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Review

Platinum drugs, copper transporters and copper chelators

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ABSTRACT

Cisplatin has been used since the end of 1970s as chemotherapeutic agent for a variety of tumors; however, its efficacy is limited by severe side effects and the rapid onset of resistance mechanisms, which include reduced drug uptake and increased efflux and sequestration.

A yeast genetic screen for cisplatin-resistant mutants identified the copper transporter CTR1 as a mediator of cisplatin uptake. However, cisplatin binding to methionine-rich motifs, located in the N-terminal extracellular region of the protein, is accompanied by fast displacement of ammine ligands that are considered essential for the anticancer activity.

Cisplatin also binds to CxxC motifs of Cu export pumps, ATP7A and ATP7B, and undergoes ATP-dependent translocation. In the presence of both Cu and cisplatin, translocation is inhibited for both metallic species.

Increased drug resistance is associated to down-regulation of CTR1 and up-regulation of Cu-ATPases and Cu-chaperone Atox1, which is crucial for Cu secretion. The ability of Cu-lowering agents, such as tetrathiomolybdate, to increase cisplatin uptake, down-regulate Cu-ATPases and inhibit platination of Cu-Atox1 may resensitize cisplatin-resistant cells and improve the treatment efficacy of platinum drugs.

Contents

Abbreviations: Atox1, antioxidant 1; CTR1, copper transporter 1; ESI-MS, electrospray ionization mass spectrometry; EXAFS, extended X-ray absorption fine structure; GSH, glutathione; HMGB, High Mobility Group Box; MBDs, metal-binding domains; NMR, nuclear magnetic resonance; QM/MM, quantum mechanics/molecular mechanics; REST, replica exchange with solute tempering; TM, tetrathiomolybdate.

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1. Platinum drugs

1.1. Cisplatin sensitivity and resistance

Cisplatin (*cis*-[$PtCl_2(NH_3)_2$], *cis*-diamminedichloridoplatinum(II)) has been used since the end of 1970s as chemotherapeutic agent for a variety of malignancies. It is a component of first-line treatment for testicular, ovarian, cervical, endometrial, bladder, head and neck, lung, and gastroesophageal cancers [1–7]. It is also used as a second or third-line treatment for prostate and pancreatic cancers, metastatic cancer of the breast, and for melanoma and gliomas [8–10]. The dosage and consequent efficacy of cisplatin, however, are limited by its side effects, the most prominent being nephrotoxicity [11]. Another major limitation of cisplatin is the rapid onset of resistance [12], which develops through cellular adaptations resulting in reduced drug uptake, increased efflux and sequestration, and enhanced detoxification, contributing to overall treatment failure [13–15]. In addition, altered gene expression, DNA copy number changes, and considerable genomic instability contribute to cisplatin resistance [14,16,17]. This calls for alternative treatments that re-sensitize cells to platinum (Pt) drugs.

1.2. Mechanism of action

Cisplatin, along with the later developed analogs carboplatin and oxaliplatin, acts by forming DNA crosslinks. Pt crosslinks inhibit DNA replication and halt cell division. The DNA repair system is activated and, if the damage is not repaired in a reasonable time, apoptosis is activated [18,19]. Cisplatin may also lead to cell death by damaging cytoplasmic proteins and inducing apoptosis at the execution phase level [16,20,21]. Damaged DNA can be recognized by specific chromosomal non-histone nucleoproteins containing the High Mobility Group Box (HMGB) [22,23]. *In vivo*, the HMGB/Pt-DNA binding is further regulated by post-translational modifications (PTMs) [24]. How PTMs can affect the stability of the HMGB/Pt-DNA complex has recently been addressed by a theoretical investigation [25].

A correlation between higher binding affinity of HMGB proteins towards cisplatin-DNA lesions and lower DNA-repair efficiency has been observed in several experiments [22–30].

This is a pharmaceutically relevant event, since it can inhibit the damage repair favoring tumor cell death.

2. Copper transporters

2.1. Cisplatin uptake by copper transporter *1*

There has been considerable interest in understanding the cellular entry mechanism of Pt drugs, as their effectiveness depends on the amount of drug delivered to the tumor cells [31]. Cisplatin is a neutral molecule and, as such, can enter cells by passive diffusion. It becomes positively charged when its chlorido ligands are displaced by water in the cytoplasm. In this active form, cisplatin is able to crosslink DNA strands and selectively bind sulfur-containing amino acids (e.g. cysteine and methionine) [32–37]. Studies of cisplatin resistant cell lines have revealed that drug uptake is a critical step that governs cisplatin sensitivity in vitro [38]. A yeast genetic screen for cisplatin-resistant mutants identified the copper transporter CTR1 as a mediator of cisplatin uptake in yeast and mammalian cells [39]. Upon exposure to excess copper (Cu), CTR1 protein undergoes endocytosis and degradation [40,41]. Thus, pretreatment of cells with high Cu results in decreased cisplatin uptake and increased resistance to this drug [39], whereas Cu chelation has opposite effects [42]. A peculiar Cu chelator is tetrathiomolybdate (TM), which has been granted orphan designation in the United States and Europe for the treatment of Wilson's disease, an autosomal recessive disorder characterized by excess Cu deposition in various organs. TM reduces bioavailable Cu levels, primarily by forming a ternary complex (TM, Cu and a protein such as albumin) which cannot be absorbed by intestinal mucosal cells and is eliminated in the feces [43–45]. Using a mouse model of human cervical cancer, Ishida et al. demonstrated that combined treatment with a Cu chelator and cisplatin increases cisplatin-DNA adduct levels in cancerous but not in normal tissues, impairs angiogenesis, and improves therapeutic efficacy. The Cu chelator also enhances the killing of cultured human cervical and ovarian cancer cells with cisplatin [42]. All these results support the notion that CTR1 can represent a therapeutic target, which can be manipulated with Cu chelating drugs to selectively enhance the benefits of Pt chemotherapeutics (Fig. 1) [46].

In an attempt to clarify at a molecular level the mechanism of Pt uptake mediated by the Cu transporter CTR1, which is located on the plasma membrane and contains functionally essential methionines (Mets), one of the Met-rich motifs of yeast CTR1 (Mets7 peptide) was reacted with cisplatin (Fig. 2) and its *trans* isomer [47]. A striking difference between *cis*- and *trans*-[$PtCl_2(NH_3)_2$] was that, upon reaction with Mets7, only the latter retains its ammine ligands while cisplatin is degraded with the complete loss of the carrier ligands in the time of minutes [47–49].

Although it can be argued that on a shorter time scale, still compatible with cellular processes, the ammine ligands can be retained and cisplatin not degraded, it is unlikely that the rather bulky moiety $[Pt(NH_3)_2]^{2+}$ may enter cells through an ion channel as for Cu^+ [31]. Cell entry may rather occur via endocytosis that could eventually be assisted by cisplatin binding to Met-rich motifs of CTR1 [47,50,51].

The endocytic vesicles containing CTR1 may fuse with a lysosome or may be recycled to the plasma membrane.

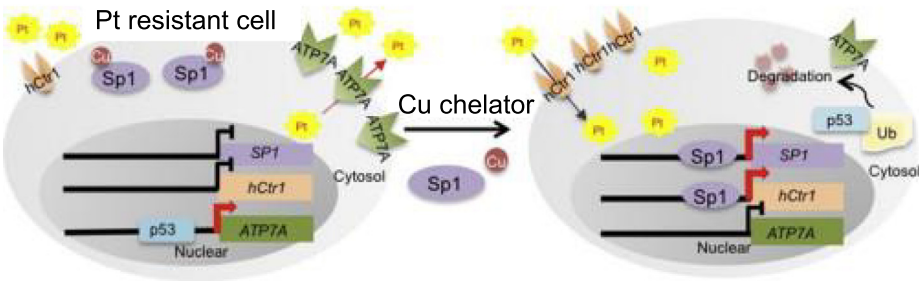

Fig. 1. Regulation of human CTR1 (hCTR1) expression by copper bioavailability. Copper-replete conditions prevent binding of Cu-responsive transcription factor Sp1 to hCtr1 promoter region. Under copper deficiency, hCTR1 expression is up-regulated by enhanced binding of Sp1 to GC boxes of Sp1 and hCTR1 promoters. Sp1 overexpression induces p53 translocation from nucleus to cytosol and causes p53 degradation through ubiquitination, which subsequently suppresses the expression of ATP7A. Adapted with permission from [46].