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Laboratory Investigation

Metallothionein and anticancer agents: the role of metallothionein in cancer chemotherapy

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Summary

Metallothioneins (MTs) are intracellular proteins containing the highest amount of thiol groups within the cytoplasm. These thiol groups are able to bind several cytotoxic agents, such as platinum compounds and alkylating agents. Increased levels of MT are one mechanism of resistance to these anticancer drugs, as intracytoplasmic binding of MT prevents the active molecules from reaching their target, the intranuclear DNA of tumor cells. MT synthesis can easily be induced by physiologic heavy metals such as zinc and copper. Pharmacological modulation of MT levels has been used to increase the MT pool in normal tissues and decrease their susceptibility to the toxicity of anticancer drugs. In the case of tumors arising in the brain, where the inducibility of MT synthesis is low, this approach would allow protection of normal tissues without decreasing the antitumor activity of the cytotoxic agents. The interaction of MT with cytotoxic agents is not limited to covalent binding. A correlation between MT synthesis and amplification of oncogenes such as *ras* has been reported. Furthermore, the cytotoxic drugs are bound by MT after competition with zinc and copper; these metals are cofactors of numerous metalloenzymes, some of which are involved in the metabolism of nucleic acids. Competitive displacement of these metals might modify nucleic acid metabolism and influence cellular proliferation. On the other hand, increased MT levels could provide a zinc cofactor reserve that increases the cell's reparative potential when faced by DNA damage by cytotoxic agents. Although the physiologic role of MT in resistance to the cytotoxic effects of anticancer drugs remains unclear, a better understanding of the interaction between MT and chemotherapeutic agents may be important in the treatment of cancer.

Introduction

The metallothioneins (MTs) are intracellular proteins of low molecular weight (6000–7000 D) characterized by an abundance of thiol groups (30% cysteine) [1] (Fig. 1). MTs are, in fact, the principal source of thiol groups in eukaryotic cells. These proteins are found in all animal species, and their structure has largely been conserved during evolution. By virtue of their thiol groups, MTs can bind heavy metals. MTs are involved in the physiologic metabolism of zinc [2] and copper [3], and they have been implicated in detoxication of heavy metals such as cadmium and mercury [4]. There are at least five types of MTs in humans, all of similar biochemical structure [5].

MT is synthesized in most body tissues. The highest concentrations are found in the kidney and liver; a brain-specific MT, MT III, has recently been described [6]. In addition to the heavy metals men-

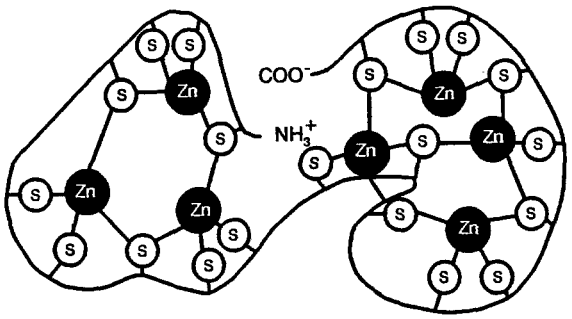

Fig. 1. Zinc thiolate clusters of metallothionein. From Kagi JH, Kojima: Chemistry and biochemistry of metallothionein. *Experientia Suppl* 52: 25–61, 1987. Reproduced with permission.

tioned above, MT synthesis can be induced by a wide variety of compounds [1], including the glucocorticoids [7], progesterone, estrogens, interleukin 1 [8], ethanol, dextran, and phorbol ester tumor promotor [9], as well as by conditions such as infection [10], fasting [11], and stress [1, 12]. The abundance of thiol radicals in MTs has prompted speculation that they interact with electrophilic cytotoxic agents, such as the alkylating agents and platinum compounds, since their affinity for thiol groups, such as intracellular glutathione in the form of reduced glutathione (GSH), is known.

Previously it was thought that MT and cytotoxic agents interacted through simple covalent binding by the thiol groups [13]. Studies of pharmacologic manipulation of the intracellular pool of MT by metals or other compounds have provided an experimental basis for attempts to reduce the toxicity of these drugs [14]. More recently, it has been suggested that the interaction between MT and cytotoxic agents is more complex than previously thought. First, it appears that the intracellular pool of MT can be modulated by oncogenes involved in cellular proliferation [15]. Second, the effect of the interaction of MT and cytotoxic agents on the intracellular pool of zinc and copper [16] may also affect the proliferation kinetics of tumor cells [17].

Metallothioneins and resistance to drugs

Studies of MT and resistance to alkylating agents and platinum compounds in cell culture

In cell culture, an increased intracellular concentration of MT, most often induced by exposure to cadmium or zinc, has been associated with greater resistance to alkylating agents [18–21] and platinum compounds [20, 22–26]. Direct binding of cisplatin by MT has been documented [13, 16, 27, 28], probably after competition with zinc and copper [16]. Intracytoplasmic covalent binding of alkylating agents and platinum compounds by MT reduces the proportion of these drugs that can interact with nuclear DNA and thereby exert their cytotoxic effect.

The role of MT in resistance to platinum compounds and alkylating agents is controversial. Kelly *et al.* [20] have demonstrated that the overexpression of MT confers resistance to alkylating agents and platinum derivatives. Through chronic exposure to cisplatin or cadmium, cellular populations made resistant to cisplatin exhibited cross resistance to other alkylating agents (chlorambucil and melphalan) and were found to have elevated intracytoplasmic levels of MT. Moreover, transfection of mouse cells with a bovine papilloma virus that expressed DNA encoding human MT II conferred resistance to these same drugs [20, 21]. However, other authors have shown no alteration in intracellular concentrations of MT in cell lines made resistant to these drugs by chronic exposure to progressively increasing doses [29–31].

The contradictory nature of these findings does not necessarily indicate that they are incompatible. MTs may well represent a potential factor in the resistance to this family of cytotoxic agents, but their role in this resistance may vary from one type of tumor to another or among individual tumors of the same type. The effect of tissue heterogeneity on the role of MTs in resistance to these drugs is complicated by the fact that different types of MT are probably heterogeneous in their inducibility and in their reactivity to these drugs [6]. Increasingly there is contradictory evidence concerning the inducibility of MT by the cytotoxic agents themselves. In certain experimental systems, cytotoxic drugs have

been shown to induce MT [20, 32, 33], whereas in others the increase in the intracellular pool of MT reduced the toxicity of the drugs, but the drugs did not directly induce MT [20].

Besides its role in the binding of these cytotoxic agents, MT may also affect resistance by interacting with intracellular GSH. The role of GSH in the resistance to platinum compounds and alkylating agents is well established [34]. In platinum-resistant tumor cell lines with elevated GHS levels, resistance is partially reversible by administration of buthionine sulfoximine to deplete GSH. Interestingly, however, it has been shown in cell culture that depletion of intracellular GSH by buthionine sulfoximine may be associated with an increase in MT synthesis [35]. This interaction might partially limit the effect of GSH depletion on increasing drug activity.

Pharmacologic modulation of intracellular metallothionein: effects on the toxicity and activity of platinum compounds and alkylating agents in experimental animals

Although the physiologic role of MT in the resistance to chemotherapeutic drugs remains uncertain, it is nevertheless possible to envisage an experimental strategy for increasing drug resistance in normal tissue by inducing MT synthesis pharmacologically. The goal of chemomodification is to reduce the toxicity of a drug without altering its activity [34]. The reduction of toxicity has been reported for platinum compounds and alkylating agents. The experimental strategy used in animals is to induce MT synthesis, usually with a nontoxic heavy metal, before treatment with the cytotoxic drug and study the effect of MT induction on the toxicity of the treatment. In this fashion it has been shown that systemic injection of copper, zinc, or bismuth reduces the renal toxicity of subsequent treatment with cisplatin [36, 37]. A reduction in cisplatin's hematologic toxicity [37, 38] and its toxicity to Sertoli cells [39] has also been demonstrated. These protective effects might also be due solely to pharmacodynamic interaction, especially in the renal handling of these metals.

The theoretical risk of this therapeutic strategy is that MT synthesis might be induced in the cancer cells, possibly conferring resistance to the cytotoxic drugs used. However, Naganuma *et al.* [37] found that MT induction did not diminish the antitumor activity of cytotoxic drugs in mice inoculated with tumor ascites (leukemia P388) or those with subcutaneously implanted tumors. Despite such encouraging results in experimental animals, it is not known why MT synthesis would be less inducible in tumor tissue than in normal tissue and therefore the possibility that it could increase resistance in tumor cells cannot be dismissed [20, 23]. Nevertheless, pharmacologic induction of MT synthesis could be a very interesting approach to the treatment of tumors of tissues in which induction of MT synthesis is limited or impossible.

We recently began to study the experimental basis for increasing the therapeutic index of carboplatin in the treatment of cerebral tumors by a high-zinc diet. Systemic administration of zinc or other nontoxic heavy metals does not induce MT synthesis in brain parenchyma [40–42]. This appears to be linked more to anatomic criteria, e.g., the poor penetration of the blood-brain barrier by zinc [43], than to any intrinsic property of cerebral tissue, as intraventricular administration of zinc can induce MT synthesis in rat brain [41]; and mouse brain-specific MT-III is not inducible by zinc [5]. A high-zinc diet increases the MT concentration in erythrocytes [44], which suggests that MT synthesis can be induced in bone marrow [45]. Carboplatin, a cisplatin analogue that is an attractive drug for treating cerebral tumors [46] because of its proportionately better brain penetration, has a dose-limiting hematotoxicity. Using a clonogenic assay (colony-forming unit/granulocyte-monocyte [CFU-GM]), we have shown that pretreatment with a high-zinc diet reduced the hematologic toxicity of carboplatin and induced the production of mRNA of MT in mouse bone marrow [14]. The high-zinc diet did not reduce the activity of carboplatin against 9L rat gliosarcoma implanted intracerebrally and did not induce MT synthesis in normal brain parenchyma or in experimental cerebral tumors [14].

Our most recent studies [47] have shown that exposure to high zinc levels does not affect the sub-

sequent sensitivity of human glioma cell lines U251 MG and SF763 to carboplatin, as determined by a 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay, and that pretreatment with zinc protected mouse bone marrow cells from subsequent treatment with carboplatin in cell culture and in experimental animals, as demonstrated by a CFU-GM assay. Similarly, exposure to high levels of zinc did not affect the sensitivity of human glioma cell lines U251 MG, U87 MG, SF126, SF188, SF763, and SF767 to 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), the most effective and most widely used chemotherapeutic agent against brain tumors, but did protect bone marrow cells from the cytotoxic effects of subsequent BCNU treatment. Thus, in this specific tumor site, increasing the intracellular pool of MT in healthy tissues appears to carry little risk of increasing tumor resistance to platinum compounds or alkylating agents.

Further studies are needed to clarify and improve the pharmacologic induction of MT synthesis to reduce the toxicity of drugs. The intracellular MT pool is known to vary significantly according to the type and duration of the treatment used to induce MT [40]. However, most of the reported studies have analyzed the effect of a single method of inducing MT. To obtain the maximal protective effect, it is therefore necessary to find a means to optimize the induction of MT synthesis in normal tissues.

Metallothionein and other anticancer agents

The thiol groups in MT can also react with the free radicals that are one of the cytotoxic mechanisms of anthracyclines. Reduction of the cardiac and hematologic toxicity of adriamycin by prior induction of MT synthesis has been shown in experimental animals [38, 48]. The detoxification of free radicals [49, 50] has been implicated as a mechanism of resistance to ionizing radiation as well, and induction of MT synthesis has been described as a factor in resistance to radiation therapy in animals [51–54]. However, in other studies, induction of MT synthesis did not protect against radiation [21]. Thus, the role of MT in resistance to radiation therapy as well as chemotherapy remains controversial.

Mattern *et al.* [55] reported that certain inhibitors of topoisomerase II (novobiocin and merbarone but not the epipodophylotoxins) can inhibit cadmium-induced MT synthesis. If confirmed in tumor tissues, this finding has interesting clinical implications as it suggests a novel type of drug synergy: inhibitors of topoisomerase II would not only exert their own cytotoxic effect, but would also reduce one factor of resistance to alkylating agents and platinum compounds.

Metallothioneins and control of cellular proliferation: other types of interactions with cytotoxic drugs

MTs are not only 'reservoirs' of heavy metals. They are distributed throughout the cytoplasm and are found in the nucleus as well. Their interactions with DNA have not been elucidated, but it is thought that they play an important role in the control of cellular proliferation. Increased expression of genes encoding for MT has been correlated with amplification of the *ras* oncogene in two human tumor cell lines that contain the activated gene *c-Ha-ras* as well as in 3T3 mouse cells transformed by the *v-Ha-ras* oncogene [15]. Interestingly, *ras*-transformed 3T3 mouse cells show increased resistance to cisplatin [56]. In normal rat cells (6m2) transformed by the *v-mos* oncogene, MT synthesis induced by dexamethasone is reduced and the protective effect of glucocorticoids against the toxicity of cisplatin is suppressed [57].

The existence of competition in binding of cisplatin and binding of copper and zinc by MT [16] could also affect the kinetics of cell proliferation. Zinc is indispensable to the functioning of more than 200 metalloenzymes, including thymidine kinase, DNA polymerases, and ribonucleases, which have important roles in nucleic acid metabolism. The eventual liberation of zinc by MT as a result of covalent binding of a cytotoxic drug such as a platinum compound or an alkylating agent could therefore have indirect effects on nucleic acid metabolism [17]. Moreover, certain oncogenes such as *gli* are 'zinc finger' proteins [58] that can bind with DNA. Modulation of the intracellular or intranuclear pool of

zinc as a result of a liberation by MT could have consequences for the control of cellular proliferation.

Conclusions

The physiologic role of MT in resistance to cytotoxic agents remains controversial and probably varies according to the type of tumor. Pharmacologic modulation of the cellular pool of MT shows promise as an experimental approach to increase the therapeutic index of anticancer drugs, especially in the case of brain tumors because of the low inducibility of MT in the brain. The interaction of these drugs with MT involves more than simple covalent binding, but the effects of this interaction on cell growth remain obscure.

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References

1. Kägi JHR, Schaffer A: Biochemistry of metallothionein. Biochemistry 27: 8509–8515, 1988
2. Fosmire GJ: Zinc toxicity. Am J Clin Nutr 51: 1225–1227, 1990
3. Felix K, Weser U: Release of copper from yeast copper-thionein after S-alkylation of copper-thiolate clusters. Biochem J 252: 577–588, 1988
4. Webb M: Toxicological significance of metallothionein. Experientia Suppl 52: 109–113, 1987
5. Lazo JS, Bahnson RR: Pharmacological modulators of DNA-interactive antitumor drugs. Trends Pharmacol Sci 10: 369–373, 1989
6. Palmiter RD, Findley SD, Whitmore TE, Durnam DM: MT-III, a brain specific member of the metallothionein gene family. Proc Natl Acad Sci USA 89: 6333–6337, 1992
7. Mayo KE, Palmiter RD: Glucocorticoid regulation of metallothionein-I mRNA in cultured mouse cells. J Biol Chem 256: 2621–2624, 1981
8. Cousins RJ, Leinart AS: Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J 2: 2884–2890, 1988
9. Imbra RJ, Karin M: Metallothionein gene expression is regulated by serum factors and activators of protein kinase C. Mol Cell Biol 7: 1358–1363, 1987
10. Abe S, Matsumi M, Tsukioki M, Mizukawa S, Takahashi T, Iijima Y, Itano Y, Kosaka F: Metallothionein and zinc metabolism in endotoxin shock in rats. Experientia Suppl 52: 587–594, 1987
11. Bremner I, Davies NT: The induction of metallothionein by zinc injection and restriction of food intake. Biochem J 149: 733–738, 1975
12. Hidalgo J, Borràs M, Garvey JS, Armario A: Liver, brain, and heart metallothionein induction by stress. J Neurochem 55: 651–654, 1990
13. Zelazowski AJ, Garvey JS, Hoeschele JD: *In vivo* and *in vitro* binding of platinum to metallothionein. Arch Biochem Biophys 229: 246–252, 1984
14. Doz F, Berens ME, Deschepper CF, Dougherty DV, Bigornia V, Rosenblum ML: Experimental basis for increasing the therapeutic index of cis-diamminedicarboxylato-cyclobutaneplatinum (II) in brain tumor therapy by a high-zinc diet. Cancer Chemother Pharmacol 29: 219–226, 1992
15. Schmidt CJ, Hamer DH: Cell specificity and an effect of *ras* on human metallothionein gene expression. Proc Natl Acad Sci USA 83: 3346–3350, 1986
16. Sharma RP: Interactions of cis-platinum with cellular zinc and copper in rat liver and kidney tissues. Pharm Res Commun 17: 197–206, 1985
17. Shimizu R, Kubota M, Tanizawa A, Sano H, Kasai Y, Hashimoto H, Akiyama Y, Mikawa H: Inhibition of both etoposide-induced DNA fragmentation and activation of poly (ADP-ribose) synthesis by zinc ion. Biochem Biophys Res Commun 169: 1172–1177, 1990
18. Endresen L, Bakka A, Rugstad HE: Increased resistance to chlorambucil in cultured cells with a high concentration of cytoplasmic metallothionein. Cancer Res 43: 2918–2926, 1983
19. Endresen L: Comparison of the cytotoxic effect of prednimustine in cultured cells with high or low levels of metallothionein. Acta Pharmacol Toxicol 54: 49–57,1984
20. Kelly SL, Basu A, Teicher BA, Hacker MP, Hamer DH, Lazo JS: Overexpression of metallothionein confers resistance to anticancer drugs. Science 241: 1813–1815, 1988
21. Lohrer H, Robson T: Overexpression of metallothionein in CHO cells and its effect of cell killing by ionizing radiation and alkylating agents. Carcinogenesis 10:2279–2284, 1989
22. Endresen L, Rugstad HE: Protective function of metallothionein against certain anticancer agents. Experientia Suppl 52: 595–602 1987
23. Andrews PA, Murphy MP, Howell SB: Metallothionein-mediated cisplatin resistance in human ovarian carcinoma cells. Cancer Chemother Pharmacol 19: 149–154, 1987
24. Bakka A, Endresen L, Johnsen ABS, Edminson PD, Rugstad HE: Resistance against cis-dichloro-diammineplati-

num in cultured cells with a high content of metallothionein. Toxicol Appl Pharmacol 61: 215–226, 1981
25. Endresen L, Schjerven L, Rugstad HE: Tumours from a cell strain with a high content of metallothionein show enhanced resistance against cis-dichlorodiammineplatinum. Acta Pharmacol Toxicol 55: 183–187, 1984
26. Lazo JS, Basu A, Schultze RL, Reffner BE: Human metallothionein isoforms and resistance to X-irradiation and cis-diamminedichloroplatinum (Abstract). Proc Am Assoc Cancer Res 31: 335, 1990
27. Kraker A, Schmidt J, Krezoski S, Petering DH: Binding of cis-dichlorodiammine platinum (II) to metallothionein in Ehrlich cells. Biochem Biophys Res Commun 130: 786–792, 1985
28. Litterst CL, Bertolero F, Uozumi J: The role of glutathione and metallothionein in the toxicity and subcellular binding of cisplatin. In: McBrien DCH, Slater TF (eds) Mechanisms of Platinum Antitumour Drugs. IRL Press, Oxford, 1986, pp 227–248
29. Farnworth P, Hillcoat B, Roos IAG: Metallothionein-like proteins and cell resistance to cis-dichlorodiammineplatinum (II) in L1210 cells. Cancer Chemother Pharmacol 25: 411–417, 1990
30. Schilder RJ, Hall L, Monks A, Handel LM, Fornace AJ Jr, Ozols RF, Fojo AT, Hamilton TC: Metallothionein gene expression and resistance to cisplatin in human ovarian cancer. Int J Cancer 45: 416–422, 1990
31. Tobey RA, Enger MD, Griffith JK, Hildebrand CE: Zinc-induced resistance to alkylating agents: lack of correlation between cell survival and metallothionein content. Toxicol Appl Pharmacol 64: 72–78, 1982
32. Farnworth PG, Hillcoat BL, Roos IAG: Metallothionein induction in mouse tissues by cis-dichlorodiammineplatinum (II) and its hydrolysis products. Chem Biol Interact 69: 319–332, 1989
33. Kotsonis N, Klaassen CD: Increase in hepatic metallothionein in rats treated with alkylating agents. Toxicol Appl Pharmacol 51: 19–27, 1979
34. Coleman CN, Bump EA, Kramer RA: Chemical modifiers of cancer treatment. J Clin Oncol 6: 709–733, 1988
35. Kang YJ, Clapper JA, Enger MD: Enhanced cadmium cytotoxicity in A549 cells with reduced glutathione levels is due to neither enhanced cadmium accumulation nor reduced metallothionein synthesis. Cell Biol Toxicol 5: 249–258, 1989
36. Naganuma A, Satoh M, Koyama Y, Imura N: Protective effect of metallothionein-inducing metals on lethal toxicity of cis-diamminedichloroplatinum in mice. Toxicol Lett 24: 203–207, 1985
37. Naganuma A, Satoh M, Imura N: Prevention of lethal and renal toxicity of cis-diamminedichloroplatinum (II) by induction of metallothionein synthesis without compromising its antitumor activity in mice. Cancer Res 47: 983–987, 1987
38. Satoh M, Naganuma A, Imura N: Metallothionein induction prevents toxic side effects of cisplatin and adriamycin used in combination. Cancer Chemother Pharmacol 21: 176–178, 1988
39. Pogach LM, Lee Y, Giglio W, Naumoff M, Huang HFS: Zinc acetate pretreatment ameliorates cisplatin-induced Sertoli cell dysfunction in Sprague-Dawley rats. Cancer Chemother Pharmacol 24: 177–180, 1989
40. Blalock RL, Dunn MA, Cousins RJ: Metallothionein gene expression in rats: Tissue-specific regulation by dietary copper and zinc. J Nutr 118: 222–228, 1988
41. Ebadi M, Swanson S: Characterization of metallothionein-like protein in rat brain. Experientia Suppl 52: 289–291, 1987
42. Lee DY, Brewer GJ, Wang Y: Treatment of Wilson's disease with zinc. VII. Protection of the liver from copper toxicity by zinc-induced metallothionein in a rat model. J Lab Clin Med 114: 639–645, 1989
43. Pullen RGL, Franklin PA, Hall GH: ^{65}Zn uptake from blood into brain in the rat. J Neurochem 56: 485–489, 1991
44. Grider A, Bailey LB, Cousins RJ: Erythrocyte metallothionein as an index of zinc status in humans. Proc Natl Acad Sci USA 87: 1259–1262, 1990
45. Harley CB, Menon CR, Rachubinski RA, Nieboer E: Metallothionein mRNA and protein induction by cadmium in peripheral blood leucocytes. Biochem J 262: 873–879, 1989
46. Doz F, Berens ME, Dougherty DV, Rosenblum ML: Comparison of the cytotoxic activities of cisplatin and carboplatin against glioma cell lines at pharmacologically relevant drug exposures. J Neurooncol 11: 27–35, 1991
47. Roosen N, Graham TW, Doz F, Berens ME, Dougherty DV, Uriu-Hare JY, Keen CL, Rosenblum ML: Bone marrow protection by zinc pretreatment can increase the therapeutic index of carmustine and carboplatin glioma chemotherapy. Proc Am Assoc Cancer Res 33: 418, 1992 (Abstr)
48. Naganuma A, Satoh M, Imura N: Specific reduction of toxic side effects of adriamycin by induction of metallothionein in mice. Jpn J Cancer Res 79: 406–411, 1988
49. Abel J, de Ruiter N: Inhibition of hydroxyl radical generated DNA degradation by metallothionein. Toxicol Lett 47: 191–196, 1989
50. Thomas JP, Bachowski GJ, Girotti AW: Inhibition of cell membrane lipid peroxidation by cadmium and zinc metallothioneins. Biochim Biophys Acta 884: 448–461, 1986
51. Matsubara J, Tajima Y, Karasawa M: Promotion of radioresistance by metallothionein induction prior to irradiation. Environ Res 43: 66–74, 1987
52. Matsubara J, Shida T, Ishioka K, Egawa S, Inada T, Machido K: Protective effect of zinc against lethality in irradiated mice. Environ Res 41: 558–567, 1986
53. Thornalley PJ, Vasak M: Possible role for metallothionein in protection against radiation induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxylls. Biochim Biophys Acta 827: 36–44, 1985
54. Bakka A, Johnsen AS, Endresen L, Rugstad HE: Radioresistance in cells with high content of metallothionein. Experientia 38: 381–383, 1982
55. Mattern MR, Tan KB, Zimmerman JP, Mong SM, O'Leary Bartus O, Hofmann GA, Drake FH, Johnson RK, Crooke ST, Mirabelli CK: Evidence for the participation of topoisomerases I and II in cadmium-induced metallothionein ex-

pression in Chinese hamster ovary cells. Anticancer Drug Des 4: 107–124, 1989
56. Sklar MD: Increased resistance to cis diamminedichloroplatinum (II) in NIH 3T3 cells transformed by *ras* oncogenes. Cancer Res 48: 793–797, 1988
57. Basu A, Lazo JS: Suppression of dexamethasone-induced metallothionein expression and cis-diamminedichloroplatinum (II) resistance by *v-mos*. Cancer Res 51: 893–896, 1991
58. Kinzler KW, Ruppurt JM, Bigner SH, Volgelstein B: The *gli* gene is a member of the Kruppel family of zinc finger proteins. Nature 332: 371–374, 1988

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