Copper-Chelator Exerts Synergistic Interaction with Platinum Drugs through Modulating Copper Transporters in Oxaliplatin-resistant Human Gastric Cancer Cell

Su Chien1, CC Kuo1, HY Pan2 and YC Chang1
1 Institute of Clinical Pharmacy and Pharmaceutical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
2 National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan.
3 Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan, Taiwan.

Abstract

Although platinum-based chemotherapy drugs including cisplatin, carboplatin, and oxaliplatin are commonly used in the treatment of solid tumor, patients ultimately develop drug-resistance and result in treatment failure. Combination copper-chelator with platinum drugs has been reported to successfully treat a subset of cisplatin-resistant ovarian cancer patients. However, the underlying mechanisms of action remain unknown. We have previously established an oxaliplatin-resistant sub-clone of human gastric adenocarcinoma TSGH cells, S3. The mechanisms responsible for oxaliplatin resistance of S3 cells included down-regulation of hCtr1 and over-expression of ATP7A. In contrast to displaying additive to antagonistic interaction with platinum drugs in parental TSGH cells, our studies showed that copper-chelator, D-penicillamine, exerts synergistic interaction with platinum drugs through increasing platinum-DNA adduct formations in S3 cells. D-penicillamine promotes copper uptake transporter hCtr1 expression via activation of transcription factor Sp1. In addition, Sp1 overexpression promotes p53 translocation from nucleus to cytosol and binds to ubiquitin, and finally causes p53 degradation, which further suppressed the expression of copper efflux transporter, ATP7A. Combination of D-penicillamine and oxaliplatin significantly inhibited S3 cell growth in animal model. Immunohistochemical analysis showed that up-regulation of hCtr1 and down-regulation of ATP7A were found in both D-penicillamine- and D-penicillamine/Oxaliplatin-treated S3 tumors, which were consistent to in vitro results. In conclusion, our results showed that D-penicillamine increases hCtr1 expression through regulating Sp1 level. Notably, D-penicillamine did not down-regulate ATP7A expression through regulation of p53. Our finding demonstrated a new treatment strategy for cancer patient resistant to oxaliplatin treatment with low expression of hCtr1 and overexpression of ATP7A in tumor tissues.

Result

Table1. Evaluation of anti-proliferative activity of platinum drugs toward S3 and TSGH

<table>
<thead>
<tr>
<th>Drugs</th>
<th>IC50 (μM)</th>
<th>Resistance Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>0.78 ± 0.05</td>
<td>68.8</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>0.17 ± 0.06</td>
<td>15.4</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>0.01 ± 0.00</td>
<td>6.8</td>
</tr>
</tbody>
</table>

In order to clarify the underlying mechanisms of platinum chemonoresistance, an oxaliplatin-resistant sub-clone of human gastric adenocarcinoma TSGH, was generated from parental TSGH by drug selection by increasing the concentrations of oxaliplatin. We evaluated the resistance of both S3 and TSGH cells to platinum drugs by growth inhibitory assay and calculated the IC50 value. The result showed that S3 cells were more resistant to oxaliplatin, cisplatin and carboplatin than TSGH cells, respectively (table 1). D-penicillamine had no cytotoxicity toward both cell lines at concentration of greater than 100 μM.

Figure 1. Changes of intracellular copper homeostasis involved to copper uptake transporter hCtr1 expression

A, expression level of copper uptake transporter, hCtr1, and efflux transporter, ATP7A, is higher and lower, respectively, in parental TSGH cells and S3 cells. B, Copper-chelator, D-penicillamine treatment increased expression level of both Sp1 and hCTR1 in S3 cells after D-penicillamine treatment, whereas ATP7A expression level was decreased significantly (p < 0.05; Student t test). C, S3 cells were transfected with scramble or Sp1 siRNA, respectively. RT-PCR showed that the level of Sp1 on hCtr1 promoter region was increased and led to promoting the transcription of hCtr1 in RNA level as S3 cells after D-penicillamine (D) treatment, but not in TSGH cells. B, RT-PCR showed that after D-penicillamine (D) treatment, both Sp1 and hCtr1 RNA expression levels were increased 1.4 fold in S3 cells. Cells without any treatment were served as negative control (NC) (p value < 0.05; Student t test). D-penicillamine failed to induce Sp1 and hCtr1 expression in RNA mediated-siRNA knockdown in S3 cells.

Figure 2. Changes of intracellular copper homeostasis involved to copper uptake transporter hCtr1 expression

A, A, expression level of copper uptake transporter, hCtr1, and efflux transporter, ATP7A, is higher and lower, respectively, in parental TSGH cells and S3 cells. B, Western blot result showed the expression of ATP7A was decreased after D-penicillamine (D) treatment, but not in TSGH cells. ATP7A protein level after D-penicillamine treatment significantly reduced the level of ubiquitination and degradation of p53. p53 and ATP7A promoter had no difference after Sp1 knockdown. E, ATP7B protein level after D-penicillamine (D) treatment. Cells without D-penicillamine treatment were served as negative control (NC). F, Both transcription factors Sp1 and p53 have binding regions on ATP7B promoter.

Acknowledgement

This work was partially supported by the following grants: Department of Health, Taiwan DOH99-TD-C-111-901, National Health Research Institutes CA-101-PP-22, National Research Program for Biopharmaceuticals 100CAP015-5 and National Science Council 102-2325-B-400-007.