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The Role of the Methionines and Histidines in the Transmembrane Domain of Mammalian Copper Transporter 1 in the Cellular Accumulation of Cisplatin

Christopher A. Larson, Preston L. Adams, Brian G. Blair,[1] Roohangiz Safaei, and Stephen B. Howell

Rebecca and John Moores Cancer Center and Department of Medicine, University of California, San Diego, La Jolla, California

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ABSTRACT

Mammalian copper transporter 1 (CTR1) is a high-affinity copper influx transporter that also mediates the uptake of platinum-containing chemotherapeutic agents including cisplatin (cDDP). Methionines 150, 154, and histidine 139 have been proposed to form a series of stacked rings in the pore formed by the CTR1 homotrimer, each of which is required for maximal copper transport. To examine the mechanism by which hCTR1 also transports cDDP, variant forms of hCTR1 in which methionines 150 and 154 were converted to isoleucines or in which histidine 139 was converted to alanine were re-expressed in cells in which both alleles of CTR1 had been knocked out. Each of these conversions disabled copper transport and increased cellular resistance to the cytotoxic effect of copper. In contrast, conversion of the methionines increased the uptake and cytotoxicity of cDDP well above that attained with wild-type hCTR1. Conversion of His139 to alanine did not impair cDDP uptake and actually enhanced cytotoxicity. Thus, although Met150 and Met154 facilitate the movement of copper through the pore, they serve to obstruct the passage of cDDP. None of the modifications altered the ability of cDDP to trigger the degradation of hCTR1, indicating that cDDP must interact with hCTR1 at other sites as well. Although both copper and cDDP may rely on a series of transchelation reactions to pass through the hCTR1 trimeric complex, the details of the molecular interactions must be different, which provides a potential basis for selective pharmacological modulation of copper versus cDDP cytotoxicity.

Introduction

Copper is an essential trace metal required for multiple normal cell functions. It plays a key role in regulating metabolic pathways, controls the redox potential of the cell, serves as a cofactor with transcription factors such as p53, and may even be important in controlling cell trafficking (Madsen and Gitlin, 2007). The cellular level of copper is maintained within a narrow range by a limited number of influx and efflux transporters. The influx transporters pass copper to a series of intracellular chaperones that ensure that it is delivered to specific copper-requiring enzymes in various compartments of the cell. The binding of copper to chaperones also serves to protect the cell against the toxic effects of free copper, which is otherwise easily oxidized and capable of generating free radicals. Mutations that disable copper efflux cause Menkes and Wilson diseases.

CTR1 is the major copper influx transporter. Each monomer contains a 67-amino acid hydrophilic extracellular N-terminal domain, three transmembrane segments, and a short intracellular C-terminal tail. It assembles in the plasma membrane as a homotrimer, in which it forms a pore through which copper is believed to pass (De Feo et al., 2009). The inner faces of the pore contain methionines, cysteines, and histidine residues that facilitate the movement of copper into the cell down-concentration gradient. Interactions between copper and methionines 150 and 154 and histidine 139 are important determinants of the high affinity of CTR1 for copper. These amino acids are believed to form a series of stacked rings, each of which can chelate Cu^{+} that is then handed down through the pore in a series of transchelation reactions (De Feo et al., 2009; Howell et al., 2010). The requirement for sequential transchelation reactions seems to be the basis for the high selectivity of CTR1, which transports Cu^{+} but not Cu^{2+}.

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[1] Current affiliation: Johns Hopkins University, Baltimore, Maryland.

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ABBREVIATIONS: CTR1, copper transporter 1; cDDP, cisplatin [*cis*-diamminedichloroplatinum(II)]; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; MEF, mouse embryonic fibroblast; DMEM-RS, Dulbecco's modified Eagle's medium reduced serum.

CTR1 is not just important for copper transport; it is also of interest because it regulates the cellular accumulation of the platinum-containing chemotherapeutic agents. Previous work from this (Holzer et al., 2004; Larson et al., 2009) and other laboratories (Song et al., 2004) has shown that CTR1 mediates the uptake of cisplatin and is an important determinant of its cytotoxicity both in vitro and in vivo. Knockout of both alleles of CTR1 reduces initial cDDP influx (Larson et al., 2009), enhances efflux (Blair et al., 2009), and renders cells highly resistant to cDDP. Re-expression of hCTR1 in cells lacking endogenous CTR1 restores cDDP uptake and sensitivity (Larson et al., 2009). Copper can trigger the down-regulation of CTR1, but in several types of cells, cDDP does so at much lower concentrations and substantially more rapidly that copper (Holzer et al., 2006; Jandial et al., 2009). A key unanswered question is how the CTR1 homotrimer transports both the small Cu^{+} ion and the much larger cDDP molecule.

As shown in Fig. 1, prior studies have established the importance of Met150, Met154, and His139 to the transport of copper. These amino acids are of particular interest because structural studies suggest that they lie in the narrowest part of the pore formed by homotrimeric CTR1 and that methionines and histidines are often involved in binding copper in other proteins. To examine the mechanism by which hCTR1 transports both copper and cDDP, variant forms of hCTR1 in which both methionines were converted to isoleucines, or in which the histidine was converted to alanine, were re-expressed in cells in which both alleles of CTR1 had been knocked out. Modification of Met150 and Met154 or His139 disabled copper transport and increased cellular resistance to the cytotoxic effect of copper. However, these same modifications of Met150 and Met154 had the opposite effect on the transport of cDDP. cDDP accumulation was increased to an even greater extent than attained with wild-type hCTR1, and this was accompanied by a further enhancement of cell kill. In contrast to the effect of converting His139 to alanine on copper transport, there was little effect on cDDP uptake. None of these changes affected the ability of cDDP to trigger the degradation of hCTR. These results provide further evidence of the ability of hCTR1 to transport cDDP as well as copper and identify Met150 and Met154 as targets for the potential pharmacological enhancement of cDDP uptake and efficacy.

Materials and Methods

Drugs and Reagents. cDDP was acquired from the Rebecca and John Moores Cancer Center pharmacy, containing cDDP at a concentration of 3.33 mM in 0.9% NaCl. The cDDP was diluted into DMEM Reduced Serum (DMEM-RS; HyClone Laboratories, Logan, UT) to a final concentration of 30 μM. Bradford reagent was obtained from Bio-Rad Laboratories (Hercules, CA), and sulforhodamine B was purchased from Sigma-Aldrich (St. Louis, MO) and was solubilized in 1% acetic acid (v/v) at a final concentration of 0.4% sulforhodamine B (w/v). Anti-myc primary antibody, clone 9B11, was obtained from Cell Signaling Technology, Inc. (Danvers, MA). Secondary anti-mouse, horseradish peroxidase-conjugated antibody was purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). Hoechst 33342 nuclear stain and anti-mouse Alexa Fluor 488-conjugated secondary antibody were obtained from Invitrogen (Carlsbad, CA).

Cell Types, Culture, and Engineering. Mouse embryonic fibroblasts containing wild-type alleles of CTR1 [CTR1(+/+)] and a line in which both copies of CTR1 had been somatically knocked out [CTR1(−/−)] were kindly provided by Dr. Dennis Thiele (Lee et al., 2002). The myc-CTR1(−/−)^{wt} subline was constructed by infecting the CTR1(−/−) cells with a lentivirus expressing wild-type human CTR1 cDNA, N-terminally tagged with the myc epitope, using the ViraPower Lentiviral Induction kit (Invitrogen). Point mutations were created with the GeneTailor Site-Directed Mutagenesis Kit (Invitrogen) using the following primers: for the myc-CTR1(−/−)^{H139A} mutation, tcctcacctcctgcaaacagtgctggccatcatccaggtggtcataagctac (forward); and gtagcttatgaccacctggatgatggccagcactgtttgcaggaggtgagga (reverse); for the myc-CTR1(−/−)^{M150,154I} mutation, aggtggtcataagctacttcctcatactcatcttcataacctacaacgggtacctctgcattg (forward) and caatgcagaggtacccgttgtaggttatgaagatgagtatgaggaagtagcttatgaccacct (reverse).

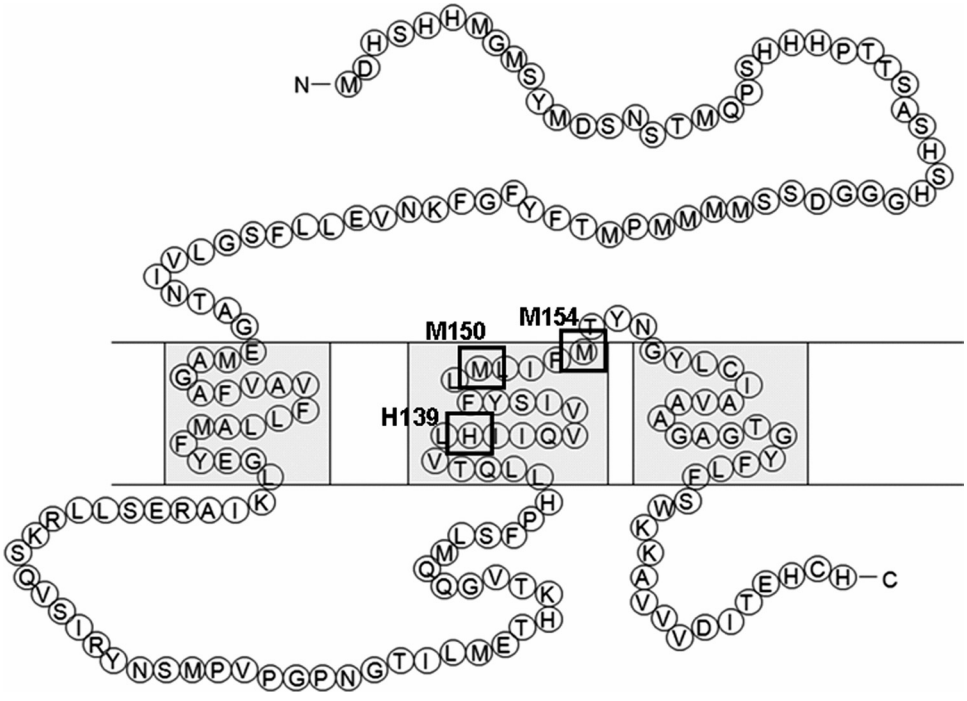

Fig. 1. Schematic diagram of the amino acid sequence of hCTR1. Boxes highlight the His139, Met150, and Met154 amino acids.

Cell Survival Assay. The sulforhodamine B assay system (Monks et al., 1991) was used to determine cell survival after exposure to increasing concentrations of drug. Five thousand cells were seeded into the wells of a 96-well tissue culture plate. Cells were incubated overnight at 37°C/5% CO_2 and then exposed to cDDP for 5 min at 37°C by the addition of 200 μl of platinum drug containing DMEM-RS medium. After 5 min, the drug-containing media were removed, cells were washed once with PBS at 37°C, PBS was aspirated off, and cells were covered in 200 μl of complete medium. Cells were allowed to grow for 5 days, after which the media were removed, the plate was washed three times with PBS, and the protein was precipitated with 50% trichloroacetic acid and stained using 100 μl of 0.4% sulforhodamine B in 1% acetic acid at room temperature for 15 min. After washing of the plate, the absorbance of each well at 515 nm was recorded using a Versamax Tunable Microplate Reader (Molecular Devices, Sunnyvale, CA). All experiments were repeated at least three times using three cultures for each drug concentration.

Immunocytochemistry. All images were visualized using a DeltaVision Deconvolution Microscope System using a Nikon TE-200 Microscope (Applied Precision, Inc., Issaquah, WA). Deconvolution and subsequent analysis was done using the softWoRx software suite (Applied Precision).

Measurement of Cellular Drug Accumulation. Drug accumulation was measured by inductively coupled plasma mass spectroscopy as described previously (Larson et al., 2009); the only change made was the use of 1 ml of medium and DMEM-RS in place of Opti-MEM.

qRT-PCR. myc-CTR1 mRNA was measured via qRT-PCR. First-strand cDNA was generated from TRIzol-isolated mRNA using oligo$(dT)_{20}$ priming and the SuperScript III First-Strand kit (both from Invitrogen). qRT-PCR was performed using a Bio-Rad MyIQ qPCR machine. The forward and reverse primers for hCTR1, mCTR1, and glyceraldehyde-3-phosphate dehydrogenase were, respectively, gatgatgatgcctatgacct, tcttgagtccttcatagaac, actgttgggcaacagatgct, ctgctgctactgcaatgcag, tcaccaccatggagaaggc, and gctaagcagttggtggtgca. Reactions used iQ SYBR Green Supermix (Bio-Rad), according to the manufacturer's recommendations. Samples were prepared in quadruplicate with a minimum of three independent RNA isolates used in independent experiments. Analysis was done using the Bio-Rad iQ5 system software.

Statistical Analysis. All two-group comparisons used Student's *t* test with the assumption of unequal variance. Data are presented as mean ± S.E.M.

Results

Expression of CTR1 in CTR1(−/−) Mouse Embryo Fibroblasts. The histidines and methionines in the transmembrane section of the pore formed by homotrimeric hCTR1 potentially chelate both copper and cDDP. The importance of Met150 and Met154 for the transport of copper has been well established; however, prior studies of the requirement for these amino acids in the transport of cDDP have been confounded by the presence of endogenous CTR1. To avoid this problem, wild-type and variant forms of hCTR1 were constitutively re-expressed in mouse embryo fibroblasts in which both alleles of CTR1 had been knocked out [CTR1(−/−) cells]. Lentiviral vectors containing a blasticidin resistance marker were constructed to express either wild-type hCTR1, a variant in which Met150 and Met154 were converted to isoleucines, or a variant in which the His139 was converted to alanine, and these were used to generate CTR1$(-/-)^{WT}$, CTR1$(-/-)^{M150,154I}$, and CTR1$(-/-)^{H139A}$ cells. All three forms of hCTR1 contained an myc tag at the N-terminal end. Cells were infected, selected with blasticidin, and the resulting population was characterized with respect to the expression of each form of exogenous CTR1 by qRT-PCR. Figure 2A shows the relative levels of CTR1 mRNA expressed in the cell lines. The two variant forms of hCTR1 were expressed at 70% of the level of the wild-type hCTR1. To further validate the system, the amount of hCTR1 in the plasma membrane was determined by exposing the cells to sulfo-NHS-SS-biotin, which labels cell surface proteins. The proteins were recovered onto streptavadin beads and subjected to Western blot analysis using antibodies that reacted with either the myc tag or α-subunit of Na^+/K^+ ATPase. As shown in Fig. 2B, the CTR1 monomer was detected at 37 kDa in all three transduced cell lines and the α-subunit at ~110 kDa. When normalized to the level of the α-subunit, the level of expression of CTR1 in the myc-CTR1$(-/-)^{H139A}$ cells was 94 ± 5% of that in the CTR1$(-/-)^{WT}$ cells, whereas expression in the myc-CTR1$(-/-)^{M150,154I}$ cells was 93 ± 4%. As shown in Fig. 2C, immunohistochemical analysis established that there were no appreciable differences in the subcellular distribution of hCTR1 in the three cell types. Thus, conversion of neither Met150 and Met154 to isoleucines nor His139 to alanine altered the trafficking of CTR1 from endoplasmic reticulum and Golgi to the cell surface or other vesicular structures.

CTR1 Regulation of Copper Uptake and Cytotoxicity. The steady-state basal level of copper was determined while the cells were growing in complete DMEM containing ~0.3 μM copper. Figure 3A shows that basal copper was 1.3-fold ($p = 0.05$) higher in the CTR1$(-/-)^{WT}$ than in the CTR1(−/−) cells. Expression of the M150,154I variant reduced the basal copper level to 89% of that found in the CTR1(−/−) cells, whereas expression of the H139A variant reduced basal copper to just 32% ($p = 0.05$) of that in the CTR1(−/−) cells. Thus, expression of these two variant forms of CTR1 perturbed either influx, efflux, or the copper binding capacity of the cells to reduce steady-state copper content.

Rates of copper accumulation were analyzed by exposing the cells to media containing 100 μM copper for 1 h. This relatively high concentration of copper allowed for the measurement of cellular copper content by inductively coupled plasma-mass spectrometry and has been shown previously to permit ready detection of copper transport abnormalities (Samimi et al., 2004). As shown in Fig. 3B, re-expression of the wild-type hCTR1 resulted in a 2-fold increase in the rate of copper accumulation compared with uptake in the CTR1(−/−) cells ($p = 0.002$). In contrast, neither of the hCTR1 variants was able to increase copper uptake at all. Thus, the integrity of either or both Met150 and Met154 and of His139 is essential for copper transport to occur in this system.

To determine whether the differences in copper accumulation translated into different tolerances to the cytotoxic effect of copper, the growth rate of the CTR(−/−), CTR1$(-/-)^{M150,154I}$, and CTR1$(-/-)^{H139A}$ cells was measured during a 96-h exposure to increasing concentrations of copper. As shown in Fig. 3C, re-expression of the wild-type CTR1 resulted in a 1.2-fold increase in sensitivity relative to the CTR1(−/−) cells (IC_{50}: 37 ± 1 versus 46 ± 2 μM; $p = 0.003$). Neither of the CTR1 variants produced a biologically significant increase in sensitivity consistent with their failure to enhance copper uptake.

CTR1 Regulation of cDDP Uptake and Cytotoxicity. To analyze the effect of the M150,154I and the H139A substitutions on the initial influx of cDDP, the four types of cells

were exposed to 30 μM cDDP for 5 min, washed thoroughly, and the platinum content was measured by inductively coupled plasma-mass spectrometry. As shown in Fig. 4A, re-expression of wild-type CTR1 resulted in a 4.2-fold increase in cDDP accumulation ($p = 0.0001$). Expression of the M150,154I variant resulted in a 7.2-fold increase in cDDP accumulation relative to that in the CTR1(−/−) cells ($p = 0.0002$) and a 1.8-fold increase relative to that in the CTR1(−/−)^{WT} cells ($p = 0.0002$). The H139A variant also increased the initial influx of cDDP, in this case by a factor 3.5-fold greater than that in the CTR1(−/−) cells ($p = 0.009$), which was 85% of the increase mediated by the wild-type hCTR1. Thus, rather than impairing the transport of cDDP, converting Met150 and Met154 to a type of amino acid not known to chelate cDDP resulted in an increase rather than a decrease in cDDP influx.

To determine whether the changes in cDDP influx translated into changes in the cytotoxicity of this drug, cells were treated with increasing concentrations of cDDP for 5 min,

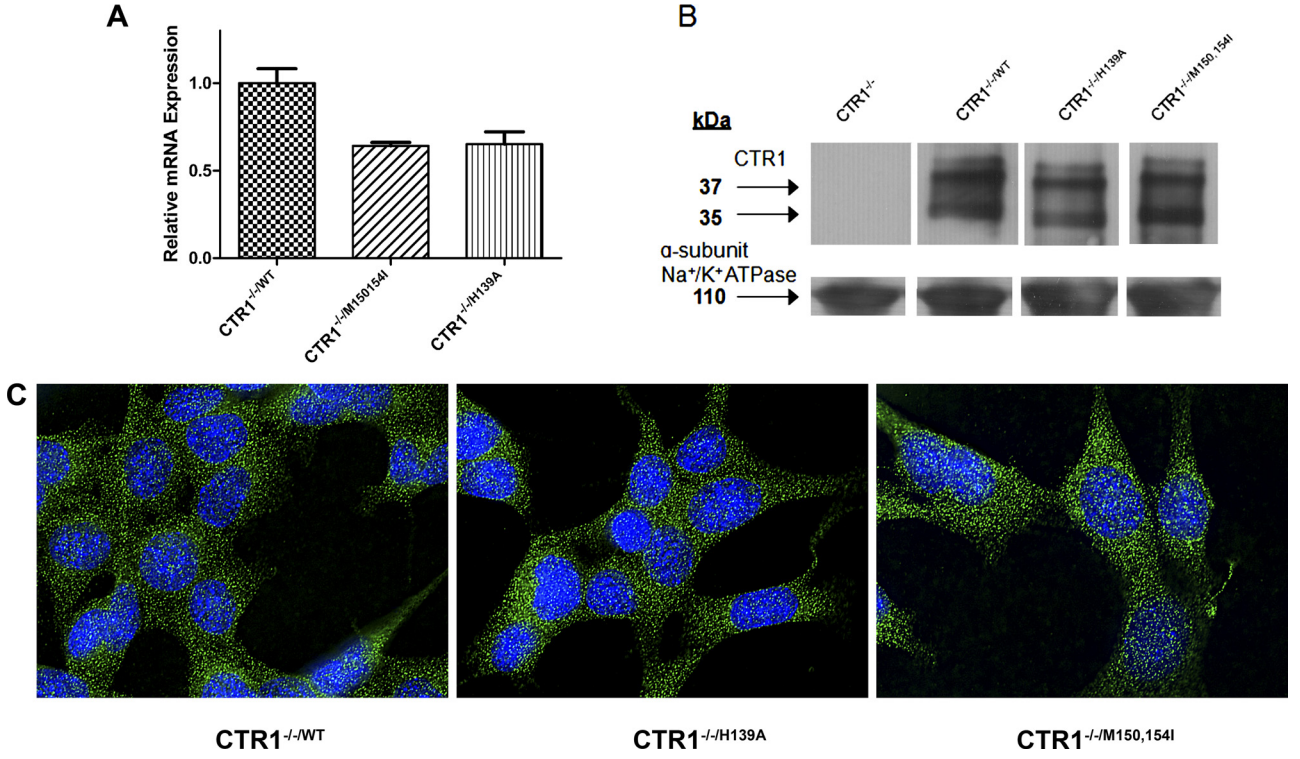

Fig. 2. Re-expression of myc-hCTR1 in CTR1(−/−) cells. A, relative hCTR1 mRNA levels as determined by qRT-PCR. B, representative Western blot showing the expression of myc-tagged CTR1. C, micrographs demonstrating uniform expression of the myc-tagged CTR1 protein (original magnification, 60×).

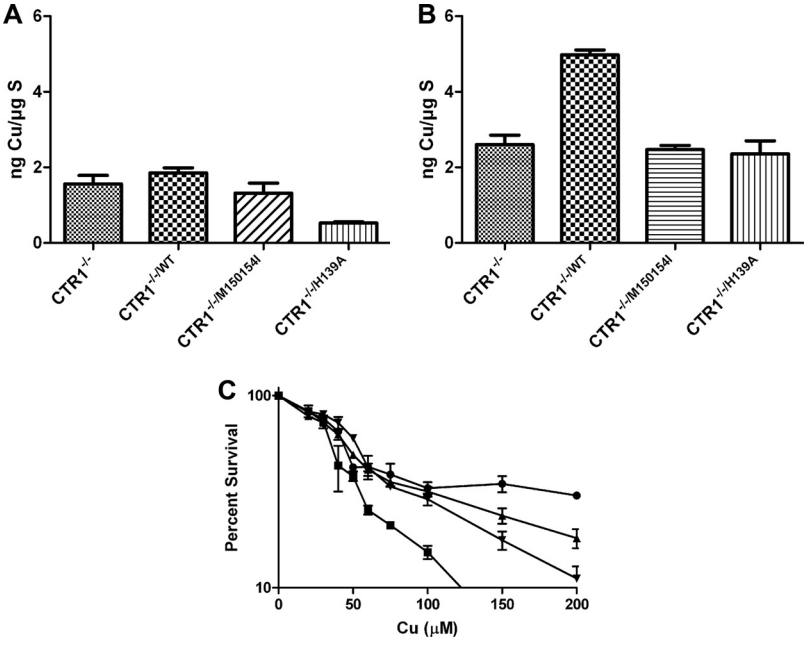

Fig. 3. Copper accumulation and cytotoxicity. A, total basal copper. B, total copper after 1-h exposure to 100 μM copper. C, inhibition of the growth of MEF cells during 96 h of continuous exposure to varying concentrations of copper. Each value represents the mean of at least three independent experiments, each performed with triplicate cultures. Vertical bars, S.E.M. ●, CTR1(−/−); □, myc-CTR1(−/−)^{WT}; ▲, myc-CTR1(−/−)^{M150,154I}; ▼, myc-CTR1(−/−)^{H139A}.

and their growth rate was assessed over the ensuing 96 h. The re-expression of wild-type CTR1 resulted in a 2.2-fold increase in cytotoxicity relative to the CTR1(−/−) cells (IC_{50}: 291 ± 7 versus 655 ± 36 μM; $p = 0.002$). Consistent with its ability to increase cDDP uptake to an even greater extent, the M150,154I variant increased the cytotoxicity of cDDP by a factor of 3.1-fold ($p = 0.001$; IC_{50} 211 ± 7 μM) or 1.6-fold more than the wild type CTR1 ($p = 0.001$). Of interest, even though the H139A variant increased cDDP uptake somewhat less than the wild-type hCTR1, it actually did a better job of enhancing cDDP cytotoxicity than the wild-type hCTR1. The IC_{50} for the CTR1(−/−)^{H139A} cells was 181 ± 3 μM, reflecting a 3.6-fold increase in cytotoxicity relative to the CTR1(−/−) cells ($p = 0.002$) and a 1.6-fold increase over that produced by wild-type CTR1 ($p = 0.0005$). Because of the discrepancy between the effect of the H139A variant on cDDP uptake versus cytotoxicity, this set of experiments was repeated a total of five times, all with consistent results. Thus, whereas the M150,154I and H139A variants were unable to transport copper, they actually mediated the enhanced uptake of cDDP.

Effect on cDDP-Induced Down-Regulation of CTR1. In mouse embryo fibroblasts, cDDP triggers the rapid degradation of CTR1 via ubiquitination and subsequent degradation in the proteosome (Jandial et al., 2009). To determine whether this reaction was a result of the interaction of cDDP with Met150, Met154, or His139, the ability of cDDP to down-regulate the expression of CTR1 in the CTR1(−/−)^{WT}, CTR1(−/−)^{M150,154I}, and CTR1(−/−)^{H139A} cells was analyzed by immunocytochemistry. The top images in Fig. 5 show that the distribution of hCTR1 was normal before cDDP exposure, and the bottom show that a 15-min exposure to 30 μM cDDP caused disappearance of nearly all of the signal. Thus, Met150, Met154, or His139 is not essential to the down-regulation of hCTR1, indicating that cDDP must be interacting with some other part of the molecule.

Discussion

Electron crystallographic analysis of CTR1 suggests that when the monomers assemble into a homotrimer in the plasma membrane, Met150, Met154, and His139 are positioned such that they can form three stacked rings in the pore (De Feo et al., 2007, 2009). These have been envisioned as mediating Cu^+ influx through a series of transchelation reactions in which Cu^+ is handed from one ring to the next down its concentration gradient. Based on the fact that the interaction of cDDP with methionines and histidines is very similar to that of Cu^+, we have proposed that hCTR1 transports cDDP in a similar manner (Howell et al., 2010). However, the results reported here indicate that, although the transchelation concept may be correct, Met150, Met154, and His139 play quite different roles in the transport of copper and cDDP.

The re-expression of wild-type and variant forms of hCTR1 in cells in which endogenous CTR1 is completely absent because the knockout of both alleles provides a powerful way of identifying structural components essential for function. Despite small differences in the level of expression at the mRNA level, all three forms of hCTR1 protein assessed in this study were expressed at equal levels at the plasma membrane. In addition, the distribution of hCTR1 in other parts of the cell seemed normal, indicating that Met150, Met154, and His139 are not important determinants of the normal trafficking of hCTR1. This model system was further validated by the observation that re-expression of wild-type hCTR1 increased cop-

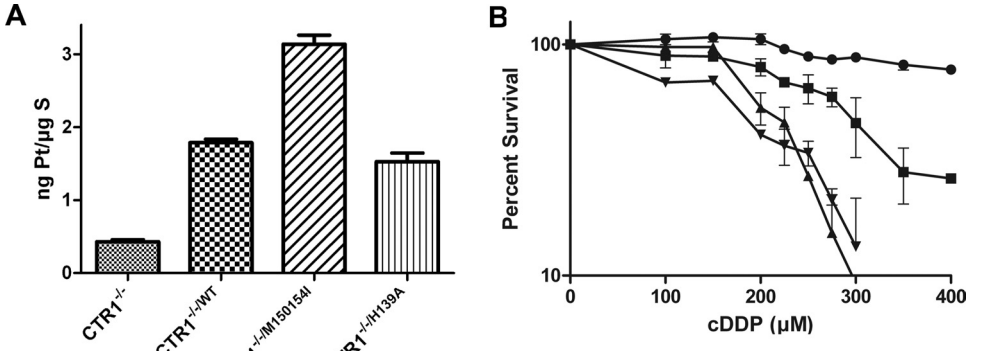

Fig. 4. cDDP accumulation and cytotoxicity. A, net accumulation of platinum in MEF cells after 5-min exposure to 30 μM cDDP. B, inhibition of growth of MEF cells after 5-min exposure to varying concentrations of cDDP. All values represent the means of six independent experiments each performed with triplicate cultures. Vertical bars, S.E.M. ●, CTR1(−/−); □, myc-CTR1(−/−)^{WT}; ▲, myc-CTR1(−/−)^{M150,154I}; ▼, myc-CTR1(−/−)^{H139A}.

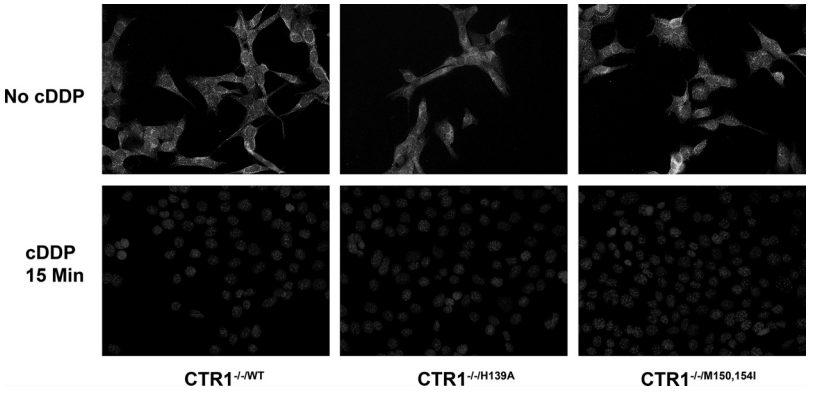

Fig. 5. Effect of modifying Met150, Met154, and His139 on the ability of cDDP to trigger CTR1 degradation. Original magnification, 20×. Top, no cDDP treatment. Bottom, 30 μM CDDP treatment for 15 min before fixing.

per and cDDP uptake and enhanced sensitivity to the cytotoxic effect of both of these drugs.

The fact that the M150,154I variant of hCTR1 failed to increase the uptake of copper and was unable to significantly enhance the cytotoxicity of copper is consistent with prior studies of copper transport in yeast, insect, and mammalian cells that established the importance of these methionines under conditions of low environmental copper (Puig et al., 2002; Eisses and Kaplan, 2005; Liang et al., 2009). Likewise, the observation that the H139A variant failed to transport copper confirms the results of a prior study that reported that conversion of His139 to alanine reduced copper uptake when the variant hCTR1 was expressed in insect cells (Eisses and Kaplan, 2005). The most intriguing observation to emerge from this study is that, instead of disabling cDDP transport, conversion of Met150 and Met154 to isoleucines significantly increased cDDP uptake well above that attained with wild-type hCTR1, and that this was accompanied by a further enhancement of the cytotoxicity of cDDP. This result indicates that the stacked rings of methionines putatively formed by Met150 and Met154 serve to obstruct the flow of cDDP through the pore. In the M150,154I variant, the stacked rings potentially formed by His139 and Cys189 in the lower part of the pore remain intact and may continue to serve an essential transchelation role, but these data suggest that the interaction of cDDP with Met150 and/or Met154 in some way controls the rate of transport. A previous study performed in small-cell lung cancer cells that contained endogenous hCTR1 found that the expression of an exogenous form of hCTR1 in which either Met150 or Met154 was converted to glutamine reduced the uptake of cDDP and rendered the cells less sensitive to cDDP. However, this was attributed to a trans-dominant-negative effect of the mutant hCTR1 on the wild-type endogenous hCTR1 mediated by the inability to form correctly assembled trimeric complexes in the plasma membrane. The design of the prior study did not permit the detection of the transport-enhancing effect of completely removing the stacked rings formed by Met150 and Met154.

Conversion of His139 to alanine did not significantly impair the ability of hCTR1 to mediate the uptake of cDDP, but it also did not enhance its uptake. This indicates that cDDP interaction with His139 is not essential to its transport and is consistent with the concept that the rings of methionines formed by Met150 and Met154 are more important determinants of flux. Of more interest is the fact that whereas wild-type hCTR1 and the H139A variant accumulated similar amounts of cDDP, the CTR1(−/−)^{H139A} cells were substantially more sensitive to the cytotoxic effect of cDDP, indicating that the potency of the accumulated drug was greater. The explanation for this is not readily apparent but may be related to alteration in the efficiency of transfer of cDDP to intracellular chaperones or a change in the ratio of cDDP that enters the cell via hCTR1 versus the other routes that must exist because CTR1(−/−) cells still accumulate measurable amounts of cDDP. The idea that the H139A variant affects other possible transport routes is bolstered by its effect on basal copper levels. Despite the fact that conversion of His139 to alanine disabled its ability to mediate copper uptake, it nevertheless markedly reduced the basal intracellular copper level in the CTR1(−/−) cells.

In the mouse embryo fibroblasts, cDDP triggers rapid degradation of endogenous CTR1, and the results of this study indicate that it does the same thing to exogenous hCTR1 when re-expressed in the CTR1(−/−) cells. Previous studies have documented that CTR1 becomes ubiquitinated (Safaei et al., 2009) and that inhibition of proteosome function blocks the cDDP-induced degradation of CTR1 (Holzer and Howell, 2006) and enhances the uptake and cytotoxicity of the drug (Jandial et al., 2009). The ability cDDP to trigger CTR1 degradation is compromised in cells that lack the copper chaperone ATOX1 (Safaei et al., 2009). How cDDP initiates CTR1 ubiquitination is unknown, but the results of this study support the conclusion that it does not require interaction of cDDP with Met150, Met54, or His139, indicating that cDDP must bind to some other domain in hCTR1 as well.

In summary, the opposite effects of converting Met150 and Met154 to isoleucines on the transport of copper and cDDP indicate that although they play a facilitating role in moving copper through the pore, they serve to obstruct the passage of cDDP. Thus, although both copper and cDDP may rely on a series of transchelation reactions to pass through the hCTR1 trimeric complex, the details of the molecular interactions must be different, which provides a potential basis for selective pharmacological modulation of copper versus cDDP cytotoxicity.

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Address correspondence to: Dr. Stephen B. Howell, Moores UCSD Cancer Center, Mail Code 0819, 3855 Health Sciences Drive, #0819, La Jolla, CA 92093. E-mail: showell@ucsd.edu