and Cousins 2000.

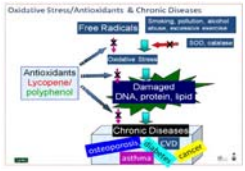
MT and Oxidative Stress

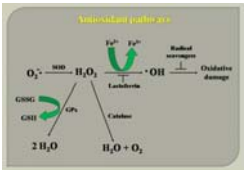
Various forms of oxygen radicals which are formed for example in an intensive mitochondrial activity have to be destroyed by the cell

For these purposes antioxidants are synthesized, which are subjects to oxidation, instead of important cellular components. These include MT and glutathione, that form redox environment and under certain conditions are able to oxidize or reduce each other.

That is the reason why an effect of oxidation factors induces the expression of MT gene in cells. From the physical point of view here belong X-rays what was confirmed in several studies. In mice whose bodies were irradiated with X-rays, an increased incidence of MT1 mRNA was found (Shibuya, Satoh et al. 1995) .

It is assumed that the MT acts as a scavenger of free radicals or a donor of zinc to the enzymes involved in the repair processes in a cell.





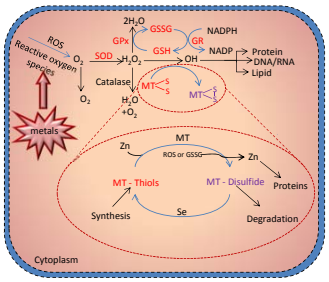
Source:http://www.redlabs.be/red-labs/our-science/oxidative-stress.php, http://www.intechopen.com/books/topics-in-osteoporosis/oxidative-stress-and-antioxidants-in-the-risk-of-osteoporosis-role-of-the-antioxidants-lycopene-and-

Antioxidant Activity of MT in Publications

- Thornalley and Vasak (Thornalley and Vasak 1985) observed, that MT-1 isolated from rabbit liver, which contained Zn^{2+} or Cd^{2+} ions scavenged free hydroxyl (•OH) and superoxid radicals (O^{2-}•) produced in vitro by the reaction of xanthine with xanthine oxidase.
- Under conditions of oxidative stress in the presence of elevated concentrations of reactive oxygen species or NO, zinc is released from MT. Incubation of lung fibroblasts with NO resulted in an increase in intracellular concetrations of zinc, which was not observed in MT null fibroblasts (without MT gene expression) (St Croix, Wasserloos et al. 2002) .
- Zn-MT induced promyelocytic leukemia cells (HL-60) were more resistant to oxidative stress caused by hydrogen peroxide than normal cells (Quesada, Byrnes et al. 1996).
- Chubatsu (Chubatsu and Meneghini 1993) investigated the role of MT in protection against oxidative damage to DNA on V79 Chinese hamster cells. An increase in MT content of V79 Chinese hamster cells was induced by zinc without concomitant increase in the GSH level. These induced cells were more resistant to the production of DNA-strand scission by H_2O_2 than the parental cells.
- In another study, Sato et al. (Sato 1991) determined dose-dependent changes in the concentration of MT-1 in rat tissues following subcutaneous administration of paraquat (PQ), a superoxide radical-generating agent. Twenty four hours after injection, MT-1 concentrations in the lung increased linearly with PQ dose.

MT Redox Cycle

MT Scavenging of ROS. Presence of redox metals (Cu, Fe, Pb, As, Cd) in a cell can produce ROS, leading to damaging of DNA and cell structures. The cell protects itself using various molecules as scavengers of the radicals. One of the most crucial cell pathways to scavenge the radicals is the glutathione redox complex. However, free –SH moieties of MT can be also involved in the scavenging of ROS in the MT redox cycle. Under physiologic conditions, zinc bound to MT is released through oxidation of the thiolate cluster when the environment becomes oxidized.



Formation of MT-disulfide would be subjected to degradation; however, when the oxidized environment became reduced—through, for example, an increase in the glutathione (GSH)/glutathione disulfide (GSSG) ratio—MT disulfide is reduced to MT-thiol. In the presence of zinc, MT is quickly reconstituted. This process constitutes the MT redox cycle, which plays a crucial role in the biologic function of MT. Adopted and modified according to Eckschlager, Adam et al. 2009 a Kang 2006.

Conclusion

Metallothioneins (MTs) appears as multifunctional molecules. Several physiological functions have been identified so far:

First, MTs are the significant transporters of metal ions. The greatest affinity they have for Cu^{+}, but most offen they bind Zn^{2+}, thereby are intensively involved in homeostasis of these metal ions in an organism.

At intoxication of a cell with heavy metals such as Cd^{2+}, Pb^{2+}, či Hg^{2+}, MTs are able to bind these metals (releasing Zn^{2+}), and thus defuse them for a cell. Subsequent detoxification will then take place probably in kidneys.

MTs also have an important antioxidant role. MT together with GSH produces a redox pair, which controls the occurrence of free oxygen radicals. They create a reducing environment, which helps to protect the nucleic acids, phospholipid membranes and cell protein apparatuses against effects of ionising radiation and chemo-oxidative effects of toxic agents.

Lately also increasingly points to the ability of MT to regulate expression of other genes for cellular proteins. As a tray for zinc it can transfer essential metals (especially zinc) to transcription factors and thus activate them. The activated transcription factors bind to regulatory sequences of DNA and they trigger DNA transcription.

Due to the increased MT expression in some tumors, MTs may be used as potential tumor markers.

Literature

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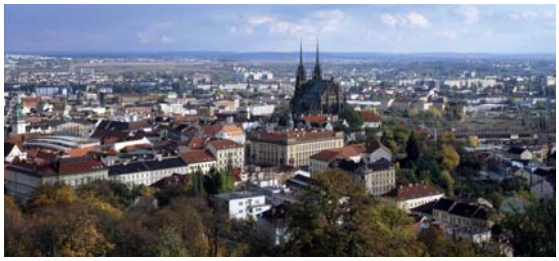
Thank You For Your Attention



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Poděkování:

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