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REVIEW

Molecular mechanisms of cisplatin resistance in cervical cancer

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Abstract: Patients with advanced or recurrent cervical cancer have poor prognosis, and their 1-year survival is only 10%–20%. Chemotherapy is considered as the standard treatment for patients with advanced or recurrent cervical cancer, and cisplatin appears to treat the disease effectively. However, resistance to cisplatin may develop, thus substantially compromising the efficacy of cisplatin to treat advanced or recurrent cervical cancer. In this article, we systematically review the recent literature and summarize the recent advances in our understanding of the molecular mechanisms underlying cisplatin resistance in cervical cancer.

Keywords: cisplatin, epithelial–mesenchymal transition, microRNA, molecular mechanism, resistance

Introduction

Cervical cancer remains to be one of the leading causes of cancer-related death in women despite advances in screening, diagnosis, prevention, and treatment. It accounts for ~4% of the total newly diagnosed cancer cases and 4% of the total cancer deaths according to the GLOBOCAN 2012 estimates.[1] The prognosis of patients with advanced/recurrent cervical cancer is particularly poor, and their chance of 1-year survival is only 10%–20%.[2] Chemotherapy is currently the standard treatment for those patients. The chemotherapeutic agent cisplatin, which is a small-molecule platinum compound and was originally found to inhibit bacterial growth and later identified as an anticancer agent, appears to most effectively treat advanced/recurrent cervical cancer.[3] The molecular mechanism underlying cisplatin-mediated anticancer effect is associated with multiple intertwined signaling pathways.[4] When the concentration of cytoplasmic chloride ion reduces, the chloride ligands of cisplatin are gradually replaced by water. The resulting aquated cisplatin is highly reactive and covalently binds to DNA to form DNA–cisplatin adducts, which in turn induce DNA damage. When the cisplatin-induced DNA damage is beyond repair, the cells undergo apoptosis and die.

Combination therapy of cisplatin and paclitaxel is a standard chemotherapeutic regimen to treat recurrent or metastatic cervical cancer. The overall response rate is 29.1%–67%, and the median overall survival is 12.87 months in patients with recurrent or advanced cervical cancer receiving the combination chemotherapy.[3,5] However, resistance to cisplatin, either intrinsic or acquired resistance, may develop, seriously compromising the efficacy of cisplatin. In this article, we summarize recent advances in our understanding of the mechanisms underlying cisplatin resistance (CPR) in cervical cancer and propose strategies to overcome CPR in cervical cancer.

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Mechanisms underlying CPR

The molecular mechanisms underlying CPR are complex and usually associated with the following features: 1) reduction in the intracellular accumulation of the platinum compounds; 2) increase in DNA damage repair; 3) inactivation of apoptosis; 4) activation of epithelial–mesenchymal transition (EMT); 5) alteration in DNA methylation, microRNA profile, cancer stem cell characteristics, and expression of stress-response chaperones (Figure 1).

Reduced intracellular accumulation and CPR

Reduced intracellular accumulation of cisplatin may be the predominant cause for CPR. Decrease in uptake, increase in efflux, and inactivation by thiol-containing proteins contribute to reduction in intracellular cisplatin accumulation, which results in reduction in cisplatin–DNA adduct formation and ultimately leads to resistance to cisplatin.[4]

Reduced uptake

Reduced cisplatin uptake has been observed in cervical cancer cells with acquired CPR. The cisplatin-resistant HeLa cells (HeLa-CPR)[6] and A431 (A431/Pt)[7] cells show 50% and 77% reduction in cisplatin uptake, respectively, compared with the parental cell lines. In HeLa-CPR cells, the human cervical adenocarcinoma cells with CPR, the amount of cisplatin–DNA adducts is two- to threefold less than that in the parental cells,[6] but the HeLa-CPR cells have a similar rate of cisplatin–DNA adduct removal as the parental HeLa cells. Similarly, in A431/Pt cells, which are CPR cervix squamous carcinoma cells, platinum accumulation, DNA-bound platinum, and interstrand cross-link frequency are also

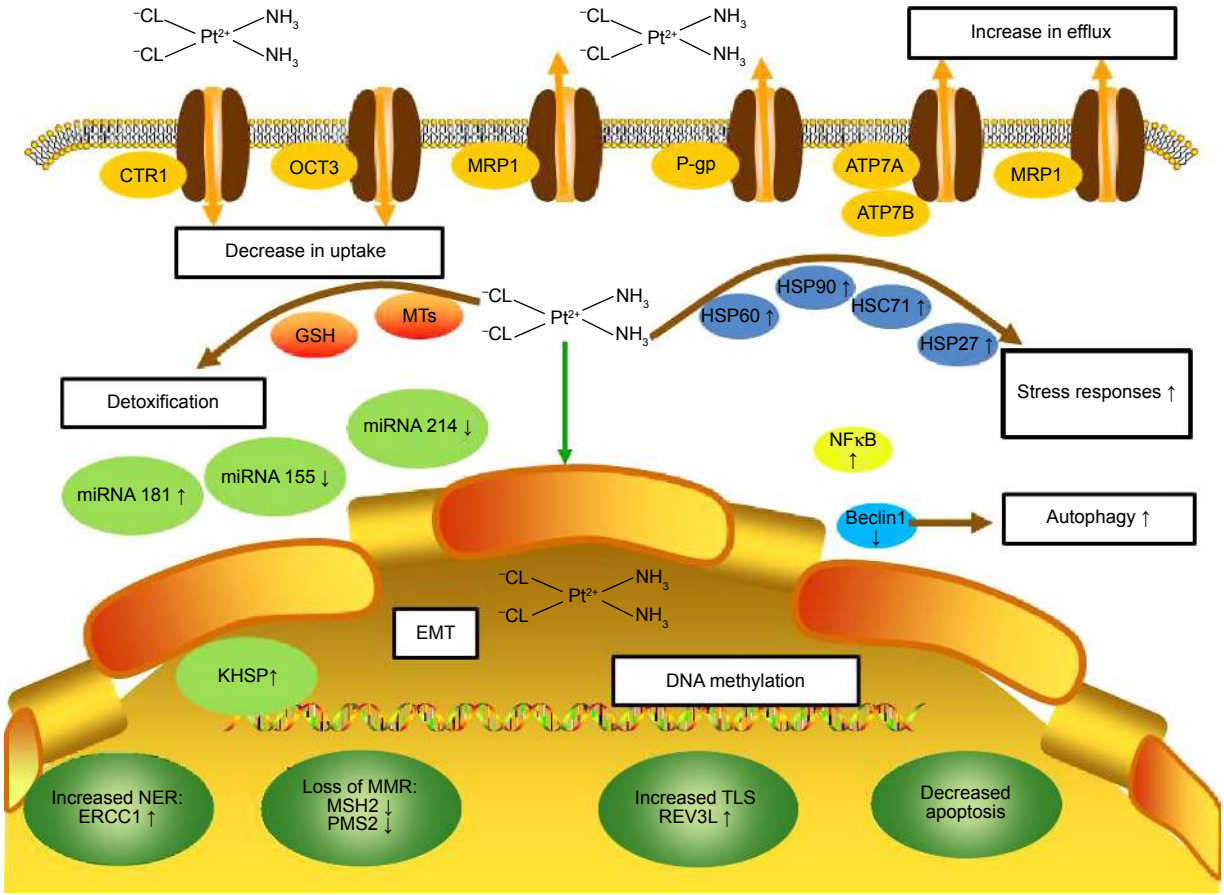

Figure 1 Molecular mechanisms of CPR in cervical cancer.

Notes: The molecular mechanisms underlying cisplatin resistance in cervical cancer are complex and associated with the following features: 1) reduction in the intracellular accumulation of the platinum compounds (decrease in uptake, increase in efflux, and increased drug detoxification by cellular thiols); 2) increase in DNA damage repair (increased NER, loss of MMR, and increased TLS); 3) inactivation of apoptosis; 4) activation of EMT; 5) alteration in DNA methylation, microRNA profile, cancer stem cell characteristics, and expression of stress-response chaperones.

Abbreviations: CPR, cisplatin resistance; CTR1, copper transporter 1; EMT, epithelial–mesenchymal transition; ERCC1, excision repair cross-complementing; GSH, glutathione; HSC71, heat-shock cognate protein 71; HSP, heat-shock protein; MMR, mismatch repair; MRP1, multidrug resistance protein 1; MSH2, MutS homolog 2; MTs, metallothioneins; NER, nucleotide excision repair; NF-κB, nuclear factor-κB; OCT3, organic cation transporter 3; P-gp, P-glycoprotein; PMS2, post-meiotic segregation 2; TLS, translesion synthesis.

reduced compared with the parental cells after a short-term drug exposure.[8] Thus, impaired uptake may contribute to the development of CPR in cervical cancer cells.

Up to now, the complex molecular mechanism by which cisplatin enters cells remains poorly understood. Cisplatin is generally believed to pass the cell membrane via passive diffusion, and the diffusion rate is associated with cisplatin lipophilicity.[9,10] Recently, copper transporter 1 (CTR1), which is a transmembrane protein and involved in the maintenance of copper homeostasis, has been recognized to regulate the influx of cisplatin and its analogs into the cells. CTR1 is downregulated in various CPR cell lines including HeLa-CPR cells.[11] HeLa cells overexpressing CTR1 show 2.2-fold increase in cisplatin accumulation compared with the mock-transfected cells.[12] Du et al[12] found that the C-terminus of CTR1 protein was required for cisplatin uptake in HeLa cells. In a mouse model of cervical cancer, Ishida et al[13] demonstrated that the level of DNA–cisplatin adducts correlated with CTR1 mRNA level in various organs, suggesting that CTR1 may regulate cisplatin uptake in vivo. In contrast, overexpression of CTR1 in the parental cells A431 and the CPR cells A431/Pt does not affect cisplatin uptake and the sensitivity of the cells to cisplatin.[7,13] These results suggest that the role of CTR1 in cisplatin transmembrane transport may vary in different types of cervical cancer cells.

Increased efflux

Previous studies suggested that adenosine 5′-triphosphate (ATP)-binding cassette (ABC) transporters, including multidrug resistance proteins (MRPs), MRP1, MRP2, MRP3, and MRP5, might mediate CPR by increasing cisplatin export.[14,15] MRP1 overexpression has been found to be associated with CPR in some cervical cancer cells.[16] MRP2 contributes to an increased cisplatin efflux in CPR human hepatic cancer cells, embryonic kidney cells, and melanoma cells.[17–19] Contrarily, MRP2 expression is reduced significantly in CPR cervical cancer KB-CP20 cells,[20] but increased in the cisplatin-sensitive KB-8-5-11 cells,[21] indicating that MRP2 is inversely associated with CPR in cervical cancer cells.

In addition to MRPs, P-glycoprotein (P-gp, ABCB1), which is also an ABC transporter, mediates the efflux of cisplatin conjugates and consequently promotes CPR. P-gp is overexpressed in CPR cervical cancer cell line SiHaR.[16] P-gp expression increases rapidly when HeLa cells are exposed to cisplatin,[22] and overexpression of P-gp attenuates cisplatin-induced apoptosis in HeLa cells. However, Takara et al[23] found that P-gp activity and expression were reduced in CPR HeLa subline. Similarly, Okada et al[24] showed that neither the non-P-gp-specific inhibitor probenecid (an inhibitor of multiple ABCs) nor the P-gp-specific inhibitor verapamil affected the sensitivity to cisplatin in CPR HeLa cells. These results suggest that P-gp may not substantially contribute to CPR in HeLa cells.

ATP7A and ATP7B, which are copper-transporting ATPases, are also involved in cisplatin efflux. Beretta et al[7] found that CPR A431 cells had higher ATP7A expression than the parental cells. In addition, upregulation of ATP7A and ATP7B was associated with acquired platinum resistance in CPR HeLa subline.[11] Contrarily, the cisplatin-sensitive KB-8-5-11 cells had reduced ATP7A protein expression and *ATP7B* gene expression.[21]

Organic cation transporter 3 (OCT3), a widely expressed transporter for endogenous and exogenous organic cations, is also found to be associated with cisplatin transport. The CPR cervical adenocarcinoma KB-CP20 cells express extremely less OCT3 than the parental cell lines. OCT3 overexpression significantly increases intracellular cisplatin accumulation and cytotoxicity, while downregulation of *OCT3* by small interfering RNA or chemical inhibitors increases the resistance of cervical cancer cells to cisplatin.[20]

Lysosome-associated protein transmembrane 4β-35 (LAPTM4B-35) is a member of the mammalian 4-tetratransmembrane spanning protein superfamily. Previous studies have shown that LAPTM4B-35 was overexpressed in malignant tissue specimens and significantly correlates with poor prognosis of cervical cancer.[25] Recently, Li et al[26] found that LAPTM4B-35 interacted with P-gp to inhibit apoptosis by activating PI3K/AKT signaling and induced multidrug resistance, such as resistance to doxorubicin, paclitaxel, and cisplatin, in cervical cancer cells by promoting drug efflux.

Thiol-containing protein-mediated inactivation

Cisplatin–DNA adducts induce DNA damage, which in turn leads to cytotoxicity. After cisplatin enters the cells, some cisplatin molecules bind to DNA and thus activate the DNA damage-induced apoptosis cascade,[15] while others can avidly bind to cytoplasmic nucleophilic species, such as glutathione (GSH), methionine, metallothioneins (MTs), and thiol-containing proteins. The binding of cisplatin to the thiol-containing nucleophilic species or thiol-containing proteins not only depletes intracellular antioxidant reserves to promote oxidative stress but also reduces the availability of reactive cisplatin.[15]

GSH, which is a thiol-containing tripeptide (Glu-Cys-Gly), can bind to cisplatin to prevent cisplatin from binding to DNA and other targets, quench proapoptotic reactive oxygen

species that are often generated by cisplatin, and reduce the sensitivity of the cells to cell death signals.[15] A large body of evidence suggests that an increased expression of enzymes that promote GSH synthesis and conjugation, such as GSH-S-transferase (GST), gamma-glutamyl cysteine synthase, and gamma-glutamyl transferase, may contribute to the development of CPR. The association between the expression of GSH conjugates and CPR in cervical cancer appears controversial. GSH has been shown to positively correlate with CPR in several cervical cancer cell lines.[24,27,28] KB-8-5-11 cells, which carry platinum-sensitive phenotype, have reduced expression of GSTA4, GSTK1, and GSTP1. Correspondingly, increased gene expression of *GSTP1*, *GSTA4*, and *GSTK1* is associated with the development of CPR in the cervical cancer cell line.[21] In contrast, Konishi et al[29] found that in patients with locally advanced or bulky cervical carcinoma, poor response to cisplatin significantly correlated with the expression of P-gp but not with GST-pi. Similarly, Chao et al[30] showed that neither intracellular GSH level nor GST activity was elevated in CPR HeLa cells. Roy and Mukherjee[16] also found that GSH level remained unaltered in the CPR clone SiHaR, which derived from SiHa. Thus, the role of GSH and its metabolism in CPR in cervical cancer remains to be determined.

MTs are low-molecular-weight thiol-containing proteins and regulate metal homeostasis and detoxification. MTs can bind to cisplatin, thus leading to the development of CPR phenotype.[31] Mellish et al[27] investigated the role of MTs in CPR in five human cervical squamous carcinoma cell lines and found a significant correlation between an increased expression of MTs and CPR.

Increased DNA repair and CPR

In CPR cancer cells, inter- and intra-strand DNA adducts often fail to trigger apoptotic cascade for many reasons.[15] Tumor cells with acquired CPR show an enhanced capability to repair cisplatin-induced DNA lesions or to tolerate high level of unrepaired DNA lesions compared with their parental cisplatin-sensitive counterparts.[15] A high level of repair-associated DNA strand breaks and an enhanced activity of DNA excision repair have been found in CPR HeLa cells.[6,32] Cisplatin-induced DNA lesions, which often cause DNA distortion, can be identified by multiple DNA repair pathways, among which nucleotide excision repair (NER) and mismatch repair (MMR) are the predominant DNA repair mechanisms.

Nucleotide excision repair

NER, which is a highly conserved DNA repair pathway and a major pathway for the repair of DNA–cisplatin adducts,[33] usually targets on the DNA damages that change the DNA helical structure and interfere in DNA replication and transcription.[34] More than 20 proteins participate in NER, including excision repair cross-complementation group 1 (ERCC1). ERCC1 is a single-strand DNA endonuclease and forms a tight heterodimer with ERCC4 to incise DNA on the 5′ side of bulky lesions such as DNA–cisplatin adducts.[15] ERCC1 expression is upregulated in CPR cervical cancer cells HCA-1R,[35] and in patients with locally advanced cervical squamous cell carcinoma. ERCC1 expression negatively correlates with responsiveness to cisplatin.[36] Thus, the level of ERCC1 expression may predict responsiveness to cisplatin in patients with cervical cancer. Furthermore, low ERCC1 expression is an independent prognostic factor and associated with a survival benefit in patients receiving adjuvant cisplatin chemotherapy or chemoradiotherapy with cisplatin.[37,38]

DNA mismatch repair

DNA MMR, an evolutionarily conserved process, corrects mismatches that are generated during DNA replication and escape from DNA proofreading.[34] A properly functioned MMR system is required to detect cisplatin-induced DNA lesions. Thus, MMR deficiency may lead to the development of DNA damage tolerance and CPR. Among MMR proteins, MutS homolog 2 (MSH2) protein has been shown to contribute to the development of CPR in cervical cancer cells.[39] Lanzi et al[8] found that MSH2 protein expression in CPR A431 cells was significantly less than that in the parental cells. Post-meiotic segregation 2 (PMS2) is also a major component of the MMR system. Zhang et al[40] found a marked downregulation of PMS2 in human cervical carcinoma tissue, and over-expression of PMS2 in HeLa cells dramatically increased cisplatin-induced apoptosis and caspase-3 activity. Thus, upregulation of PMS2 appears to enhance the sensitivity of HeLa cells to cisplatin. Additionally, REV3L, the catalytic subunit of DNA polymerase ζ (Polζ), plays a key role in skipping DNA damage during translesion synthesis. Yang et al[41] demonstrated that REV3L conferred resistance to cisplatin in cervical cancer cells by regulating apoptosis rate and the expression of antiapoptotic proteins, such as B-cell lymphoma 2 (Bcl-2), myeloid cell leukemia sequence 1 (Mcl-1), Bcl-extra large (Bcl-xL), and proapoptotic Bcl-2-associated x protein (Bax).

Inactivation of apoptosis pathway and CPR

Cisplatin-induced apoptosis is essential for the anticancer effect of cisplatin. Cisplatin stimulates apoptosis by

triggering the extrinsic death receptor pathway or the intrinsic mitochondrial pathway. Multiple proteins such as the Bcl-2 family proteins and p53 and several signaling pathways including mitogen-activated protein kinase (MAPK) pathway and nuclear factor-κB (NF-κB) pathway are involved in the extrinsic and intrinsic apoptosis pathways. Dysfunction of these proteins and signaling pathways may lead to the development of CPR (Figure 2). Brozovic et al[42] found that HeLa cells with acquired CPR showed lower level of cisplatin-induced apoptosis and lower levels of Bcl-2 and p-Bad than the parental cells. In addition, SiHa cells, which are more resistant to cisplatin than HeLa cells, showed reduced activity of caspase-3, caspase-8, and caspase-9 and cisplatin-induced cleavage of poly(adenosine diphosphate [ADP]-ribose) polymerase compared with HeLa cells.[43]

Caspases

Caspases play critical roles in apoptosis. The activity of caspase-3, caspase-8, and caspase-9 is decreased in CPR cells.[44] Compared with drug-sensitive parental cells, the multidrug-resistant endocervical HEN-16-2/CDDP cells are more resistant to apoptosis and exhibit reduced caspase-3 activation.[45]

Bcl-2 protein family

Exposure to cisplatin initiates programmed cell death. Bcl-2 protein family contains three functionally and structurally distinct subfamilies: 1) anti-apoptotic proteins, including Bcl-2, Bcl-xL, Mcl-1, Bcl-w, Bag-1, and A1; 2) proapoptotic effector proteins Bax and Bak; 3) proapoptotic BH3-only proteins. These proteins tightly control apoptotic process by regulating the permeability of the mitochondrial outer membrane and the release of cytochrome c and other proapoptotic factors. The release of proapoptotic factors activates caspases.[46]

CPR cervical cancer cells frequently overexpress anti-apoptotic proteins.[47] For example, Brozovic et al[42] found that Bcl-2 was upregulated in CPR HeLa cells compared with the parental cells. The overexpression of Bcl-2 was related to poorer clinical outcome in patients with cervical cancer receiving CDDP-based chemoradiotherapy.[48] The multidrug-resistant endocervical HEN-16-2/cisplatin cells show significantly higher level of Bcl-xL and Bag-1 than the drug-sensitive parental cells.[45] Similarly, overexpression of Bag-1L prevents cisplatin-induced apoptosis by affecting Raf/Ras signaling and the expression of Mcl-1 and heat-shock proteins (HSPs) in HeLa cells.[49] Furthermore, stable overexpression of Bag-1 promotes resistance to cisplatin-induced apoptosis in C33A cells.[47] In addition to the upregulation of anti-apoptotic proteins, suppression of proapoptotic effector proteins also contributes to the development of CPR.[44]

MAPK pathway

MAPKs play critical roles in the complex intracellular signaling network, which regulates gene expression in response to various extracellular stimuli.[50] In mammalian cells, there are mainly three types of MAPKs: stress-activated protein kinase/c-Jun-N-terminal kinase (SAPK/JNK), p38 kinase, and extracellular signal-regulated kinase (ERK). The association of MAPK activation and CPR has recently been recognized. CPR cancer cells often have reduced MAPK activity. Cisplatin activates SAPK/JNK, p38 kinase, and ERK dose dependently in cisplatin-sensitive HeLa cells, whereas

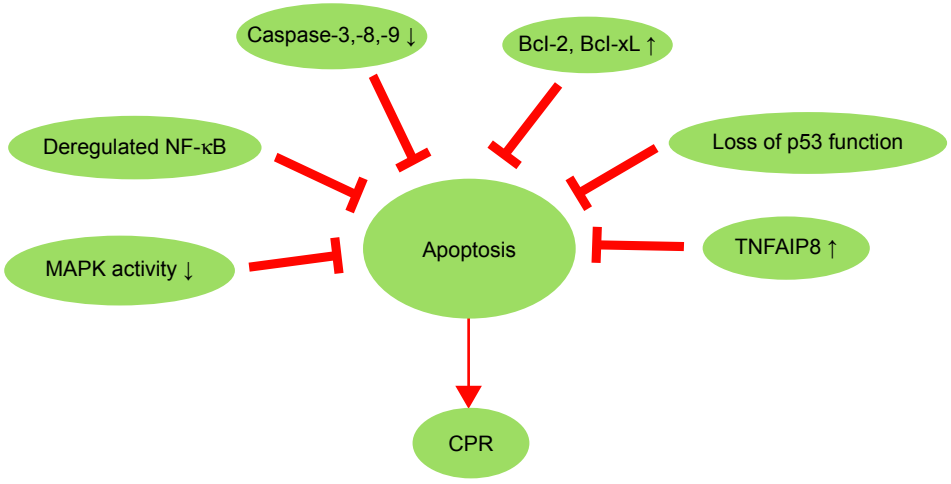

Figure 2 Inactivation of apoptosis pathway and CPR in cervical cancer.
Note: Multiple molecules and signaling pathways that inhibit apoptosis can lead to CPR.
Abbreviations: CPR, cisplatin resistance; MAPK, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; TNFAIP8, tumor-necrosis-factor-α-induced protein 8.

cisplatin-mediated activation of SAPK/JNK is significantly reduced in the CPR subline.[42,51,52] In addition, inhibition of JNK, p38 kinase, or ERK attenuates cisplatin-induced apoptosis and cell death.[42,51,52] Similarly, blockade of MEK–ERK by MEK inhibitor PD98059 induces CPR in human cervical carcinoma SiHa cells.[53] Thus, sufficient MAPK activation appears to be required for cisplatin-induced apoptosis.

P53 signaling pathway

Stabilization and activation of wild-type *p53* are critical for cisplatin-induced apoptosis. Thus, loss of p53 can impair apoptosis process and lead to tolerance of DNA damage, consequently promoting drug resistance.[44] Sultana et al[54] demonstrated that chemosensitivity was associated with p53-Bax-mediated apoptosis in cervical cancer. Cisplatin-based chemotherapy for cervical cancer is more beneficial to patients harboring wild-type *p53* than to patients with mutated *p53*.[55] Additionally, patients who respond to cisplatin well show a higher proportion of p53-positive cells than nonresponders.[56] Overexpression of p53 is a predictive factor for chemoresistance in adenocarcinoma of the uterine cervix.[57]

NF-κB pathway

NF-κB, usually in the form of heterodimer or homodimer formed by the NF-κB family members, is ubiquitously expressed and regulates >500 genes that are involved in immunoregulation, inflammation, growth regulation, apoptosis, and carcinogenesis.[58] Numerous in vitro and in vivo studies, including ours, have demonstrated that constitutive activation of NF-κB inhibits chemotherapy-induced apoptosis in different types of cancer, including cervical cancer.[43,59] We found that NF-κB contributed to CPR in human cervical cancer SiHa cells and inhibition of NF-κB sensitized SiHa cells to cisplatin-induced apoptosis.[59] This study suggests that combination of cisplatin and NF-κB inhibitors may have therapeutic potential.

Others

The tumor-necrosis-factor-α-induced protein 8 (TNFAIP8) family, which is a newly identified and poorly characterized group of proteins, is found to play a role in the maintenance of immune homeostasis and inhibition of apoptosis. Shi et al[60] reported that high protein level of TNFAIP8 was significantly associated with CPR and poor clinical outcomes in patients with cervical cancer.

Activation of EMT and CPR

In recent years, the role of EMT in acquired CPR has been increasingly recognized. Our previous study found that 7-day low-concentration cisplatin (1 μM) treatment induced EMT in cervical cancer HeLa and C4-1 cell lines by activating transforming growth factor-β pathway. *TWIST1*, which is a highly conserved transcription factor and belongs to the basic helix-loop-helix protein family, plays a key role in EMT. In cervical cancer, TWIST1 expression appears to correlate with MDR1/P-gp expression positively. In HeLa cells, silencing *TWIST1* expression by RNAi downregulates MDR1/P-gp expression, suppresses cell proliferation, inhibits rhodamine 123 efflux, and sensitizes the cells to cisplatin.[61] In addition to TWIST1, other proteins that are involved in EMT have also been found to be associated with CPR. Shen et al[4] found that human cervical cancer cells with high CPR overexpressed SNAIL1 and E-cadherin, whereas the cells with mild CPR did not. These results indicate that overexpression of SNAIL1 and E-cadherin may occur at late stage of CPR development and thus facilitate cell survival under high-dose platinum.[4] Astrocyte elevated gene-1 (AEG-1), which can be induced by human immunodeficiency virus-1 (HIV-1), was initially identified from human fetal astrocytes.[62] Knockdown of AEG-1 blocks EMT and reduces CPR in cervical cancer cells.[63] These findings indicate that EMT may contribute to the development of CPR in cervical cancer.

MicroRNA and CPR

MicroRNAs have been found to regulate multiple pathways that are involved in the cellular response to cisplatin.[64] The effects of microRNAs on the development of CPR in cervical cancer have been investigated. Pouliot et al[65] found that the miR-181 family members were overexpressed in CPR cells KB-CP5 and KB-CP20 compared with the parental KB-3-1 cells, and silencing the proteins that are essential for microRNA synthesis, such as DICER and TRBP2, reversed CPR in the cells. Chen et al[66] found that in cervical squamous cell carcinoma, upregulation of miR-181 appeared to correlate with CPR significantly and miR-181a inhibited apoptosis and enhanced CPR by targeting protein kinase C-δ (PRKCD). In contrast to miR-181, other microRNAs can increase drug sensitivity. Lei et al[67] found that miR155 suppressed epithelial-growth-factor-induced EMT, decreased migration/invasion, inhibited cell proliferation, and increased the sensitivity to cisplatin in human CaSki cervical cancer cells. Wang et al[68] showed that miR-214 increased the sensitivity of HeLa cells to cisplatin by directly inhibiting Bcl2l2 expression and increasing the expression of Bax, caspase-9, caspase-8, and caspase-3. In addition, KH-type splicing regulatory protein, which interacts with Drosha and increases the binding and subsequent processing of specific pri-microRNAs such as pri-let7a-1 and pri-miR-21,[64] is upregulated in the nucleus

of HeLa cells that are exposed to cisplatin.[69] These results indicate that the levels of some microRNAs may predict patient response to cisplatin.

Cancer stem cells and CPR

Cancer stem cells are highly resistant to various chemotherapeutic agents, because they express abundant levels of multiple drug resistance transporters such as MDR1/P-gp and mitoxantrone resistance protein (MXR)/breast cancer resistance protein-1 (BCRP-1).[50] Liu and Zheng[70] demonstrated that aldehyde dehydrogenase (ALDH) was a marker for cervical cancer stem cells and high ALDH activity was associated with an increased resistant to cisplatin in cervical cancer cells. In addition, the CPR clone R-ME-180 can form three-dimensional multicellular spheroids, which show positive expression of the cancer stem cell marker ALDH, but the parental ME-180 cells do not express the marker.[71] Wang et al[72] enriched cervical cancer stem cells by using a nonadhesive culture system and found that the cervical cancer stem cells had high resistance to cisplatin.

DNA methylation and CPR

DNA methylation plays a critical role in the development of CPR in several cell culture models. Aberrant DNA methylation may alter the expression of genes that are critical to drug response and thus affect the sensitivity of cancer cells to cisplatin.[4] Epigenetic drugs such as 5-azacytidine have been shown to block aberrant DNA methylation and thus reverse CPR.[64] Combination of cisplatin and 5-aza-2′-deoxycytidine, a demethylating agent, significantly enhances the sensitivity to cisplatin in ME180 parent cells and the CPR subclones, and the removal of 5-aza-2′-deoxycytidine restores the CPR of the subclones rapidly.[73]

Stress response chaperones and CPR

Molecular chaperones that are involved in the general stress responses, such as autophagy and HSPs, can promote CPR via multiple, most often indirect, mechanisms.[10,15] Autophagy, a process by which cells digest their own damaged organelles, has been recently shown to contribute to CPR in some cell types and inhibition of autophagy can enhance the cytotoxicity of chemotherapeutic agents.[74] Cisplatin induces autophagy in HeLa cells, and inhibition of autophagy induces endoplasmic reticulum (ER) stress and thus enhances cisplatin cytotoxicity.[75] Beclin-1, an autophagy-related molecule, plays a critical role in the regulation of response to chemotherapeutic agents. In CaSki cells, overexpression of Beclin-1 promotes apoptosis signaling and thus sensitizes the cells to cisplatin.[76] NH_4Cl, an autophagy inhibitor, blocks the activation of lysosomal enzymes and the degradation of autolysosome components. NH_4Cl has been found to increase DNA damage and consequently enhance cisplatin cytotoxicity in HeLa cells.[77] Similarly, pretreatment with the autophagy inhibitor bafilomycin sensitizes both cervical adenocarcinoma HeLa cells and squamous cell carcinoma CaSki cells effectively to cisplatin.[78]

The function of HSPs is to adapt the cells to high temperature or other stressful conditions, and HSPs promote conformational change in target proteins and protect the cells from oxidant-induced DNA damage and apoptosis.[4] Because cisplatin induces DNA damage and consequently results in apoptosis, HSPs are probably affected during the development of CPR.[4] Shen et al[79] used two-dimensional gel electrophoreses and amino acid micro-sequencing and found HSP 60 overexpression in human cervical CPR cells. Castagna et al[80] further confirmed that the expression of heat shock cognate protein 71 (HSC71) and HSP 60 was significantly increased in the CPR cells, A431/Pt cells, compared with the parental A431 cells. Furthermore, Zhang and Shen[31] demonstrated that HSP 27 suppressed cisplatin-induced ASK1/p38 and Akt activation, which consequently impaired the cytotoxic response of HeLa cells to cisplatin. The HSP 90 inhibitor PU-H71 (8-[(6-iodo-1,3-benzodioxol-5-yl) sulfanyl]-9-[3-(propan-2-ylamino)propyl]purin-6-amine) induces ER stress and thus promotes apoptosis in HeLa cells, and HeLa cells treated with PU-H71 overcome the CPR conferred by Bcl-2.[81] These results indicate that HSPs may contribute to CPR.

System biology of CPR

System biological approach has been used to investigate CPR.[10] In our previous studies on proteomics profiling, we found that protein levels of S100A8 and S100A9 were increased and annexin A2 level was decreased in patients with cervical cancer who failed to response to cisplatin-based neoadjuvant chemotherapy treatment.[82,83] S100A8, S100A9, and annexin A2 are Ca^{2+}-binding proteins and play roles in immune response, vesicle trafficking, cell division, apoptosis, and calcium signaling. Chavez et al[84] conducted quantitative proteomics, analyzed protein interaction network, and found that 374 proteins were differentially expressed in CPR versus cisplatin-sensitive HeLa cells. Those differentially expressed proteins are involved in DNA binding, DNA damage repair, energy-producing metabolic pathways, and stress response.[84] Castagna et al[80] used a similar approach to discover that calmodulin, microtubule-associated protein, and stathmin were downregulated in CPR A431 subline. Wu et al[85] performed gene expression profiling on CPR HeLa cells and

Table 1 Agents to overcome CPR in cervical cancer

Compound	Type of study	Mode of action	References
Genistein	In vitro	Inhibition of NF-κB and Akt/mTOR pathways	Sahin et al[93]
Curcumin	In vitro	Modulation of multidrug-resistant proteins such as MRP1 and P-gp1	Roy and Mukherjee[16]
Tea polyphenols	In vitro	Induction of apoptosis	Singh et al[94]
Melatonin	In vitro	Induction of apoptosis	Pariente et al[95]
Mifepristone	In vitro, in vivo	Inducing apoptosis, increasing cisplatin, accumulating and upregulating p53	Segovia et al[96] Jurado et al[97] Li and Ye[98]
Epigallocatechin gallate	In vitro	Regulating NF-κB p65, COX-2, p-Akt, and p-mTOR pathways	Kilic et al[100]
Ursolic acid	In vitro	Inhibiting NF-κB activation	Li et al[101]
Morinda citrifolia	In vitro	Altering oxidative stress marker and antioxidant activity, inducing apoptosis	Gupta and Singh[102] Gupta et al[103]
Wogonin	In vitro	Accumulation of intracellular reactive oxygen species and induced apoptosis	He et al[104]
Pyrrolidine dithiocarbamate	In vitro	Blocking cisplatin-induced activation of NF-κB, enhancing apoptosis	Zheng et al[59]

Abbreviations: CPR, cisplatin resistance; MRP1, multidrug resistance protein 1; NF-κB, nuclear factor-κB; P-gp1, P-glycoprotein 1.

identified nine genes (*NAPA, CITED2, CABIN1, ADM, HIST1H1A, EHD1, MARK2, PTPN21*, and *MVD*), which were consistently upregulated in two CPR HeLa cell lines.

Conclusion and future direction

Cisplatin has been used to treat cervical cancer since the early 1980s[86] and remains to be the most effective anticancer agent for advanced/recurrent cervical cancer. However, chemoresistance may develop, and thus seriously hinders the use of cisplatin in clinical practice. Over the past 3 decades, great efforts have been devoted to characterize the molecular mechanisms underlying CPR in cervical cancer cells. Starting with the early 1990s, GSH[87] and NER[88] were identified as causative of resistance to cisplatin. In the early 2000s, CTR1 turned out to play a critical role in the uptake of cisplatin.[89] In the mid-2000s, EMT and microRNA have been implicated in the development of drug resistance. In the last decade, the advent of high-content and high-throughput screening technologies has accelerated the discovery of the molecular mechanisms underlying CPR at cell-intrinsic level.[10] Ca^{2+}-binding proteins such as S100A8, S100A9, and annexin A2 and energy-producing metabolic pathways have been identified to be related to CPR in cervical cancer.[82] NAPA, a protein found in the ER, and CITED2, a transcriptional modulator, have been identified to confer resistance to cisplatin in HeLa cells.[85] Further investigation is required to obtain additional insights into CPR at protein, DNA, RNA, mRNA, and microRNA levels.

Based on the mechanisms underlying CPR, the following strategies have been proposed to overcome the resistance in cervical cancer: 1) develop new platinum drugs;[90,91] 2) improve cisplatin delivery to tumor;[71] 3) specifically target CPR mechanisms; and 4) combine cisplatin with other drugs.[92] The greatest challenge is that CPR often exhibits a multifactorial nature. Strategies that target one mechanism at a time may not sufficiently enhance the sensitivity to cisplatin. Thus, combination therapies targeting multiple mechanisms underlying CPR should be exploited. In our previous study, we found that a combination of pyrrolidine dithiocarbamate and cisplatin synergistically increased apoptosis and inhibited cell growth by suppressing NF-κB in human cervical cancer cells. Additionally, agents including genistein,[93] curcumin,[16] tea polyphenols,[94] melatonin,[95] mifepristone,[96–98] epigallocatechin gallate,[99,100] ursolic acid,[101] *Morinda citrifolia*,[102,103] and wogonin[104] have been shown to be beneficial to overcome CPR via targeting the molecular pathways that are involved in CPR (Table 1). Understanding the molecular mechanisms underlying CPR may be helpful to identify patients with a potential to develop CPR, and thus oncologists may be able to provide an effective therapy for those patients.

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Disclosure

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