DOI: https://doi.org/10.1016/j.semcancer.2021.05.023

ACCEPTED VERSION

Interference Between Copper Transport Systems and Platinum Drugs

Fabio Arnesano[a], Giovanni Natile[a]*

*

[a] Department of Chemistry, University of Bari, via Orabona, 4, 70125 Bari, Italy

* E-mail: giovanni.natile@uniba.it

Keywords: Cisplatin, Cancer, Copper transporters, Copper homeostasis, Platinum trafficking, Cisplatin resistance, Sensitization therapy, Pt-interference with copper metabolism, CTR1, ATP7A, ATP7B, ATOX1.

Abstract. Cisplatin, or cis-diamminedichloridoplatinum(II) *cis*-[$PtCl_2(NH_3)_2$], is a platinum-based anticancer drug largely used for the treatment of various types of cancers, including testicular, ovarian and colorectal carcinomas, sarcomas, and lymphomas. Together with other platinum-based drugs, cisplatin triggers malignant cell death by binding to nuclear DNA, which appears to be the ultimate target. In addition to passive diffusion across the cell membrane, other transport systems, including endocytosis and some active or facilitated transport mechanisms, are currently proposed to play a pivotal role in the uptake of platinum-based drugs. In this review, an updated view of the current literature regarding the intracellular transport and processing of cisplatin will be presented, with special emphasis on the plasma membrane copper permease CTR1, the Cu-transporting ATPases, ATP7A and ATP7B, located in the trans-Golgi network, and the soluble copper chaperone ATOX1. Their role in eliciting cisplatin efficacy and their exploitation as pharmacological targets will be addressed.

Introduction. Copper (Cu) transporters have emerged as key factors at the basis of the biological response to antitumor platinum (Pt) drugs, e.g. cisplatin, carboplatin and oxaliplatin (Figure 1), which are among the most potent and broadly used chemotherapeutics worldwide. With respect to the entry into the cell, it is widely accepted that the intact neutral drugs cross the plasma membrane by passive diffusion [1]. In the case of cisplatin, this assumption is supported by the observed linear cellular uptake that occurs without saturation up to 1 mM concentration. Furthermore, cisplatin analogues fail to inhibit its absorption, demonstrating the absence of competition [2]. However, accumulated evidence suggests that, together with passive diffusion, active mechanisms can contribute to cisplatin uptake and efflux [3–5]. In this scenario, the primary actors appear to be the organic cation transporters (OCTs), belonging to the SoLute Carrier superfamily (SLC), and the major copper influx transporter CTR1. Moreover, other non-saturable systems, such as fluid-phase endocytosis mediated by membrane invagination, have been proposed [6]. In contrast, the Menkes and Wilson disease proteins (ATP7A and ATP7B, respectively) were found to be involved in the efflux and sequestration of cisplatin, thus increasing tumor cell resistance to the platinum therapy [7–11]. Also the soluble Cu chaperone ATOX1, which physiologically brings Cu^{+} from CTR1 to the metal-binding domains (MBDs) of ATP7A and ATP7B, appears to be implicated in the intracellular distribution of the drug resulting in cisplatin sensitivity being dependent upon the expression levels of ATOX1 [12]. A better understanding of the Pt drug processing by Cu trafficking proteins under physiological conditions may help to answer key issues, such as drug availability in tumor cells and induction of resistance after an initial responsiveness, and to orchestrate a pharmacological sensitization of tumor cells to the platinum therapy.

What is cisplatin. Cisplatin is the prototype of platinum-based antitumor drugs. It is a platinum coordination compound synthesized for the first time by Michele Peyrone, an Italian medical doctor, in 1844 while visiting the Justus von Liebig laboratory in Giessen [13]. Only in 1893 Alfred Werner [14] proposed for this compound a square-planar structure which could account for the obtainment of a *cis* (Peyrone's compound) and a *trans* isomer (Reiset's compound reported somewhat earlier [15]). In the 1960s, Rosenberg and colleagues, while investigating the role of an electric field on the viability of *E. coli* cells, discovered that accidental electrolysis of a platinum electrode resulted in the formation of a small amount of a platinum compound which inhibited cell division and caused filamentous cell growth [16] (a phenomenon already observed for the antitumoral drug nitrogen mustard in use since the 1940s [17]). Later the same group tested the effects of several platinum complexes on rat sarcomas with exciting outcomes for cisplatin [18]. In 1971 cisplatin was applied to a cancer patient [19] for the first time and it became available for clinical use in 1978 as Platinol® (Bristol-Myers Squibb). Cisplatin was very effective against

several types of solid tumors; however, the efficacy of cisplatin was often accompanied by toxic side effects and tumor resistance, which in turn lead to secondary malignancies [19,20]. Beside cisplatin, many other platinum derivatives have been tested in clinical trials, however only few of them (e.g. carboplatin, approved by FDA in 1989, and oxaliplatin, approved by FDA in 2002) have received worldwide approval for clinical practice, while some others (nedaplatin, lobaplatin and heptaplatin) have received only regional approval for clinical use [21,22]. Other platinum drugs (such as satraplatin, picoplatin and multinuclear BBR3464), developed with the intent to overcome the side effects, the drug resistance, and the poor oral bioavailability of cisplatin, carboplatin and oxaliplatin [22,23], despite encouraging preclinical in vitro and in vivo results, proved to be below expectations at the stage of clinical trials (Figure 1). Presently, cisplatin, carboplatin, and oxaliplatin are routinely used in frontline cancer therapy [24] and are estimated to be administered to half of all cancer patients at some stage during their treatment regimens [25]. Platinum drugs are specifically used for treating some types of cancers including testicular, ovarian, bladder, head and neck, esophageal, small and non-small cell lung, breast, cervical, stomach and prostate cancers, as well as Hodgkin's and non-Hodgkin's lymphomas, neuroblastoma, sarcomas, multiple myeloma, melanoma, and mesothelioma [23]. Despite the undoubtedly positive effects of platinum compounds, patients receiving these agents experience severe side effects that limit the dose that can be administered. Such side effects include general cell-damaging effects (e.g. nausea and vomiting), decreased blood cell and platelet production in bone marrow (myelosuppresion) and decreased response to infection (immunosuppression). More specific side effects include damage to the kidney (nephrotoxicity), damage of neurons (neurotoxicity) and hearing loss [26,27]. Key management strategies include renoprotection and enhancing drug elimination with intravenous hydration together with additional therapies including antiemetics and hematopoietic colony stimulating factor [27].

Mechanism of action of cisplatin. Low concentrations of several cis-platinum compounds suppress DNA synthesis in vitro while affecting protein and RNA synthesis to a much lower extent [28,29] (Figure 2); thus cisplatin is considered to be primarily a DNA-damaging anticancer drug, forming different types of bifunctional adducts [30,31]. Such adducts are recognized by a number of cellular proteins, e.g. mismatch repair proteins and some damage-recognition proteins [32]. The presence of cisplatin adducts in DNA, if not repaired in a reasonable time, is thought to trigger cell cycle arrest and apoptosis (Figure 3) [33]. It is common belief that abortive attempts to repair the DNA lesions play a key role in the cytotoxicity of the drug, and loss of the mismatch repair activity is known to cause cisplatin resistance, a major problem in antineoplastic therapy [34]. Cisplatin may also lead to

cell death by damaging cytoplasmic proteins and inducing apoptosis at the execution phase level [35–37].

Of particular relevance was the early discovery by Lippard that platinum-damaged DNA can be recognized by specific chromosomal non-histone nucleoproteins containing the High Mobility Group Box (HMGB) [38,39]. This is believed to be a pharmaceutically relevant event, since it can inhibit the damage repair favoring tumor cell death. On this ground the hypersensitivity of testicular germ cell tumors (TGCT) to cisplatin treatment could be explained with the presence of HMGB-containing protein 4 (HMGB4), preferentially expressed in testes, which uniquely blocks excision repair of cisplatin-DNA adducts (particularly the 1,2-intrastrand cross-links) so potentiating the sensitivity of TGCT to cisplatin therapy [39]. The correlation between higher binding affinity of HMGB proteins towards cisplatin-DNA lesions and consequent lowering of the DNA-repair efficiency can also account for the selection of only the *RR* enantiomeric form of the cyclohexane diamine ligand for the synthesis of oxaliplatin [40,41].

In vivo, the HMGB-protein/Pt-DNA binding is further regulated by post-translational modifications (PTMs) [42]. It was experimentally found that PTMs increase the binding affinity between the platinated DNA and the protein, notwithstanding acetylation of lysines and phosphorylation of serines both would be expected to reduce the positive charges on the HMGB protein and hence to diminish the electrostatic attraction for the negatively charged DNA [42]. This apparent contradiction was addressed in a theoretical investigation [43], where the simulation suggested that PTMs can increase the hydrophobic contacts between the platinated DNA and the protein and, specifically, can contribute to stabilize a key contact between the two Pt-crosslinked guanines and the intercalating phenyl group of phenylalanine 37 (Figure 4) [44]. The increased hydrophobic interactions can not only compensate for the loss of electrostatic interactions but also result in an overall increase of binding affinity on passing from native to post-transcriptional modified protein [43].

It is also worth mentioning that, besides interacting with specific chromosomal non-histone nucleoproteins such as the HMGB-containing proteins, cisplatin can also interact with histone proteins. Nuclear DNA is tightly assembled with histone proteins to form nucleosomes in chromatin, and this can prevent the drug from accessing the DNA. However, the linker histone H1, due to its exposed location and dynamic conformation, can constitute a gate to nucleosomal DNA and favor the platination of DNA. In a recent study, the reaction of cisplatin with histone H1 was investigated together with the interaction of the H1/cisplatin adduct with DNA [45]. The results showed that H1 readily reacts with cisplatin and generates bidentate and tridentate adducts, with methionine and glutamate residues as preferential binding sites. Moreover, platinated H1 can form

H1–cisplatin–DNA ternary complexes (Figure 5) [45]. The formation of H1–cisplatin–DNA ternary complexes could also inhibit the DNA repair. The crucial role of nuclear proteins in modulating the pharmacological activity of cisplatin is also shared by non-nuclear proteins [46], some implications will be seen in the following sections.

Mechanisms of resistance to cisplatin therapy. Several mechanisms are involved in cisplatin resistance [37,47] that include decreased intracellular drug accumulation and/or increased drug efflux, drug inactivation by increased levels of cellular thiols, alterations in drug target, processing of drug-induced damage by increased nucleotide excision-repair activity, and decreased mismatch-repair activity and evasion of apoptosis [31,48,49]. In addition, altered gene expression, DNA copy number changes, and increased genomic instability can contribute to cisplatin resistance [37,50,51]. Among transporters, the ATP-binding cassette (ABC) transporters, the solute carriers (SLCs), and the ATPase membrane protein superfamilies, have proved to influence the pharmacology of platinum drugs [52,53]. In resistant cells, changes in membrane transporters can affect the accumulation of platinum drugs by increasing drug efflux or decreasing drug uptake [54,55].
To gain information about the intracellular distribution of cisplatin, a fluorescent probe (such as the carboxyfluorescein-diacetate, CFDA, developed by Reedjik and co-workers) was attached to cisplatin (F-cisplatin) and its cellular localization in human osteosarcoma (U2-OS) cells followed as a function of time. It was found that platinum rapidly enters tumor cells and is initially localized throughout the whole cytoplasm. After 2–3 h of exposure, the probe accumulates also in the nucleus, but 6-8 h after incubation a punctate staining of a cytoplasmic region was observed, that persisted and became more pronounced after 24 h. The overall fluorescence in the cell decreased over time, implying a secretion of the platinum complex. Co-localization experiments with a Golgi apparatus-selective stain indicated the involvement of Golgi vesicles in intracellular processing of cisplatin [56]. Quite similar results were obtained for the subcellular location of F-cisplatin in 2008 ovarian carcinoma cells. F-cisplatin in the nucleus exhibited a finely granular pattern, whereas elsewhere in the cell the drug was distributed in discrete vesicular structures rather than being diffusely localized throughout the cytoplasm (Figure 6) [57]. F-cisplatin accumulated in the Golgi, in vesicles belonging to the secretory export pathway, and in lysosomes but not in early endosomes. Moreover, F-cisplatin extensively colocalized with vesicles expressing the copper efflux protein ATP7A, whose expression does appear to modulate also the cellular pharmacology of cisplatin. It is interesting to note that the compartmental distribution of F-cisplatin in the drug-sensitive (2008) and drug-resistant (2008/C13*5.25) cells is quite different (Figure 6) [57]. Indeed, several studies of the cellular pharmacology of cisplatin have suggested that specialized membrane-bound proteins

mediate the uptake and efflux of the drug, and nearly all cell lines selected for resistance to cisplatin exhibit alterations in drug accumulation [2,58]. Overall, the copper importer CTR1 [59,60] and the copper efflux transporters ATP7A [61] and ATP7B [62,63] appear to play an important role in the pharmacokinetics and cytotoxicity of cisplatin together with the copper chaperone ATOX1 that mediates the transfer of copper from CTR1 to ATP7A and ATP7B [64,65].

Copper homeostasis and tumor growth. Copper is an enzymatic cofactor that in mammals is necessary, inter alia, for cellular respiration, iron homeostasis, melanogenesis, biosynthesis of neurotransmitter, and connective tissue formation. Copper has the ability to switch between 1+ and 2+ oxidation states conferring to cuproenzymes the ability to catalyze redox reactions. However, if copper concentration surpasses the capacity of cellular detoxification mechanisms, it can potentiate the toxicity of ROS by catalyzing Fenton-like reactions [66]. Pathways of copper homeostasis also appear to regulate tumor growth. For instance, serum copper levels are elevated in cancer patients, which correlate with disease outcome and response to therapies [67,68]. Copper also appears to be rate limiting for mitogenic signaling pathways necessary for tumor growth (such as RAS/MAPK signaling) at least in part due to a direct role for copper ions in regulating MAP2K1 activity [69]. Studies have also shown that copper-dependent superoxide dismutase is necessary for receptor tyrosine kinase signaling by producing hydrogen peroxide, which blocks downstream inhibitory phosphatases [70].

In contrast, copper-chelating drugs, such as tetrathiomolybdate or penicillamine, are known to have anti-angiogenic and anti-neoplastic activity, confirming that copper is rate limiting for tumor growth [71–74]. Copper chelation therapy has also shown to reduce ATP levels and suppress pancreatic tumors in mice due to reduced activity of the copper-dependent mitochondrial cytochrome *c* oxidase [75]. Nuclear factor kappa B (NFkappaB)-dependent tumorigenesis appears also to be impaired by copper chelation [73].

Overall these studies indicate that cancer cells are vulnerable to disturbances in copper homeostasis, and some therapeutic strategies could be based on restricting copper delivery to one or more key enzymes with roles in cancer biology.

Regulators of copper homeostasis in cells. The main regulators of copper homeostasis in cells include CTR1, a plasma membrane copper importer, and the copper exporting P-type ATPases ATP7A and ATP7B (the Menkes and Wilson disease proteins, respectively). The intracellular activity of ATP7A/B includes delivery of copper to essential cuproenzymes of the secretory pathway and copper excretion across the plasma membrane [76–78]. Thus, loss of ATP7A in

cultured cells results in the accumulation of copper in the cytosol and hypoactivity of various enzymes that require copper to be transported into the secretory pathway [79–82]. The Cu-chaperone ATOX1 (antioxidant-1 protein) chattels the Cu^+ ion between CTR1 and ATP7A/B. Human CTR1 is organized into i) three transmembrane (TM) domains, ii) an N-terminal extracellular domain rich in methionines and histidines, iii) a large intracellular loop and iv) a short intracellular C-terminal domain. Conserved methionine (Met)-rich motifs and individual methionines, histidines and cysteines, essential for Cu transport, are located within the extracellular domain, within the second and third transmembrane domains and in the C-terminal tail [83].
Cu-ATPases ATP7A and ATP7B share about 54% amino acid sequence identity and contain eight highly conserved putative transmembrane helices and a long N-terminal region containing six putative metal binding domains (MBD) having similar structures. Each MBD has a ferredoxin like fold and a conserved metal-binding site, $MetX_1CysX_2X_3Cys$ (X_i = any residue), in a solvent-exposed loop, which contributes to the metal transport.
ATOX1 is a small protein with a ferredoxin-like fold ($\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$) and a metal-binding motif, CXXC, located in the solvent-exposed β_1-α_1 loop, which binds a single Cu^+ ion. This structure is highly conserved among metallochaperones and soluble domains of Cu-transporting ATPases and appears to be a requirement for the proteins to be able to exchange copper ion among them [84].
In addition to ATOX1, that delivers Cu^+ to ATP7A and ATP7B for injection into the Trans-Golgi-Network (TGN), at least two other pathways are involved in the intracellular distribution of copper: COX17, which transfers copper to the subunits of cytochrome c oxidase in the mitochondria, and CCS, which incorporates Cu into superoxide dismutase 1 (SOD1) (Figure 7) [85,86].
What renders all copper transporting proteins very likely to interact with the platinum drugs is the high affinity for platinum of the copper binding motifs, as supported by NMR, crystallographic and mass spectrometry experiments [87–89] .

Efficacy of cisplatin related to CTR1. There has been considerable interest in understanding the cellular entry mechanism of Pt drugs, as their effectiveness determines the amount of drug delivered to the tumor cells [90,91]. Cisplatin is a neutral molecule and, as such, can enter cells by passive diffusion. Once in the cytosol, where the concentration of free chloride is much lower than in the extracellular fluid, it becomes positively charged via substitution of its chlorido ligands by water molecules. In this active form, cisplatin can easily bind amino acids with sulfur-containing side chains (e.g. cysteine and methionine) and also enter the nucleus and crosslink DNA strands [92–97].

A yeast genetic screen for cisplatin-resistant mutants identified the copper transporter CTR1 as a mediator of cisplatin uptake in yeast cells [98]. Moreover, CTR1-deficient mouse embryonic fibroblasts exhibited reduced influx of cisplatin, carboplatin, and oxaliplatin [99], indicating that CTR1 represents a significant pathway for the import of the platinum drug into the cell [59,100–102]. Additionally, lower CTR1 levels are usually associated with increased cisplatin resistance in tumors whereas higher CTR1 expression is associated with higher sensitivity to the cisplatin treatment [103]. For instance, this is the case of non-small cell lung cancer, where better survival rates have been reported for patients having higher expression of CTR1 [104]. Results contrasting with those of CTR1 have been reported for the second copper transporter CTR2. Low levels of CTR2 have been found to be associated with higher success rate of platinum treatment in cancer patients [103,105] and experimental studies have also shown that cells lacking CTR2 exhibit increased whole-cell platinum accumulation [106,107]. Furthermore, in a panel of ovarian carcinoma cell lines, an inverse correlation between CTR2 levels and sensitivity to platinum has been found [108]. The CTR2/CTR1 ratio was found to be higher in chemo-resistant patients so that patients with higher CTR2 or with CTR2/CTR1 ratio greater than 1 had an adverse disease prognosis [105]. To account for the different effects of the two copper transporters, it has been proposed that CTR2 affects platinum accumulation via a CTR1-dependent mechanism involving the generation of a cleaved form of CTR1 lacking the metal binding ecto-domain. As a consequence, when CTR2 levels are high, the cells have more cleaved form of CTR1 which, in turn, has lower affinity for both Cu and platinum and, hence, imports less metal [109]. From this point of view, CTR2 and CTR1, and their ratio, could be prognostic markers for individual responsiveness to platinum-based therapy (Figure 8) [109].

Mechanism of CTR1-mediated Pt uptake. The copper-transporter CTR1 is located on the plasma membrane and contains at the extracellular N-terminus functionally essential Met-rich motifs. In an attempt to clarify, at a molecular level, the mechanism of Pt uptake mediated by this transporter, one of the Met-rich motifs of yeast CTR1 (Mets7 peptide) was reacted with cisplatin [87,110]. Upon reaction with Mets7, cisplatin was degraded with the complete loss of the carrier ligands in the time of minutes. Since the ammine ligands are present in the DNA adducts formed by clinically effective cisplatin, the potential displacement of the carrier ligands by the Met-rich motifs of CTR1 cannot be without consequences on the efficacy of the drug [111,112]. Similar results were obtained by using peptides corresponding to the two Met-rich motifs of human CTR1: two 8mer encompassing residues 7–14 and 40–47 [113], a 20mer encompassing residues 1-20 [114], and a 55mer encompassing residues 1–55 [88].

Although it could be argued that on a shorter time scale, still compatible with cellular processes, the ammine ligands could be retained and cisplatin not degraded, the rather bulky $[Pt(NH_3)_2]^{2+}$ moiety is unlikely to enter the cells via the same ion channel used by the much smaller Cu^+ ion [90], but rather via a vesicle trafficking mechanism that could be assisted by binding of cisplatin to the Met-rich motifs of CTR1 on the vesicle membrane [87,115]. Thus, while CTR1 transports Cu^+ through a pore formed by a CTR1 multimer in the plasma membrane, on the contrary, the cisplatin transport occurs via a quite different mechanism that depends upon endocytosis [99].
Pinocytic vesicles are constantly formed irrespective of the availability of substrates [116]. Consistent with this view, cisplatin exposure has no influence on the subcellular localization of CTR1and the entry pathway is not saturable at elevated concentrations of platinum drugs [90,100]. Moreover, co-localization between CTR1 and a fluorescent cisplatin analogue is observed in vesicular structures when continuous recycling of the protein between perinuclear region and cell membrane is inhibited [117,118] (Figure 9A). Endocytic vesicles containing CTR1 may fuse with a lysosome or may be recycled back into the membrane by exocytosis [119].

CTR1 as a therapeutic target. Upon exposure to excess copper, the CTR1 protein undergoes endocytosis and degradation [120,121]. Thus, pretreatment of cells with high Cu levels results in decreased cisplatin uptake and increased resistance to the platinum drug [59], whereas addition of the Cu chelator bathocuproine disulphonate has opposite effects [98]. A peculiar Cu chelator is ammonium tetrathiomolybdate ($[(NH_4)_2MoS_4]$, TTM), 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). TTM reduces bioavailable Cu, primarily by forming a ternary complex (TTM, Cu and a protein such as albumin) which cannot be absorbed by intestinal mucosal cells and is eliminated in the feces [122–124]. 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 [98]. All these results support the view that CTR1 can represent a therapeutic target that can be manipulated with Cu chelating drugs to selectively enhance the benefits of Pt chemotherapeutics [125,126].

Cisplatin resistance mediated by Cu-ATPases. Apart from down-regulation of CTR1, also up-regulation of ATP7A and ATP7B (both are Cu export pumps [127]) has been associated to increased drug resistance [128,129] (Figure 10). For instance, increased levels of ATP7A or ATP7B

were reported to correlate with cisplatin resistance in several human cancer cell lines [58,63]. Elevated expression of ATP7A are also associated with worse outcomes in patients with ovarian or non-small cell lung cancers who are treated with platinum-containing drugs [61,130,131]. Tumor grafts lacking ATP7A were found to be markedly more sensitive to cisplatin chemotherapy compared to ATP7A-expressing control tumors. Similar correlations have also been found between cisplatin resistance and levels of ATP7B [63].
Several hypotheses have been proposed to explain the ATP7A/B dependent cisplatin resistance, these include direct transport and sequestration of the drug into intracellular vesicles, or sequestration through interactions between cisplatin and metal-binding domains of these proteins [9,132,133]. Studies also showed that ATP7A can sequester cisplatin into subcellular compartments such as lysosomes (Figure 9B) [57,118,134].

Mechanism of Pt efflux and sequestration mediated by Cu-ATPases. To gain information, at the molecular level, on the possibility that Pt drugs can be translocated by human Cu-ATPases, an investigation was performed in which purified microsomes, grown in COS-1 cell and enriched with recombinant ATP7A, ATP7B or selected mutants, were adsorbed on a solid supported membrane (SSM) and subjected to a constant potential difference (ΔV) applied across the whole system. When charged species are translocated into the microsomes by the ATPases, a compensating current I(t) flows along the external circuit and can be monitored. The experimental results indicated that Pt drugs undergo ATP-dependent translocation in a fashion similar to that of Cu^+. Moreover, co-presence of Cu^+ and cisplatin inhibits translocation of both metallic species (Figure 11) [135]. Since the N-terminal metal-binding domains of ATP7A/B contribute to the metal transporting mechanism, the coordination of the platinum drugs to these N-terminal metal-binding domains of ATP7A/B was investigated by NMR spectroscopy and ESI-MS [129]. Interestingly, the various metal-binding domains (MBDs) of these ATPases show different reactivity towards cisplatin and in all cases pre-loading with copper favors the platination of the MBDs, but to different extents. Furthermore, platinum binding weakens the Cu^+ coordination, but does not expel the copper ion from the MBDs. The latter result can explain the inhibitory effect of Cu^+ upon the cisplatin translocation promoted by the Cu-ATPases [136]. Altogether, these investigations have clearly shown that Cu-ATPases are able to translocate aquated cisplatin across a membrane, but have also shown that the co-presence of Cu^+ ion (the physiological substrate) inhibits translocation. As far as this last point is concerned, it should be kept in mind that the conditions used in the *in vitro* experiments are not those occurring in vivo where the Cu^+ ion is firmly bound to chaperones and the free ion is estimated to be around 10^{-17} M. This consideration highlights the complexity of Pt-loading reactions occurring in vivo, and

how only a careful molecular investigation of the speciation occurring under physiological conditions can elucidate the mechanism of Pt transport [137].

Cu-ATPase-based therapeutic strategies. Growing tumors actively consume Cu and employ ATP7A/B to regulate the availability of this metal for Cu-dependent enzymes such as mitogen-activated kinase 1 [71, 140], superoxide dismutase 1 [138], cytochrome c oxidase [75], and lysyl oxidase (LOX) group of proteins [139], whose role in tumor growth and/or metastasis has been documented. Simultaneously, ATP7A/B activity and trafficking allow tumor cells to detoxify platinum-based drugs (like cisplatin), which are used for the chemotherapy of different solid tumors (Figure 7) [140]. These findings identify ATP7A/B at the nexus between tumorigenesis and cisplatin resistance pathways, underscoring its potential as a therapeutic target for regulating both tumor growth and the efficacy of cisplatin treatment. Interestingly, to circumvent resistance mechanisms against platinum drugs without heavily affecting copper homeostasis, a long list of already approved drugs was screened to identify molecules able to maintain Cu transport to the Golgi apparatus while inhibiting platinum excretion [141]. Using a synthetic lethality approach, were identified three hits (Tranilast, Telmisartan, and Amphotericin B) that increased cisplatin efficacy. All three drugs induced Pt-mediated DNA damage and inhibited either expression or tracking of ATP7B in a tumor-specific manner. Global transcriptome analyses showed that Tranilast and Amphotericin B affect expression of genes operating in several pathways that confer tolerance to cisplatin. In the case of Tranilast, these comprised key Pt-transporting proteins, including ATOX1, whose suppression affected the ability of ATP7B to traffic in response to cisplatin. It was concluded that Tranilast, Telmisartan, and Amphotericin B could be effective drugs that selectively promote cisplatin toxicity in Pt-resistant ovarian cancer cells and can be rapidly repurposed to overcome resistance of tumors to Pt-based chemotherapy [141]. Also the orphan drug TTM has been exploited for targeting Cu-transporting ATPases and found to be able to reduce the levels of ATP7A and hence increase the cytotoxicity of cisplatin [142].

Cisplatin resistance mediated by Cu chaperone ATOX1. The Cu chaperone ATOX1 receives cuprous ions from CTR1 and delivers them to ATP7A and ATP7B for injection into the TGN. ATOX1 is a small protein with a ferredoxin-like fold and a CXXC metal-binding motif highly conserved among metallochaperones and soluble domains of Cu-transporting ATPases [143] and therefore able to exchange copper ion with them [84]. Moreover, a lysine-rich region (KKTGK), located at the C-terminus, could represent a nuclear localization signal needed for ATOX1 translocation to the nucleus. Evidence exists that ATOX1 can act as a Cu-dependent nuclear

transcription factor implicated in cell proliferation (over-expression of cyclin-D1) and oxidative stress modulation in the cardiovascular system (over-expression of extracellular SOD3) [144,145]. Moreover, ATOX1 can promote inflammatory neovascularization via chaperone and transcription factor function [146]. It is also noteworthy that ATOX1-deficient cells possess higher levels of proteasome activity, which could be linked to its transcriptional activity. In fact, many of the subunits constituting the proteasome contain the putative ATOX1 binding site GAAAGA within their promoter regions [12]. However, other results indicate that, whereas ATOX1 is present in the nucleus in HeLa cells, it does not bind to DNA *in vitro* [147], but can still mediate transcriptional regulation via additional proteins [148]. In addition to Cu transport for loading of Cu-dependent enzymes, ATOX1 can modulate the cell cycle by interacting with subunits of the anaphase-promoting complex (APC). Due to its key role in the cell cycle, APC represents a putative target for anticancer agents. In the absence of ATOX1, cells have prolonged G2/M phases and a slower proliferation rate. [149]. In patients with metastatic colon cancer, there is a significant increase in the expression of nuclear ATOX1 [150] and single-cell tracking experiments demonstrate that ATOX1 is required for breast cancer cell migration [151].

Several reports indicate that the ATOX1 levels in cells have an evident influence on their sensitivity to cisplatin [64,65]. For instance, cancer cell lines with higher levels of ATOX1 have, correspondingly, higher resistance to cisplatin than cell lines with lower levels of ATOX1. Using solution and in-cell NMR spectroscopy methods, the interaction of cisplatin with ATOX1 overexpressed in *E. coli* was proven (in this experimental setup the intracellular environment provides itself suitable conditions for the preservation of the protein in its active form). It was found that cisplatin reacts nearly quantitatively with ATOX1 and that over expression of ATOX1 reduces the amount of DNA platination and, consequently, the degree of cell filamentation [152]. The apparently contradicting result that Drosophila ATOX1-/- mutants [65] and mouse ATOX1-/- fibroblast cell lines [12] display lower sensitivity to cisplatin than the wild-type controls can have a different explanation: ATOX1-/- cells could have reduced ability to accumulate cisplatin due to their inability to trigger the endocytotic process that putatively brings cisplatin into the cell and that accompanies the down regulation of CTR1 during cisplatin exposure. A lower uptake and accumulation of cisplatin in vesicular compartments would result in a reduced DNA platination [83]. Interestingly, it has been reported that platinum anticancer agents (cisplatin, carboplatin, oxaliplatin, pyriplatin, etc.) by binding to ATOX1 can promote ATOX1 unfolding followed by aggregation of the protein that increases as a function of the incubation time [64,153]. The similarities between the Cu^{+}-binding site of ATOX1, on one side, and the soluble domains of Cu-ATPases and metallothioneins (MTs), on the other side, all characterized by CXXC sequences,

support the notion that all of them can sequester Pt in cells as already shown in *E. coli* for an ATP7B recombinant variant containing four out of six Cu^+-binding domains [132] and for ATOX1 [152], thus reducing the abnormal filamentous growth promoted by cisplatin. That resistance to cisplatin chemotherapy associated to over expression of Cu transporters is just due to sequestration of cisplatin, with undesired beneficial effect on cancer cell viability, is not the only explanation. For instance, by binding to ATOX1 in the cytoplasm, the drug could be easily targeted for ATP7A/B-mediated cell export [64] or inhibit the ATOX1-triggered endocytotic process that putatively brings cisplatin into the cell [83]. Furthermore, cisplatin bound to ATOX1 may alter Cu homeostasis with severe consequences on several other cellular functions and, *in primis*, cellular defense against reactive oxygen species, hence providing alternative routes leading to cell killing.

Modes of interaction of ATOX1 with platinum drugs. Based on the high affinity of Pt^{2+} for S-donor ligands, it is expected that ATOX1 binds cisplatin at the CXXC Cu-binding motif. Two early crystal structures of ATOX1 bound to cisplatin were reported [89]. In one structure, Pt was stripped of all its original ligands and bound to Cys12 (N,S) and Cys15 (S) of ATOX1with a molecule of tris(2-carboxyethyl)phosphine (TCEP, a reducing agent used in the preparation of the apoprotein) completing the square planar coordination. The second structure contained molecules of stoichiometry cis-$Pt(NH_3)_2(ATOX1)_2$ with each protein molecule bound to Pt through the sulfur atom of Cys15. It is of interest to note that the crystal structure of $Hg(ATOX1)_2$ also involves metal ion coordination by the two CXXC motifs [154]. Thus, like Cu and Hg, also Pt can be accommodated within the CXXC metal binding domain. The mode of binding of cisplatin to a single molecule of ATOX1, in solution, was established by NMR spectroscopy measurements that showed how the Pt atom binds to Cys12 and Cys15 while retaining the two ammine groups. Moreover, the structural determinants of the whole protein, in the adduct, were disclosed by hybrid Car–Parrinello density functional theory-based QM/MM simulations which showed how the coordination of the platinum moiety modifies only minimally the fold of the protein (Figure 12) [155]. Unexpectedly, it was found that preloading of the ATOX1 protein with Cu^+ favors its reaction with cisplatin, notwithstanding the platinum moiety binds to the same coordination site as copper. In addition, preloading with Cu^+ enhances the competition of ATOX1, with respect to dithiothreitol (DTT, a small-molecule redox reagent frequently used in biology and containing a six-member chain with two terminal thiols) for binding to cisplatin [156]. Similarly, it was shown that apo ATOX1 is unable to bind cisplatin in the presence of excess GSH but, in contrast, preloading with Cu^+ renders the ATOX1 protein competitive with GSH for binding to cisplatin [137]. Remarkably, a mixture of cisplatin with preformed Cu^+-ATOX1 exhibits near-UV circular

dichroism signals indicative of Cu-Pt interactions as if both copper and cisplatin occupy the same metal-binding site [64]. These results indicate that cuprous ions can directly intervene in regulating the cellular trafficking of cisplatin.

Platinum can inhibit copper trafficking between ATOX1 and ATPases. As already underlined, the Cu^+-binding site of ATOX1 is remarkably similar to those of the soluble domains of Cu-ATPases as required for ATOX1- mediated Cu^+ delivery to ATP7A and ATP7B, which are responsible for Cu release to the secretory pathway and efflux of excess Cu from the cell. Cu^+ handover is believed to occur through the formation of three-coordinate intermediates where the metal ion is simultaneously linked to ATOX1 and to a soluble domain of Cu-ATPases [143]. Recently it has been shown that platinum drugs can specifically bind to the heterodimeric complex ATOX1-Cu^+-MNK1 (MNK1 is the first soluble domain of ATP7A), thus leading to a kinetically inert adduct that has been structurally characterized by solution NMR and X-ray crystallography (Figure 13) [157]. In the X-ray structure, two alternate configurations for this heterodimer were found: one configuration comprising Pt1 and Cu1 and the other configuration comprising Cu2. The latter configuration, comprising just one Cu^+ ion (Cu2), had a coordination environment very similar to that found in an analogous heterodimeric species containing just a Cd^{2+} ion, supporting the notion that this mode of interaction is that occurring in the physiological handover of Cu^+ between ATOX1 and soluble domains of ATPases. The former configuration, comprising the Pt1 and Cu1 atoms, indicates that a Cu^+ ion and a Pt moiety can be accommodated in the cage of four sulfur atoms (two from each protein molecule) which forms in the metal-handover process. It is interesting to note that the Cu^+ ion, retained after Pt^{2+} coordination, can be released to glutathione (a physiological thiol) or to other complexing agents. Remarkably, the Pt^{2+}-supported heterodimeric complex does not form if Zn^{2+} is used in place of Cu^+ and transplatin (the *trans* isomer of cisplatin) is used instead of cisplatin. These results thus indicate that cis-platinum drugs can specifically affect Cu^+ homeostasis by interfering with the rapid exchange of Cu^+ between ATOX1 and MBDs of Cu-ATPases with consequences on cancer cell viability and migration [157].

Cisplatin handover between copper transporters. Pt-loading reactions occurring in vivo are rather complex and different platinophiles are likely to influence each other. In the absence of other platinofiles, it has been found that Pt-loaded ATOX1 is unable to handover Pt to MNK1 (the terminal MBD of ATP7A) [137]. The latter result could appear in contrast with other reports that showed that Pt can be unidirectionally transferred from cisplatin-loaded ATOX1 to MBD2 of ATP7A [158]. However, the latter reaction was performed in the presence of TCEP which was

shown by the same authors to react with Pt, therefore, it is conceivable that TCEP could coordinate to platinum and, with its strong trans-labilizing effect, confer a peculiar reactivity to the Pt-protein adduct. Similarly, it was reported that cisplatin can be transferred from ATOX1 to MBD4 of ATP7B [159]. However the same authors also noted that increasing the time of incubation of ATOX1 with cisplatin, less Pt was transferred to MBD4 and suggested that this trend could be linked to changes in the Pt coordination with time (i.e., from monodentate to bidentate binding). Moreover, also in this latter case was used an exogenous reducing agent (DTT), which also has good coordinating ability towards Pt and could modify the reactivity of the formed complex. Finally, it was found that platinum, once complexed to GSH, the most abundant reducing agent in the cytoplasm, does not react with the Cu transport proteins, which is in line with the idea that GSH can intercept the great majority of the active cisplatin entering the cell, thus buffering its cytotoxic potential [137]. However there are indications that Cu pre-loaded ATOX1 [156] and MNK1 [135] can behave quite differently from the apo proteins as, for instance, shown by the copper-loaded ATOX1 that, differently from apo ATOX1, can compete successfully with GSH for binding to cisplatin [137]. In conclusion, chelate binding of Pt to ATOX1 and to MBDs of ATPases appears to make these adducts rather inert, so that a physiologically relevant exchange appears to be quite improbable; however, most reactions have been carried out in the absence of Cu, the physiological substrate for these transporters, which implies that the reaction occurring *in vivo* could be quite different. It is foremost desirable that the crucial influence of Cu in prompting Pt affinity and metal exchange between this array of transporters could be fully unraveled.

ATOX1-based therapeutic strategies. The already mentioned copper chelator TTM, an orphan drug used in the clinic for the treatment of Wilson's disease [160], has proved to inhibit tumor growth via its anti-angiogenic activity [161]. Mechanistic in vitro and in vivo investigations also indicate that TTM modulates copper levels by binding to copper proteins, such as serum albumin, ceruloplasmin and metallothioneins [162–164]. An X-ray crystal structure showed that TTM binds to ATX1 (the analogue of ATOX1 in yeast) and forms a stable [TTM-Cu-$(Cu–ATX1)_3$] complex, containing a TTM-bridged Cu cluster which corresponds to the molybdenum cluster detected in a kidney sample extracted from LPP rats (animal model of Wilson's disease) treated with TTM [165]. More recently, it has been reported that TTM can inhibit the reaction of cisplatin with Cu-ATOX1 and prevent the protein unfolding and aggregation induced by cisplatin. Interestingly, although Ag^+ ion binds to ATOX1 in a way similar to Cu^+-ATOX1, TTM does not prevent the reaction of Ag^+-ATOX1 with cisplatin (Figure 14) [166]. These results indicate that the formation of a Mo-centered trimeric

protein cluster in the TTM-Cu-ATOX1 system can indeed enhance the cytotoxic efficacy of cisplatin and help to circumvent cisplatin resistance of tumor cells.

Copper-based anticancer strategies that involve biochemical pathways other than copper-transport. The targeting of Cu with TTM has been found to induce antitumor effects in cells with BRAF V600E-kinase mutation, which provided a strong rationale for further studies aiming at disrupting the central role of Cu in other BRAF V600E-positive malignancies, such as thyroid, lung, and colorectal cancers or hairy cell leukemia [167]. The TTM chelator inhibited the growth of melanoma cell lines resistant to BRAF or MEK1/2 inhibitors and increased the antineoplastic activity of these inhibitors [168]. In addition, in CRC cells carrying BRAF V600E mutation, Cu depletion induced by pharmacological treatment with TTM reduced the growth of BRAF V600E cells in colon cancers that were resistant to BRAF inhibitors [169]. Pretreatment with TTM was also found to significantly enhance cisplatin sensitivity by promoting p38 activation and cisplatin-induced degradation of the epidermal growth factor receptor (EGFR) [170]. Currently, this chelator is evaluated as an adjuvant therapy in various cancer clinical trials.

Apart from TTM, other copper chelators have been exploited. Bleomycin (a glycopeptidic antibiotic produced by Streptomyces verticillus) and curcumin (a phytochemical agent) are chelators that gave promising results in oncology [171,172]. Bleomycin is regularly used in combination with other therapeutic agents such as cisplatin and etoposide in testicular cancer [173]. Curcumin may be used in monotherapy or in combination with other anticancer agents for the prevention of cancer [174].

Not only copper chelators, but also copper ionophores have been exploited for anticancer therapy. Copper ionophores are molecules that transport Cu ions through cellular membranes. Ionophores increase and/or redistribute intracellular Cu levels, often allowing Cu to become bioavailable [175]. These molecules have high affinity for Cu^{2+} but low affinity for Cu^{+}; therefore, in the reducing environment of cytosol the Cu^{2+} entering the cell will be reduced to the Cu^{+} form and released by the ionophore. Such a release of Cu^{+} will poison the cell [176].

In the family of ionophores, several compounds such as docosahexaenoic acid (DHA), disulfiram (DSF), thiosemicarbazones, and clioquinol have been exploited. It has to be noted that only the Cu-complexed form of these ionophores is active as a cancer treatment because the ligands alone (metal-free compounds) have a minimal anticancer effect [177]. Once more, the metabolism of copper has proved to play a primary role in cancer progression and therapy thus representing a versatile pharmacological target [178].

Future perspectives of copper transporters for anticancer therapy. The potential of copper transporters for anticancer research is yet beginning to unfold and further studies of the molecular mechanisms regulating copper-transporting activities, interactions, and tracking in tumor cells will definitely help to determine how Cu and Pt metabolic pathways are intertwined and can be manipulated to suppress oncogenic processes. Moreover, a better knowledge of the complexity of Pt-loading reactions occurring in vivo, and how different metallic metabolic pathways can influence each other, will offer new opportunities for the further development and improvement of Pt-based anticancer therapeutic strategies.

Acknowledgments

This work was supported by the Italian Ministero dell'Istruzione, dell'Università e della Ricerca [Project “ICHEMFLY” PRIN 2017WBZFHL], the University of Bari and the Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (CIRCMSB).

Figure Legends

Figure 1. Structures of the clinically active Pt-drugs in use worldwide (*first row*) or regionally, plus satraplatin, picoplatin, and trinuclear BBR3464 subjected to advanced clinical trials.

Figure 2. The selective inhibition of DNA synthesis in human AV3 cells grown in tissue culture by exposure to 5 μM (1 ppm) cis-dichlorodiammineplatinum(II) as measured by the rate of uptake of tritiated thymidine. The RNA synthesis, measured by the rate of uptake of tritiated uridine, and the protein synthesis, measured by the rate of uptake of tritiated leucine, are close to the control values (the horizontal line at 100 per cent). Reproduced with permission from Ref. [28].

Figure 3. Schematic representation of the mechanism of action of cisplatin and resistance to the drug. Reprinted by permission from Ref. [179]. Copyright © 2016 Springer-Verlag Berlin Heidelberg.

Figure 4. An HMG-domain protein (HMGB1; domain A shown as gray ribbon) inserts a phenyl group (yellow) into the groove created when cisplatin (platinum shown in red) forms a complex with DNA, causing it to bend. Reproduced with permission from Ref. [44]. Copyright © 2005 American Chemical Society.

Figure 5. The histone H1 and cisplatin form a ternary complex with DNA. Reproduced with permission from Ref. [45]. Copyright © 2019 The Royal Society of Chemistry.

Figure 6. Distribution of F-cisplatin in 2008 ovarian carcinoma cells. Cells were incubated with 2 μmol/L of F-cisplatin for 1 hour in parallel, and then stained for filamentous actin (red) with Alexa Flour 647 phalloidin and with Hoechst 33342 for nuclei (blue) prior to being imaged for F-cisplatin (green) accumulation. A, distribution of F-cisplatin in cisplatin-sensitive 2008 cells; B, distribution of F-cisplatin in cisplatin-resistant 2008/C13*5.25 cells; C, distribution of F-cisplatin in a single 2008 cell; D, distribution of F-cisplatin in a single 2008/C13*5.25 cell. Adapted with permission from Ref. [57]. Copyright © 2005, American Association for Cancer Research.

Figure 7. Schematic depiction of copper and platinum distribution in tumor cell. Copper (Cu) and cisplatin (Pt) taken up via CTR1 (red) are transferred to chaperones ATOX1, CCS and COX17, which ferry Cu or Pt derivates (black dash arrows) to ATP7A/B (blue) in the Golgi, to Cu–Zn superoxide dismutase (magenta) in the cytosol and to cytochrome c oxidase (green) in the

mitochondria, respectively. In the Golgi ATP7A/B load Cu on newly synthesized cuproenzyme lysyl oxidase (LOX, orange ball), which traffics along the biosynthetic pathway (orange arrows). Metallated LOX is secreted to the cell exterior (orange arrows), where it promotes migration and invasion (orange dash arrow) of the malignant cell. A significant increase in intracellular Pt induces export of ATP7A/B (blue arrow) towards vesicular compartments and cell membrane, where ATP7A/B drives the sequestration and efflux of excessive Pt. Bold black arrows indicate Pt/Cu translocation across the membrane. Reproduced with permission from Ref. [140].

Figure 8. A, schematic figure depicting CTR1 and CTR2 homotrimers, and a possible way for their interaction (heterotrimerization). B, model for CTR1-dependent cisplatin import and the modulation of Pt uptake by truncation of CTR1. Adapted with permission from Refs. [109] (Copyright © 2014 Elsevier GmbH) and [59] (Copyright © 2013, National Academy of Sciences).

Figure 9. A, Co-localization of F-cisplatin (CFDA-Pt, green) and the marker for CTR1 (red) in A2780 and A2780cis cells upon endocytosis inhibition with methyl-β-cyclodextrin. Yellow, the structure is positive for CFDA-Pt and the protein marker. Scale bar, 10 μm. B, subcellular localization of ATP7A and ATP7B in A2780 and A2780cis cells. Immunofluorescence localization of ATP7A and ATP7B (both green) in A2780 and A2780cis cells. Cell nuclei were stained with propidium iodide (red). Ovals indicate cell periphery. Scale bar, 10 μm. Adapted with permission from Refs. [117] (Copyright © 2012 Elsevier Inc.) and [118].

Figure 10. ATP7A/B structure. A, schematic structure of the ATP7A/B. The proteins have eight transmembrane (TM) domains, which form a pore for transport of Cu and Pt across the membrane. ATP7A/B contain ATP-binding (red), phosphatase (green), and phosphorylation (blue) domains, which regulate the catalytic activity of the protein. The N-terminal tail comprises six metal-binding sites (MBSs, depicted as cyan balls) that interact with Cu or Pt and regulate the protein conformation. In addition, the Cysteine-Proline-Cysteine (CPC) motif in the sixth transmembrane domain plays a key role in the metal translocation along the channel. N-terminal apical sorting signal of ATP7B is highlighted in yellow, while basolateral targeting signal in C-tail of ATP7A is highlighted in orange. B, three-dimensional structures of ATP7B based on the existing structures of soluble domains and the previously determined structure of the homologous LpCopA from the bacterium *Legionella pneumophila*. Only MBSs 5 and 6 are shown in the model (cyan). Other domains of the protein are depicted with the same color as in panel A: ATP-binding (red), phosphatase (green), and phosphorylation (blue). Reproduced with permission from Ref. [140].

Figure 11. A, diagram of a microsome containing ATP7A/ATP7B adsorbed on an SSM. When charge displacement occurs, a compensating current I(t) flows along the external circuit (the spheres represent electrons) to keep constant the potential difference (ΔV) applied across the whole system. RE = reference electrode. B, current measurements on ATP7A in the presence of cisplatin.

Representative current transients, induced by ATP (100 μM) concentration jumps, in the presence of: $CuCl_2$ (5 μM), Pt complex (5 μM), a mixture of $CuCl_2$ and Pt complex (5 μM each), and a mixture of $CuCl_2$ and Pt complex (5 μM each) with BCS (1 mM). The insets show the charges related to ATP-induced current transients obtained under the various experimental conditions. Adapted with permission from Ref. [135]. Copyright © 2014, John Wiley and Sons.

Figure 12. Structural models of apo and platinated ATOX1 emerging from QM/MM simulations based on NMR, and CD spectra. ATOX1 backbone along with Cys12 and Cys15 side chains (*left*); close up on the Cys12/Cys15 region in apo ATOX1 (*center*); close up on the Cys12/Cys15 region in the Pt-adduct (*right*). The backbone of the protein is represented by a red ribbon. Cys12 and Cys15 residues, along with the Pt moiety, are represented in balls and sticks with the following color code: platinum in brown, sulfur in yellow, carbon in cyan, nitrogen in blue, and hydrogen in white. Water molecules are represented by lines, the oxygen is colored in red and the hydrogen in white. Reproduced with permission from Ref. [155]. Copyright © 2014, John Wiley and Sons.

Figure 13. A, crystal structure of the heterodimeric complex (green) crystallized in the presence of $[Pt(SO_4)(H_2O)(1,2\text{-R,R-DACH})]$, superimposed to that of the ATOX1−Cd(II)−Mnk1 complex (light gray, PDB entry 3CJK). ATOX1 (chain A) and MNK1 (chain B) molecules are shown in cartoon representation. Pt, Cu, and Cd atoms are shown as magenta, red, and gray spheres, respectively. B, platinum binding site Pt1. Protein residues within 3 Å from Pt and Cu are shown as sticks, with C, O, N, and S atoms colored in green, red, blue, and yellow, respectively. Red and black dashed lines indicate the two different metallic cores (one comprising Pt1 and Cu1 and the other Cu2) of the crystallized heterodimer. Adapted with permission from Ref. [157]. Copyright © 2019, American Chemical Society.

Figure 14. Tetrathiomolybdate inhibits the platination of Cu–ATOX1 and prevents protein unfolding and aggregation induced by cisplatin. Reproduced with permission from Ref. [166]. Copyright © 2018, The Royal Society of Chemistry.

References

[1] S.P. Binks, M. Dobrota, Kinetics and mechanism of uptake of platinum-based pharmaceuticals by the rat small intestine, Biochem. Pharmacol. 40 (1990) 1329–1336. https://doi.org/10.1016/0006-2952(90)90400-F.

[2] D.P. Gately, S.B. Howell, Cellular accumulation of the anticancer agent cisplatin: A review, Br. J. Cancer. 67 (1993) 1171–1176. https://doi.org/10.1038/bjc.1993.221.

[3] A.K. Holzer, G. Samimi, K. Katano, W. Naerdemann, X. Lin, R. Safaei, S.B. Howell, The copper influx transporter human copper transport protein 1 regulates the uptake of cisplatin in human ovarian carcinoma cells, Mol. Pharmacol. 66 (2004) 817–823. https://doi.org/10.1124/mol.104.001198.

[4] R. Safaei, S.B. Howell, Copper transporters regulate the cellular pharmacology and sensitivity to Pt drugs, Crit. Rev. Oncol. Hematol. 53 (2005) 13–23. https://doi.org/10.1016/j.critrevonc.2004.09.007.

[5] R. Safaei, Role of copper transporters in the uptake and efflux of platinum containing drugs, Cancer Lett. 234 (2006) 34–39. https://doi.org/10.1016/j.canlet.2005.07.046.

[6] X.J. Liang, D.W. Shen, K.G. Chen, S.M. Wincovitch, S.H. Garfield, M.M. Gottesman, Trafficking and localization of platinum complexes in cisplatin-resistant cell lines monitored by fluorescence-labeled platinum, J. Cell. Physiol. 202 (2005) 635–641. https://doi.org/10.1002/jcp.20253.

[7] G. Samimi, K. Katano, A.K. Holzer, R. Safaei, S.B. Howell, Modulation of the cellular pharmacology of cisplatin and its analogs by the copper exporters ATP7A and ATP7B, Mol. Pharmacol. 66 (2004) 25–32. https://doi.org/10.1124/mol.66.1.25.

[8] G. Samimi, R. Safaei, K. Katano, A.K. Holzer, M. Rochdi, M. Tomioka, M. Goodman, S.B. Howell, Increased Expression of the Copper Efflux Transporter ATP7A Mediates Resistance to Cisplatin, Carboplatin, and Oxaliplatin in Ovarian Cancer Cells, Clin. Cancer Res. 10 (2004) 4661–4669. https://doi.org/10.1158/1078-0432.CCR-04-0137.

[9] K. Katano, R. Safaei, G. Samimi, A. Holzer, M. Tomioka, M. Goodman, S.B. Howell, Confocal microscopic analysis of the interaction between cisplatin and copper transporter ATP7B in human ovarian carcinoma cells, Clin. Cancer Res. 10 (2004) 4578–4588. https://doi.org/10.1158/1078-0432.CCR-03-0689.

[10] R. Safaei, S. Otani, B.J. Larson, M.L. Rasmussen, S.B. Howell, Transport of cisplatin by the copper efflux transporter ATP7B, Mol. Pharmacol. 73 (2008) 461–468. https://doi.org/10.1124/mol.107.040980.

[11] L.S. Mangala, V. Zuzel, R. Schmandt, E.S. Leshane, J.B. Halder, G.N. Armaiz-Pena, W.A. Spannuth, T. Tanaka, M.M.K. Shahzad, Y.G. Lin, A.M. Nick, C.G. Danes, J.W. Lee, N.B. Jennings, P.E. Vivas-Mejia, J.K. Wolf, R.L. Coleman, Z.H. Siddik, G. Lopez-Berestein, S. Lutsenko, A.K. Sood, Therapeutic targeting of ATP7B in ovarian carcinoma, Clin. Cancer Res. 15 (2009) 3770–3780. https://doi.org/10.1158/1078-0432.CCR-08-2306.

[12] R. Safaei, M.H. Maktabi, B.G. Blair, C.A. Larson, S.B. Howell, Effects of the loss of Atox1 on the cellular pharmacology of cisplatin, J. Inorg. Biochem. 103 (2009) 333–341. https://doi.org/10.1016/j.jinorgbio.2008.11.012.

[13] M. Peyrone, Ueber die Einwirkung des Ammoniaks auf Platinchlorür, Ann. Der Chemie Und Pharm. 51 (1844) 1–29. https://doi.org/10.1002/jlac.18440510102. Translated into English by S. Muspratt as:'Action of Ammonia upon the Protochloride of Platinum', Med. Times 10 (1844) 381.

[14] A. Werner, Beitrag zur Konstitution anorganischer Verbindungen, Zeitschrift Für Anorg. Chemie. 3 (1893) 267–330. https://doi.org/10.1002/zaac.18930030136. Translated into English as:'Contribution to the Constitution of Inorganic Compounds', in G. B. Kauffman, "Classics in Coordination Chemistry, Part I: The Selected Papers of Alfred Werner", Classics of Science, Volume 4, Dover Publications, New York, NY (1968) 5–88 B.

[15] J. Reisei, Neue Verbindung von Platinchlorür mit Ammoniak; Radikal der Gros' schen Salze, Ann. Der Chemie Und Pharm. 36 (1840) 111–112. https://doi.org/10.1002/jlac.18400360113. Translated into English by J. Reiset, J. Compt. Rend. 11 (1840) 711.

[16] B. Rosenberg, L. Van Camp, T. Krigas, Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode, Nature. 205 (1965) 698–699. https://doi.org/10.1038/205698a0.

[17] B.A. Chabner, T.G. Roberts, Chemotherapy and the war on cancer, Nat. Rev. Cancer. 5 (2005) 65–72. https://doi.org/10.1038/nrc1529.

[18] B. Rosenberg, Platinum complexes for the treatment of cancer, in: T.G. Spiro (Ed.), Nucleic Acid-Metal Ion Interact., John Wiley & Sons Inc., New York, NY, USA, 1980: pp. 1–29.

[19] D. Lebwohl, R. Canetta, Clinical development of platinum complexes in cancer therapy: An historical perspective and an update, Eur. J. Cancer. 34 (1998) 1522–1534. https://doi.org/10.1016/S0959-8049(98)00224-X.

[20] D. Chen, V. Milacic, M. Frezza, Q. Dou, Metal Complexes, their Cellular Targets and Potential for Cancer Therapy, Curr. Pharm. Des. 15 (2009) 777–791. https://doi.org/10.2174/138161209787582183.

[21] B. Desoize, C. Madoulet, Particular aspects of platinum compounds used at present in cancer treatment, Crit. Rev. Oncol. Hematol. 42 (2002) 317–325. https://doi.org/10.1016/S1040-8428(01)00219-0.

[22] U. Olszewski, G. Hamilton, A Better Platinum-Based Anticancer Drug Yet to Come?, Anticancer. Agents Med. Chem. 10 (2012) 293–301. https://doi.org/10.2174/187152010791162306.

[23] N. Shah, D.S. Dizon, New-generation platinum agents for solid tumors, Futur. Oncol. 5 (2009) 33–42. https://doi.org/10.2217/14796694.5.1.33.

[24] T.C. Johnstone, K. Suntharalingam, S.J. Lippard, The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs, Chem. Rev. 116 (2016) 3436–3486. https://doi.org/10.1021/acs.chemrev.5b00597.

[25] M. Galanski, M. Jakupec, B. Keppler, Update of the Preclinical Situation of Anticancer Platinum Complexes: Novel Design Strategies and Innovative Analytical Approaches, Curr. Med. Chem. 12 (2005) 2075–2094. https://doi.org/10.2174/0929867054637626.

[26] A.M. Florea, D. Büsselberg, Occurrence, use and potential toxic effects of metals and metal compounds, BioMetals. 19 (2006) 419–427. https://doi.org/10.1007/s10534-005-4451-x.

[27] R.Y. Tsang, T. Al-Fayea, H.J. Au, Cisplatin overdose: Toxicities and management, Drug Saf. 32 (2009) 1109–1122. https://doi.org/10.2165/11316640-000000000-00000.

[28] B. Rosenberg, Some biological effects of platinum compounds. New Agents for the Control of Tumours., Platin. Met. Rev. 15 (1971) 42–51.

[29] H.C. Harder, B. Rosenberg, Inhibitory effects of anti-tumor platinum compounds on DNA, RNA and protein syntheses in mammalian cells in vitro, Int. J. Cancer. 6 (1970) 207–216. https://doi.org/10.1002/ijc.2910060207.

[30] J. Reedijk, P.H.M. Lohman, Cisplatin: Synthesis, antitumour activity and mechanism of action, Pharm. Weekbl. Sci. Ed. 7 (1985) 173–180. https://doi.org/10.1007/BF02307573.

[31] Y. Sedletska, M.J. Giraud-Panis, J.M. Malinge, Cisplatin is a DNA-damaging antitumour compound triggering multifactorial biochemical responses in cancer cells: Importance of apoptotic pathways, Curr. Med. Chem. - Anti-Cancer Agents. 5 (2005) 251–265. https://doi.org/10.2174/1568011053765967.

[32] S.G. Chaney, S.L. Campbell, E. Bassett, Y. Wu, Recognition and processing of cisplatin- and oxaliplatin-DNA adducts, Crit. Rev. Oncol. Hematol. 53 (2005) 3–11. https://doi.org/10.1016/j.critrevonc.2004.08.008.

[33] M. Duan, J. Ulibarri, K.J. Liu, P. Mao, Role of nucleotide excision repair in cisplatin resistance, Int. J. Mol. Sci. 21 (2020) 1–13. https://doi.org/10.3390/ijms21239248.

[34] P. Jordan, M. Carmo-Fonseca, Molecular mechanisms involved in cisplatin cytotoxicity, Cell. Mol. Life Sci. 57 (2000) 1229–1235. https://doi.org/10.1007/PL00000762.

[35] V.M. Gonzalez, M. a Fuertes, C. Alonso, J.M. Perez, Is cisplatin-induced cell death always produced by apoptosis?, Mol. Pharmacol. 59 (2001) 657–663. https://doi.org/10.1124/mol.59.4.657.

[36] R.P. Perez, Cellular and molecular determinants of cisplatin resistance, Eur. J. Cancer. 34 (1998) 1535–1542. https://doi.org/10.1016/S0959-8049(98)00227-5.

[37] L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo, G. Kroemer, Molecular mechanisms of cisplatin resistance, Oncogene. 31 (2012) 1869–1883. https://doi.org/10.1038/onc.2011.384.

[38] S. Park, S.J. Lippard, Binding interaction of HMGB4 with cisplatin-modified DNA, Biochemistry. 51 (2012) 6728–6737. https://doi.org/10.1021/bi300649.

[39] S.G. Awuah, I.A. Riddell, S.J. Lippard, Repair shielding of platinum-DNA lesions in testicular germ cell tumors by high-mobility group box protein 4 imparts cisplatin hypersensitivity, Proc. Natl. Acad. Sci. 114 (2017) 950–955. https://doi.org/10.1073/pnas.1615327114.

[40] J. Malina, O. Novakova, M. Vojtiskova, G. Natile, V. Brabec, Conformation of DNA GG intrastrand cross-link of antitumor oxaliplatin and its enantiomeric analog, Biophys. J. 93 (2007) 3950–3962. https://doi.org/10.1529/biophysj.107.116996.

[41] J. Kasparkova, M. Vojtiskova, G. Natile, V. Brabec, Unique properties of DNA interstrand cross-links of antitumor oxaliplatin and the effect of chirality of the carrier ligand, Chem. - A Eur. J. 14 (2008) 1330–1341. https://doi.org/10.1002/chem.200701352.

[42] Y. He, Y. Ding, D. Wang, W. Zhang, W. Chen, X. Liu, W. Qin, X. Qian, H. Chen, Z. Guo, HMGB1 bound to cisplatin-DNA adducts undergoes extensive acetylation and phosphorylation in vivo, Chem. Sci. 6 (2015) 2074–2078. https://doi.org/10.1039/c4sc03650f.

[43] W. Lyu, F. Arnesano, P. Carloni, G. Natile, G. Rossetti, Effect of in vivo post-translational modifications of the HMGB1 protein upon binding to platinated DNA: a molecular simulation study, Nucleic Acids Res. (2018).

[44] S. Trzaska, Chemical & Engineering News: Top Pharmaceuticals: Cisplatin, Chem. Eng. News. 83 (2005) 52. https://pubsapp.acs.org/cen/coverstory/83/8325/8325cisplatin.html.

[45] L. Cheng, C. Li, Z. Xi, K. Wei, S. Yuan, F. Arnesano, G. Natile, Y. Liu, Cisplatin reacts with histone H1 and the adduct forms a ternary complex with DNA, Metallomics. 11 (2019) 556–564. https://doi.org/10.1039/c8mt00358k.

[46] L. Messori, A. Merlino, Cisplatin binding to proteins: A structural perspective, Coord. Chem. Rev. 315 (2016) 67–89. https://doi.org/10.1016/j.ccr.2016.01.010.

[47] A.M. Florea, D. Büsselberg, Cisplatin as an anti-tumor drug: Cellular mechanisms of activity, drug resistance and induced side effects, Cancers (Basel). 3 (2011) 1351–1371. https://doi.org/10.3390/cancers3011351.

[48] V. Brabec, J. Kasparkova, Modifications of DNA by platinum complexes: Relation to resistance of tumors to platinum antitumor drugs, Drug Resist. Updat. 8 (2005) 131–146. https://doi.org/10.1016/j.drup.2005.04.006.

[49] M. Kartalou, J.M. Essigmann, Mechanisms of resistance to cisplatin, Mutat. Res. - Fundam. Mol. Mech. Mutagen. 478 (2001) 23–43. https://doi.org/10.1016/S0027-5107(01)00141-5.

[50] L. Amable, Cisplatin resistance and opportunities for precision medicine, Pharmacol. Res. 106 (2016) 27–36. https://doi.org/10.1016/j.phrs.2016.01.001.

[51] D.-W. Shen, L.M. Pouliot, M.D. Hall, M.M. Gottesman, Cisplatin Resistance: A Cellular Self-Defense Mechanism Resulting from Multiple Epigenetic and Genetic Changes, Pharmacol. Rev. 64 (2012) 706–721. https://doi.org/10.1124/pr.111.005637.

[52] M. Ohmichi, J. Hayakawa, K. Tasaka, H. Kurachi, Y. Murata, Mechanisms of platinum drug resistance, Trends Pharmacol. Sci. 26 (2005) 113–116. https://doi.org/10.1016/j.tips.2005.01.002.

[53] K.F. Ejendal, C.A. Hrycyna, Multidrug Resistance and Cancer: The Role of the Human ABC Transporter ABCG2, Curr. Protein Pept. Sci. 3 (2005) 503–511. https://doi.org/10.2174/1389203023380521.

[54] O. Kis, K. Robillard, G.N.Y. Chan, R. Bendayan, The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters, Trends Pharmacol. Sci. 31 (2010) 22–35. https://doi.org/10.1016/j.tips.2009.10.001.

[55] H. Glavinas, P. Krajcsi, J. Cserepes, B. Sarkadi, The Role of ABC Transporters in Drug Resistance, Metabolism and Toxicity, Curr. Drug Deliv. 1 (2005) 27–42. https://doi.org/10.2174/1567201043480036.

[56] C. Molenaar, J.M. Teuben, R.J. Heetebrij, H.J. Tanke, J. Reedijk, New insights in the cellular processing of platinum antitumor compounds, using fluorophore-labeled platinum complexes

and digital fluorescence microscopy, J. Biol. Inorg. Chem. 5 (2000) 655–665. https://doi.org/10.1007/s007750000153.

[57] R. Safaei, K. Katano, B.J. Larson, G. Samimi, A.K. Holzer, W. Naerdemann, M. Tomioka, Intracellular Localization and Trafficking of Fluorescein-Labeled Cisplatin in Human Ovarian Carcinoma Cells Intracellular Localization and Trafficking of Fluorescein-Labeled Cisplatin in Human Ovarian Carcinoma Cells, Clin. Cancer Res. 11 (2005) 756–767.

[58] K. Katano, A. Kondo, R. Safaei, A. Holzer, G. Samimi, M. Mishima, Y.M. Kuo, M. Rochdi, S.B. Howell, Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper, Cancer Res. 62 (2002) 6559–6565. https://doi.org/10.1158/0008-5472.can-06-1710.

[59] S. Ishida, J. Lee, D.J. Thiele, I. Herskowitz, Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals, Proc. Natl. Acad. Sci. 99 (2002) 14298–14302. https://doi.org/10.1073/pnas.162491399.

[60] X. Lin, T. Okuda, A. Holzer, S.B. Howell, The copper transporter CTR1 regulates cisplatin uptake in Saccharomyces cerevisiae, Mol. Pharmacol. 62 (2002) 1154–1159. https://doi.org/10.1124/mol.62.5.1154.

[61] G. Samimi, N.M. Varki, S. Wilczynski, R. Safaei, D.S. Alberts, S.B. Howell, 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 (2003) 5853–9. http://www.ncbi.nlm.nih.gov/pubmed/14676106 (accessed January 9, 2019).

[62] K. Katano, R. Safaei, G. Samimi, A. Holzer, M. Rochdi, S.B. Howell, The copper export pump ATP7B modulates the cellular pharmacology of carboplatin in ovarian carcinoma cells, Mol. Pharmacol. 64 (2003) 466–473. https://doi.org/10.1124/mol.64.2.466.

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

[64] M.E. Palm, C.F. Weise, C. Lundin, G. Wingsle, Y. Nygren, E. Bjorn, P. Naredi, M. Wolf-Watz, P. Wittung-Stafshede, Cisplatin binds human copper chaperone Atox1 and promotes unfolding in vitro, Proc. Natl. Acad. Sci. 108 (2011) 6951–6956. https://doi.org/10.1073/pnas.1012899108.

[65] H. Hua, V. Günther, O. Georgiev, W. Schaffner, Distorted copper homeostasis with decreased sensitivity to cisplatin upon chaperone Atox1 deletion in Drosophila, in: BioMetals, 2011: pp. 445–453. https://doi.org/10.1007/s10534-011-9438-1.

[66] S.D. Aust, L.A. Morehouse, C.E. Thomas, Role of metals in oxygen radical reactions, J. Free Radicals Biol. Med. 1 (1985) 3–25. https://doi.org/10.1016/0748-5514(85)90025-X.

[67] M.C. Linder, K. Wright, J.R. Moor, Ceruloplasmin assays in diagnosis and treatment of human lung, breast, and gastrointestinal cancers, J. Natl. Cancer Inst. 67 (1981) 263–275. https://doi.org/10.1093/jnci/67.2.263.

[68] E. Pagliardi, E. Giangrandi, Clinical significance of the blood copper in hodgkin's disease, Acta Haematol. 24 (1960) 201–212. https://doi.org/10.1159/000206463.

[69] M.L. Turski, D.C. Brady, H.J. Kim, B.-E. Kim, Y. Nose, C.M. Counter, D.R. Winge, D.J. Thiele, A Novel Role for Copper in Ras/Mitogen-Activated Protein Kinase Signaling, Mol. Cell. Biol. 32 (2012) 1284–1295. https://doi.org/10.1128/mcb.05722-11.

[70] C.Y. Tsai, J.C. Finley, S.S. Ali, H.H. Patel, S.B. Howell, Copper influx transporter 1 is required for FGF, PDGF and EGF-induced MAPK signaling, Biochem. Pharmacol. 84 (2012) 1007–1013. https://doi.org/10.1016/j.bcp.2012.07.014.

[71] S.S. Brem, D. Zagzag, A.M.C. Tsanaclis, S. Gately, M.P. Elkouby, S.E. Brien, Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the depletion of copper an angiogenic cofactor, Am. J. Pathol. 137 (1990) 1121–1142. /pmc/articles/PMC1877678/?report=abstract (accessed February 25, 2021).

[72] J. Yoshii, H. Yoshiji, S. Kuriyama, Y. Ikenaka, R. Noguchi, H. Okuda, H. Tsujinoue, T. Nakatani, H. Kishida, D. Nakae, D.E. Gomez, M.S. De Lorenzo, A.M. Tejera, H. Fukui, The copper-chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells, Int. J. Cancer. 94 (2001) 768–773. https://doi.org/10.1002/ijc.1537.

[73] Q. Pan, C.G. Kleer, K.L. Van Golen, J. Irani, K.M. Bottema, C. Bias, M. De Carvalho, E.A. Mesri, D.M. Robins, R.D. Dick, G.J. Brewer, S.D. Merajver, Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis, Cancer Res. 62 (2002) 4854–4859. https://doi.org/10.1002/(sici)1097-4644(19980601)69:3<326::aid-jcb10>3.0.co.

[74] C. Cox, T.N. Teknos, M. Barrios, G.J. Brewer, R.D. Dick, S.D. Merajver, The role of copper suppression as an antiangiogenic strategy in head and neck squamous cell carcinoma, Laryngoscope. 111 (2001) 696–701. https://doi.org/10.1097/00005537-200104000-00024.

[75] S. Ishida, P. Andreux, C. Poitry-Yamate, J. Auwerx, D. Hanahan, Bioavailable copper modulates oxidative phosphorylation and growth of tumors, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 19507–19512. https://doi.org/10.1073/pnas.1318431110.

[76] J.R. Prohaska, Role of copper transporters in copper homeostasis, in: Am. J. Clin. Nutr., American Society for Nutrition, 2008: pp. 826S-829S. https://doi.org/10.1093/ajcn/88.3.826s.

[77] S. La Fontaine, J.F.B. Mercer, Trafficking of the copper-ATPases, ATP7A and ATP7B: Role in copper homeostasis, Arch. Biochem. Biophys. 463 (2007) 149–167. https://doi.org/10.1016/j.abb.2007.04.021.

[78] J.F.B. Mercer, R.M. Llanos, Molecular and cellular aspects of copper transport in developing mammals, in: J. Nutr., American Institute of Nutrition, 2003: pp. 1481S-1484S. https://doi.org/10.1093/jn/133.5.1481s.

[79] W. Masson, H. Hughes, D. Papworth, Y. Boyd, N. Horn, Abnormalities of copper accumulation in cell lines established from nine different alleles of mottled are the same as those found in Menkes disease, J. Med. Genet. 34 (1997) 729–732. https://doi.org/10.1136/jmg.34.9.729.

[80] Y. Qian, E. Tiffany-Castiglioni, J. Welsh, E.D. Harris, Copper efflux from murine microvascular cells requires expression of the Menkes disease CU-ATPase, J. Nutr. 128 (1998) 1276–1282. https://doi.org/10.1093/jn/128.8.1276.

[81] S. La Fontaine, S.D. Firth, J. Camakaris, A. Englezou, M.B. Theophilos, M.J. Petris, M.

Howie, P.J. Lockhart, M. Greenough, H. Brooks, R.R. Reddel, J.F.B. Mercer, Correction of the copper transport defect of Menkes patient fibroblasts by expression of the menkes and Wilson ATPases, J. Biol. Chem. 273 (1998) 31375–31380. https://doi.org/10.1074/jbc.273.47.31375.

[82] M.J. Petris, D. Strausak, J.F.B. Mercer, The Menkes copper transporter is required for the activation of tyrosinase, Hum. Mol. Genet. 9 (2000) 2845–2851. https://doi.org/10.1093/hmg/9.19.2845.

[83] F. Arnesano, M. Losacco, G. Natile, CHAPTER 15. Platinum, in: W. Maret, A.G. Wedd (Eds.), Bind. Transp. Storage Met. Ions Biol. Cells, Royal Society of Chemistry, 2014: pp. 429–460. https://doi.org/10.1039/9781849739979-00429.

[84] I. Anastassopoulou, L. Banci, I. Bertini, F. Cantini, E. Katsari, A. Rosato, Solution structure of the apo and copper(I)-loaded human metallochaperone HAH1, Biochemistry. 43 (2004) 13046–13053. https://doi.org/10.1021/bi0487591.

[85] N.J. Robinson, D.R. Winge, Copper metallochaperones, Annu. Rev. Biochem. 79 (2010) 537–562. https://doi.org/10.1146/annurev-biochem-030409-143539.

[86] V.C. Culotta, M. Yang, T. V. O'Halloran, Activation of superoxide dismutases: Putting the metal to the pedal, Biochim. Biophys. Acta - Mol. Cell Res. 1763 (2006) 747–758. https://doi.org/10.1016/j.bbamcr.2006.05.003.

[87] F. Arnesano, S. Scintilla, G. Natile, Interaction between platinum complexes and a methionine motif found in copper transport proteins, Angew. Chemie - Int. Ed. 46 (2007) 9062–9064. https://doi.org/10.1002/anie.200703271.

[88] C.M. Sze, G.N. Khairallah, Z. Xiao, P.S. Donnelly, R.A.J. O'Hair, A.G. Wedd, Interaction of cisplatin and analogues with a Met-rich protein site, J. Biol. Inorg. Chem. 14 (2009) 163–165. https://doi.org/10.1007/s00775-008-0452-x.

[89] A.K. Boal, A.C. Rosenzweig, Crystal structures of cisplatin bound to a human copper chaperone, J. Am. Chem. Soc. 131 (2009) 14196–14197. https://doi.org/10.1021/ja906363t.

[90] K.D. Ivy, J.H. Kaplan, A Re-Evaluation of the Role of hCTR1, the Human High-Affinity Copper Transporter, in Platinum-Drug Entry into Human Cells, Mol. Pharmacol. 83 (2013) 1237–1246. https://doi.org/10.1124/mol.113.085068.

[91] M.D. Hall, M. Okabe, D.-W. Shen, X.-J. Liang, M.M. Gottesman, The Role of Cellular Accumulation in Determining Sensitivity to Platinum-Based Chemotherapy, Annu. Rev. Pharmacol. Toxicol. 48 (2008) 495–535. https://doi.org/10.1146/annurev.pharmtox.48.080907.180426.

[92] P.J. Stone, A.D. Kelman, F.M. Sinex, Specific binding of antitumour drug cis-Pt(NH3) 2Cl2 to DNA rich in guanine and cytosine, Nature. 251 (1974) 736–737. https://doi.org/10.1038/251736a0.

[93] L.A. Zwelling, M.O. Bradley, N.A. Sharkey, T. Anderson; Kurt W. Kohn, Mutagenicity, cytotoxicity and DNA crosslinking in V79 Chinese hamster cells treated with cis- and trans-Pt(II) diamminedichloride, Mutat. Res. Toxicol. 67 (1979) 271–280. https://doi.org/10.1016/0165-1218(79)90021-1.

[94] H. Kurokawa, T. Ishida, K. Nishio, H. Arioka, M. Sata, H. Fukumoto, M. Miura, N. Saijo, γ-

glutamylcysteine synthetase gene overexpression results in increased activity of the ATP-dependent glutathione S-conjugate export pump and cisplatin resistance, Biochem. Biophys. Res. Commun. 216 (1995) 258–264. https://doi.org/10.1006/bbrc.1995.2618.

[95] J. Holford, P.J. Beale, F.E. Boxall, S.Y. Sharp, L.R. Kelland, Mechanisms of drug resistance to the platinum complex ZD0473 in ovarian cancer cell lines, Eur J Cancer. 36 (2000) 1984–1990. https://doi.org/S0959-8049(00)00192-1 [pii].

[96] A.K. Godwin, A. Meister, P.J. O'Dwyer, C.S. Huang, T.C. Hamilton, M.E. Anderson, High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis., Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 3070–3074. https://doi.org/10.1073/pnas.89.7.3070.

[97] X. Wang, Z. Guo, The Role of Sulfur in Platinum Anticancer Chemotherapy, Anticancer. Agents Med. Chem. 7 (2007) 19–34. https://doi.org/10.2174/187152007779314062.

[98] S. Ishida, F. McCormick, K. Smith-McCune, D. Hanahan, Enhancing Tumor-Specific Uptake of the Anticancer Drug Cisplatin with a Copper Chelator, Cancer Cell. 17 (2010) 574–583. https://doi.org/10.1016/j.ccr.2010.04.011.

[99] S.B. Howell, R. Safaei, CTR1 as a Determinant of Platinum Drug Transport, in: A. Bonetti, R. Leone, F.M. Muggia, S.B. Howell (Eds.), Platin. Other Heavy Met. Compd. Cancer Chemother., Humana Press, Totowa, NJ, 2009: pp. 89–94. https://doi.org/10.1007/978-1-60327-459-3_12.

[100] Y. Guo, K. Smith, M.J. Petris, Cisplatin stabilizes a multimeric complex of the human Ctr1 copper transporter: Requirement for the extracellular methionine-rich clusters, J. Biol. Chem. 279 (2004) 46393–46399. https://doi.org/10.1074/jbc.M407777200.

[101] A.K. Holzer, G.H. Manorek, S.B. Howell, Contribution of the major copper influx transporter CTR1 to the cellular accumulation of cisplatin, carboplatin, and oxaliplatin, Mol. Pharmacol. 70 (2006) 1390–1394. https://doi.org/10.1124/mol.106.022624.

[102] I.S. Song, N. Savaraj, Z.H. Siddik, P. Liu, Y. Wei, C.J. Wu, M.T. Kuo, Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells, Mol. Cancer Ther. 3 (2004) 1543–1549. https://mct.aacrjournals.org/content/3/12/1543 (accessed February 25, 2021).

[103] Y.Y. Lee, C.H. Choi, I.G. Do, S.Y. Song, W. Lee, H.S. Park, T.J. Song, M.K. Kim, T.J. Kim, J.W. Lee, D.S. Bae, B.G. Kim, Prognostic value of the copper transporters, CTR1 and CTR2, in patients with ovarian carcinoma receiving platinum-based chemotherapy, Gynecol. Oncol. 122 (2011) 361–365. https://doi.org/10.1016/j.ygyno.2011.04.025.

[104] X. Xu, H. Ren, B. Zhou, Y. Zhao, R. Yuan, R. Ma, H. Zhou, Z. Liu, Prediction of copper transport protein 1 (CTR1) genotype on severe cisplatin induced toxicity in non-small cell lung cancer (NSCLC) patients, Lung Cancer. 77 (2012) 438–442. https://doi.org/10.1016/j.lungcan.2012.03.023.

[105] H. Yoshida, M. Teramae, M. Yamauchi, T. Fukuda, T. Yasui, T. Sumi, K.I. Honda, O. Ishiko, Association of copper transporter expression with platinum resistance in epithelial ovarian cancer, Anticancer Res. 33 (2013) 1409–1414. https://ar.iiarjournals.org/content/33/4/1409 (accessed February 25, 2021).

[106] H. Öhrvik, Y. Nose, L.K. Wood, B.E. Kim, S.C. Gleber, M. Ralle, D.J. Thiele, Ctr2 regulates

biogenesis of a cleaved form of mammalian Ctr1 metal transporter lacking the copper- And cisplatin-binding ecto-domain, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) E4279–E4288. https://doi.org/10.1073/pnas.1311749110.

[107] B.G. Blair, C. a Larson, P.L. Adams, P.B. Abada, R. Safaei, S.B. Howell, Regulation of copper transporter 2 expression by copper and cisplatin in human ovarian carcinoma cells., Mol. Pharmacol. 77 (2010) 912–921. https://doi.org/10.1124/mol.109.062836.

[108] B.G. Blair, C. Larson, R. Safaei, S.B. Howell, Copper transporter 2 regulates the cellular accumulation and cytotoxicity of cisplatin and carboplatin, Clin. Cancer Res. 15 (2009) 4312–4321. https://doi.org/10.1158/1078-0432.CCR-09-0311.

[109] H. Öhrvik, D.J. Thiele, The role of Ctr1 and Ctr2 in mammalian copper homeostasis and platinum-based chemotherapy, J. Trace Elem. Med. Biol. 31 (2015) 178–182. https://doi.org/10.1016/j.jtemb.2014.03.006.

[110] T.H. Nguyen, F. Arnesano, S. Scintilla, G. Rossetti, E. Ippoliti, P. Carloni, G. Natile, Structural determinants of cisplatin and transplatin binding to the met-rich motif of Ctr1: A computational spectroscopy approach, J. Chem. Theory Comput. 8 (2012) 2912–2920. https://doi.org/10.1021/ct300167m.

[111] A. Gupta, S. Lutsenko, Human copper transporters: Mechanism, role in human diseases and therapeutic potential, Future Med. Chem. 1 (2009) 1125–1142. https://doi.org/10.4155/fmc.09.84.

[112] P. Abada, S.B. Howell, Regulation of cisplatin cytotoxicity by Cu influx transporters, Met. Based. Drugs. 2010 (2010) 317581. https://doi.org/10.1155/2010/317581.

[113] S.E. Crider, R.J. Holbrook, K.J. Franz, Coordination of platinum therapeutic agents to met-rich motifs of human copper transport protein1, Metallomics. 2 (2010) 74–83. https://doi.org/10.1039/b916899k.

[114] Z. Wu, Q. Liu, X. Liang, X. Yang, N. Wang, X. Wang, H. Sun, Y. Lu, Z. Guo, Reactivity of platinum-based antitumor drugs towards a Met- and His-rich 20mer peptide corresponding to the N-terminal domain of human copper transporter 1, J. Biol. Inorg. Chem. 14 (2009) 1313–1323. https://doi.org/10.1007/s00775-009-0576-7.

[115] A.K. Holzer, S.B. Howell, The internalization and degradation of human copper transporter 1 following cisplatin exposure, Cancer Res. 66 (2006) 10944–10952. https://doi.org/10.1158/0008-5472.CAN-06-1710.

[116] K. Williams, J.B. Lloyd, Non-specific adsorptive pinocytosis, Biochem. Soc. Trans. 12 (1983) 527–528. https://doi.org/10.1042/bst0120527.

[117] G. V. Kalayda, C.H. Wagner, U. Jaehde, Relevance of copper transporter 1 for cisplatin resistance in human ovarian carcinoma cells, J. Inorg. Biochem. 116 (2012) 1–10. https://doi.org/10.1016/j.jinorgbio.2012.07.010.

[118] G. V. Kalayda, C.H. Wagner, I. Buß, J. Reedijk, U. Jaehde, Altered localisation of the copper efflux transporters ATP7A and ATP7B associated with cisplatin resistance in human ovarian carcinoma cells, BMC Cancer. 8 (2008) 175. https://doi.org/10.1186/1471-2407-8-175.

[119] F. Arnesano, M. Losacco, G. Natile, An updated view of cisplatin transport, Eur. J. Inorg. Chem. (2013) 2701–2711. https://doi.org/10.1002/ejic.201300001.

[120] C.E. Ooi, E. Rabinovich, A. Dancis, J.S. Bonifacino, R.D. Klausner, Copper-dependent degradation of the Saccharomyces cerevisiae plasma membrane copper transporter Ctr1p in the apparent absence of endocytosis, EMBO J. 15 (1996) 3515–3523.

[121] M.J. Petris, K. Smith, J. Lee, D.J. Thiele, Copper-stimulated endocytosis and degradation of the human copper transporter, hCtr1, J. Biol. Chem. 278 (2003) 9639–9646. https://doi.org/10.1074/jbc.M209455200.

[122] C.F. Mills, T.T. El-Gallad, I. Bremner, G. Wenham, Copper and molybdenum absorption by rats given ammonium tetrathiomolybdate, J. Inorg. Biochem. 14 (1981) 163–175. https://doi.org/10.1016/S0162-0134(00)80037-9.

[123] S.R. Gooneratne, J.M. Howell, J.M. Gawthorne, An investigation of the effects of intravenous administration of thiomolybdate on copper metabolism in chronic Cu-poisoned sheep, Br. J. Nutr. 46 (1981) 469–480. https://doi.org/10.1079/BJN19810055.

[124] G.J. Brewer, S.D. Merajver, Cancer therapy with tetrathiomolybdate: Antiangiogenesis by lowering body copper - A review, Integr. Cancer Ther. 1 (2002) 327–337. https://doi.org/10.1177/1534735402238185.

[125] S.J. Chen, C.C. Kuo, H.Y. Pan, T.C. Tsou, S.C. Yeh, J.Y. Chang, Mechanistic basis of a combination d-penicillamine and platinum drugs synergistically inhibits tumor growth in oxaliplatin-resistant human cervical cancer cells in vitro and in vivo, Biochem. Pharmacol. 95 (2015) 28–37. https://doi.org/10.1016/j.bcp.2015.03.006.

[126] F. Arnesano, M.I. Nardella, G. Natile, Platinum drugs, copper transporters and copper chelators, Coord. Chem. Rev. 374 (2018) 254–260. https://doi.org/10.1016/j.ccr.2018.07.003.

[127] G. Inesi, R. Pilankatta, F. Tadini-Buoninsegni, Biochemical characterization of P-type copper ATPases, Biochem. J. 463 (2014) 167–176. https://doi.org/10.1042/BJ20140741.

[128] D. Kilari, E. Guancial, E.S. Kim, Role of copper transporters in platinum resistance, World J. Clin. Oncol. 7 (2016) 106–113. https://doi.org/10.5306/wjco.v7.i1.106.

[129] V. Calandrini, F. Arnesano, A. Galliani, T.H. Nguyen, E. Ippoliti, P. Carloni, G. Natile, Platination of the copper transporter ATP7A involved in anticancer drug resistance, Dalt. Trans. 43 (2014) 12085–12094. https://doi.org/10.1039/c4dt01339e.

[130] Z.H. Li, M.Z. Qiu, Z.L. Zeng, H.Y. Luo, W.J. Wu, F. Wang, Z.Q. Wang, D.S. Zhang, Y.H. Li, R.H. Xu, Copper-transporting P-type adenosine triphosphatase (ATP7A) is associated with platinum-resistance in non-small cell lung cancer (NSCLC), J. Transl. Med. 10 (2012) 21. https://doi.org/10.1186/1479-5876-10-21.

[131] K. Nakayama, A. Kanzaki, K. Terada, M. Mutoh, K. Ogawa, T. Sugiyama, S. Takenoshita, K. Itoh, N. Yaegashi, K. Miyazaki, N. Neamati, Y. Takebayashi, Prognostic Value of the Cu-Transporting ATPase in Ovarian Carcinoma Patients Receiving Cisplatin-Based Chemotherapy, Clin. Cancer Res. 10 (2004) 2804–2811. https://doi.org/10.1158/1078-0432.CCR-03-0454.

[132] N. V. Dolgova, D. Olson, S. Lutsenko, O.Y. Dmitriev, The soluble metal-binding domain of the copper transporter ATP7B binds and detoxifies cisplatin, Biochem. J. 419 (2009) 51–56. https://doi.org/10.1042/BJ20081359.

[133] R. Safaei, P.L. Adams, M.H. Maktabi, R.A. Mathews, S.B. Howell, The CXXC motifs in the

metal binding domains are required for ATP7B to mediate resistance to cisplatin, J. Inorg. Biochem. 110 (2012) 8–17. https://doi.org/10.1016/j.jinorgbio.2012.02.016.

[134] R. Safaei, Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells, Mol. Cancer Ther. 4 (2005) 1595–1604. https://doi.org/10.1158/1535-7163.MCT-05-0102.

[135] F. Tadini-Buoninsegni, G. Bartolommei, M. Rosa Moncelli, G. Inesi, A. Galliani, M. Sinisi, M. Losacco, G. Natile, F. Arnesano, Translocation of platinum anticancer drugs by human copper ATPases ATP7A and ATP7B, Angew. Chemie - Int. Ed. 53 (2014) 1297–1301. https://doi.org/10.1002/anie.201307718.

[136] T. Fang, Y. Tian, S. Yuan, Y. Sheng, F. Arnesano, G. Natile, Y. Liu, Differential Reactivity of Metal Binding Domains of Copper ATPases towards Cisplatin and Colocalization of Copper and Platinum, Chem. - A Eur. J. 24 (2018) 8999–9003. https://doi.org/10.1002/chem.201801894.

[137] A. Galliani, M. Losacco, A. Lasorsa, G. Natile, F. Arnesano, Cisplatin handover between copper transporters: The effect of reducing agents, J. Biol. Inorg. Chem. 19 (2014) 705–714. https://doi.org/10.1007/s00775-014-1138-1.

[138] L. Papa, G. Manfredi, D. Germain, SOD1, an unexpected novel target for cancer therapy, Genes and Cancer. 5 (2015) 15–21. https://doi.org/10.18632/genesandcancer.4.

[139] H.E. Barker, T.R. Cox, J.T. Erler, The rationale for targeting the LOX family in cancer, Nat. Rev. Cancer. 12 (2012) 540–552. https://doi.org/10.1038/nrc3319.

[140] R. Petruzzelli, R.S. Polishchuk, Activity and Trafficking of Copper-Transporting ATPases in Tumor Development and Defense against Platinum-Based Drugs, Cells. 8 (2019) 1080. https://doi.org/10.3390/cells8091080.

[141] M. Mariniello, R. Petruzzelli, L.G. Wanderlingh, R. La Montagna, A. Carissimo, F. Pane, A. Amoresano, E.Y. Ilyechova, M.M. Galagudza, F. Catalano, R. Crispino, L. V. Puchkova, D.L. Medina, R.S. Polishchuk, Synthetic lethality screening identifies fda-approved drugs that overcome atp7b-mediated tolerance of tumor cells to cisplatin, Cancers (Basel). 12 (2020) 608. https://doi.org/10.3390/cancers12030608.

[142] C.L. Chisholm, H. Wang, A. Hang-Heng Wong, G. Vazquez-Ortiz, W. Chen, X. Xu, C.-X. Deng, Ammonium tetrathiomolybdate treatment targets the copper transporter ATP7A and enhances sensitivity of breast cancer to cisplatin, Oncotarget. 7 (2016) 84439–84452. https://doi.org/10.18632/oncotarget.12992.

[143] F. Arnesano, L. Banci, I. Bertini, S. Ciofi-Baffoni, E. Molteni, D.L. Huffman, T. V. O'Halloran, Metallochaperones and metal-transporting ATPases: A comparative analysis of sequences and structures, Genome Res. 12 (2002) 255–271. https://doi.org/10.1101/gr.196802.

[144] S. Itoh, W.K. Ha, O. Nakagawa, K. Ozumi, S.M. Lessner, H. Aoki, K. Akram, R.D. McKinney, M. Ushio-Fukai, T. Fukai, Novel role of antioxidant-1 (Atox1) as a copper-dependent transcription factor involved in cell proliferation, J. Biol. Chem. 283 (2008) 9157–9167. https://doi.org/10.1074/jbc.M709463200.

[145] S. Itoh, K. Ozumi, H.W. Kim, O. Nakagawa, R.D. McKinney, R.J. Folz, I.N. Zelko, M. Ushio-Fukai, T. Fukai, Novel mechanism for regulation of extracellular SOD transcription

and activity by copper: Role of antioxidant-1, Free Radic. Biol. Med. 46 (2009) 95–104. https://doi.org/10.1016/j.freeradbiomed.2008.09.039.

[146] G.F. Chen, V. Sudhahar, S.W. Youn, A. Das, J. Cho, T. Kamiya, N. Urao, R.D. McKinney, B. Surenkhuu, T. Hamakubo, H. Iwanari, S. Li, J.W. Christman, S. Shantikumar, G.D. Angelini, C. Emanueli, M. Ushio-Fukai, T. Fukai, Copper Transport Protein Antioxidant-1 Promotes Inflammatory Neovascularization via Chaperone and Transcription Factor Function, Sci. Rep. 5 (2015) 14780. https://doi.org/10.1038/srep14780.

[147] D. Kahra, T. Mondol, M. Niemiec, P. Wittung-Stafshede, Human Copper Chaperone Atox1 Translocates to the Nucleus but does not Bind DNA In Vitro, Protein Pept. Lett. 22 (2015) 532–538. https://doi.org/10.2174/0929866522666150506094546.

[148] H. Öhrvik, P. Wittung-Stafshede, H. Öhrvik, P. Wittung-Stafshede, Identification of New Potential Interaction Partners for Human Cytoplasmic Copper Chaperone Atox1: Roles in Gene Regulation?, Int. J. Mol. Sci. 16 (2015) 16728–16739. https://doi.org/10.3390/ijms160816728.

[149] M. Matson Dzebo, S. Blockhuys, S. Valenzuela, E. Celauro, E.K. Esbjörner, P. Wittung-Stafshede, Copper Chaperone Atox1 Interacts with Cell Cycle Proteins, Comput. Struct. Biotechnol. J. 16 (2018) 443–449. https://doi.org/10.1016/j.csbj.2018.10.018.

[150] A. Jana, A. Das, N.L. Krett, G. Guzman, A. Thomas, G. Mancinelli, J. Bauer, M. Ushio-Fukai, T. Fukai, B. Jung, Nuclear translocation of Atox1 potentiates activin A-induced cell migration and colony formation in colon cancer, PLoS One. 15 (2020) e0227916. https://doi.org/10.1371/journal.pone.0227916.

[151] S. Blockhuys, X. Zhang, P. Wittung-Stafshede, Single-cell tracking demonstrates copper chaperone Atox1 to be required for breast cancer cell migration, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 2014–2019. https://doi.org/10.1073/pnas.1910722117.

[152] F. Arnesano, L. Banci, I. Bertini, I.C. Felli, M. Losacco, G. Natile, Probing the interaction of cisplatin with the human copper chaperone Atox1 by solution and in-cell NMR spectroscopy, J. Am. Chem. Soc. 133 (2011) 18361–18369. https://doi.org/10.1021/ja207346p.

[153] M.E. Palm-Espling, P. Wittung-Stafshede, Reaction of platinum anticancer drugs and drug derivatives with a copper transporting protein, Atox1, Biochem. Pharmacol. 83 (2012) 874–881. https://doi.org/10.1016/J.BCP.2012.01.018.

[154] A.K. Wernimont, D.L. Huffman, A.L. Lamb, T. V. O'Halloran, A.C. Rosenzweig, Structural basis for copper transfer by the metallochaperone for the Menkes/Wilson disease proteins, Nat. Struct. Biol. 7 (2000) 766–771. https://doi.org/10.1038/78999.

[155] V. Calandrini, T.H. Nguyen, F. Arnesano, A. Galliani, E. Ippoliti, P. Carloni, G. Natile, Structural biology of cisplatin complexes with cellular targets: The adduct with human copper chaperone atox1 in aqueous solution, Chem. - A Eur. J. 20 (2014) 11719–11725. https://doi.org/10.1002/chem.201402834.

[156] Z. Xi, W. Guo, C. Tian, F. Wang, Y. Liu, Copper binding promotes the interaction of cisplatin with human copper chaperone Atox1, Chem. Commun. 49 (2013) 11197–11199. https://doi.org/10.1039/c3cc45905e.

[157] A. Lasorsa, M.I. Nardella, A. Rosato, V. Mirabelli, R. Caliandro, R. Caliandro, G. Natile, F. Arnesano, Mechanistic and Structural Basis for Inhibition of Copper Trafficking by Platinum

Anticancer Drugs, J. Am. Chem. Soc. 141 (2019) 12109–12120. https://doi.org/10.1021/jacs.9b05550.

[158] N. V. Dolgova, S. Nokhrin, C.H. Yu, G.N. George, O.Y. Dmitriev, Copper chaperone Atox1 interacts with the metal-binding domain of Wilson's disease protein in cisplatin detoxification, Biochem. J. 454 (2013) 147–156. https://doi.org/10.1042/BJ20121656.

[159] M.E. Palm-Espling, C.D. Andersson, E. Björn, A. Linusson, P. Wittung-Stafshede, Determinants for Simultaneous Binding of Copper and Platinum to Human Chaperone Atox1: Hitchhiking not Hijacking, PLoS One. 8 (2013) e70473. https://doi.org/10.1371/journal.pone.0070473.

[160] G.J. Brewer, F. Askari, R.B. Dick, J. Sitterly, J.K. Fink, M. Carlson, K.J. Kluin, M.T. Lorincz, Treatment of Wilson's disease with tetrathiomolybdate: V. control of free copper by tetrathiomolybdate and a comparison with trientine, Transl. Res. 154 (2009) 70–77. https://doi.org/10.1016/j.trsl.2009.05.002.

[161] G. Brewer, Copper Lowering Therapy With Tetrathiomolybdate as an Antiangiogenic Strategy in Cancer, Curr. Cancer Drug Targets. 5 (2005) 195–202. https://doi.org/10.2174/1568009053765807.

[162] M. V. Chidambaram, G. Barnes, E. Frieden, Inhibition of ceruloplasmin and other copper oxidases by thiomolybdate, J. Inorg. Biochem. 22 (1984) 231–239. https://doi.org/10.1016/0162-0134(84)85008-4.

[163] K.T. Suzuki, Y. Ogra, Formation of copper-metallothionein/tetrathiomolybdate complex is the first step in removal of Cu from LEC rats, Res. Commun. Mol. Pathol. Pharmacol. 88 (1995) 187–195. https://europepmc.org/article/med/7670850 (accessed March 1, 2021).

[164] E.K. Quagraine, R.S. Reid, UV/visible spectrophotometric studies of the interactions of thiomolybdates, copper(II) and other ligands, J. Inorg. Biochem. 85 (2001) 53–60. https://doi.org/10.1016/S0162-0134(00)00223-3.

[165] H.M. Alvarez, Y. Xue, C.D. Robinson, M.A. Canalizo-Hernández, R.G. Marvin, R.A. Kelly, A. Mondragón, J.E. Penner-Hahn, T. V. O'Halloran, Tetrathiomolybdate Inhibits Copper Trafficking Proteins Through Metal Cluster Formation, Science. 327 (2010) 331–334. https://doi.org/10.1126/SCIENCE.1179907.

[166] Y. Tian, T. Fang, S. Yuan, Y. Zheng, F. Arnesano, G. Natile, Y. Liu, Tetrathiomolybdate inhibits the reaction of cisplatin with human copper chaperone Atox1, Metallomics. 10 (2018) 745–750. https://doi.org/10.1039/c8mt00084k.

[167] H. Davies, G.R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M.J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, H. Jayatilake, B.A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G.J. Riggins, D.D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J.W.C. Ho, S.Y. Leung, S.T. Yuen, B.L. Weber, H.F. Seigler, T.L. Darrow, H. Paterson, R. Wooster, M.R. Stratton, P.A. Futreal, Mutations of the BRAF gene in human cancer, Nature. 417 (2002) 949–954. https://doi.org/10.1038/nature00766.

[168] D.C. Brady, M.S. Crowe, D.N. Greenberg, C.M. Counter, Copper chelation inhibits BRAFV600E-driven melanomagenesis and counters resistance to BRAFV600Eand MEK1/2 inhibitors, Cancer Res. 77 (2017) 6240–6252. https://doi.org/10.1158/0008-5472.CAN-16-

1190.

[169] S. Baldari, G. Di Rocco, M.C. Heffern, T.A. Su, C.J. Chang, G. Toietta, Effects of copper chelation on BRAFv600e positive colon carcinoma cells, Cancers (Basel). 11 (2019) 1–17. https://doi.org/10.3390/cancers11050659.

[170] K.K. Kim, A. Han, N. Yano, J.R. Ribeiro, E. Lokich, R.K. Singh, R.G. Moore, Tetrathiomolybdate mediates cisplatin-induced p38 signaling and EGFR degradation and enhances response to cisplatin therapy in gynecologic cancers, Sci. Rep. 5 (2015) 1–11. https://doi.org/10.1038/srep15911.

[171] S.M. Hecht, Bleomycin: New perspectives on the mechanism of action, J. Nat. Prod. 63 (2000) 158–168. https://doi.org/10.1021/np990549f.

[172] J. Chen, J.A. Stubbe, Bleomycins: Towards better therapeutics, Nat. Rev. Cancer. 5 (2005) 102–112. https://doi.org/10.1038/nrc1547.

[173] L.H. Einhorn, Curing metastatic testicular cancer, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4592–4595. https://doi.org/10.1073/pnas.072067999.

[174] J.G. Devassy, I.D. Nwachukwu, P.J.H. Jones, Curcumin and cancer: Barriers to obtaining a health claim, Nutr. Rev. 73 (2015) 155–165. https://doi.org/10.1093/nutrit/nuu064.

[175] C.M. Weekley, C. He, Developing drugs targeting transition metal homeostasis, Curr. Opin. Chem. Biol. 37 (2017) 26–32. https://doi.org/10.1016/j.cbpa.2016.12.011.

[176] M.E. Helsel, K.J. Franz, Pharmacological activity of metal binding agents that alter copper bioavailability, Dalt. Trans. 44 (2015) 8760–8770. https://doi.org/10.1039/C5DT00634A.

[177] M.A. Cater, H.B. Pearson, K. Wolyniec, P. Klaver, M. Bilandzic, B.M. Paterson, A.I. Bush, P.O. Humbert, S. La Fontaine, P.S. Donnelly, Y. Haupt, Increasing intracellular bioavailable copper selectively targets prostate cancer cells, ACS Chem. Biol. 8 (2013) 1621–1631. https://doi.org/10.1021/cb400198p.

[178] P. Lelièvre, L. Sancey, J.L. Coll, A. Deniaud, B. Busser, The multifaceted roles of copper in cancer: A trace metal element with dysregulated metabolism, but also a target or a bullet for therapy, Cancers (Basel). 12 (2020) 1–25. https://doi.org/10.3390/cancers12123594.

[179] S. Dilruba, G. V. Kalayda, Platinum-based drugs: past, present and future, Cancer Chemother. Pharmacol. 77 (2016) 1103–1124. https://doi.org/10.1007/s00280-016-2976-z.

Figures

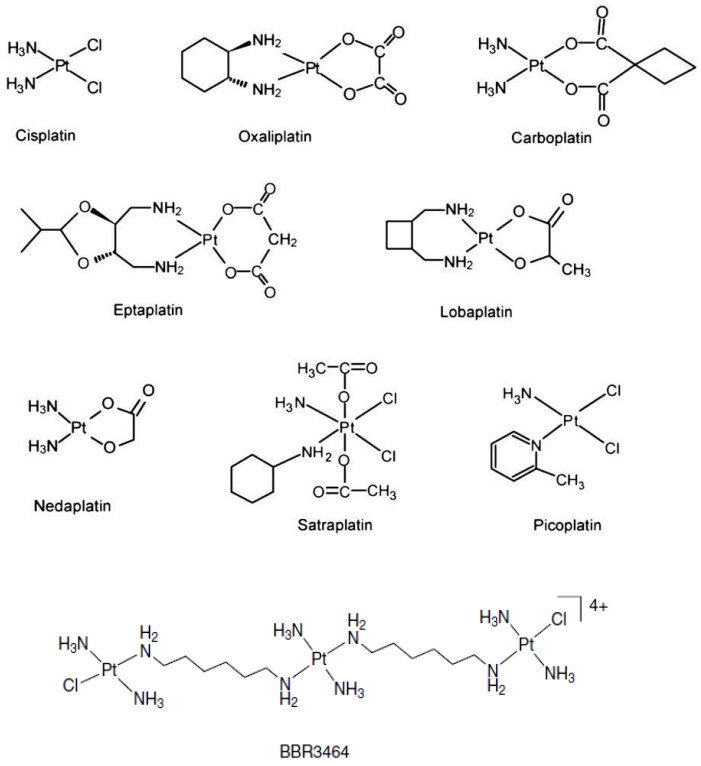

Figure 1

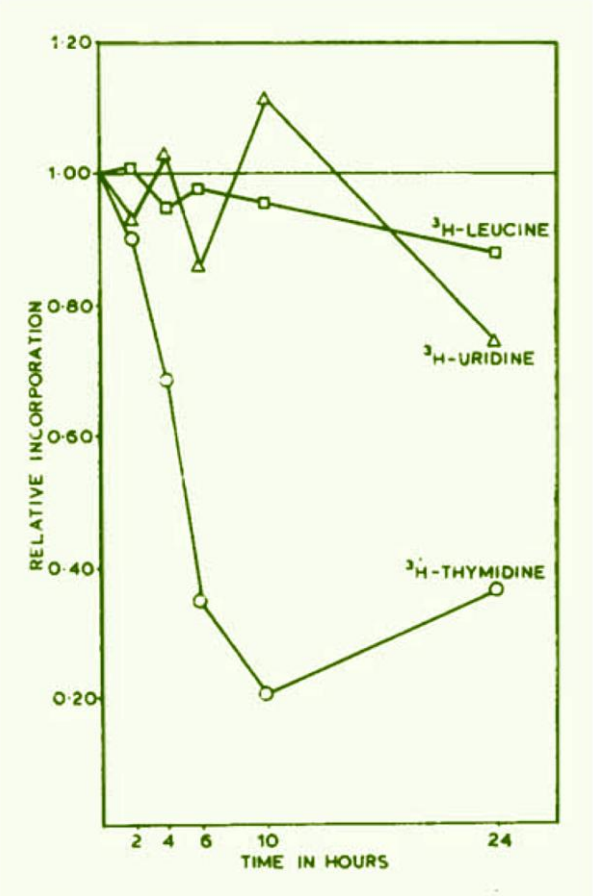
1·20
1·00
0·80
0·60
0·40
0·20
RELATIVE INCORPORATION
³H-LEUCINE
³H-URIDINE
³H-THYMIDINE
2
4
6
10
24
TIME IN HOURS

Figure 2

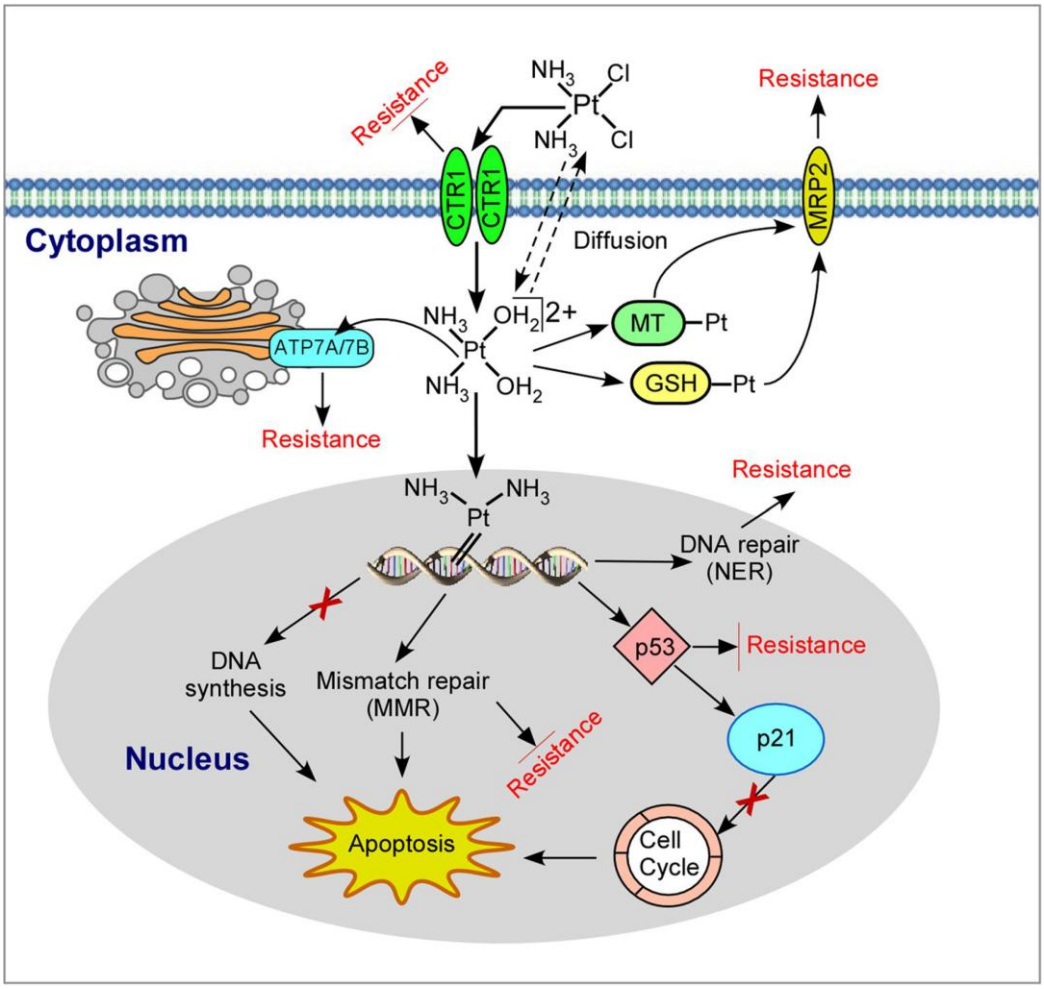

Figure 3

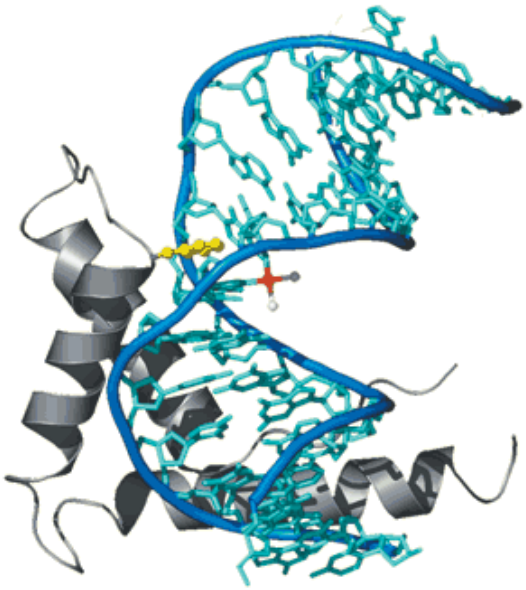

Figure 4

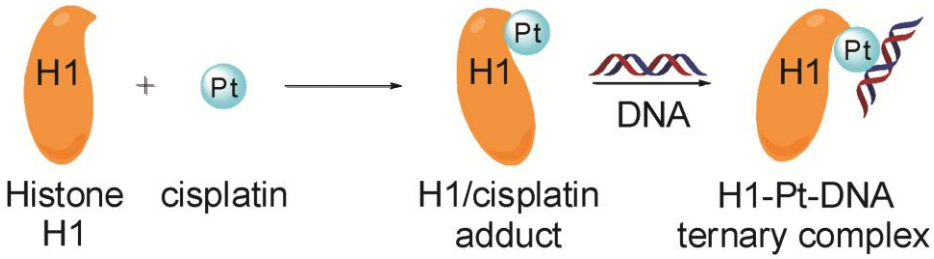

Figure 5

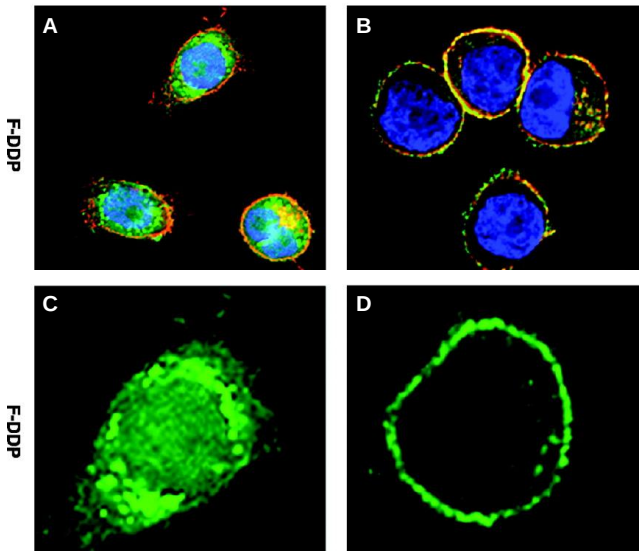

Figure 6

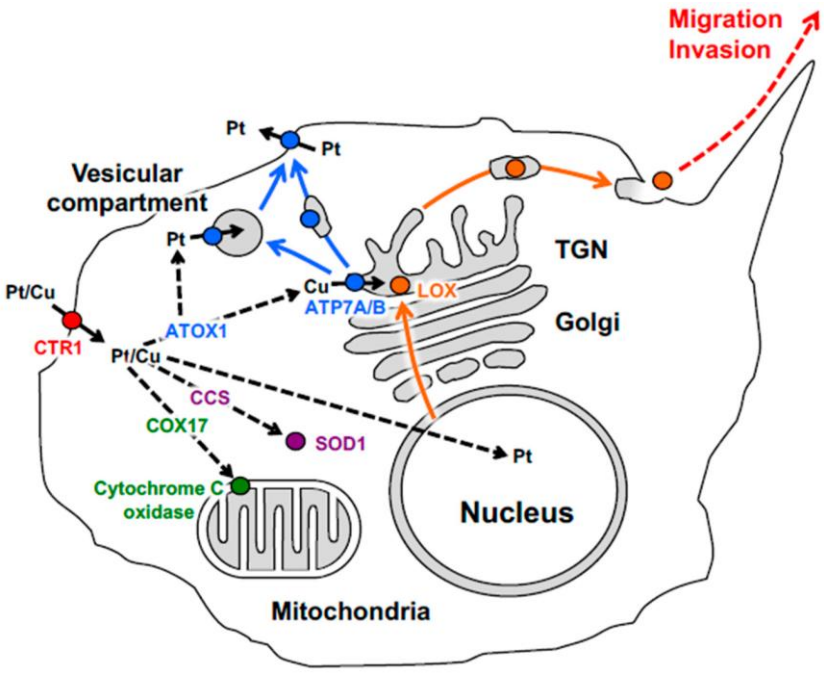

Figure 7

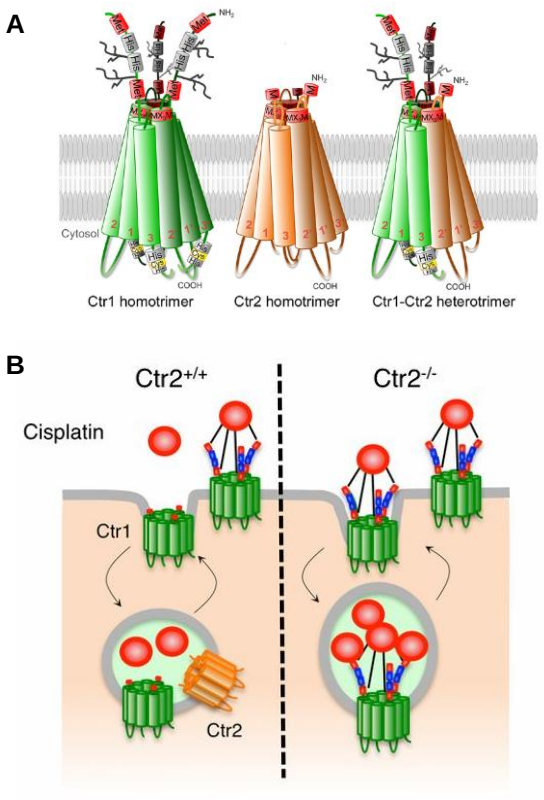

Figure 8

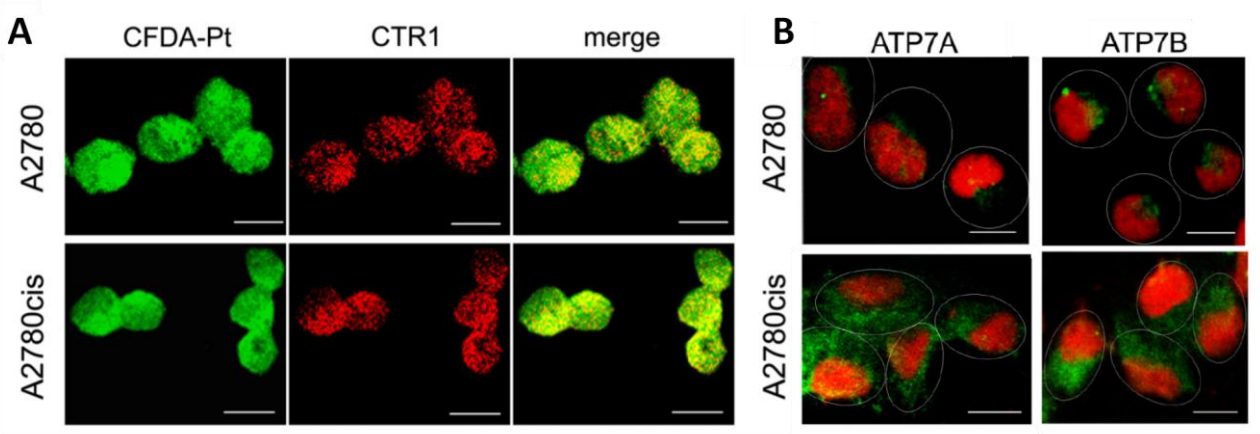

Figure 9

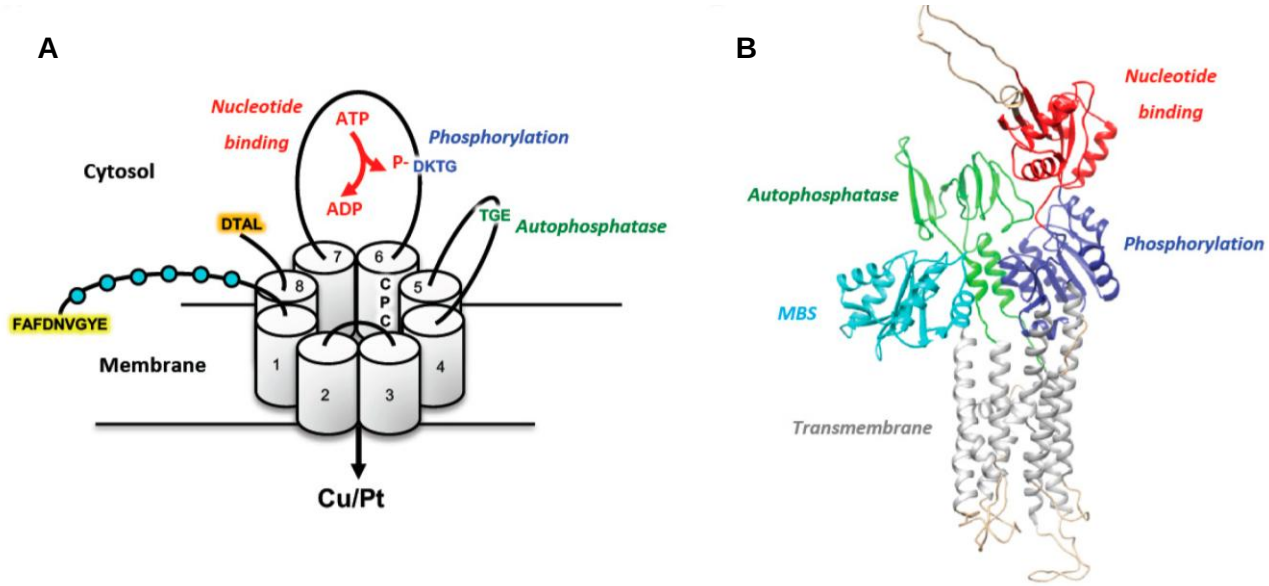

Figure 10

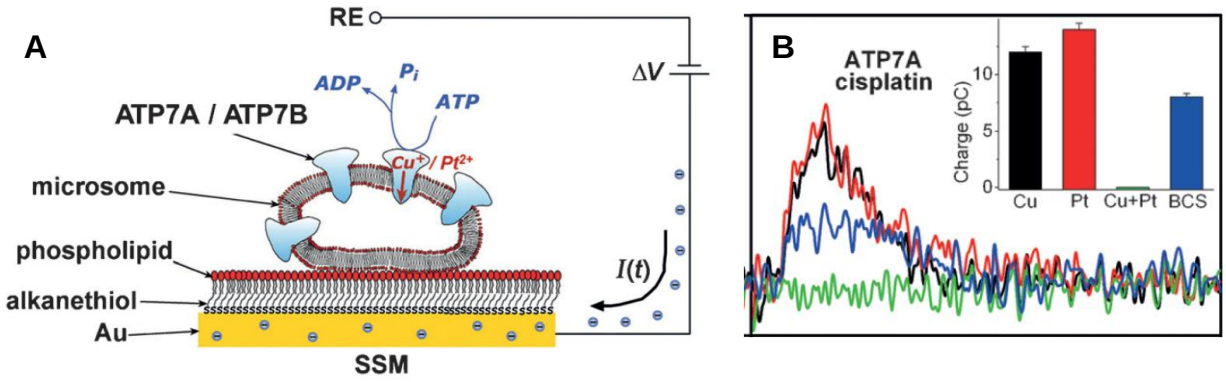

Figure 11

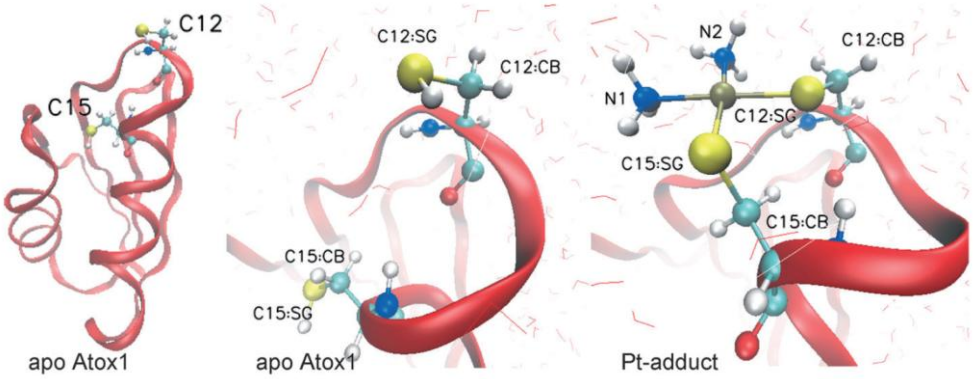

Figure 12

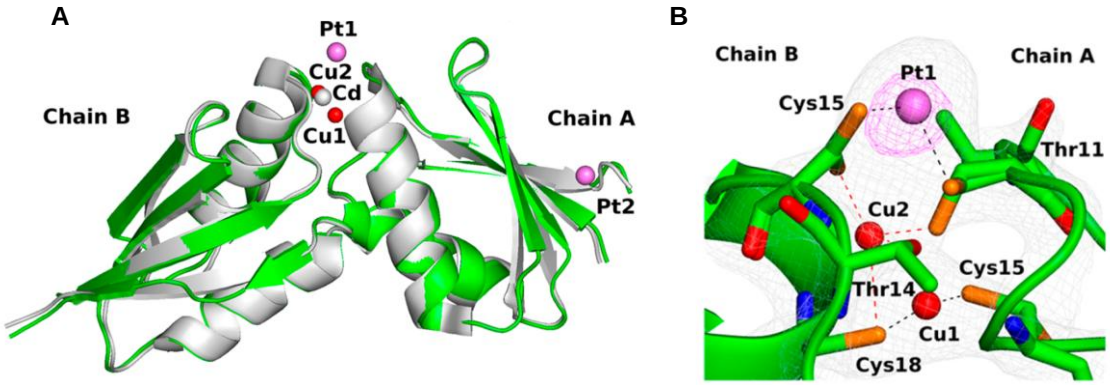

Figure 13

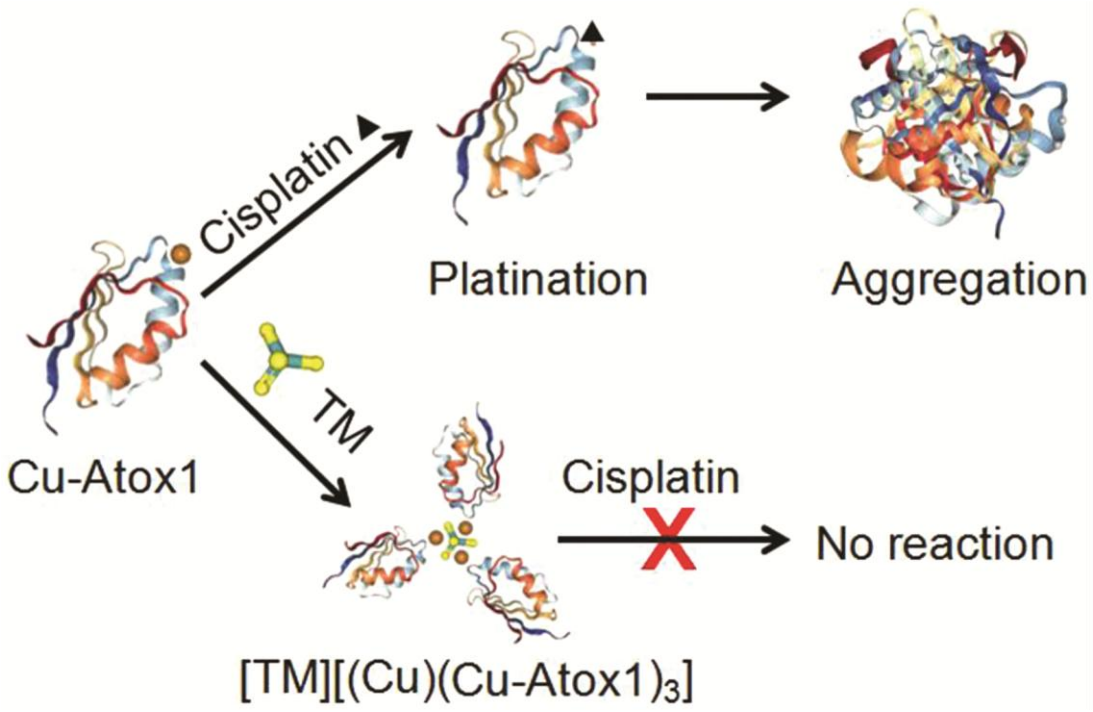

Figure 14