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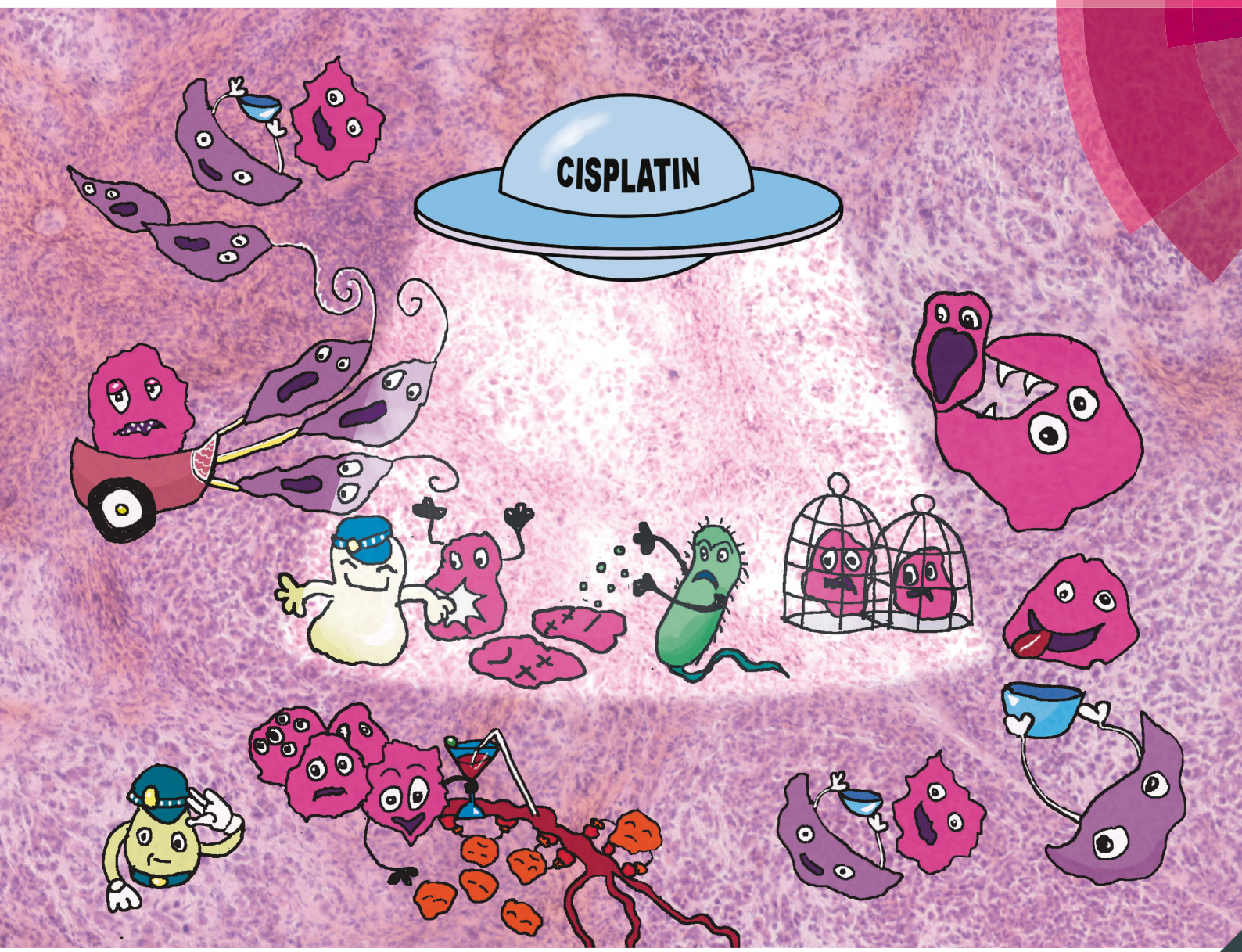

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Unexpected therapeutic effects of cisplatin

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Cisplatin is a widely used chemotherapeutic agent that is clinically approved to fight both carcinomas and sarcomas. It has relatively high efficiency in treating ovarian cancers and metastatic testicular cancers. It is generally accepted that the major mechanism of cisplatin anti-cancer action is DNA damage. However, cisplatin is also effective in metastatic cancers and should, therefore, affect slow-cycling cancer stem cells in some way. In this review, we focused on the alternative effects of cisplatin that can support a good therapeutic response. First, attention was paid to the effects of cisplatin at the cellular level such as changes in intracellular pH and cellular mechanical properties. Alternative cellular targets of cisplatin, and the effects of cisplatin on cancer cell metabolism and ER stress were also discussed. Furthermore, the impacts of cisplatin on the tumor microenvironment and in the whole organism context were reviewed. In this review, we try to reveal possible causes of the unexpected effectiveness of this anti-cancer drug.

Introduction

cis-Diamminedichloroplatinum(II) (NSC 119875), more often called cisplatin, is a platinum coordination compound with a planar geometry. Platinum is bound to two amine groups and two chloride ions. Cisplatin is called the "penicillin of cancer" because it is widely used in clinics and it was also the first relevant chemotherapy drug in cancer therapy (FDA approved cisplatin under the name of Platinol® for cancer treatment in 1978). Cisplatin has a water solubility of 2.53 g L^{-1} at 25 °C, a melting point of 270 °C, a molecular weight of 300.01 mg mol^{-1}, and a density of 3.74 g cm^{-3}.[1] Cisplatin is clinically approved to fight both carcinomas and sarcomas and has relatively high efficiency in treating ovarian cancers and metastatic testicular cancers. Nevertheless, other tumor types such as head and neck cancer, bladder cancer, lung cancer, or breast cancer also benefit from a therapeutic regimen that includes this drug.[1–3] Cellular accumulation of cisplatin happens by different mechanisms, including passive diffusion and multiple transport systems such as high-affinity copper uptake transporter 1 (hCTR1/*SLC31A1*) or some members of the *SLC22* family.[4]

It is generally accepted that the major mechanism of cisplatin anti-cancer action is binding of platinum to DNA by forming intra-stranded and inter-stranded crosslinks. This DNA damage then arrests the cell cycle and initiates cell death in fast proliferating cells.[2] Nevertheless, before interaction with DNA, cisplatin has to undergo the activation step which consists of the chloro-ligand(s) replacement, usually by water molecules. Depending on the pH value of the environment, the hydrated complexes can be subsequently stabilized by deprotonation of the aqua ligands or again passivated in the alkaline solution.[219] Various values of pK_a were determined for cisplatin hydrates in several studies. For example, the pK_a value of *cis*-$[PtCl(H_2O)(NH_3)_2]^+$ was 6.41 in ref. 220 or 6.6 in ref. 219, the pK_a value of *cis*-$[Pt(H_2O)2(NH_3)_2]^{2+}$ was 5.37 in ref. 220 or 5.5 in ref. 219, and the pK_a value of *cis*-$[PtCl(H_2O)(OH)(NH_3)_2]^+$ was 7.21 in ref. 220 or 7.3 in ref. 219. Furthermore, when cisplatin is administered as an anti-cancer treatment, it is exposed to various endogenous sulfur-containing molecules such as glutathione, metallothioneins and thioredoxins, and it has been found that in 180 minutes almost all the ligands are substituted by sulfur ligands.[221] In the cell, cisplatin is co-localized with sulfur-rich and phosphorus-rich regions in the nucleus and cytoplasm. In the nucleus, most of the platinum was associated with the nucleolus. Within the cytoplasm, platinum mainly accumulated in the acidic organelles.[222] Probably only 1–10% of intracellular cisplatin ends up in the nucleus and reacts with DNA which leads to the cell cycle arrest and initiates cell death in fast proliferating cells.[71,222,223] However, cancer stem cells (CSCs) maintain a quiescent slow-cycling state which protects them from the type of therapy targeting fast proliferating cells. Quiescent CSCs have been proven in many human malignancies and are probably the major cause of

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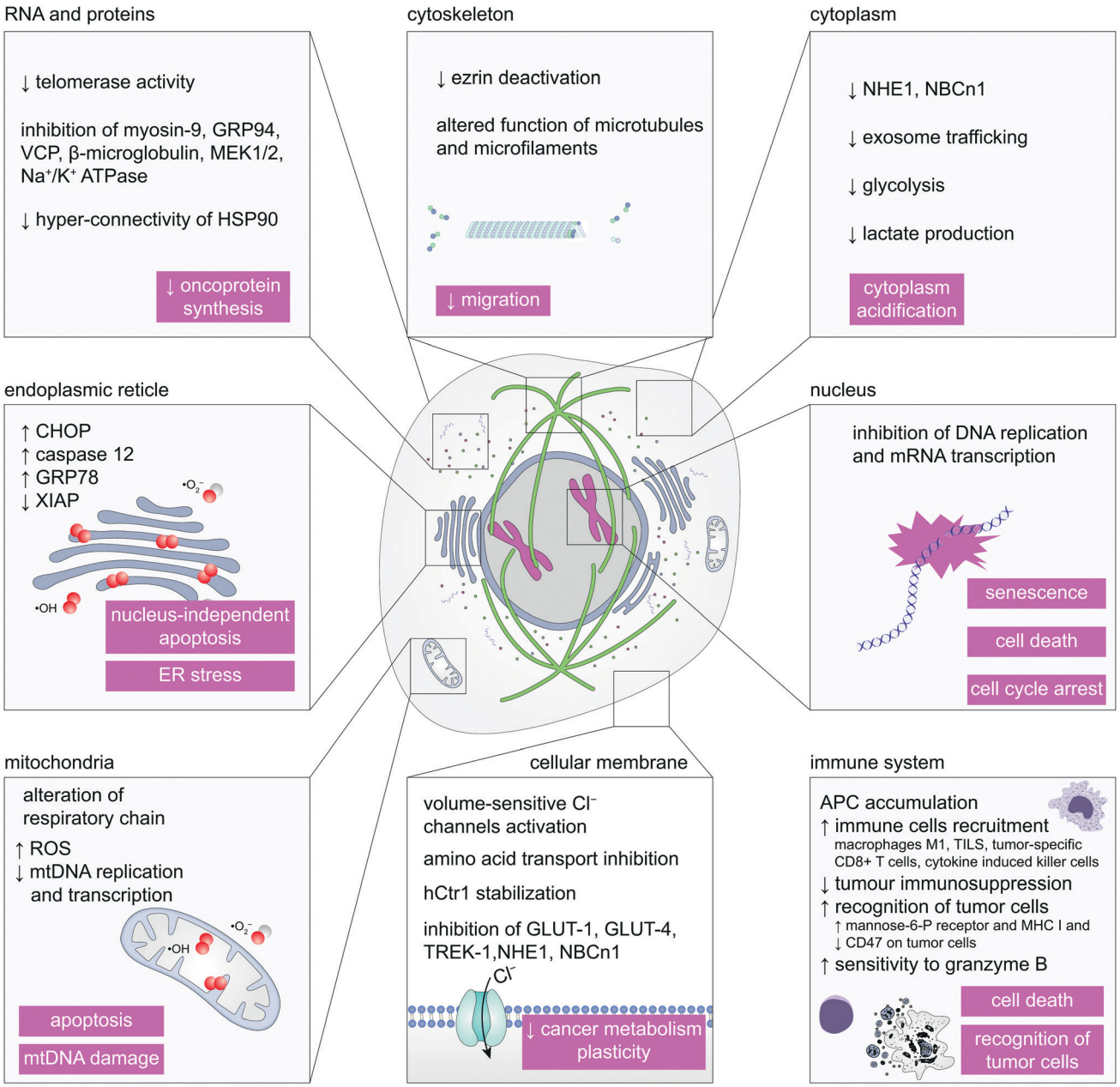

Fig. 1 Alternative effects of cisplatin. Cisplatin is known as a cytotoxic drug which kills cancer cells by damaging DNA. However, other interesting mechanisms such as acidification of the cytoplasm, ER stress, disruption of RNA transcription, inhibition of important oncogenic proteins and decrease in metabolic plasticity of cancer cells as well as changes in their mechanobiology should be considered as cisplatin has a pleiotropic effect on cellular proteins significantly affecting their conformation and function. Cisplatin may also exert antitumor immunomodulation.

treatment resistance in metastatic cancers[5–7] because CSCs can survive chemotherapy with increased tumorigenic and invasive potential.[8] Nevertheless, about 80% of patients with metastatic testicular germ-cell tumors can be cured using cisplatin-based chemotherapy.[3,9] Why is cisplatin so effective? Recent studies suggest that cisplatin could have other mechanisms of action and more variable cellular targets beyond nuclear DNA. Therefore, we will focus on the alternative effects of cisplatin that can reflect its good therapeutic response in this review. First, we will pay attention to the effects of cisplatin at the cellular level; then the whole organism context will be reviewed. Uncovering of the cellular pathways that could be influenced by cisplatin may provide an important clue for the design of new cancer treatment strategies. The alternative effects of cisplatin discussed in this review are summarized in Fig. 1.

Cisplatin and intracellular pH of tumor cells

The acid–base balance of tumor tissues is fundamentally different from that in healthy tissues. Cancer cells tend to have

more alkaline intracellular pH (pH_i = 7.12–7.7) compared to healthy cells (pH_i = 6.99–7.05) while producing acidic extracellular pH (pH_e = 6.2–6.9). In healthy tissues, the pH_e value is in the range of 7.3–7.4.[10] This situation leads to an inverse pH gradient (ΔpH_i to ΔpH_e) between the outside and inside space of the cell, which can enhance proliferation, metabolic adaptation, apoptosis resistance, migration, and invasion of cancer cells.[11] Moreover, the inverse pH_e/pH_i gradient influences the effectivity of antineoplastic drugs. Many of them, such as doxorubicin, mitoxantrone, paclitaxel, and vinblastine are weak bases which are inactivated by protonation in the acidic microenvironment surrounding the cancer cells. On the other hand, cisplatin which is a weak acid has a better chance to concentrate on the more alkaline intracellular space of tumor cells.[12,13]

The aberrant regulation of hydrogen ion dynamics in tumor tissues can be considered as one of the hallmarks of cancer.[13] Rapid cytoplasmic alkalization seems to be an oncogene-dependent early event in the malignant transformation and accounts for the increased activity of hydrogen ion extruders such as the Na^+/H^+ exchangers of the *SLC9* family.[14–16] An increase in pH_i above 7.2 facilitates the cell cycle progression through the S and G2/M phases by increasing the activity of the key mitotic regulators such as cyclin-dependent kinase 1 (*CDK1*) and cyclin B1 (*CCNB1*).[17,18] Changes in pH_i seem to be a conserved evolutionary mechanism for the regulation of mitosis and meiosis.[19] Furthermore, an alkaline pH_i promotes glycolysis,[20] which may depend on the pH-sensitive activity of several glycolytic enzymes. Optimal pH at 30 °C (pH_{30} optimum) for the activity of lactate dehydrogenase (LDH) by conversion of pyruvic acid to lactate is within the range of 7.20–7.40 for all the LDH isoenzymes.[21] The LDH activity is elevated in many types of cancers and has been linked to tumor growth and invasion.[22,23] Moreover, intracellular alkaline pH prevents the progression of apoptosis because pH_i acidification is essential for the activation of caspases and endonucleases.[24,25]

Recently, it was shown that cisplatin could significantly affect the intracellular pH of cancer cells. Acidification of the cytoplasm was described as a result of cisplatin treatment, as demonstrated by *in vitro* and *in vivo* experiments.[26,27] Although the exact causes of this cytoplasmic acidification are yet to be clarified, it is possible that this effect is associated with cisplatin-caused inhibition of the proton extrusion and seems to be independent of cisplatin–DNA adduct formation.[26,27] Indeed, non-competitive inhibition of the Na^+/H^+ exchanger 1 (NHE-1/*SLC9A1*) by cisplatin was confirmed in the HT29 cells and fibroblasts from the PS120 cell line.[27,28] The activity of NHE-1 is typically increased in cancer cells[13] and steadily increased pH_i has been shown in the cisplatin-resistant cell lines.[29–31] The role of NHE-1 in tumorigenesis may be essential because NHE-1-deficient cells showed severe acidification of pH_i and cell death due to Ras oncogene overactivation.[32] Thus, NHE-1 inhibition by cisplatin can play an important role in its antineoplastic effect.

Further evidence that cisplatin has a significant effect on the intracellular and extracellular pH of tumor cells is that proton pump inhibition,[33] and also carbonic anhydrase 9 (CAIX/*CA9*)-targeted therapy enhance the anti-cancer effects of cisplatin.[34] CAIX is a dimeric protein belonging to a family of zinc-containing enzymes that catalyze the reversible hydration of carbon dioxide (CO_2) to bicarbonate and protons ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). CAIX can effectively utilize CO_2 to produce bicarbonate that is delivered through the plasma membrane by the bicarbonate transporter protein family NBC (*NBC2*/*SLC4A5* and others).[12,35] The protons produced by CAIX stay outside and increase the acidosis of the tumor microenvironment. The intracellular bicarbonate is converted back to CO_2 by cytoplasmic carbonic anhydrase 2 (*CA2*) in a reaction that scavenges protons and helps neutralize the intracellular pH. In summary, the CAIX activity protects the cytosol from acidification, while contributing to the acidosis of the extracellular microenvironment.[36] Cisplatin can also intervene in this mechanism, as sodium bicarbonate cotransporter NBCn1 (NBC3/*SLC4A7*) might be made unfunctional by cisplatin treatment, for example, by its reduced plasma membrane localization.[37]

In conclusion, the induction and maintenance of intracellular alkalization and extracellular acidification has an important role in the progression of the neoplastic transformation.[38] Furthermore, low microenvironmental pH is a key factor for exosome trafficking in tumor cells[39,40] and some cancer cell-derived exosomes can increase treatment resistance of recipient cells.[41,42] Due to cisplatin treatment, the pH gradient reversal in cancer tissues can be abolished, and this can mean a major contribution to the treatment efficacy.

Cisplatin and cell metabolism

It is generally accepted that binding of cisplatin to the nuclear DNA is mainly responsible for its antineoplastic effect. However, cisplatin also forms a high amount of adducts in mitochondrial DNA (mtDNA), because mitochondria are not able to carry out nucleotide excision repair, and to effectively remove cisplatin–mtDNA adducts compared to the nucleus. So, the possibility shall not be excluded that mitochondrial DNA may also be an important target of cisplatin. Actually, it has been shown that the level of DNA adducts in mtDNA is higher than that in nuclear DNA. This may be a consequence of both higher initial platinum binding and inefficient removal of cisplatin–mtDNA adducts.[43,44]

Mitochondria are important for the energy supply and regulation of apoptosis. Apart from mtDNA adducts, cisplatin also stimulates ROS generation, prompting oxidative alterations in mitochondrial lipids, proteins, and mtDNA and inducing apoptosis.[45] Mitochondrial ROS generation is independent of the amount of cisplatin-induced damage of nuclear DNA. The cytotoxic effect of cisplatin varies among cells and depends on the mitochondrial redox status and integrity of mitochondrial DNA.[44] Cisplatin may induce serious mitochondrial damage[46] and tumor cells with such damaged mtDNA showed delayed tumor growth. Further tumorigenesis was conditioned by the acquisition of mtDNA from the host cells.[47]

Cancer cells were found to switch their cellular metabolism to glycolysis. Moreover, glycolysis is uncoupled from the mitochondrial tricarboxylic acid (TCA) cycle and oxidative

phosphorylation in such cells. Consequently, lactate formation increases. This metabolic phenotype is called the Warburg effect. By restricting the input of pyruvate into oxidative metabolism in mitochondria, the Warburg effect decreases the mitochondrial ROS generation and increases the cell death resistance and survival advantage for metastasis.[48] Originally, increased glycolysis in cancer cells under aerobic conditions was misinterpreted as evidence for respiration damage. However, we now understand that it reflects an altered regulation of glycolysis, not respiration damage directly. The metabolic flexibility of cancer cells allows the possibility to alternate between glycolysis and oxidative phosphorylation.[49,50] However, cisplatin generates a high level of oxidative stress which is accompanied by cytosolic and mitochondrial acidification, rapid shifts in carbon metabolism and severe decrease of cancer cell metabolic plasticity.[26,51,52] Cytosolic acidification is known to inhibit glycolysis in many ways, for example by the reduction of the glucose transporters expression, and by the inhibition of phosphofructokinase (PFK) and other glycolytic enzymes, while activating oxidative phosphorylation.[22,26,53] Forced stimulation of oxidative phosphorylation in cancer cells with cisplatin-damaged mitochondria raises ROS production and oxidative stress and can restore cancer cells' sensitivity to cell death.[48] Accordingly, antioxidants and mitochondrial uncoupling proteins neutralize cisplatin-induced cytotoxicity in tumor cells and on the contrary, lactate dehydrogenase or pyruvate dehydrogenase kinase 1 (PDK1) inhibitors can further sensitize cancer cells to cisplatin.[45,46,54–57]

Cisplatin down-regulates the expression of many glycolysis-related proteins, including hexokinases, phosphofructokinases, pyruvate kinases, glucose transporters 1 and 4 (GLUT-1/*SLC2A1* and GLUT-4/*SLC2A4*), and lactate dehydrogenase B (*LDHB*).[58,59] Consequently, lactate production is reduced after cisplatin treatment in cancer cells.[51,59] Lactate is probably a key signaling molecule in the tumor microenvironment necessary for all main hallmarks of carcinogenesis, including immune escape, angiogenesis, cell migration, metastasis and self-sufficient metabolism.[40,60] The lactate levels are highly correlated with cancer aggressiveness, and poor survival and reduction of lactate may have a beneficial effect on the cancer therapy.[61,62] The effect of cisplatin on glucose metabolism is summarized in Fig. 2.

Other key signaling molecules in the cancer microenvironment are ATP and adenosine. The ATP levels in the resting/healthy tissues are very low (in the nanomolar range), whereas it can reach hundreds of μmol L^{-1} in stimulated or cancer tissues.[63] Cell death-inducing chemotherapeutic agents such as etoposide, oxaliplatin, cisplatin, staurosporine, and doxorubicin may trigger the release of ATP from the tumor and dendritic cells.[64] Virtually, all tumor cell lines and many primary human tumors express purinergic receptors and are sensitive to ATP.[63] Nevertheless, the activation of purinergic receptors has very heterogeneous and contradictory effects on tumorigenesis.[64] Some results suggest that cisplatin can induce a Cl^- current by activating volume-sensitive chloride channels through the

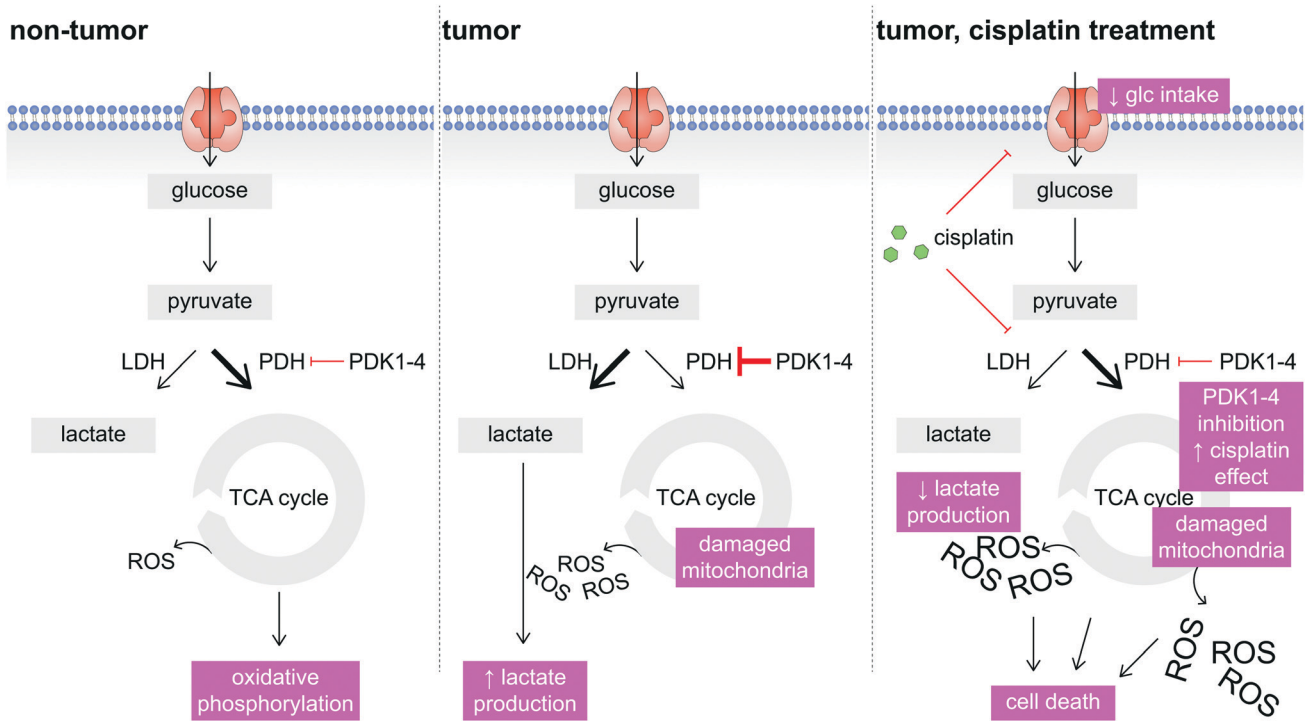

Fig. 2 Changes in cancer metabolism; effect of cisplatin. In most healthy cells with ample oxygen supply, glucose is metabolized to pyruvate which is transformed to acetyl-CoA by pyruvate dehydrogenase (PDH) for entering the respiratory chain. In proliferating cancer cells, increased expression of pyruvate dehydrogenase kinases (PDKs) shifts the pyruvate away from the tricarboxylic acid (TCA) cycle by inhibiting its conversion to acetyl-CoA. 85% pyruvate in malignant cells is fermented into lactate and only 5% pyruvate goes into the TCA cycle by the Warburg effect. Cisplatin exerts an inhibiting effect on glucose transport, glycolysis and lactate production and stimulates ROS generation by OXPHOS which contributes to the mitochondrial dysfunction and cell death. Pyruvate dehydrogenase kinase 1 (PDK1) inhibitors can sensitize cancer cells to cisplatin.

P2Y purinergic receptor pathway.[65] Volume-sensitive chloride channels are involved in the apoptotic cellular volume decrease and cell death after cisplatin treatment.[66–68] Interestingly, ATP suppresses the antiproliferative activity of paclitaxel and etoposide, while it enhances the antineoplastic effect of cisplatin in human lung epithelial tumor cells.[69]

Taken together, cisplatin exerts an inhibiting effect on glycolysis and lactate production;[51] it stimulates ROS generation and purinergic signaling and contributes to mitochondrial dysfunction and cell death.[44,70] Targeting mitochondria and lactate production seem to be an important contribution to the treatment efficacy of cisplatin.

Cisplatin, endoplasmic reticulum, and nucleus-independent apoptosis

Cisplatin can react with nucleophiles other than DNA. It was reported that cisplatin could trigger apoptotic signaling independently of nuclear DNA damage even in enucleated cells through increased cytosolic calcium and calpain-dependent activation of the ER-specific caspase-12.[71,72] Cisplatin-mediated activation of calpain protease was found to occur early in the apoptotic process and to coincide with BH3-interacting domain death agonist (*BID*) cleavage.[73] In contrast to cisplatin, etoposide, which is also a DNA-damaging agent, failed in inducing apoptosis in these enucleated cells.[71] Moreover, the inhibition of ER-specific caspase-12 with the anti-caspase-12 antibody significantly decreased cisplatin-induced apoptosis, indicating that ER stress is involved in the cisplatin-induced cell death.[74] Accordingly, the expression of chaperone glucose regulated-protein 78 (GRP-78/*HSPA5*), which is an ER stress marker, is up-regulated after cisplatin treatment.[75] This phenomenon is probably not caused by reactive oxygen species (ROS), because ROS scavenger *N*-acetyl cysteine (NAC) failed to inhibit calpain activation and apoptosis in cisplatin-treated cells and the presence of NAC did not affect the up-regulation of GRP-78 levels after cisplatin treatment.[71] These facts suggest that the endoplasmic reticulum (ER) might be a non-nuclear target of cisplatin. Disturbances in the normal functions of the ER lead to a stress response because protein folding in the ER is sensitive to various deleterious conditions such as calcium concentrations, the redox state, misglycosylation of glycoproteins, and low ATP levels.[76] The ER stress mechanism is a key response to deleterious environmental factors and triggers the unfolded protein response (UPR). A moderate UPR activation enables compensation for damage and has an anti-apoptotic role that enhances tumor cell survival and drug resistance.[77] However, the compensatory phase of the ER stress response is not limitless. When ER stress becomes severe, cell death is triggered even in the presence of high levels of GRP-78.[78] The key factor in this phenomenon is the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP/*DDIT3*). The increased expression of CHOP triggers the activation of pro-apoptotic pathways.[79] Apparently, cisplatin treatment induces significant ER stress followed by the up-regulation of pro-apoptotic signaling molecules CHOP or protein disulfide isomerase (PDI).[80–82] Furthermore, cisplatin inhibits the expression of X-chromosome-linked inhibitor of apoptosis protein (XIAP).[83,84]

Adaptation to ER stress depends not only on the activation of the UPR but also on autophagy.[85] Knockdown of p62 (adaptor for autophagic degradation, *SQSTM1*) or the autophagy inhibitors 3-methyladenine and chloroquine increases the level of ubiquitinated proteins, which elevates the ER stress and results in a higher apoptotic rate of cancer cells treated with cisplatin.[86,87] In conclusion, the activation of UPR and CHOP in various cancer cell lines indicates that cisplatin may induce apoptosis through the ER stress pathways.

Cisplatin and mechanical properties of tumor cells

The cell has a complex internal structure that changes in response to its microenvironment as well as to the physiological state. Altered cellular functions can markedly remodel the cellular biomechanical properties. The cellular shape, mechanical response, and mechanical deformability are primarily determined by the cytoskeleton. In concert with accessory proteins, the cytoskeleton also plays a key role in important cellular processes such as mechanotransduction, migration, and mitosis.[88] The structures of the cytoskeleton, cellular membrane, and extracellular matrix are transformed during cancer progression, which changes the deformability of the cancer cells. As a result, the motility of cancer cells can be different from that of normal cells, causing them to migrate through the tissue to different sites in the human body and inducing metastasis.[89,90] It was also shown that cytoskeletal remodelling is a key process in the formation of cancer stem cells (CSCs).[91] It seems that cisplatin can strongly influence the actin stress fiber formation and affects the cytoskeleton.[91–96] The actin cytoskeleton strongly influences the membrane mechanical properties and is connected to mechanosensitive channels and transporters, such as NHE-1.[97] The significance of this sodium/hydrogen exchanger in carcinogenesis was discussed in the previous text. The ability of cisplatin to modify microtubule disassembly by direct tubulin modification was also shown. In contrast to cisplatin, carboplatin did not produce microtubule disassembly abnormalities.[96] Accordingly, the treatment with cisplatin caused a significant increase in the cell stiffness of the prostate cancer cells.[92] Changes in the cell stiffness due to cisplatin treatment probably do not result from metal accumulation in the cells because no such increase was shown in the zinc-treated cells. Under cisplatin treatment, the cytoskeletal tubules and filaments, which are normally distributed as a gently organized network spreading through the whole cytoplasm and forming delicate protrusions such as filopodia, aggregated to dense areas on the leading edge of the cell or to the cap-like structures around the nucleus. These phenomena are dependent on the dose of cisplatin applied.[92,95] This effect of cisplatin was observable also in breast cancer cells, where cisplatin produced changes in the cell morphology and the actin cytoskeleton. These changes were manifested as a loss of lamellipodia/filopodia and the appearance of membrane ruffles.

The activation of acid sphingomyelinase (ASMase/*SMPD1*) was shown to be required upstream of these morphological changes.[93] Cisplatin activates ASMase and ceramide production, which triggers the redistribution of CD95 into the plasma membrane rafts. Such redistribution sensitizes tumor cells to CD95-mediated apoptosis.[98] Furthermore, cisplatin induces dephosphorylation of the actin-binding protein ezrin (*EZR*), and its relocation from the membrane to the cytosol.[93] Cytosolic non-phosphorylated ezrin represents a dormant form of ezrin,[99] while phosphorylated (active) ezrin regulates the cytoskeletal dynamics by cross-linking the actin filaments to the plasma membrane. The membrane localization of ezrin plays a pivotal role in the progression of malignant diseases.[100,101] Ezrin has been shown to support cancer dissemination by several mechanisms including changes in proliferative signaling, cell motility, and anoikis resistance. Ezrin probably regulates these processes through the influence on the expression levels of E-cadherin and CD44. The suppression of ezrin active state also sensitized cells to anti-cancer drugs.[99,100]

Nevertheless, the tumor-suppressive effect of cisplatin through cytoskeletal remodeling is probably context dependent. Some types of cancer cells such as prostate cancer cells and ovarian cancer cells surviving cisplatin treatment are stiffer with a cytoskeleton composed of long actin stress fibers created due to RhoA activation.[92,102] These stiffer cells are more resistant but less aggressive showing a significant decrease in cell migration, invasion, and formation of colonies.[92] On the other hand, cisplatin-treated melanoma cells exhibit a significant decrease in cell stiffness and the up-regulation of FAK-mediated and MAPK-mediated signaling promoting the malignancy, chemoresistance, and invasiveness of these cells.[94,103] Accordingly, cisplatin is not effective against melanoma.[103] We can speculate that highly aggressive cells need to be rather more pliable[89,90,104,105] with low levels of Rho GTPase activation and low stress fiber formation,[88,106,107] because tumor cells with high deformability and low RhoA activation preferentially engulf and outcompete neighboring cells with low deformability in heterogeneous cancer cell populations.[107] Accordingly, changes in cell stiffness may be a promising marker of the cisplatin treatment response of individual cancer cells.

Other cellular targets and binding sites of cisplatin

Recent studies suggest that cisplatin has multiple cellular targets beyond DNA. Cisplatin could inactivate essential RNA molecules such as the RNA components of ribosomes and splicing machineries, catalytic RNA motifs, or tRNA and also membrane lipids, proteins, and cellular enzymes.[27,108–112] 65–98% of cisplatin molecules have formed adducts with proteins such as hemoglobin, serum albumin, transferrin, metallothionein, and glutathione after 24 h of cisplatin administration to the patient.[113,114] However, it is possible that upon several ligand exchange reactions, cisplatin may exchange the chlorido/aquo and both ammine ligands with nucleophilic amino acids of a protein and may form a Pt-adduct while causing functional disruption of the targeted molecules. Accordingly, cisplatin reduced telomerase activity in a specific and concentration-dependent manner in human testicular tumor cells, while bleomycin, doxorubicin, methotrexate, or melphalan had no effect. The telomerase inhibition could be a decisive reason for cisplatin's success in the therapy of testicular cancer.[115,116]

In addition to telomerase, there are other cancer-associated proteins identified as cisplatin binding sites, including high-affinity copper transporter 1 (hCtr1/*SLC31A1*), non-muscle myosin IIA (Myosin-9, *MYH9*), heat shock protein 90 (HSP90), endoplasmin (GRP-94/*HSP90B1*), valosin-containing protein (*VCP*), or β_2-microglobulin (*B2M*).[117–119]

High-affinity copper transporter 1 functions as homotrimer having three transmembrane domains forming a pore through the plasma membrane. hCTr1 mediates cellular copper uptake, but has also been shown to be involved in the cellular import of cisplatin.[120–122] Whereas Cu triggers internalization of hCTr1 from the plasma membrane, cisplatin does not.[123] Conversely, cisplatin may stabilize hCTr1 trimeric pores by spanning the methionine-rich motifs of the interacting hCtr1 subunits facilitating the cisplatin cross through the membrane into the cytoplasm.[119] Cisplatin also induces the expression of hCtr1 in time- and concentration-dependent manners. Abundance and multimeric state of hCtr1 in various tumors reflect their response to cisplatin.[124] Other cisplatin binding sites were found on copper chaperone Cox17, copper chaperone Atox-1 and the Cu-ATPase ATP7B.[114,224] The ability of cisplatin to form protein dimers was observed in the case of Atox-1. Since Atox-1 is transferred to the nucleus after copper exposure, it may be also involved in the transport of cisplatin to DNA. Furthermore, cisplatin bound to Atox-1 may alter copper homeostasis and cellular defense against oxidative stress, therefore providing an alternative route to cell killing.[225] Cox17 seems to be involved in cisplatin transfer to mitochondria.[114] Cisplatin bound to ATP7B stimulates its catalytic phosphorylation with the formation of a transient acyl-phosphate intermediate (which is unstable at basic pH, but stable at acidic conditions).[224] Hyperphosphorylation is associated with the transfer of ATP7B from the trans-Golgi network to vesicles.[226]

Another cisplatin-binding protein, whose activity could be changed by cisplatin is myosin-9.[117] Myosin-9 is a class II non-muscle myosin that regulates cell motility and maintains an equilibrium between the actomyosin and microtubule systems.[125] Elevated myosin-9 expression was associated with poor prognosis, lymph node positivity, and advanced tumor stage in oesophagal squamous cell carcinoma patients.[126] Myosin-9 was also found as a key protein for the invasion of MCF-7 breast cancer cells.[127] On the other hand, p53 failed to accumulate and/or remain in the nucleus in the absence of endogenous myosin-9 activity in squamous-cell carcinoma cells.[128] Myosin-9 also interacts with the cytoplasmic tail of Golgi glycosyltransferases and creates a force for Golgi disorganization, which is typical for colon and prostate cancer progression. Myosin-9 is more stably associated with the Golgi of androgen-refractory prostate cancer cells than androgen-sensitive cells

and inhibition of myosin-9 restored compact Golgi morphology in prostate and colon cancer cells.[129] Myosin-9 inhibitors (such as cisplatin) could also block the development of tolerogenic dendritic cells.[130]

Cisplatin also binds heat shock protein 90 (HSP90) and inhibits its activity.[117] HSP90 is a molecular chaperone that is generally thought to function in assisting protein folding, and degradation of misfolded proteins. Nevertheless, a critical role of HSP90 in cancer was also revealed. HSP90 can protect mutated and overexpressed oncoproteins from degradation, facilitating cancer cell survival.[131] HSP90 was also identified as an inhibitor of the mammalian pro-apoptotic protein inositol hexakisphosphate kinase 2 (*IP6K2*) in cancer cells. Consequently, HSP90 inhibition should be cytotoxic for cancer cells.[132] Furthermore, HSP90 inhibition caused by cisplatin halts adipogenesis and differentiation of adipocytes.[133,134] Adipocytes secrete a great number of pro-inflammatory adipokines, which support tumorigenesis and metastasis.[135] Also, the adipocyte mediated conversion of androgens to estrogen contributes to the development of endometrial cancer.[136] Cisplatin binds to the C-domain and N-domain of the human HSP90 and inhibits HSP90 chaperone activity.[137,138] Because the HSP90 N-domain is the binding site of the aryl hydrocarbon receptor (AhR), AhR is dissociated from the HSP90 chaperone complex in the presence of cisplatin and is degraded through the 26S proteasome. AhR is a transcription factor and induces an enzyme of the cytochrome P450 family, CYP1A1. In the presence of cisplatin, the CYP1A1 mRNA level was strongly reduced.[139] CYP1A1 was shown to regulate breast cancer proliferation and survival, and its knockdown decreased colony formation and cell proliferation, and increased apoptosis associated with a reduction of survival.[140] HSP90 is stable as a dimer; however, oncogene-induced stress, such as MYC hyperactivation, can lead to chaperone oligomerization and hyper-connectivity. The oligomerization may activate functions that are normally silent including NF-κB signaling and autophagy. Cisplatin could disrupt oligomerization by binding to HSP90 and impair the formation of signaling loops in cancer cells that enable resistance to kinase inhibitors.[141]

Another cisplatin binding protein is GRP-94 (*HSP90B1*), the HSP90-like chaperone functioning in the lumen of the endoplasmic reticulum.[117] An elevated level of GRP-94 has been reported in many types of cancer such as breast cancer,[142] lung cancer,[143] esophageal adenocarcinoma,[144] and colon, and gastric cancer.[145,146] GRP94 overexpression is probably involved in the migration and proliferation of cancer cells.[147]

Cisplatin further binds valosin-containing protein (VCP; or p97). VCP is an ATPase belonging to the AAA family which is involved in the ubiquitin/proteasome degradation pathways.[148] VCP influences both increased cell proliferation and the attenuation of cell death in cancer cells by regulating NF-κB signaling.[149] High VCP expression in tumor tissue was correlated with poor prognosis in patients with non-small cell lung carcinoma, hepatocellular carcinoma, gastric carcinoma, follicular thyroid and prostate cancer.[150–154]

Cisplatin binding sites also contain β_2-microglobulin. β_2-microglobulin is a component of major histocompatibility complex class 1 molecules and can act as a growth factor and signaling molecule inducing epithelial to mesenchymal transition in cancer.[155] β_2-microglobulin expression increases during the progression of many human cancers such as breast cancer,[156] prostate cancer,[157] lung cancer,[158] or colon cancer.[159] β_2-microglobulin was also proved as a proaging factor that impairs cognitive functions and neurogenesis.[160] Inhibition of β_2-microglobulin improved radiation sensitivity in prostate cancer cells.[161]

Other important cisplatin binding sites were found on cytochrome *c*, calmodulin, insulin, ribonuclease A, cytochrome *c* oxidase, insulin growth factor, α_2-macroglobulin, α_1-anti-trypsin, apolipoprotein A1 and A2, superoxide dismutase, specificity protein 1 (SP1), or on ribosomal protein L5.[114,117,162–164] Furthermore, cisplatin binds to the CXXC motif of proteins containing a ferrodoxin-like fold.[165] Cisplatin is also able to induce the formation of higher oligomers of proteins *via* crosslinking. The ability of cisplatin to form protein dimers was observed in the case of human serum albumin and is suggested for insulin.[114] Furthermore, acute inhibition of mechanosensitive transporters and channels such as Na^+/H^+ exchanger NHE-1 and K^+ channel TREK-1 (*KCNK2*) was observed after cisplatin treatment.[28] TREK-1 is abundantly expressed in the PC-3 and LNCaP prostate cancer cell lines but is not detectable in healthy prostate epithelial cells. The overexpression of TREK-1 resulted in a significant increase in cell proliferation in normal prostate epithelial cells and Chinese hamster ovary cells.[166] TREK-1 overexpression was also related to shorter castration resistance free survival in prostate cancer patients.[167] Cisplatin also interacts with proteins that comprise high-mobility-group domains, such as upstream binding factor (UBF), and in this way influences ribosomal RNA transcription by RNA polymerase I. Cisplatin causes a redistribution of UBF, TATA-binding protein (TBP), TBP-associated factors for RNA polymerase I, and RNA polymerase I. Consequently, cisplatin blocks the synthesis of ribosomal RNA, while the activity of RNA polymerase II stays intact.[168] Furthermore, clinically relevant concentrations of cisplatin inhibit MEK1 and MEK2 activity.[165] MEK1 and MEK2 are protein kinases that are the gatekeepers of ERK1/2 activity.[169] Many types of tumor cells exhibit hyperactivation of ERK, and a range of MEK inhibitors are in late-stage clinical trials.[170] Moreover, cisplatin can inhibit the Na^+/K^+-ATPase (NKA).[171] Several reports suggest that the alpha subunits of the NKA could be interesting anti-cancer targets.[172,173]

In conclusion, cisplatin has a pleiotropic effect on cellular proteins significantly affecting their conformation and function and takes part in the disruption of rRNA synthesis, which is stimulated in proliferating cells. Cisplatin can also influence the transport of amino acids, which are the basic building units of proteins.[174] As some cancer cells are auxotrophic for special amino acids, the inhibition of essential amino acid transporters by cisplatin may be an important part of the clinical success of cisplatin as well as the inhibition of key oncoproteins. Nevertheless, in the above-mentioned studies, cisplatin or cisplatin derivatives were usually prepared by challenging the drug with one purified protein. This is not a realistic situation because cisplatin is simultaneously challenged with a huge variety of different

proteins in a real organism. Consequently, the biological actions of cisplatin will be most probably the result of thousands of cisplatin–protein interactions and their functional consequences. Competitive binding experiments can give valuable insight into the selectivity of platinum-based drugs with mixtures of small molecules, proteins or peptides and oligonucleotides.[227]

Cisplatin and the immune system

According to the FDA prescribing information (https://www.drugs.com/pro/cisplatin.html), platinum concentrations in tumors after cisplatin treatment are usually somewhat lower than the concentrations in the organ where a tumor is located. Consequently, some effect exceeding simple damage of tumor cells should be considered. Recent studies suggest that an important part of the antitumor effect of cisplatin occurs through mechanisms counteracting cancer immune evasion.[175,176] It is well known that an immune escape of tumor cells is associated with the major histocompatibility complex class I (MHC-I) downregulation and the capacity to induce upregulation of MHC class I cell surface expression is a critical step in the tumor rejection.[177] Some studies have recently demonstrated that cisplatin may upregulate the tumor cells' MHC-I expression and may boost CD8+ T cell-mediated anti-cancer immunity.[178–181] Such MHC class I recovery might well synergize with some forms of immunotherapy.[177,178,182] Interestingly, cisplatin chemotherapy broadened the range of tumor antigens recognized by cytotoxic CD8+ T cells.[183] The cancer immune editing of the host's immune system represents one of the major mechanisms by which tumors evade anti-cancer immunity. The cancer immune editing includes T cell anergy, regulatory T cells and their immune suppressive mediators, and systemic defects of antigen presenting cells. The ability of the immune system to fight against tumor cells is highly dependent on the accumulation and activation of immune effector cells.[184] Some studies suggest that low-dose cisplatin could promote the accumulation of antigen presenting cells such as CD11c+ dendritic cells in tumor loci[185] and support the recruitment and proliferation of immune effector cells such as M1 macrophages, tumor-specific CD8+ T cells,[181,186] and cytokine-induced killer cells.[187,188] Cisplatin can also activate murine peritoneal macrophages to the tumoricidal state[189] and cisplatin-treated monocytes enhance the proliferation of CD4+ T cells by the increased production of IFN-β. No such effect was seen in dexamethasone, doxorubicin, or irinotecan-treated monocytes.[190] Cisplatin can also enhance the immunostimulatory potential of dendritic cells (DCs) and decrease the immunosuppressive capability of tumor cells.[191] This immunomodulatory activity is based on the inhibition of STAT6-mediated expression of co-inhibitory molecule PD-L2. Decreased PD-L2 expression led to the increased activation and proliferation of T cells by DCs and enhanced recognition of tumor cells by T cells.[192] Cisplatin also sensitizes tumor cells to attack of cytotoxic T cells.[193] This attack may be mediated by the up-regulation of mannose-6-phosphate receptors on the surface of tumor cells, which makes the tumor cells sensitive to granzyme B,[194] or by enhanced expression of death receptor Fas/CD95 on the cancer cells.[195] Stimulation of the anti-cancer immune response is also mediated by the exposure of calreticulin, which is a dominant pro-phagocytic signal, on the surface of the cancer cells facilitating their uptake by dendritic cells and the following presentation of tumor-associated antigens to T lymphocytes.[196] In contrast to oxaliplatin, cisplatin probably fails to induce the translocation of calreticulin to the cell surface.[196] Nevertheless, the calreticulin effect is counterbalanced by CD47 in multiple human cancers[197] and a significant reduction in CD47 surface expression occurs after cisplatin treatment.[198] The enhanced expression of the CD47 molecule on cancer cells has been found in many cancers, including malignant blood tumors.[199]

Cisplatin is also able to modulate immune-suppressive milieu of tumor tissues. Treatment with cisplatin significantly reduced the levels of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) in the tumor microenvironment.[181,182,186–188,200] Inflammatory mediators including cytokines can participate in tumor progression, as the important component of the tumor microenvironment, inflammatory mediators including cytokines can participate in tumor promotion and progression. Cisplatin inhibits the growth, migration, and invasion of cervical cancer cells by downregulating the IL-17E/IL-17RB pathway[201] and enhances the tumoricidal activity of bone marrow-derived macrophages through the production of extracellular and membrane-associated interleukin-1 (IL-1) and tumor necrosis factor (TNF-α).[189] Furthermore, cisplatin-treated phytohemagglutinin-stimulated human peripheral blood lymphocytes displayed enhanced IL-2, IL-2R, IFN-γ and TNF-α mRNA levels compared to non-treated controls.[202] IL-1 is known to be required for tumor eradication mediated by tumor-specific Th1 cells and was also shown to synergize with IFN-γ for induction of tumoricidal activity in tumor-infiltrating macrophages.[203] Simultaneous administration of TNF-α with IFN-γ resulted in synergistic effects manifested by the retardation of tumor growth[204] and local combined treatments with IL-1 and IL-2 can induce T cell-mediated anti-cancer effects.[205]

In conclusion, cisplatin promotes the antigen presentation and function of effector immune cells while simultaneously counteracting numerous immune-suppressive mechanisms which stay behind cancer immune evasion. Although cisplatin does not induce immunogenic cell death *per se*, which is probably caused by the lack of calreticulin exposure after cisplatin treatment, cisplatin could still potentiate immunogenic cell death by coadministration with another kind of therapy, such as radiotherapy. Radiotherapy is a potent inducer of calreticulin exposure and combining cisplatin with induced calreticulin exposure consequently leads to immunogenic cell death.[175,196]

Cisplatin and the microbiome

In the previous chapter, we have shown that cisplatin may exert its anti-cancer effect by influencing the immune system. Some studies indicate that the ROS required for platinum toxicity *in vivo* is mostly derived from tumor-associated inflammatory

cells.[206] Recently, it was shown that anti-cancer immunity could also be significantly influenced by the intervention of the intestinal microbiota.[207,208] Compared with controls with a normal gut microbiome, the antibiotic-treated and germ-free mice reacted poorly to immunotherapy and chemotherapy by cisplatin. It is possible that cisplatin influences gut microbes to make immune cells ready to produce reactive oxygen species (ROS), which then kill tumor cells.[209] Accordingly, the absence of gut microbiota in mice was shown to prevent the paracrine production of ROS by tumor-infiltrating myeloid cells.[206] Conversely, administration of antibiotic-treated mice with *Lactobacillus acidophilus* renews the cisplatin anti-cancer effect and restores some of the cisplatin-induced inflammatory gene expression that is observed in ordinarily raised mice.[210] The ROS production in intra-tumor myeloid cells after platinum treatment seems to be managed by signaling through myeloid differentiation primary response 88 (*MYD88*)-associated innate immune receptors also known as pattern recognition receptors (PRRs) which detect molecules typical for the pathogens.[206,211] Microbes highly influence the effect of cisplatin, but this effect is two-sided. Cisplatin can also modify proliferation and the resulting effect of microbes. It was shown that cisplatin protects macrophages from lysis by *Bacillus anthracis* lethal toxin (LT).[212] Cisplatin also inhibits protein splicing in *Mycobacteria* by decreasing the activation of inteins. In addition to *M. tuberculosis*, self-splicing inteins are critical proteins in *Mycobacterium leprae*, *Coxiella burnetii*, and *Cryptococcus neoformans*. Moreover, cisplatin is a potent inhibitor of RecA intein splicing and DNA gyrase in *Escherichia coli*.[213,214] It has been shown that *Escherichia coli* (B2 phylogenetic group) promotes the pro-tumoral activities of macrophages in colon cancer by inducing sustained COX-2 expression.[215] Furthermore, *E. coli* (B2 phylogenetic group) harbours the pks island (pks+ *E. coli*) coding colibactin.[216] Colibactin is a bacterial genotoxin promoting colon tumor growth by inducing a senescence-associated secretory phenotype while simultaneously making the epithelial cells that line the gut more prone to DNA damage.[217] Colonization of mice with the pks+ strain of *E. coli* was sufficient to drive tumorigenesis, whereas germ-free mice were protected,[218] hence inhibition of *E. coli* by cisplatin may also contribute to its treatment effect.

Conclusion

Cisplatin is one of the most effective anti-cancer drugs extensively used for the cure of different types of neoplasms (ovarian, head and neck, lung, breast, leukaemia, brain, kidney, and testicular cancers). Generally, cisplatin is known as a cytotoxic drug which kills cancer cells by damaging DNA, inhibiting mitosis, and triggering cell death. However, other interesting mechanisms should be considered such as immunomodulation and interference in the communication between the tumor cells and their microenvironment. Cisplatin can also change the mechanical properties of cancer cells and significantly encroach on cancer cell metabolism. Recently the modulating effect of cisplatin on the intestinal microbiome was also proved. Uncovering of the cellular pathways that could be influenced by cisplatin may provide us with an important clue for designing new cancer treatment strategies by finding new potential targets for therapeutic intervention. The mechanisms of cisplatin action in the context of the whole body are weakly studied in humans and need further elucidation and deep cooperation between biologists, chemists and clinicians. Study of cisplatin may also benefit from competitive binding experiments and from global omics studies which can give valuable insight into the non-DNA binding sites of cisplatin.

List of abbreviations

AhR	Aryl hydrocarbon receptor
ASMase/SMPD1	Acid sphingomyelinase
Atox-1	Antioxidant 1 copper chaperone
ATP	Adenosine triphosphate
B2M	β_2-Microglobulin
BID	BH3-interacting domain death agonist
CA2	Cytoplasmic carbonic anhydrase 2
CAIX/CA9	Carbonic anhydrase 9
CCNB1	Cyclin B1
CD47	Cluster of differentiation 47
CD95	Cluster of differentiation 95; fas receptor, also known as apoptosis antigen 1
CDK1	Cyclin-dependent kinase 1
CHOP/DDIT3	CCAAT-enhancer-binding protein homologous protein
Cl^- current	Chloride current
Cox17	Cytochrome *c* oxidase copper chaperone
CSCs	Cancer stem cells
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1
DCs	Dendritic cells
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
EZR	Ezrin
FAK	Focal adhesion kinase
GLUT-1/SLC2A1	Glucose transporters 1
GLUT-4/SLC2A4	Glucose transporters 4
GRP-78/HSPA5	Glucose regulated-protein 78
GRP-94/HSP90B1	Heat shock protein 90 kDa beta member 1 also known as endoplasmin
hCtr1/SLC31A1	High affinity copper transporter 1
HSP90	Heat shock protein 90
IFN	Interferon
IFN-β	Interferon beta
IL	Interleukin
IP6K2	Inositol hexakisphosphate kinase 2
LDH	Lactate dehydrogenase
LDHB	Lactate dehydrogenase B
MAPK	Mitogen-activated protein kinase
MDSC	Myeloid-derived suppressor cells
MEK	Mitogen-activated protein kinase kinase
MHC-I	Histocompatibility complex class I

mtDNA	Mitochondrial DNA
MYC	Myelocytomatosis proto-oncogene
MYD88	Myeloid differentiation primary response 88
MYH9	Non-muscle myosin IIA
NAC	*N*-Acetyl cysteine
NBC	Sodium bicarbonate cotransporter family
NBC2/*SLC4A5*	Sodium bicarbonate cotransporter, member 5
NBCn1 (NBC3/*SLC4A7*)	Sodium bicarbonate cotransporter, member 7
NHE-1/SLC9A1	Na^+/H^+ exchanger 1
NKA	Na^+/K^+-ATPase
P2Y receptors	Purinergic G protein-coupled receptors
PDI	Disulfide isomerase
PDK1	Pyruvate dehydrogenase kinase
PD-L2	Programmed death ligand-2
PFK	Phosphofructokinase
pH_i	Intracellular pH
RhoA	Ras homolog gene family, member A
ROS	Reactive oxygen species
SLC22	Solute carrier family 22
SLC9	Solute carrier family 9
SP1	Specificity protein 1
STAT6	Signal transducer and activator of transcription 6
TBP	TATA-binding protein
TCA cycle	Tricarboxylic acid cycle
TNF	Tumor necrosis factor
Tregs	Regulatory T cells
TREK-1/KCNK2	Potassium two pore domain channel subfamily K member 2
UBF	Upstream binding factor
UPR	Unfolded protein response
VCP	Valosin-containing protein
XIAP	X-Chromosome-linked inhibitor of apoptosis protein

Authors' contributions

MR review design, writing; JB writing, mechanobiology section; MF writing, cisplatin targets; JG pathway analyses, illustrations; MM review design.

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Conflicts of interest

None declared.

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