INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Metalothionein a jeho vztah k rakovině

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Reg.č.projektu: CZ.1.07/2.4.00/31.0023
Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu
NanoBioMetalNet

Content

I. What are metallothioneins?

II. The biologically important roles of metallothioneins

III. Determination of metallothioneins at patients with a tumour disease

Metallothioneins as a new potential tumour marker

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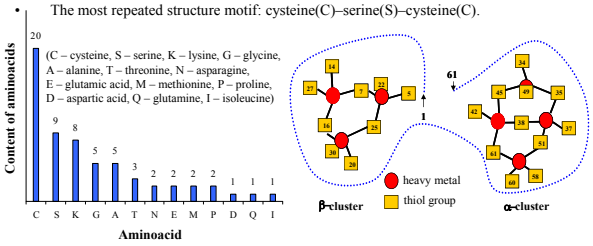
Metallothioneins = proteins

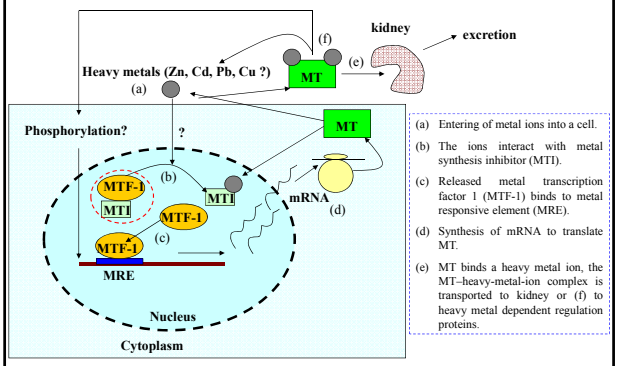
- Intracellular, low molecular and cysteine-rich proteins with molecular weight from 6 to 10 kDa
- MTs consist of two binding domains – **α** and **β**.
- N-terminal part of protein – β-domain; three binding places for divalent ions.
- C-terminal part of protein – α-domain; four binding places for divalent ions.
- The most repeated structure motif: cysteine(C)–serine(S)–cysteine(C).



Adopted from J. Petrlova, et al. Attomole voltammetric determination of metallothionein, Electrochim. Acta 51 (2006) 5112-5119.

Content

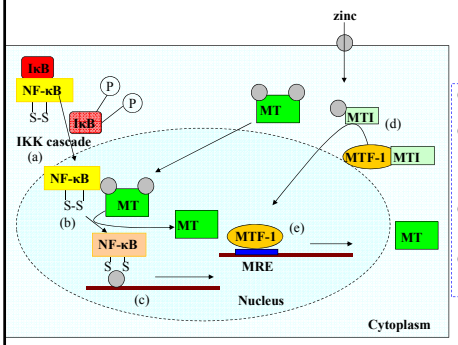
Detoxification of heavy metals



(a) Entering of metal ions into a cell.
(b) The ions interact with metal synthesis inhibitor (MTI).
(c) Released metal transcription factor 1 (MTF-1) binds to metal responsive element (MRE).
(d) Synthesis of mRNA to translate MT.
(e) MT binds a heavy metal ion, the MT–heavy-metal-ion complex is transported to kidney or (f) to heavy metal dependent regulation proteins.

Adopted from Eckschlager, et al. (2009). **Metallothioneins and Cancer.** Curr. Protein Pept. Sci., 10, 360-375.

Metallothioneins and NF-κB?



(a) Activation of NF-κB by IKK cascade.

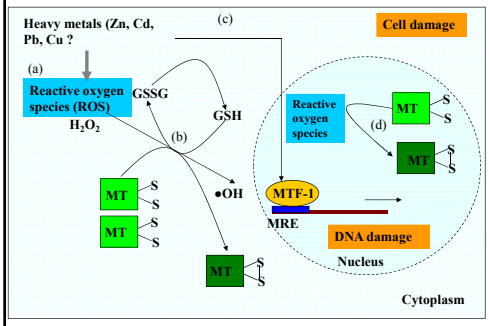
(b) NF-κB is transported to nucleus, where it can interact with metallothionein-Zinc complex.

(c) Zinc is transferred to NF-κB, which can thus bind on regulation sequences.

(d) Zinc level is regulated by MTF-1, (e) which binds on MRE.

Adopted from Eckschlager, et al. (2009). **Metallothioneins and Cancer.** Curr. Protein Pept. Sci., 10, 360-375.

Metallothioneins as scavengers of reactive oxygen species



(a) Presence of heavy metals in a cell can produce reactive oxygen species (ROS).

(b) To scavenge ROS glutathione and MT can be used.

(c) Free heavy metal ions lead to activation of MTF-1 → to synthesis of MT.

(d) MT can bind metal ions and scavenge ROS.

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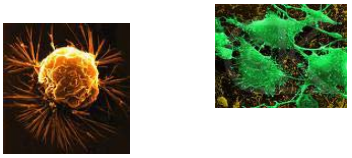
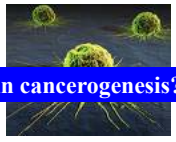
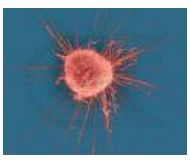
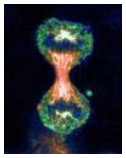




Can metallothioneins play a key role in cancerogenesis?





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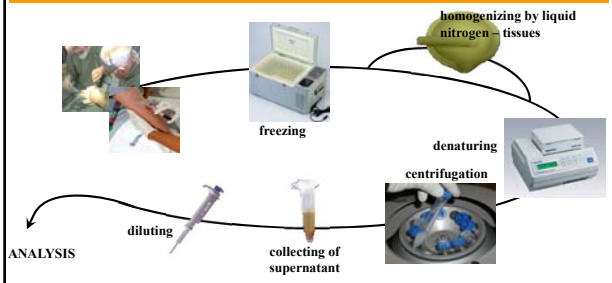
Homogenization of the samples

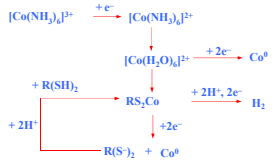


- Briefly, the sample was kept at 99 °C for 15 min. with occasional stirring, and then cooled to 4 °C. The denatured homogenates were centrifuged at 4 °C, 15 000 *g* for 30 min. Heat treatment and solvent precipitation effectively denature and remove high molecular weight proteins out from samples; metallothionein belongs to thermo stable proteins.
- The prepared samples are analysed by **Adsorptive Transfer Stripping Technique coupled with Differential Pulse Voltammetry Brdicka Reaction.**

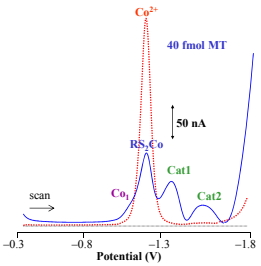
Brdicka reaction

- Brdicka reaction – the hydrogen evolution from supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer (NH_3(aq) + NH_4Cl, pH = 9.6) in the presence of peptides and/or proteins containing thiol group.
- limit of quantification: **50 pM** (1 fmol MT in 5 µl).

Scheme of Brdicka reaction



Signals of Cat1 a Cat2 correspond to the reduction of hydrogen at the mercury electrode. Another signal, which is appeared at the potential about –1.0 V, relates with the reduction of the RS_2Co complex. In addition the signal called Co_1 could result from reduction of $[Co(H_2O)_6]^{2+}$.



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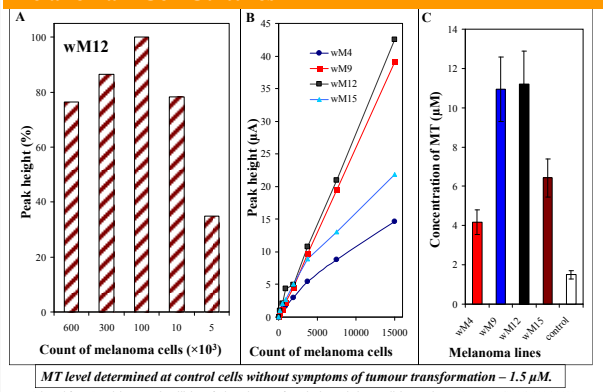
Melanoma – Cell Cultures



MT level determined at control cells without symptoms of tumour transformation – 1.5 μM.

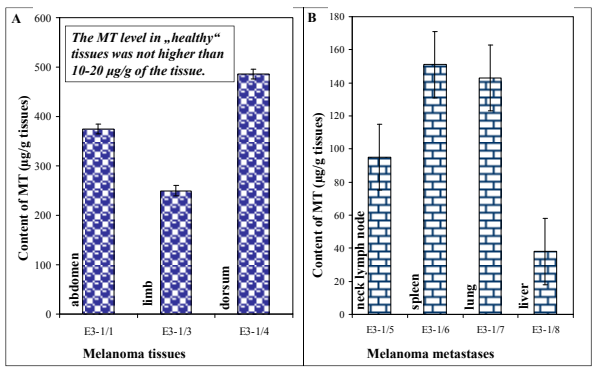
Adopted from S. Krizkova, et al. Utilizing of adsorptive transfer stripping technique Brdicka reaction…, Sensors 8 (2008) 3106-3122..

Melanoma – Tissue

- Using directed selection an original cancer model was established in the Institute of Animal Physiology and Genetics in Liběchov - a strain of miniature pigs that was designated with acronym MeLiM (Melanoma-bearing Libechov Minipig).
- Melanoma in this strain is heritable.
- Multiple skin nodular tumours (i.e. the most aggressive form of melanoma) appear on various parts of body in about a half of piglets.
- Their histological, immunohistochemical and biochemical characterization and a broad melanoma cell dissemination document similarities with human melanoma and malignant behaviour of this porcine cancer.

Adopted from S. Krizkova, et al. Utilizing of adsorptive transfer stripping technique Brdicka reaction…, Sensors 8 (2008) 3106-3122..

Melanoma – Tissue



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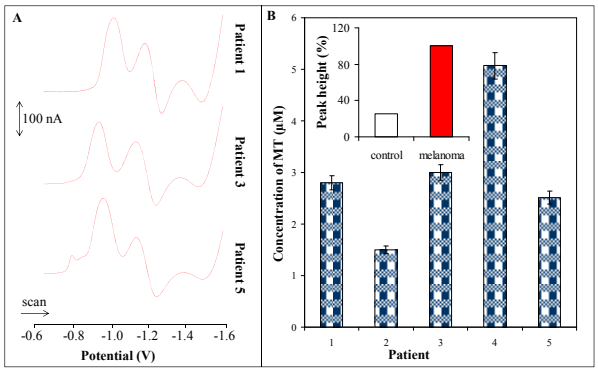
III. Determination of metallothioneins at patients with a tumour disease

Blood Serum Samples from Patients with Melanoma



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Thank you very much

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