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The role of copper in drug-resistant murine and human tumors

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Abstract Multidrug resistance (MDR) is still a major threat to successful clinical application of cancer chemotherapy. Copper plays an important role in biological systems, and copper is also involved in carcinogenesis. In the present investigation, we addressed the question whether metal copper might be involved in drug resistance of murine and human tumors. By means of atomic absorption spectroscopy, we determined serum copper concentrations. We found that the blood serum of tumor-bearing mice contained higher amounts of copper than healthy mice with tumors. Secondly, mice bearing doxorubicin-resistant Ehrlich ascites carcinoma- or cyclophosphamide-resistant Lewis lung carcinoma contained more copper in their serum than mice bearing the corresponding drug-sensitive parental tumors. Furthermore, the analysis of patients with breast cancer, colon carcinoma or lung cancer showed that the serum copper contents were higher in patients not responding to chemotherapy when compared to patients whose tumors responded to treatment. The copper levels in serum of healthy volunteers were lower than in cancer patients irrespective of their response to chemotherapy. Our results imply that the level of serum copper may be considered as a biomarker for treatment response.

Keywords Biomarker · Copper · Multidrug resistance · Serum copper

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Introduction

Multidrug resistance (MDR) is still a major threat to successful clinical application of cancer chemotherapy and also is a topic in basic cancer research. Drug resistance occurs at the cellular level, and a number of molecular mechanisms account for this phenomenon. Drug-resistant cells differ from the drug-sensitive cells in many ways, e.g., by (1) reduced accumulation of cytotoxic drugs, due to decreased drug influx and or increased drug efflux; (2) altered expression and activity of certain cellular detoxification proteins and (3) physiological changes that alter the intracellular milieu (German 1996). Several

proteins have been found to be over-expressed in multidrug-resistant human cancer cells, including the multidrug resistant *mdr1* gene product *P*-glycoprotein (Juliano and Ling 1976), the multi-drug resistance-associated protein (MRP) (Cole et al. 1992) and enzymes associated with the glutathione (GSH) metabolism (O'Brien and Tew 1996; Eijdems et al. 1995; Broxterman et al. 1993; Edincott and Ling 1989; Roninson 1991). Moreover atypical multi-drug resistance has been ascribed to decreased expression or altered activity of topoisomerase II (Efferth and Volm 2005). Although each of these proteins has been associated with a unique profile of cellular drug resistance, the drug resistance patterns may be partially overlapping. In many human tumors, several mechanisms are involved in drug resistance (Volm et al. 2004).

Copper plays an important role in human and other biological systems, e.g., copper is essential for the proper functioning of copper-dependent enzymes (cytochrome C oxidase, superoxide dismutase, tyrosinase, dopamine hydroxylase, lysyl oxidase, clotting factor V, ceruloplasmin) (Shing 1998; Watanbe et al. 1990). Copper (Cu^{2+}) is also involved in carcinogenic (Hu 1986; Raju et al. 1982) as well as anticarcinogenic (Hu 1986; Brem et al. 2005) processes. Copper stimulates endothelial cell proliferation (Hu 1986). Copper reduction inhibits the antiangiogenic response (Brem et al. 2005). Copper administration suppresses the development of rat hepatoma induced by chemical carcinogens (Raju et al. 1982). Copper (II) complexes induce the re-differentiation of tumor cells to normal cells (Kawamoto et al. 1973). Although the role of copper in physiological systems is controversial, there is no doubt that copper is an essential component of several endogenous antioxidant enzymes (Sorenson 1987; Yosmi et al. 2001).

Although copper transporters have been found to transport anti-neoplastic drugs such as cisplatin (Kuo et al. 2007), the role of copper itself for drug resistance is still poorly understood (Engleka and Maciag 1994; Majumder et al. 2005). We have reported that a copper chelate overcomes drug resistance in vivo (Choudhuri and Chatterjee 1998; Majumder et al. 2006). In the present investigation, we addressed the question for the first time whether metal copper might be involved in drug resistance of murine and human tumors. For this reason, we first analyzed serum copper levels in mice bearing drug-sensitive or drug-resistant tumors and compared the copper concentrations with healthy mice without tumors. Then, we determined the levels of copper in the serum of patients suffering from breast, colon, or lung carcinoma. Again, copper concentrations in the serum of healthy subjects were measured for comparison.

Material and methods

Biological materials

All animals (male Swiss albino and male C57BL6/J mice) were collected from our animal colony. All experiments were done in accordance with the legal regulations for animal experimentation in India and with official permission of the Chittaranjan National Cancer Institute (Kolkatta, India).

Cell lines

The Ehrlich ascites carcinoma (EAC) cell line was maintained in male Swiss albino mice, weighing 18–20 g (6–8 weeks old) and water was supplied ad libidum. A doxorubicin-resistant sub-line (EAC/DOX) was developed by sequential transfer of EAC cells to subsequent generation of host mice with continuous doxorubicin (Dox) treatment as described previously (Majumder et al. 2005; Choudhuri and Chatterjee 1998; Majumder et al. 2006; Friche et al. 1992). The treatment regime consisted of 2.0 mg/kg/week Dox intraperitoneally (i.p.). The daily treatment dose was 0.4 mg/kg for 5 days. The drug was applied 24 h after inoculation of 1×10^6 ascites tumor cells i.p. to mice (Majumder et al. 2005).

The Lewis lung carcinoma (3LL) cell line is a gift from Professor Per H. Basse, University of Pittsburg, Cancer Institute, Pittsburg, USA. 3LL cells were maintained in C57BL6/J mice weighing 18–20 g (6 weeks old). A cyclophosphamide (CTX)-resistant sub-line was developed following the method of Teicher et al. (Teicher et al. 1990) by sequential transfer of 1×10^6 3LL cells subcutaneously (s.c.) in the dorsal hind scapula. The treatment regime consisted of 300 mg/kg/week CTX i.p. (single dose). The drug was injected 24 h prior to cell collection. Primary subcutaneous tumors were observed within 24 ± 1.9 days (number of animals, $n = 20$) with cell

Fig. 1 Cyclophosphamide-resistant 3LL/CTX primary tumor (48 days growth) in C57BL6/J mouse

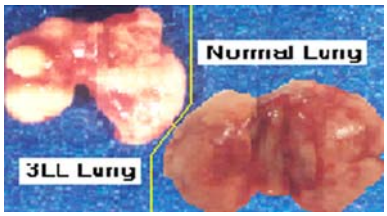

Fig. 2 Cyclophosphamide-resistant 3LL/CTX metastases (48 days growth) in the lung of C57BL6/J mouse

yields of 26.5 $\times$ 10^7 ($\pm$2.69). A representative image of a C57BL/6J mouse bearing a 3LL/CTX primary tumor s.c. is shown in Fig. 1. Secondary tumors (metastatic tumors) of 3LL/CTX-bearing mice appeared in the lung. Fig. 2 shows 3LL/CTX metastases in the lung. Normal lung is shown for comparison.

Selection of patients

Patients with cancer either in the breast, colon or lung in the advanced stage (histologically confirmed stage II–IV) were treated by Dr. J. Biswas (Hospital of Chittaranjan National Cancer Institute, Kolkatta, India). The median age of the patients was 54 years (range 28–70 years). Patients with significant peripheral neuritis or congestive heart failure were excluded from this study.

Collection of serum from normal and cancerous human beings

Blood (2 ml) was collected directly from the heart and kept for clotting. Serum was collected from blood of cancerous patients undergoing routine check-ups in the Department of Clinical Biochemistry (Hospital of Chittaranjan National Cancer Institute, Kolkatta, India). At least 2 weeks have elapsed from the application of radiotherapy or chemotherapy, before samples have been collected for this study, in order to avoid interferences of cancer therapy with copper level determination. Number of patients and treatment schedule are shown in Table 1. After chemotherapy, patients were grouped as responders (complete or partial response) or non-responders (no change, progression). In addition, blood samples (peripheral blood) were collected from five healthy volunteers of age group 20–40 years.

Measurement of serum copper

Aliquots of 100 µl serum were added to 3.9 ml of nitric acid (2.5%) and vortexed for 5 min. The solutions were kept at 37°C for 6 h with occasional shaking. The mixture was centrifuged at 2,800 rpm for 5 min. Copper was measured in the clear supernatant by means of flame atomic absorption spectrophotometry (AAS) (Varian Spectra 200 FS, Varian Inc, Californa, USA) (hollow cathode lamp, Flame type: Air acetylene; replicate 3; wavelength 324.8 nm) as described (US EPA 1994).

Results

As a first step, we measured serum copper concentrations in Swiss albino mice bearing sensitive EAC/S or doxorubicin-resistant EAC/DOX tumors. High concentrations of copper were found in EAC/DOX-bearing mice compared with EAC/S-bearing mice or healthy mice without tumors (Fig. 3).

Next, we determined serum copper levels in C57BL/6J mice bearing sensitive 3LL/S or cyclophosphamide-resistant 3LL/CTX tumors. The serum copper levels of 3LL/CTX-bearing C57BL/6J mice were 146% higher than in normal C57BL/6J mice without tumors (Fig. 4). The copper concentrations in the serum were 29% higher in 3LL/S-bearing mice than in non-tumor-bearing ones (Fig. 4).

Then, we determined the serum copper levels in cancer patients and healthy volunteers. Sixty percent of human breast cancer patients contained higher levels of copper in their serum (average: 3.25 µg/ml) than the normal healthy individuals having 2 µg/ml copper in the serum. These patients responded to chemotherapy and were, therefore, categorized as being sensitive. They contained 63% more serum copper than normal subjects without cancer (Fig. 5).

Table 1 Clinical data and serum copper determination of cancer patients

Serial number	Age/ sex	Histology	Treatment	Chemotherapy	Clinical response	Serum copper concentration (μg/ml)
Breast Ca						
1	25/F	IDC, stage II	S + RT + CT	CTX, MTX, 5FU	Responder	3.66
2	35/F	IC in mammary tissue, stage III	S + RT + CT	CTX	Non-responder	5.22
3	58/F	IDC, stage II	CT	5FU, DOX, CTX	Responder	3.76
4	30/F	IDC, stage II	S + RT + CT	CTX	Non-responder	4.21
5	70/F	ET, stage II	S + RT + CT	CTX, MTX, 5FU	Responder	1.9
6	54/F	IDC, stage II	S + RT + CT	CTX, MTX, 5FU	Responder	2.00
7	37/F	IDC, stage III	S + RT + CT	5FU, DOX, CTX	Non-responder	3.76
8	56/F	IDC, stage III	S + RT + CT	CTX, DOX	Non-responder	4.12
9	28/F	IDC, stage III	CT	CTX	Non-responder	3.9
10	44/F	IDC, stage III	S + RT + CT	CTX, MTX, 5FU	Non-responder	3.78
Lung Ca						
1	65/F	Adeno Ca, stage II	RT + CT	DDP, DOX, CTX	Responder	3.88
2	66/M	PD SCC, stage II	RT + CT	CTX, VBL, 5FU	Non-responder	3.76
3	55/M	Adeno Ca, stage III	CT	CTX, DDP, DOX	Responder	2.14
4	35/M	SCC, stage III	RT + CT	CTX, VCR, MTX	Non-responder	5.38
5	56/F	SCC, stage II	RT + CT	CTX, DOX, DDP	Responder	3.7
6	55/M	SCC, stage III	CT	DDP, ETO	Non-responder	5.36
Colon Ca						
1	54/M	Adeno Ca, stage III	CT	5FU, Bleo	Non-responder	4.88
2	36/M	Adeno Ca, stage III	S + RT + CT	5FU, Bleo	Non-responder	6.1
3	32/M	PD IC (adeno) Ca, stage II	CT	5FU, Bleo	Responder	3.66
4	28/M	MD IC(Adeno), stage III	CT	5FU, Bleo	Non-responder	5.42
5	43/M	AdenoCa, stage III	CT	5FU, Bleo	Non-responder	5.32
6	42/M	PD IC (Adeno Ca, stage II)	CT	5FU, Bleo	Responder	3.65

Bleo bleomycin; *Ca* Carcinoma; *CT* chemotherapy; *CTX* cyclophosphamide; *DDP* cisplatin; *DOX* doxorubicin; *ET* epithelial tumor; *ETO* etoposide; *5FU* 5-fluorouracil; *IC* infiltrating carcinoma; *IDC* infiltrating adenocarcinoma; *MD* moderately differentiating tumor; *MTX* methotrexate; *PD* poorly differentiating tumor; *R* radioptherapy; *S* surgery; *SCC* small cell lung carcinoma; *VBL* vinblastine; *VCR* vincristine

Student *t*-test for responders versus non responders disclose that: Group I Breast Ca $P = 0.026$; Group II Lung Ca $P = 0.107$; Group III Colon Ca $P = 0.005$

The other 40% patients did not respond to chemotherapy. The serum of these drug-resistant breast cancer patients revealed 161% more serum copper than normal human beings (Fig. 5).

Fifty-three percent of patients with colon carcinoma were non-responders (no change or progression). These patients showed 171% higher serum copper levels than healthy control subjects, whereas colon cancer patients, who responded to chemotherapy (complete or partial responders) had only 110% more serum copper than normal human beings (Fig. 5).

Eighty-five percent of patients having non-small cell lung cancer did not respond to chemotherapeutic drugs. These resistant patients had 169% more copper in their serum than healthy volunteers (Fig. 5). The 15% patients with drug-sensitive lung cancer had 109% more serum-copper than normal individuals.

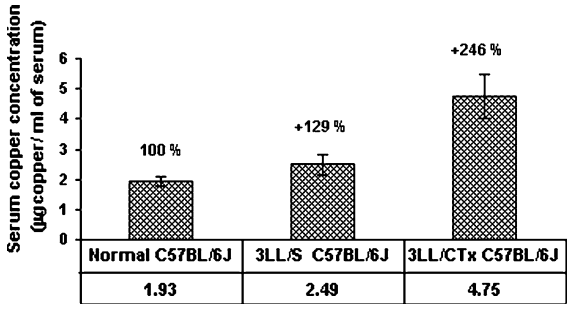

Fig. 3 Serum-copper level of healthy normal and Ehrlich carcinoma-bearing Swiss albino mice

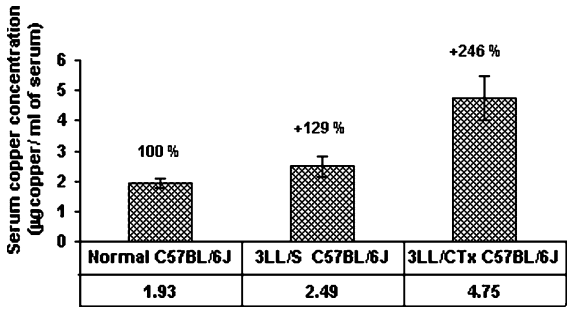

Fig. 4 Serum-copper level of healthy normal and Lewis lung carcinoma-bearing C57BL/6J mice

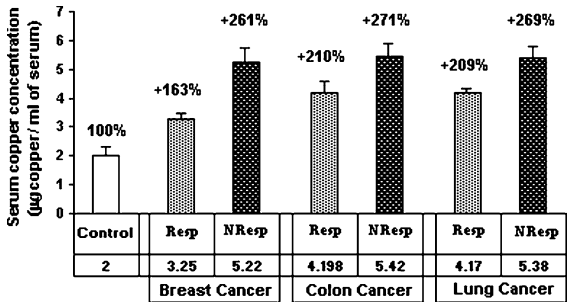

Fig. 5 Serum-copper concentration in healthy volunteers (control) and cancer patients. *Resp* Responder; *NResp* Non responder

The clinical response to chemotherapy as well as the copper levels is given in Table 1. Student *t*-test for responders versus non responders disclose that in Group I for breast Ca $P = 0.026$; in Group II for lung Ca $P = 0.107$ and in Group III for colon Ca $P = 0.005$. To prove the statistical significance of the results, we subjected the clinical response to chemotherapy with the serum copper levels of the patients to Fisher's exact test. As shown in Table 2,

Table 2 Relationship of serum copper of cancer patients and response to chemotherapy

	Responder	Non-responder
<4 µg/ml Cu	8	4
>4 µg/ml Cu	0	9

$P = 0.002$ (Fisher exact test)

patients responding to chemotherapy had significant lower serum copper levels than non-responders ($P = 0.002$) indicating that copper is associated with drug resistance.

Discussion

In the present investigation, we analyzed the association between copper and drug resistance with a view to identify drug resistant patients for better treatment. Firstly, we found that the blood serum of tumor-bearing mice contained higher amounts of copper than healthy mice with tumors. Secondly, mice bearing doxorubicin-resistant EAC- or cyclophosphamide-resistant 3LL tumors contained more copper in their serum than mice bearing the corresponding drug-sensitive parental tumors. Thirdly, we studied the level of copper in healthy volunteers and cancer patients. This indicates that copper is linked both to tumor growth and to drug resistance.

Animal models of drug resistance have the advantage that the level of copper can be measured in each generation (Majumder et al. 2005). In human cancer patients, this kind of measurements is not feasible. Therefore, we measured the level of serum copper only in advanced stages of human cancers. Our analysis showed that the concentration of copper in the serum of patients correlated with the response of the tumors to chemotherapy. Therefore, we conclude that higher levels of serum copper are associated with drug resistance and refractoriness. The reasons are not yet known, but it can be hypothesized that proteins involved in the transport of copper may also be causatively linked to drug resistance. Indeed, it has been shown previously that metallothioneins confers drug resistance to multiple anti-neoplastic drugs (Bahnson et al. 1991; Lazo et al. 1998; Volm et al. 2002; Efferth and Volm 2004). The stress-induced expression of metallothioneins depends on the transcription factor MTF-1, and

MTF-1 is also involved in the development of drug resistance (Yang et al. 2007). The copper transporters ATP7A, ATP7B, and CTR1 have recently been identified as mediators of cisplatin resistance (Kuo et al. 2007; Matsumoto et al. 2007; Yoshizawa et al. 2007; Konkimalla et al. 2008). Further work is warranted to study the defect or role of copper-binding proteins and copper transporters in drug resistance of human tumors.

Apart from copper's role in carcinogenesis and cancer drug resistance, copper is an essential trace element and is involved in many enzymatic reactions in prokaryotic and eukaryotic systems. Copper is metabolized in the liver of human being, and excess levels of copper are transported to the extracellular environment by an energy-dependent system. Copper is stored in the living body mostly as metallothionein (MT)-copper complexes and unbound free Cu^{2+} is almost nil in the living system. When copper is injected to living system, GSH binds to it before the metal complexes with MT (Engleka and Maciag 1994). Defects in copper transport cause a number of diseases like Wilson's disease (WND) or Menkes disease, which are characterized by a chronic liver and kidney damage (Petrukhin et al. 1993). The *WND* gene encodes a copper-transporting P-type adenosine triphosphate (ATP7B), whereas Menkes disease is caused by mutations in the *ATP7A* gene. Its close relative, *ATP7B* is also a member of a class of heavy metal transporting P-type ATPase that pump out Cu, Cd, Zn, Ag and Pb (Terada et al. 1998).

Can withdrawal of copper from serum cure cancer or sensitize resistant cells to drugs? Conflicting results have been obtained by lowering the level of copper in the serum. In some cases withdrawal of copper by using copper chelator, tetrathiomolybdate (TM) inhibited the process of angiogenesis and led to the partial decrease in the size of the tumor (Pan et al. 2002). The molecular mechanism by which copper deficiency regulates angiogenesis remains unknown (Brem et al. 2005; Ziche et al. 1982).

In many cases no significant tumor regression has been found by lowering copper (Brem et al. 2005; Ziche et al. 1982); Brem et al. have shown that lowering of copper in the serum (hypocupremea) by penicillamin and diet did not significantly increase the survival of glioma patients.

Substantial evidence supports the view that application copper (as copper chelate) cures cancer and overcomes drug resistance (Raju et al. 1982; Engleka and Maciag 1994; Majumder et al. 2005). High level of copper in the serum of cancer bearing animal is an expression of the stage of the disease and not the cause. The increase in copper level may be secondary to the development of resistance based on other prior molecular changes, e.g., change copper transporters, enzyme system (Safaei et al. 2004). Where from copper comes in the serum or how Cu is increased in the serum of cancer patients? We have previously reported in animal model that with concomitant rise of Cu in the serum, the level of copper is decreased in the liver (Medicicinal Chem, 2005). Although the underlying mechanism behind elevation of copper level in cancer patients is not yet understood, the level of copper may be an indication of the change in copper transporter like ATP7B in the cancer patients (Terada et al. 1998; Petrukhin et al. 1993).

We have observed that intra peritoneal (i.p.) application of copper complex (CuNG) in mouse model can overcome MDR by deactivating MRP (Majumder et al. 2006). CuNG also generates ROS (Mookerjee et al. 2006) and induces apoptosis to resistant cells by releasing IFN-γ when applied intramuscularly (im). Therefore, we did not make any further attempt to lower copper concentration to sensitizing resistant cells.

In conclusion, in the present investigation we showed that serum copper was elevated in tumor-bearing animals and patients and that the serum of animals or patients with drug-resistant tumors contained more copper than of those with drug-sensitive ones. This implies that the level of serum copper may be considered as a biomarker of response of cancer patients to chemotherapy. It merits further investigation, whether novel treatment strategies can be developed by utilizing the copper level as marker of drug resistance.

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References

Bahnson RR, Basu A, Lazo JS (1991) The role of metallothioneins in anticancer drug resistance. Cancer Treat Res 57:251–260

Brem S, Grossman, Carson KA, New P, Phuphanich S, Alvi JB, Mikkelsen T, Joy D (2005) Phase 2 trial of copper

depletion and penicillamine as antiangiogenesis therapy of glio blsatoma, new approaches to brain teory CNS consortium. Neuro-oncology 7(3):246–253

Broxterman HJ, Versantvoort CHM, Linn SC (1993) Multidrug resistance in lung cancer. In: Hansen HH (ed) Lung cancer-biological and clinical aspects. Kluwer, Norwell

Choudhuri SK, Chatterjee A (1998) Reversal of resistance against doxorubicin by a newly developed compound, oxalyl bis(*N*-phenyl) hydroxamic acid in vitro. Anticancer Drugs 9:825–832. doi:10.1097/00001813-199810000-00013

Cole SPC, Bhardwaj G, Gerlach JH, Machie JE, Grant CE (1992) Overexpression of a transporter gene in multidrug resistant human lung cancer cell line. Science 258:1650–1654. doi:10.1126/science.1360704

Edincott JA, Ling V (1989) The biochemistry of *P*-glycoprotein-mediated multidrug resistance. Annu Rev Biochem 58:137–171. doi:10.1146/annurev.bi.58.070189.001033

Efferth T, Volm M (2004) Protein expression profiles indicative for drug resistance of kidney carcinoma. Cancer Genomics Proteomics 1:17–22

Efferth T, Volm M (2005) Glutathione-related enzymes contribute to resistance of tumor cells and low toxicity in normal organs to artesunate. In Vivo 66:225–232

Eijdems EWHM, Dettass M, Timmerman AJ, Vander Schans GP, De Nooy J (1995) Reduced topoisomerase II activity in multidrug resistant human non-small-cell lung cancer cell lines. Br J Cancer 71:40–47

Engleka KA, Maciag T (1994) Inactivation of human fibroblast growth factor (FGF-1) activity by interaction with copper ions involves FGF-1 dimmer formation induced by copper catalyzed oxidation. J Biol Chem 267:11307–11315

Friche E, Danks MK, Beck WT (1992) Characterization of tumor cell resistance to 4-deoxy-4-iododoxorubicin developed in Ehrlich ascites cells in vivo. Cancer Res 52:5701–5706

German UA (1996) P-glycoprotein-a modulator of multidrug resistance in tumor cells. Eur J Cancer 32A:927–944. doi:10.1016/0959-8049(96)00057-3

Hu GF (1986) Copper stimulates proliferation of human endothelial cells under culture. J Cell Biochem 69:326–335. doi:10.1002/(SICI)1097-4644(19980601)69:3<326::AID-JCB10>3.0.CO;2-A

Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese Hamster ovary cell. Biochem Biophys Acta 455:152–156. doi:10.1016/0005-2736(76)90160-7

Kawamoto Y, Makiura S, Sugihara S (1973) The inhibitory effect of copper on DL-ethionine carcinogenesis in rats. Cancer Res 33:1129–1135

Konkimalla VB, Kaina B, Efferth R (2008) Role of transporter genes in cisplatin resistance. In Vivo 22(3):279–283

Kuo MT, Chen HH, Song IS, Savaraj N, Ishikawa T (2007) The roles of copper transporters in cisplatin resistance. Cancer Metastasis Rev 26:71–83. doi:10.1007/s10555-007-9045-3

Lazo JS, Kuo SM, Woo ES, Pitt BR (1998) The protein thiol metallothionein as an antioxidant and protectant against antineoplastic drugs. Chem Biol Interact 111:255–262. doi:10.1016/S0009-2797(97)00165-8

Majumder S, Dutta P, Choudhuri SK (2005) The role of copper in development of drug resistance in murine carcinoma. Med Chem 1:563–573. doi:10.2174/157340605774598153

Majumder S, Dutta P, Mookerjee A, Choudhuri SK (2006) The role of a novel copper complex in overcoming doxorubicin resistance in Ehrlich ascites carcinoma cells in vivo. Chem Biol Interact 159:90–103. doi:10.1016/j.cbi.2005.10.044

Matsumoto S, Tanaka T, Kurokawa H, Matsuno K, Hayashida Y, Takahashi T (2007) Effect of copper and role of the copper transporters ATP7A and CTR1 in intracellular accumulation of cisplatin. Anticancer Res 27:2209–2216

Mookerjee A, Mookerjee Basu J, Dutta P, Majumder S, Bhattacharyya S, Baral RN, Das T, Mukherjee P, Efferth T, Raha S, Sa G, Biswas J, Pal S, Roy S, Choudhuri SK (2006) Resolution of drug resistant cancer by a newly developed copper chelate through host protective cytokine mediated apoptosis. Clin Cancer Res 12(14):4339–4349. doi:10.1158/1078-0432.CCR-06-0001

O'Brien ML, Tew KD (1996) Glutathione and related enzymes in multidrug resistance. Eur J Cancer 32A:967–978. doi:10.1016/0959-8049(96)00051-2

Pan Q, Kleer CG, Keneth L et al (2002) Coper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenisis. Cancer Res 62:4854–4859

Petrukhin K, Fischer SG, Pirastu M, Chen ZS, Terada K, Furukawa T et al (1993) Mapping, cloning and genetic characterization of the region containing the Wilson's disease gene. Nat Genet 5:338–343. doi:10.1038/ng1293-338

Raju KS, Alesandrii G, Zinche M et al (1982) Ceruloplasmin, copper ions and angiogenesis. J Natl Cancer Inst 69:1183–1188

Roninson IB (ed) (1991) In molecular and cellular biology of multidrug resistance in tumor cells. Plenum, New York

Safaei R, Holzer AK, Katano K, Samimi G, Howell SB (2004) The role of copper transporters in the development of resistance to Pt drug. J Inorg Biochem 98:1607–1613. doi:10.1016/j.jinorgbio.2004.05.006

Shing Y (1998) Heparin-copper biaffinity chromatography of fibroblast growth factors. J Biol Chem 263:9059–9062

Sorenson RJR (ed) (1987) In biology of copper complexes. The Humana, Clifton

Teicher BA, Herman TS, Holden SA, Wang Y, Pfeffer MR, Carwford JW, Frei E (1990) Tumor resistance to alkylating agents conferred by mechanisms operative only. Science 247:1457. doi:10.1126/science.2108497

Terada K, Schilsky ML, Miura N, Sugiama T (1998) ATP7B (WND) protein. Int J Biochem Cell Biol 30:1063–1067. doi:10.1016/S1357-2725(98)00073-9

US EPA (1994) Methods for determination of metals in environmental samples. Supplement I Washington, DC, US Environmental Protection Agency, Office of Research and Development (EPA-600/R-94-111)

Volm M, Koomägi R, Mattern J, Efferth T (2002) Protein expression profiles indicative for drug resistance of non-small cell lung cancer. Br J Cancer 87:251–257. doi:10.1038/sj.bjc.6600463

Volm M, Koomägi R, Efferth T (2004) Prediction of drug sensitivity and resistance of cancer by protein expression profiling. Cancer Genomics Proteomics 1:157–166

Watanbe T, Seno M, Sasada R et al (1990) Molecular characterization of recombinant human acidic fibroblast growth factor produced in *E*coli comparative studies with human basic fibroblast growth factors. Mol Endocrinol 4:869–879

Yang M, Kroft SH, Chitambar CR (2007) Gene expression analysis of gallium-resistant and gallium-sensitive lymphoma cells reveals a role for metal-responsive transcription factor-1, metallothionein-2A, and zinc transporter-1 in modulating the antineoplastic activity of gallium nitrate. Mol Cancer Ther 6:633–643. doi:10.1158/1535-7163.MCT-06-0557

Yoshizawa K, Nozaki S, Kitahara H, Ohara T, Kato K, Kawashiri S, Yamamoto E (2007) Copper efflux transporter (ATP7B) contributes to the acquisition of cisplatin-resistance in human oral squamous cell lines. Oncol Rep 18:987–991

Yosmi J, Yosmi H, Kuriyama S et al (2001) The copper chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells. Int J Cancer 94:768. doi:10.1002/ijc.1537

Ziche M, Jones J, Gullino PM (1982) Role of prostaglandin E1 and copper in angiogenesis. J Natl Cancer Inst 69:475–482