Metallothionein and cancer

Soňa Křížková[1], Markéta Vaculovičová[1], Vojtěch Adam[1] and René Kizek[1*,*]

[1] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic - European Union, E-Mails: kizek@sci.muni.cz (R.K.)

* Author to whom correspondence should be addressed; E-Mail: kizek@sci.muni.cz; Tel.: +420-5-4513-3350; Fax: +420-5-4521-2044.

Received: 6.5.2015/ Accepted: 13.5.2015/ Published: 1.7.2015

Metallothioneins (MT) are small (molecular weight in range 500 – 1400 Da) intracellular proteins rich in cysteine content. In humans four main isoforms of MTs have been discovered so far – MT-1, MT-2, MT-3 and MT-4. All of MTs functions – heavy metals binding, antioxidative, regulation and immunomodulation are involved in MTs roles in cancer. MTs role is double-edged in carcinogenesis; in healthy cells with high MT content it has protective effects, while in healthy cells with low MT content, carcinogenesis via environmental factors occurs, on the other hand, at cancer-transformed cell the high MT content increases the tumour malignancy and chemotherapeutics resistance. Expression of MT can be used as marker of tumour diseases. Increased level of MT in serum or full blood was found in many cancer diseases and can be used as early stage biomarker. To decrease tumour resistance, or on the other hand, to improve non-target cells tolerance to chemotherapeutic multiple approaches are tested, including specific increasing or decreasing of MT expression based on specific compounds or targeted gene therapy.

Keywords: metallothionein; cancer; biomarker; chemoresistance; epigenetics

1. Metallothionein

Metallothioneins (MT) are small (molecular weight in range 500 – 1400 Da) intracellular proteins rich in cysteine content. In 1957 MT was discovered in horse liver as cadmium binding and detoxification protein [1]. With progressing research it has been discovered, that metallothioneins are found in all kingdoms – bacteria, animals, fungi and even plants and that they are involved in numerous cell processes according their isoform, oxidation state and metals content [2].

In humans four main isoforms of MTs have been discovered so far – MT-1, and MT-2, which are ubiquitous, MT-3 and MT-4, which are specific for neuronal tissue, but their expression also in skin was found. Human MT genes are localized on chromosome 16q13 and are encoded by a multigene cluster of closely linked genes. Genes for MT consist of 11 MT-1 genes and one gene for every other MT isoform (the MT-2A gene, MT-3 gene and MT-4 gene) [3]. A gene called MT-like 5 (MTL-5) that encodes a testis-specific MT-like protein called tesmin was described in the 11q13 [4]. A number of other MT or MT-like genes and pseudogenes with significant homology to functional MT genes exist in human genome, but their functionality is unknown.

2. Roles of metallothionein in the cells

The first discovered and still not fully understood roles of MT-1 and MT-2 are transporting of essential heavy metals, detoxification of toxic ones and protection against oxidation stress. MT by interaction with other proteins fulfils its function, resulting in different effects in the organism [5]. Interaction of MT with other proteins occurs either directly or via heavy metals administration and redox reactions.

Interaction of MT with ferritin, which causes a redox reaction leads to reduction of Fe^{3+} stored in ferritin with consequent releasing of harmful Fe^{2+} [6]. Interaction of MT with GSH/GSSG modulates Zn transfer between MT and zinc-binding proteins [7]. Apo-MT is able to deactivate Zn-transcription factors and Zn--dependent enzymes, while Zn-MT can activate them. Interaction of MT with zinc-dependent enzymes such as carbonic anhydrase, Cu/Zn SOD, δ-aminolevulinic acid dehydratase via zinc binding is known [5,8]. After interaction of MT with endocytic LDL receptor megalin the uptake of CdMT occurs, resulting in disruption of proximal tubules [9]. MT is involved in many cellular processes, which are regulated by protein-protein interaction. The most known are transcription, apoptosis, immunomodulation and cancerogenesis, interacting with NF κB, p53, specificity protein 1 (Sp1), transcription factor IIA (TFIIA), estrogen receptor (ER), Gal4, and tramrack (TTK), matrix metalloproteinases and other [5,10].

MT-3 has been discovered as neuronal growth inhibition factor. It is involved in growth of neuronal tissue, but its expression has also been found in skin, and epithelial tissues. It also binds heavy metals, but its main function is regulation of neuronal development [11].

MT-4, discovered few years ago is the least known MTs isoform. It is found in stratified squamous epithelia, including the oesophagus, upper stomach, tail, footpads and neonatal skin [12]. It helps to regulate stomach acid pH, taste and texture discrimination of the tongue and help protect against sunburn and other skin traumas [13].

3. Connection of metallothionein with cancer diseases

All of MTs functions – heavy metals binding, antioxidative, regulation and immunomodulation are involved in MTs roles in cancer. MTs role is double-edged in carcinogenesis, in healthy cells with high MT content it has protective effects, while in healthy cells with low MT content, carcinogenesis via environmental factors occurs, on the other hand, at cancer-transformed cell the high MT content increases the tumour malignancy [14]. Proliferative, anti--apoptic function of MTs, (de)activation of transcription factors, ROS scavenging are beneficial for cancer cell to survive, proliferate and defend against organisms' immune system.

All isoforms, except the least known MT-4 have been found to be involved in cancer. However, according to last findings MT-4 –expression at mRNA level was found in lung cancer (NSLC) [15] and squamous cell carcinoma [16].

The most about MT up/down regulation is known about MT-1 and MT-2 [8,17-19]. Disclosed MT expression as a useful diagnostic factor for tumour progression and drug resistance was reported in a variety of malignancies e.g. leukaemia, melanoma, breast, ovarian, renal, lung, pancreatic, gall bladder, oesophageal, and basal cell carcinomas [20-27]. One may suggest that MTs may lead to a protection of tumour cells against apoptosis and support the metastatic behaviour of tumours and/or cancer cell proliferation. On the other hand in some other studies devoted to colorectal and bladder cancer and others, no significant correlation between MT expression and prognosis was observed [28,29]. In recent years, common MT polymorphisms were identified and associated with, particularly, western lifestyle diseases such as cancer, complications of atherosclerosis, and type 2 diabetes mellitus along with related complications [30]. MT is often downregulated at epigenetic level in numerous cancers [31-36].

MT-3 expression was found to be corrected with lung cancer [20], skin cancer [37], breast cancer [38], prostate cancer [39], oesophageal squamous cell carcinoma [40], bladder cancer [41] and renal carcinoma [42]. Moreover, MT-3 is a putative tumour suppressor gene, that is frequently inactivated in paediatric acute myeloid leukemia [33].

4. Metallothionein and resistance to chemotherapeutics

In MT-related chemoresistance heavy metals chelation and antioxidative functions are involved. It can contribute not only to cancer cell survival, but also to decreasing of side effect of chemotherapy, especially anthracyclines [8]. To decrease tumour resistance, or on the other

hand, to improve non-target cells tolerance to chemotherapeutic multiple approaches are tested, including specific increasing or decreasing of MT expression based on specific compounds or targeted gene therapy, including using antisense mRNA, siRNA, microRNA, and DNA methylation, can be considered [8,38,43].

5. Metallothionein as cancer biomarker

From numerous studies it is known, that expression of MT both on nucleic acids and protein level can be used as marker of tumour diseases [15,26,44,45]. Also methylation of MT promoters has been shown to be usable as a biomarker [34]. Increased level of MT in serum or full blood has been found in many cancer diseases and can be used as early stage biomarker [26,45,46]. Also changes in Zn isotopes in tumour tissues can be usable for detection of cancer. The authors hypothesize, that higher content of light isotopes in tumour tissue than in control tissues (both non tumour and healthy volunteers) is caused by preferential chelation of light isotopes by MT via SH groups [47].

6. Conclusion

MTs role is double-edged in carcinogenesis; in healthy cells with high MT content it has protective effects, while in healthy cells with low MT content, carcinogenesis via environmental factors occurs, on the other hand, at cancer--transformed cell the high MT content increases the tumour malignancy and chemotherapeutics resistance. Expression of MT both on nucleic acids and protein level can be used as marker of tumour diseases. Increased level of MT in serum or full blood has been found in many cancer diseases and can be used as early stage biomarker. To decrease tumour resistance, or on the other hand, to improve non-target cells tolerance to chemotherapeutic multiple approaches are tested, including specific increasing or decreasing of MT expression based on specific compounds or targeted gene therapy, can be considered.

Acklowledgement

The financial support from League against cancer Prague (project 18257/2014-981) and CEITEC CZ.1.05/1.1.00/02.0068 is greatly acknowledged.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Margoshes, M.; Vallee, B.L. A cadmium protein from equine kidney cortex. Journal of the American Chemical Society 1957, 79, 4813-4814.
2. Coyle, P.; Philcox, J.C.; Carey, L.C.; Rofe, A.M. Metallothionein: The multipurpose protein. Cell. Mol. Life Sci. 2002, 59, 627-647.
3. Vasak, M.; Meloni, G. Chemistry and biology of mammalian metallothioneins. J. Biol. Inorg. Chem. 2011, 16, 1067-1078.
4. Olesen, C.; Moller, M.; Byskov, A.G. Tesmin transcription is regulated differently during male and female meiosis. Molecular Reproduction and Development 2004, 67, 116-126.
5. Zalewska, M.; Trefon, J.; Milnerowicz, H. The role of metallothionein interactions with other proteins. Proteomics 2014, 14, 1343-1356.
6. Orihuela, R.; Fernandez, B.; Palacios, O.; Valero, E.; Atrian, S.; Watt, R.K.; Dominguez-Vera, J.M.; Capdevila, M. Ferritin and metallothionein: Dangerous liaisons. Chem. Commun. 2011, 47, 12155-12157.
7. Maret, W. Fluorescent probes for the structure and function of metallothionein. J. Chromatogr. B 2009, 877, 3378-3383.
8. Krizkova, S.; Ryvolova, M.; Hrabeta, J.; Adam, V.; Stiborova, M.; Eckschlager, T.; Kizek, R. Metallothioneins and zinc in cancer diagnosis and therapy. Drug Metabolism Reviews 2012, 44, 287-301.
9. Klassen, R.B.; Crenshaw, K.; Kozyraki, R.; Verroust, P.J.; Tio, L.; Atrian, S.; Allen, P.L.; Hammond, T.G. Megalin mediates renal uptake of heavy metal metallothionein complexes. American Journal of Physiology-Renal Physiology 2004, 287, F393-F403.
10. Krizkova, S.; Fabrik, I.; Adam, V.; Hrabeta, P.; Eckschlager, T.; Kizek, R. Metallothionein - a promising tool for cancer diagnostics. Bratislava Medical Journal-Bratislavske Lekarske Listy 2009, 110, 93-97.
11. Uchida, Y.; Takio, K.; Titani, K.; Ihara, Y.; Tomonaga, M. The growth inhibitory factor that is deficient in the alzheimers-disease brain is a 68-amino acid metallothionein-like protein. Neuron 1991, 7, 337-347.
12. Quaife, C.J.; Findley, S.D.; Erickson, J.C.; Froelick, G.J.; Kelly, E.J.; Zambrowicz, B.P.; Palmiter, R.D. Induction of a new metallothionein isoform (mt-iv) occurs during differentiation of stratified squamous epithelia. Biochemistry 1994, 33, 7250-7259.
13. Thirumoorthy, N.; Sunder, A.S.; Kumar, K.T.M.;

Kumar, M.S.; Ganesh, G.N.K.; Chatterjee, M. A review of metallothionein isoforms and their role in pathophysiology. World J. Surg. Oncol. 2011, 9.
14. McGee, H.M.; Woods, G.M.; Bennett, B.; Chung, R.S. The two faces of metallothionein in carcinogenesis: Photoprotection against uvr-induced cancer and promotion of tumour survival. Photochem. Photobiol. Sci. 2010, 9, 586-596.
15. Werynska, B.; Pula, B.; Muszczynska-Bernhard, B.; Gomulkiewicz, A.; Piotrowska, A.; Prus, R.; Podhorska-Okolow, M.; Jankowska, R.; Dziegiel, P. Metallothionein if and 2a overexpression predicts poor outcome of non-small cell lung cancer patients. Exp. Mol. Pathol. 2013, 94, 301-308.
16. Nakano, M.; Sogawa, C.A.; Sogawa, N.; Mishima, K.; Yamachika, E.; Mizukawa, N.; Fukunaga, J.; Kawamoto, T.; Sawaki, K.; Sugahara, T., et al. Expression pattern of cisplatin-induced metallothionein isoforms in squamous cell carcinoma. Anticancer Res. 2003, 23, 299-303.
17. Cherian, M.G.; Jayasurya, A.; Bay, B.H. Metallothioneins in human tumors and potential roles in carcinogenesis. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 2003, 533, 201-209.
18. Theocharis, S.E.; Margeli, A.P.; Klijanienko, J.T.; Kouraklis, G.P. Metallothionein expression in human neoplasia. Histopathology 2004, 45, 103-118.
19. Eckschlager, T.; Adam, V.; Hrabeta, J.; Figova, K.; Kizek, R. Metallothioneins and cancer. Current Protein & Peptide Science 2009, 10, 360-375.
20. Werynska, B.; Pula, B.; Kobierzycki, C.; Dziegiel, P.; Podhorska-Okolow, M. Metallothioneins in the lung cancer. Folia Histochemica Et Cytobiologica 2015, 53, 1-10.
21. Goldmann, T.; Ribbert, D.; Suter, L.; Brode, M.; Otto, F. Tumor characteristics involved in the metastatic behaviour as an improvement in primary cutaneous melanoma prognostics. Journal of Experimental & Clinical Cancer Research 1998, 17, 483-489.
22. Jin, R.X.; Huang, J.X.; Tan, P.H.; Bay, B.H. Clinicopathological significance of metallothioneins in breast cancer. Pathology & Oncology Research 2004, 10, 74-79.
23. Huang, G.W.; Yang, L.Y. Metallothionein expression in hepatocellular carcinoma. World Journal of Gastroenterology 2002, 8, 650-653.
24. Neville, C.S.; Smith, C.; Van Huizen, I.; Yamasaki, Y.; Moussa, M.; Xuan, J.; Leal, J.; Chin, J.L.; Izawa, J.I. Metallothionein expression as a prognostic factor for renal cell carcinoma. Journal of Urology 2005, 173, 100-100.
25. Grove, P.S.; Chang, W.W.L.; Gangarosa, L.M. Metallothionein expression in the pancreas and pancreatic cancer. Gastroenterology 1999, 116, A1128-A1128.
26. Krizkova, S.; Masarik, M.; Majzlik, P.; Kukacka, J.; Kruseova, J.; Adam, V.; Prusa, R.; Eckschlager, T.; Stiborova, M.; Kizek, R. Serum metallothionein in newly diagnosed patients with childhood solid tumours. Acta Biochimica Polonica 2010, 57, 561-566.
27. Surowiak, P.; Materna, V.; Kaplenko, I.; Spaczynski, M.; Dietel, M.; Lage, H.; Zabel, M. Augmented expression of metallothionein and glutathione s-transferase pi as unfavourable prognostic factors in cisplatin-treated ovarian cancer patients. Virchows Archiv 2005, 447, 626-633.
28. Jasani, B.; Schmid, K.W. Significance of metallothionein overexpression in human. Histopathology 1997, 31, 211-214.
29. Ofner, D.; Maier, H.; Riedmann, B.; Bammer, T.; Rumer, A.; Winde, G.; Bocker, W.; Jasani, B.; Schmid, K.W. Immunohistochemical metallothionein expression in colorectal adenocarcinoma - correlation with tumor stage and patient survival. Virchows Archiv 1994, 425, 491-497.
30. Raudenska, M.; Gumulec, J.; Podlaha, O.; Sztalmachova, M.; Babula, P.; Eckschlager, T.; Adam, V.; Kizekb, R.; Masarik, M. Metallothionein polymorphisms in pathological processes. Metallomics 2014, 6, 55-68.
31. Alkamal, I.; Ikromov, O.; Tolle, A.; Fuller, T.F.; Magheli, A.; Miller, K.; Krause, H.; Kempkensteffen, C. An epigenetic screen unmasks metallothioneins as putative contributors to renal cell carcinogenesis. Urologia Internationalis 2015, 94, 99-110.
32. Faller, W.J.; Rafferty, M.; Hegarty, S.; Gremel, G.; Ryan, D.; Fraga, M.F.; Esteller, M.; Dervan, P.A.; Gallagher, W.M. Metallothionein 1e is methylated in malignant melanoma and increases sensitivity to cisplatin-induced apoptosis. Melanoma Research 2010, 20, 392-400.
33. Tao, Y.F.; Xu, L.X.; Lu, J.; Cao, L.; Li, Z.H.; Hu, S.Y.; Wang, N.N.; Du, X.J.; Sun, L.C.; Zhao, W.L., et al. Metallothionein iii (mt3) is a putative tumor suppressor gene that is frequently inactivated in pediatric acute myeloid leukemia by promoter hypermethylation. Journal of Translational Medicine 2014, 12.
34. Jadhav, R.R.; Ye, Z.Q.; Huang, R.L.; Liu, J.; Hsu, P.Y.; Huang, Y.W.; Rangel, L.B.; Lai, H.C.; Roa, J.C.; Kirma, N.B., et al. Genome-wide DNA methylation analysis reveals estrogen-mediated epigenetic repression of metallothionein-1 gene cluster in breast cancer. Clinical Epigenetics 2015, 7.
35. Peng, D.F.; Hu, T.L.; Jiang, A.X.; Washington, M.K.; Moskaluk, C.A.; Schneider-Stock, R.; El-Rifai, W. Location-specific epigenetic regulation of the metallothionein 3 gene in esophageal adenocarcinomas. Plos One 2011, 6.
36. Tse, K.Y.; Liu, V.W.S.; Chan, D.W.; Chiu, P.M.; Tam, K.F.; Chan, K.K.L.; Liao, X.Y.; Cheung, A.N.Y.; Ngan, H.Y.S. Epigenetic alteration of the metallothionein 1e gene in human endometrial carcinomas. Tumor Biology 2009, 30, 93-99.
37. Slusser, A.; Zheng, Y.; Zhou, X.D.; Somji, S.; Sens, D.A.; Sens, M.A.; Garrett, S.H. Metallothionein isoform 3 expression in human skin, related cancers and human skin derived cell cultures. Toxicology Letters 2015, 232, 141-148.
38. Lai, Y.Y.; Yip, G.W.C.; Bay, B.H. Targeting metallothionein for prognosis and treatment of breast cancer. Recent Patents Anti-Canc. Drug Discov. 2011, 6, 178-185.
39. Wei, H.; Desouki, M.M.; Lin, S.; Xiao, D.; Franklin, R.B.; Feng, P. Differential expression

of metallothioneins (mts) 1, 2, and 3 in response to zinc treatment in human prostate normal and malignant cells and tissues. Molecular Cancer 2008, 7.
40. Smith, E.; Drew, P.A.; Tian, Z.Q.; De Young, N.J.; Liu, J.F.; Mayne, G.C.; Ruszkiewicz, A.R.; Watson, D.I.; Jamieson, G.G. Metallothionien 3 expression is frequently down-regulated in oesophageal squamous cell carcinoma by DNA methylation. Molecular Cancer 2005, 4.
41. Sens, M.A.; Somji, S.; Lamm, D.L.; Garrett, S.H.; Slovinsky, F.; Todd, J.H.; Sens, D.A. Metallothionein isoform 3 as a potential biomarker for human bladder cancer. Environmental Health Perspectives 2000, 108, 413-418.
42. Hoey, J.G.; Garrett, S.H.; Sens, M.A.; Todd, J.H.; Sens, D.A. Expression of mt-3 mrna in human kidney, proximal tubule cell cultures, and renal cell carcinoma. Toxicology Letters 1997, 92, 149-160.
43. Heger, Z.; Merlos, M.A.R.; Krizkova, S.; Ruttkay-Nedecky, B.; Zalewska, M.; Planells, E.M.P.; Pelfrene, A.; Pourrut, B.; Stiborova, M.; Eckschlager, T., et al. Metallothionein as a scavenger of free radicals – new cardioprotective therapeutic agent or initiator of tumor chemoresistance? Curr. Drug Targets 2015, in press.
44. Gumulec, J.; Raudenska, M.; Adam, V.; Kizek, R.; Masarik, M. Metallothionein - immunohistochemical cancer biomarker: A meta-analysis. Plos One 2014, 9.
45. Tariba, B.; Zivkovic, T.; Krasnici, N.; Marijic, V.F.; Erk, M.; Gamulin, M.; Grgic, M.; Pizent, A. Serum metallothionein in patients with testicular cancer. Cancer Chemotherapy and Pharmacology 2015, 75, 813-820.
46. Krejcova, L.; Fabrik, I.; Hynek, D.; Krizkova, S.; Gumulec, J.; Ryvolova, M.; Adam, V.; Babula, P.; Trnkova, L.; Stiborova, M., et al. Metallothionein electrochemically determined using brdicka reaction as a promising blood marker of head and neck malignant tumours. International Journal of Electrochemical Science 2012, 7, 1767-1784.
47. Larner, F.; Woodley, L.N.; Shousha, S.; Moyes, A.; Humphreys-Williams, E.; Strekopytov, S.; Halliday, A.N.; Rehkamper, M.; Coombes, R.C. Zinc isotopic compositions of breast cancer tissue. Metallomics 2015, 7, 107-112.