Editorial

Lustrous Insights into Cisplatin Accumulation: Copper Transporters

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As a relatively polar molecule, CDDP[1] is thought to use specific plasma membrane systems for passage into cells, although entry by passive diffusion is also likely to occur. Alterations in these plasma membrane systems have been considered to be important resistance factors because one of the most consistent features of CDDP-resistant cell lines is decreased intracellular drug levels (1). Whereas the membrane determinants of accumulation have therefore been of keen interest, their molecular identities have gone undefined for many years. An interesting and noteworthy advance in this area has been the recent determination that the same proteins that mediate transport of copper are also capable of transporting CDDP. In this issue of *Clinical Cancer Research* an analysis of the potential for a copper transporter to impact treatment of ovarian cancer patients with platinum-based regimens is reported (2).

Copper is an essential metal that functions as a cofactor for enzymes involved in diverse metabolic pathways, including cytochrome *c* oxidase (the terminal enzyme of the mitochondrial respiratory chain), superoxide dismutase (an antioxidant that represents ~1% of cellular protein), and dopamine-β-monoxygenase (catecholamine biosynthesis). Although copper is a required cofactor, it is also highly reactive in the cell and, when present in excess, exerts significant toxicity consequent to the generation of free radicals. Thus, copper is subject to complex homeostatic mechanisms that maintain appropriate intracellular concentrations and restrict its ability to inflict intracellular damage. This process is accomplished by the action of specific copper transporters and chaperones (3, 4). Copper influx into the cell is mediated by Ctr1, a high affinity copper transporter. Specific chaperones, known as COX17, CCS, and HAH1, respectively, then deliver copper from the plasma membrane to the mitochondria, to cytoplasmic superoxide dismutase, and to two copper transporters located in the *trans*-Golgi apparatus. These two copper transporters, ATP7A and ATP7B, are members of the P-type ATPase family of cation transporters and are the products of the genes affected in two disorders of copper accumulation in humans, Menkes disease and Wilson disease, respectively (5, 6). In the liver, ATP7B transports copper into the *trans*-Golgi either for incorporation into copper-requiring proteins, or for extrusion into the bile, the latter of which is accomplished by vesicular trafficking from the *trans*-Golgi to the canalicular (apical) surface of hepatocytes and represents a major route of copper elimination from the body. In most other tissues, copper transport into the *trans*-Golgi is accomplished by ATP7A. Like ATP7B, ATP7A shuttles between the *trans*-Golgi and the plasma membrane in a process that mediates copper extrusion from the cell. Trafficking of ATP7A and ATP7B to the plasma membrane is thought to be regulated by intracellular copper levels (7). Copper uptake is also regulated by copper levels, at least as determined for Ctr1p, a yeast homologue of Ctr1, in that both *CTR1* transcription and Ctr1p degradation are regulated by an usual mechanism involving a copper-sensing transcription factor (8, 9).

The first insight into the involvement of copper transporters in the cellular pharmacology of CDDP was provided by Komatsu *et al.* (10), who found that ectopic expression of ATP7B in KB-3-1 cells conferred CDDP resistance associated with decreased accumulation of this agent (Fig. 1). In addition, they reported increased expression of ATP7B in a CDDP-resistant prostate cancer cell line. This study thus indicated that ATP7B had the facility for extruding CDDP from the cell and that the pump could be induced as a resistance factor. The involvement of copper transporters in CDDP resistance was extended to copper uptake systems by Ishida *et al.* (11) who used transposon mutagenesis to identify CDDP resistance factors in yeast and found that inactivation of *CTR1* was able to confer resistance to CDDP. In accord with the notion that Ctr1p is able to mediate uptake of CDDP, Ctr1p-deficient yeast exhibited decreased levels of CDDP bound to DNA and decreased CDDP accumulation. A functional link between CDDP and copper transport in the context of Ctr1p was further elucidated in wild-type yeast by experiments showing that copper and CDDP behaved as mutual competitive inhibitors, as would be expected were the two compounds common substrates for Ctr1p. That is, copper was able to reduce CDDP accumulation and attenuate CDDP toxicity, and conversely, CDDP could reduce copper accumulation. It was also demonstrated that, like copper, CDDP is able to stimulate degradation of Ctr1p. Finally, Ishida *et al.* (11) went on to demonstrate, by analyzing the CDDP sensitivity of embryonic stem cells that are homozygous for deletion of *Ctr1*, that mammalian Ctr1 is capable of mediating the uptake of CDDP and thereby functioning as a sensitivity factor for this agent.

In combination, these surprising findings on ATP7B and Ctr1 established that mammalian transporters that mediate influx and efflux of copper are also capable of transporting CDDP. These studies have been extended by reports showing that ATP7B is also able to confer resistance to carboplatin and that human Ctr1 is able to mediate the uptake of carboplatin and oxaliplatin, in addition to CDDP (12, 13). Although an analysis of the capabilities ATP7A for conferring CDDP resistance in transfected cells has yet to be reported, the high degree of structural similarity between ATP7B and ATP7A (67%) and the finding that ATP7A is overexpressed in certain CDDP-resistant cell lines suggest that this is likely to be the case (14).

Several studies have now evaluated expression of ATP7B

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[1] The abbreviations used are: CDDP, cisplatin; MRP, multidrug resistance protein.

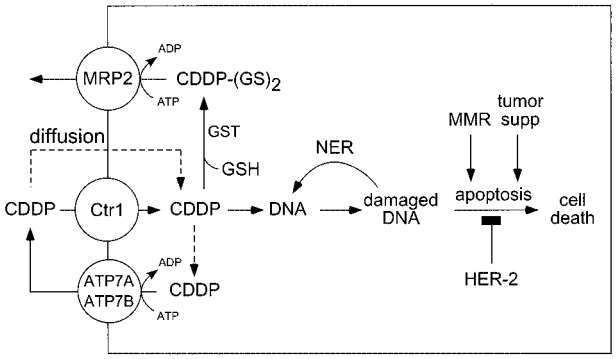

Fig. 1 Schematic summary of the molecular determinants of CDDP sensitivity. CDDP enters cells by passive diffusion and by the copper influx transporter Ctr1. CDDP is effluxed by ATP7B and probably by ATP7A (the ability of the latter pump to mediate CDDP transport has yet to be formally demonstrated). CDDP is susceptible to intracellular detoxification by reaction with nucleophiles such as glutathione and metallothioneins (the latter are not shown). CDDP-$(GS)_2$ complexes are formed spontaneously in the cell and are subject to efflux by MRP2 and probably by MRP6. On the basis of expression studies, it has been suggested that glutathione *S*-transferase π (*GST*) may enhance complex formation. CDDP damages DNA by the formation of adducts, principally at the N7 sites of purine bases. The most toxic adducts appear to be intrastrand cross-links. Damaged DNA can be repaired, primarily by the nucleotide excision repair (*NER*) pathway, or lead to cell death by apoptosis. Mismatch repair (*MMR*) system competency and tumor suppressors facilitate apoptosis, whereas oncogenes block this process. For simplicity, ATP7A and ATP7B are shown at the plasma membrane but are localized in the *trans*-Golgi network in cells. By analogy with the mechanism by which ATP7B functions to extrude copper into bile, CDDP extrusion by ATP7B, and possibly by ATP7A, may involve vesicle trafficking to the plasma membrane.

in human cancers. To date, significant levels of expression have been reported for cancers of the esophagus, stomach, breast, ovary, and oral mucosa, suggesting that ATP7B may be a factor that contributes to inherent CDDP chemoresistance (15–20). In addition, the absence of expression in adjacent normal tissues, a finding that is in accord with prior studies showing that ATP7B is largely restricted to liver, suggests that ATP7B is induced in certain cancers and also raises the possibility that cancer cells may have altered copper metabolism and/or requirements. CDDP resistance mechanisms in ovarian cancer have been intensely studied because of the striking inherent sensitivity of this cancer to CDDP-based regimens, and the fact that the majority of patients with advanced disease nevertheless relapse. This situation indicates that CDDP resistance is acquired during treatment. With respect to ovarian cancer, a study in which ATP7B expression was examined in 82 specimens is of interest, in that expression was found in 44% of samples, and high levels correlated with less differentiated cancers and with higher risk of recurrence (17). In addition, within a group of patients with moderately/poorly differentiated specimens, high ATP7B expression levels correlated with decreased survival.

In this issue of *Clinical Cancer Research*, the first description of ATP7A expression in human cancers is reported. Samimi *et al.* (2) report that ATP7A is widely expressed in human cancers, including breast, stomach, colon, ovary, lung, and prostate. Similar to the case with ATP7B, expression of ATP7A in several types of cancers was increased by comparison with expression in normal adjacent tissue. In addition to surveying a variety of cancers, a more detailed evaluation of ovarian cancer samples was undertaken by the retrospective analysis of 54 paired samples obtained before initial treatment and after treatment with at least two cycles of a CDDP- or carboplatin-based regimen. This analysis showed that ATP7A was expressed in 54% of pretreatment samples (28 samples). In addition, patients for whom expression in posttreatment samples was increased in comparison with pretreatment levels had decreased survival. Although the percentage of samples that expressed ATP7A after treatment (46%) was somewhat lower than the percentage in pretreatment samples, and the number of patients for whom expression decreased from a detectable level to undetectable (13 patients) was greater than the reverse situation (10 patients), overall this analysis suggests that induction of ATP7A expression could negatively impact the effectiveness of platinum-based chemotherapy.

These reports showing that cells can deploy energy-dependent copper pumps for defense against CDDP and carboplatin, that Ctr1 is able to mediate influx of platinum agents, and that expression of ATP7A and ATP7B correlates with worse prognosis in ovarian cancer provide intriguing insights into the cellular pharmacology of platinum compounds and into potential resistance factors associated with these agents. In addition to indicating that further investigation into the relationship between expression of copper transporters and clinical outcomes in patients treated with platinum-based chemotherapy regimens is needed, these advances also raise many important questions. Among the most important are the extent to which CDDP accumulation in the cell is attributable to copper transporters, and the extent to which alterations in the expression levels of these transporters account for the accumulation deficit that has been consistently documented in CDDP-resistant cellular models. Most studies of CDDP-resistant cell lines indicate that reduced accumulation is consequent to decreased influx, as opposed to increased efflux (1). For this reason, evaluation of Ctr1 expression in addition to ATP7A and ATP7B in patient samples will be of particular interest. Also, the mechanism by which ATP7B and presumably ATP7A depress intracellular levels of platinum compounds is unclear; like normal tissues, the pumps appear to be localized in the *trans*-Golgi apparatus in cancer cells. This suggests that the pumps may operate to sequester platinum compounds in intracellular vesicles, which may itself provide some degree of cellular protection, and that cellular efflux is accomplished by vesicular trafficking to the plasma. How CDDP affects transcriptional and posttranscriptional regulation of copper pumps and whether copper chaperones are able to interact with CDDP are also open questions. Finally, it should be borne in mind that in addition to copper transporters, other membrane systems, such as certain members of the MRP family (MRP2 and possibly MRP6; Ref. 21), have the ability to affect CDDP accumulation and that the molecular identification of other proteins involved in this process remains an important goal.

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References

1. Siddik, Z. H. Cisplatin: mode of cytotoxic action and moleclar basis of resistance. Oncogene, *22:* 7265–7279, 2003.

2. Samimi, G., Varki, N. M., Wilczynski, S., Safaei, R., Alberts, D. S., and Howell, S. B. Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. Clin. Cancer Res., *9:* 5853–5859, 2003.

3. Mercer, J. F., and Llanos, R. M. Molecular and cellular aspects of copper transport in developing mammals. J. Nutr., *133:* 1481S–1484S, 2003.

4. Shim, H., and Harris, Z. L. Genetic defects in copper metabolism. J. Nutr., *133:* 1527S–1531S, 2003.

5. Vulpe, C., Levinson, B., Whitney, S., Packman, S., and Gitschier, J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat. Genet., *3:* 7–13, 1993.

6. Bull, P. C., Thomas, G. R., Rommens, J. M., Forbes, J. R., and Cox, D. W. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat. Genet., *5:* 327–337, 1993.

7. Petris, M. J., Mercer, J. F., Culvenor, J. G., Lockhart, P., Gleeson, P. A., and Camakaris, J. Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. EMBO J., *15:* 6084–6095, 1996.

8. Yonkovich, J., McKenndry, R., Shi, X., and Zhu, Z. Copper ion-sensing transcription factor Mac1p post-translationally controls the degradation of its target gene product Ctr1p. J. Biol. Chem., *277:* 23981–23984, 2002.

9. Labbe, S., Zhu, Z., and Thiele, D. J. Copper-specific transcriptional repression of yeast genes encoding critical components in the copper transport pathway. J. Biol. Chem., *272:* 15951–15958, 1997.

10. Komatsu, M., Sumizawa, T., Mutoh, M., Chen, Z. S., Terada, K., Furukawa, T., Yang, X. L., Gao, H., Miura, N., Sugiyama, T., and Akiyama, S. Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. Cancer Res., *60:* 1312–1316, 2000.

11. Ishida, S., Lee, J., Thiele, D. J., and Herskowitz, I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc. Natl. Acad. Sci. USA, *99:* 14298–14302, 2002.

12. Lin, X., Okuda, T., Holzer, A., and Howell, S. B. The copper transporter CTR1 regulates cisplatin uptake in *Saccharomyces cerevisiae*. Mol. Pharmacol., *62:* 1154–1159, 2002.

13. Katano, K., Safaei, R., Samimi, G., Holzer, A., Rochdi, M., and Howell, S. B. The copper export pump ATP7B modulates the cellular pharmacology of carboplatin in ovarian carcinoma cells. Mol. Pharmacol., *64:* 466–473, 2003.

14. Katano, K., Kondo, A., Safaei, R., Holzer, A., Samimi, G., Mishima, M., Kuo, Y. M., Rochdi, M., and Howell, S. B. Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper. Cancer Res., *62:* 6559–6565, 2002.

15. Kanzaki, A., Toi, M., Neamati, N., Miyashita, H., Oubu, M., Nakayama, K., Bando, H., Ogawa, K., Mutoh, M., Mori, S., Terada, K., Sugiyama, T., Fukumoto, M., and Takebayashi, Y. Copper-transporting P-type adenosine triphosphatase (ATP7B) is expressed in human breast carcinoma. Jpn. J. Cancer Res., *93:* 70–77, 2002.

16. Ohbu, M., Ogawa, K., Konno, S., Kanzaki, A., Terada, K., Sugiyama, T., and Takebayashi, Y. Copper-transporting P-type adenosine triphosphatase (ATP7B) is expressed in human gastric carcinoma. Cancer Lett., *189:* 33–38, 2003.

17. Nakayama, K., Kanzaki, A., Ogawa, K., Miyazaki, K., Neamati, N., and Takebayashi, Y. Copper-transporting P-type adenosine triphosphatase (ATP7B) as a cisplatin based chemoresistance marker in ovarian carcinoma: comparative analysis with expression of MDR1, MRP1, MRP2, LRP and BCRP. Int. J. Cancer, *101:* 488–495, 2002.

18. Nakayama, K., Miyazaki, K., Kanzaki, A., Fukumoto, M., and Takebayashi, Y. Expression and cisplatin sensitivity of copper-transporting P-type adenosine triphosphatase (ATP7B) in human solid carcinoma cell lines. Oncol. Rep., *8:* 1285–1287, 2001.

19. Kanzaki, A., Nakayama, K., Miyashita, H., Shirata, S., Nitta, Y., Oubu, M., Higashimoto, M., Mutoh, M., Mori, S., Konno, S., Ogawa, K., Toi, M., and Takebayashi, Y. Mutation analysis of copper-transporting P-type adenosine triphosphatase (ATP7B) in human solid carcinomas. Anticancer Res., *23:* 1913–1915, 2003.

20. Higashimoto, M., Kanzaki, A., Shimakawa, T., Konno, S., Naritaka, Y., Nitta, Y., Mori, S., Shirata, S., Yoshida, A., Terada, K., Sugiyama, T., Ogawa, K., and Takebayashi, Y. Expression of copper-transporting P-type adenosine triphosphatase in human esophageal carcinoma. Int. J. Mol. Med., *11:* 337–341, 2003.

21. Kruh, G. D., and Belinsky, M. G. The MRP family of drug efflux pumps. Oncogene, *22:* 7537–7552, 2003.