Mechanisms of Chemotherapeutic Drug Resistance in Cancer Therapy—A Quick Review

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SUMMARY

Chemotherapy is one of the principal modes of treatment for cancer patients. Clinically, many tumors present a satisfactory response when they are first exposed to the chemotherapeutic drugs. However, drug resistance occurs sooner or later in these tumors, and the majority of the patients develop progressive disease. The mechanisms of treatment failure of chemotherapeutic drugs have been well studied. Via a unique protection system, i.e. multidrug resistance (MDR), the cancer cells can escape the toxic effect of most commonly used cancer drugs in spite of their different chemical structures and different mechanisms of intracellular activity. There are two classes of transporter proteins at the cellular surface which are responsible for MDR in tumors. One is the adenosine triphosphate-binding cassette transporter superfamily, which is an energy-requiring efflux pump with the function of extruding toxic chemotherapeutic drugs from the cancer cells. The other is the solute carrier transporter superfamily, which mediates the cellular uptake of anticancer drugs, and drug resistance may result from decreased activity of these transporters. Although transporters of MDR are responsible for the tumor resistance to many chemotherapeutic drugs currently used in cancer therapy, the mechanisms of resistance to platinum-based antitumor agents are through different pathways. In this article, the mechanisms of MDR transporters mediating resistance to the commonly used chemotherapeutic drugs and to platinum-based agents are reviewed. Finally, with the finding of cancer stem cells in more and more solid tumors, it is recognized that the cancer stem cell is spared along with its normal tissue stem cell counterparts with very subtle differences. One characteristic of the normal tissue stem cell is the self-protection ability through innate MDR transporters. Therefore, the essential self-protection property is also present in the cancer stem cells. The quiescent tumor stem cell with constitutive MDR is the main barrier to therapy. Successful cancer therapy will depend on the ability to discern the subtle differences between the tumor and normal stem cells so that approaches can be developed to eliminate the tumor stem cells without excessive toxicity to normal stem cells. [*Taiwan J Obstet Gynecol* 2009;48(3):239–244]

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Introduction

Although chemotherapy has led to improvement in the survival and quality of life of cancer patients, the majority of these patients eventually develop progressive disease after initially responding to treatment. Drug resistance represents a major obstacle to improving the overall response and survival of cancer patients. Tumors showing resistance to chemotherapy may present this character before encountering any chemotherapeutic drugs, being intrinsically resistant, such as melanoma and hepatoma. More tumors, however, may initially be sensitive to therapy and later become insensitive to similar drugs, having acquired resistance. Ovarian cancer is a prime example of these tumors. The phenomenon of acquired tumor resistance to chemotherapeutic drugs has been recognized for decades. It is known that several cell membrane transporter proteins are responsible for the resistance to many commonly used chemotherapeutic

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drugs by affecting disposition of these drugs in the tumor cells. However, resistance to another group of chemotherapeutic drugs, i.e. platinum (Pt)-based anticancer agents, is caused by different mechanisms. In this article, a concise review of the mechanisms of chemotherapeutic drug resistance is presented.

Multidrug Resistance (MDR)

MDR is a system of protection of the cell population against numerous compounds, including drugs characterized by different chemical structures and by different mechanisms of intracellular activity. There are two classes of membrane transporter proteins which influence the pharmacokinetics of drugs in cells, and their changes are responsible for MDR in tumors. One is the adenosine triphosphate-binding cassette (ABC) transporter superfamily, which is frequently associated with decreased cellular accumulation of hydrophobic anticancer chemotherapeutic drugs by extruding them from cells when these drugs diffuse down a concentration gradient into the cells. The other transporter superfamily, solute carrier transporters, commonly increases chemosensitivity by mediating the cellular uptake of hydrophilic anticancer drugs [1]. Chemotherapeutic drug resistance may result from decreased activity of these uptake transporters.

Proteins of the ABC family are characterized by the presence of a cytoplasmic adenosine triphosphate (ATP)-binding domain with a specific structure, i.e. a nucleotide-binding domain (NBD). It functions to harvest energy from ATP hydrolysis so that the transporters can act as efflux pumps to remove various intracellular chemotherapeutic drugs. ABC transporters contain another membrane-spanning component, the transmembrane domain (TMD). It is composed, in most cases, of six membrane-spanning helices. The TMD offers the binding site for substrates or chemotherapeutic drugs for translocation from the cytoplasm to the cell membrane.

Human proteins of the ABC family are divided into seven subfamilies (class A to G) based on the domain organization, namely the number and combination of TMDs and NBDs. In total, there are 49 protein members in this family, but only three are well known for their MDR [2]. They are P-glycoprotein (P-gp; MDR1/ABCB1), MDR-associated protein (MRP1; ABCC1), and breast cancer resistance protein (BCRP; ABCG2). All three extrude various hydrophobic compounds and, therefore, have broad and, to a certain extent, overlapping substrate specificities in transporting the major drugs currently used in cancer chemotherapy. In addition, MRP1 and ABCG2 can expel xenobiotics and intracellularly formed metabolic products.

P-glycoprotein (P-gp, MDR1, ABCB1)

P-gp was the first identified ABC transporter and is the product of the human *ABCB1* gene, localized to chromosome 7q21. P-gp is a membrane-bound glycoprotein consisting of 1,280 amino acids. According to sequence analysis, P-gp is constructed in the form of a so-called full transporter, i.e. it is composed of two homologous half transporters with each one containing a TMD and NBD. The two TMDs and two NBDs of P-gp are arranged in the sequence of TMD1-NBD1-TMD2-NBD2 with a linker region connecting the two half transporters [3]. The linker region plays a critical role in ensuring proper interaction of the two subunits. With deletion of the central core of the linker region, a P-gp protein is still expressed at the cell membrane at levels similar to the wild-type protein, but is not functional for either transport or drug-stimulated adenosine triphosphatase activity [4]. It is suggested that the linker region is most likely to provide communication between the two ATP sites of NBD. P-gp transports neutral and cationic hydrophobic compounds, such as the natural products vinblastine, vincristine, doxorubicin, daunorubicin, actinomycin D, etoposide, and paclitaxel. Drug transport by P-gp is coupled to two hydrolyses of ATP. The first ATP hydrolysis, converting ATP to adenosine diphosphate (ADP), is essential for nucleoside diphosphate trapping into P-gp to form a transition-state intermediate of the P-gp complex. Following the first ATP hydrolysis, the drug is extruded from P-gp and the ADP dissociates from the complex. An additional molecule of ATP then binds to the alternate ATP site on P-gp. It is hydrolyzed and the disassociation of ADP allows the conformation of P-gp to be restored to its original state, to initiate the next cycle of drug transport [5].

P-gp expression is regulated by various factors. Mutation of the p53 gene and overexpression of the p63 gene and/or the p73 gene in certain tumors may facilitate P-gp expression. *ABCB1* promoter activation by a nuclear protein, MDR1 promoter-enhancing factor, or a component of the multiprotein complex, RNA helicase A, may upregulate P-gp expression. Epigenetic methylation, in contrast, results in silence of *ABCB1*. Other factors which activate P-gp expression include random chromosomal rearrangement, stress signals, such as heat shock, inflammation and hypoxia, exposure to xenobiotics, toxic metabolites, ultraviolet radiation, and glucocorticoids. Chromatin-modifying enzymes, like histone

acetylases and histone deacetylases, were found to be involved in the regulation of the *ABCB1* gene [3].

Despite 30 years of P-gp research, there is still no clear therapeutic strategy to overcome the actions of this classical drug efflux pump in tumors. Recently, it was found that the expression of a major glycosaminoglycan in the extracellular matrix, hyaluronan (HA), and its receptor, CD44, a cell surface marker for both normal and cancer stem cells, are tightly linked to MDR and tumor progression. In breast and ovarian cancer cell lines, HA-CD44 interaction may activate the stem cell marker, Nanog, which can further activate the expression of pluripotent stem cell regulators (Rex1 and Sox2) and Stat-3-mediated *ABCB1* gene expression. In addition, HA-CD44 binding may form a complex with ankyrin, the downstream effector of CD44. This complex formation results in an efflux of chemotherapeutic drugs. Anti-CD44 antibody not only blocks HA-CD44 binding but also inhibits ABCB1-mediated efflux activity. Thus, anti-CD44 antibody may be used in combination with chemotherapy to enhance chemosensitivity [6].

MDR-associated Protein (MRP1, ABCC1)

The human *MRP1* gene is mapped to chromosome 16p13.1. It encodes a membrane-bound glycoprotein consisting of 1,531 amino acids. This protein has a similar topologic structure to that of P-gp. However, in addition to the two half transporters connected by a linker region L1 as in P-gp, MRP1 protein contains an extra N-terminal segment, i.e. TMD0, which connects TMD1 with a L0 linker region. The L0 linker region is essential for drug transport, whereas TMD0 is not required for transport [7].

Although MRP1 also requires two ATPs as the energy source to transport chemotherapeutic drugs, the mechanism in the cycle of transportation is somewhat different from that of P-gp. In P-gp, the functions of the two NBDs are "equal", and the two ATP-binding sites operate randomly but alternately. In MRP1, the function of NBD1 and NMD2 is nonequivalent, i.e. NBD1 has higher affinity than NBD2 for ATP. Therefore, when the substrate binds to TMDs of MRP1, the conformational change of MRP1 protein first induces ATP binding at NBD1. It then further alters the conformation of the protein and enhances ATP binding at NBD2. When both NBD1 and NBD2 are occupied by the two ATPs simultaneously, the bound substrate is transported out of the cell. After substrate extrusion, the ATP bound at NBD2 is hydrolyzed first. The release of ADP and inorganic phosphate from NBD2 partially brings the MRP1 protein back to its original conformation, and facilitates the dissociation of ATP bound at NBD1. Subsequent release of ADP and inorganic phosphate from NBD1 returns the MRP1 protein to its original conformation.

MRP1 is expressed almost ubiquitously in many different organs and cell types. Unlike P-gp, which is invariably located in the apical membranes of epithelial cells, MRP1 is located basolaterally and tends to pump drugs into the body, rather than excrete them into the bile, urine or gut. Cells overexpressing MRP1 protein are resistant to a wide variety of anticancer drugs, e.g. doxorubicin, epirubicin, vinblastine, vincristine, and etoposide. However, MRP1 cannot transport the unmodified anticancer drugs without the presence of glutathione (GSH). This implies that MRP1 may co-transport the anticancer drugs with GSH, or GSH may bind to the MRP1 protein to enhance the transport of these hydrophobic anticancer drugs across biological membranes [7].

Until now few MRP1-specific inhibitors have been developed. The lack of success of clinical trials attempting to reverse P-gp-mediated drug resistance left many hesitant to attempt studies to inhibit MRP1 clinically.

Breast Cancer Resistance Protein (BCRP, ABCG2)

The *ABCG2* gene is localized to chromosome 4q21–4q22. It encodes a plasma membrane glycoprotein of 655 amino acids. This gene was first cloned from a heavily drug-selected breast cancer cell line (MCF-7/AdrVp) and was named breast cancer resistance protein (BCRP) [8].

Compared with P-gp and MRP1, ABCG2 is a half transporter in structure. It contains only one TMD and one NBD and shows a reverse domain arrangement, i.e. NBD-TMD sequence. Two ABCG2 molecules form a functioning homodimer by a disulfide (S-S) bridge so that the ABCG2 can have two NBDs and two TMDs for drug transportation [3].

ABCG2 is physiologically expressed in a variety of tissues, most abundantly in the liver and intestinal epithelium, the placenta, the blood–brain barrier, and various stem cells. In tumors, overexpression of ABCG2 was documented in drug-selected cell lines from ovary, lung, breast, colon, and gastric cancer. Clinically, the chemotherapy response rate in patients with non-small cell lung cancer was found to be correlated with ABCG2 expression [3].

The substrate transport and ATP cleavage cycle of ABCG2 has not yet been investigated in as much detail

as for P-gp or MRP1, but it is speculated to have no major differences in the basic steps. ABCG2 substrates include many kinds of chemotherapeutic drugs and some molecularly targeted drugs, such as mitoxantrone, topotecan, irinotecan, methotrexate, doxorubicin, epirubicin, etoposide, gefitinib, and imatinib.

The expression of ABCG2 may be controlled by several factors. Sex hormones, such as estrogen, progesterone and testosterone, were shown to exert an effect on its expression, but conflicting data exist. ABCG2 expression is upregulated in the mammary gland during lactation. In a multiple myeloma system and a renal carcinoma system, ABCG2 promoter hypermethylation was linked to a decrease in ABCG2 expression. In addition, hypoxia can regulate ABCG2 expression. It was suggested that stem cells or tumor cells in hypoxic environments may be protected from chemotherapeutic agents because of increased levels of ABCG2 induced by hypoxia [9]. The list of reported ABCG2 inhibitors has been growing rapidly. However, none of them has been used in a clinical setting.

Mechanisms of Resistance to Pt-based Antitumor Agents

Although P-gp, MRP1, and ABCG2 are responsible for tumor resistance to many chemotherapeutic drugs currently used in cancer therapy, they play no roles in resistance to Pt-based antitumor agents such as cisplatin, carboplatin, and oxaliplatin.

Pt-based antitumor agents are taken up into the cells through a member of the solute carrier transporter superfamily, hCtr1, which is also a copper transporter at the cell membrane. There are three pathways for Pt after it is taken up into the cells. Pt can be exported by the copper efflux transporters, ATP7A and ATP7B. It may also interact with GSH in the cytoplasm to form $Pt(GS)_2$ complex and be eliminated by an ABC transporter, MDR-associated protein MRP2. Finally, a fraction of Pt may enter the nucleus and form Pt-DNA adducts [10]. The Pt atom of cisplatin in the Pt-DNA adducts binds covalently to the N-7 position of purines in the DNA to form 1,2- or 1,3-intrastrand cross-links, and interstrand cross-links [11]. However, 85–90% of DNA lesions caused by cisplatin are intrastrand cross-links.

DNA damage caused by cisplatin is recognized by DNA damage recognition proteins, such as high mobility group proteins (HMG1 and HMG2) and mismatch repair complexes (hMSH2 or hMutSα), which transduce DNA damage signals to various downstream effectors. Cell death or cell survival after DNA damage depends on the relative intensity of the signals generated and the crosstalk between the effectors involved. Among these effectors, the p53 tumor suppressor gene plays a central role in determining the final fate of the cell. DNA damage recognition proteins activate the mitogen-activated protein kinase signal transduction pathway, which then activates the function of p53 and causes cell cycle arrest at the G_2/M checkpoint for DNA repair. If the DNA damage is too excessive to repair, apoptosis occurs through the Bax and caspase system. In addition, DNA damage may also result in apoptosis through the p53-related gene, the p73 gene [12].

The regulation of Pt transport in cells is a complex network of regulation systems. It includes the following proteins which are responsible for cisplatin transportation:

1. Expression of hCtr1, ATP7A and ATP7B, which are regulated by intracellular copper homeostasis. Recent studies have shown that increased expression of ATP7A mediates resistance to cisplatin, carboplatin, and oxaliplatin in ovarian cancer cells and is associated with poor survival in ovarian cancer patients [13,14].
2. Other factors which affect intracellular copper availability, such as copper-binding proteins. The cellular thiol-containing proteins (e.g. metallothioneins and GSH) bind not only copper but also Pt; therefore, their levels in cells may influence the efficacies of Pt drugs. It has been observed that increased intracellular concentration of GSH may result in inactivation of cisplatin because of increased formation of $Pt(GS)_2$ and decreased Pt-DNA adduct formation.
3. Changes in MRP2 level. Human carcinoma cell lines with increased levels of MRP2 are associated with elevated cisplatin resistance, decreased intracellular accumulation of cisplatin, and decreased DNA adduct formation [10].

The other mechanisms involved in Pt-resistance include enhanced DNA repair capacity and increased antiapoptotic activity. Nucleotide excision repair is the major pathway for Pt adduct removal and repair of DNA damage. The nucleotide excision repair complex is composed of at least 17 proteins, but upregulation of only a few rate-limiting proteins is necessary to increase the excision repair capacity in resistant tumor cells, namely XPA, ERCC1, topoisomerase II, and BRCA1. Downregulation or gene mutation of the DNA damage recognition proteins (e.g. mismatch repair complex and p53) may enhance the replicative bypass pathway and cause post-replication DNA repair or DNA damage tolerance. The factors involving increased antiapoptotic activity include downregulation of the proapoptotic molecules

Bax or Bad, increased expression of the antiapoptotic molecules Bcl-2 or Bcl-x_L, overexpression of the apoptotic inhibitor survivin, and suppressed activity of the direct effectors of apoptosis, i.e. caspases 3, 8 and 9 [12].

Cancer Stem Cells and Chemotherapeutic Drug Resistance

The cancer stem cell hypothesis states that the cancer-initiating cell is a transformed tissue stem cell, which is spared along with its normal tissue stem cell counterparts with very subtle differences. One of the defining characteristics of normal tissue stem cells is their constitutive resistance to environmental toxins, including most chemotherapeutic agents. The constitutive drug resistance of normal tissue stem cells is mediated by MDR transporters and detoxifying enzymes. Cancer stem cells also retain the essential property of self-protection through the activity of MDR transporters [15].

Cancer stem cells have been identified in leukemias and some solid tumors. Many researchers now suspect that all cancers are composed of a mixture of stem cells and proliferative cells. These cancer stem cells make up as few as 1% of the total tumor cells, making them difficult to detect and study. Therefore, the existence of cancer stem cells provides a tumor reservoir that is the source of disease recurrence and metastasis. *ABCB1* and *ABCG2* genes are expressed in both normal stem cells and most tumor stem cells [16]. Thus, the major barrier to therapy is the quiescent tumor stem cell with constitutive MDR. In fact, dose-limiting toxicities of many antineoplastic agents occur precisely at drug concentrations that damage normal tissue stem cells. If the proposed relationships between normal and neoplastic stem cells prove correct, the inescapable conclusion is that systemic cytotoxic therapies are doomed to failure because regimens that spare resting normal stem cells will also likely spare resting tumor stem cells [15]. Similarly, inhibition of drug transporters may also cause toxicity of the patient's normal stem cells, particularly those of the bone marrow [16]. Successful therapy awaits the discernment of biological and immunologic differences between the tumor and normal stem cells so that approaches can be developed to eliminate the tumor stem cells without excessive toxicity to normal stem cells.

Conclusion

Chemotherapeutic drug resistance is a major obstacle in cancer therapy. Although the three MDR transporters have been recognized for decades and their operating mechanisms have been extensively studied, development of inhibitors of these transporters is still clinically unsuccessful. The more complicated mechanisms involved in the resistance to Pt-based anticancer agents make it more difficult to surmount. In addition to searching for potential MDR transporter inhibitors, another important strategy in cancer treatment is to ascertain the subtle differences between normal and tumor stem cells in order to develop therapies specifically targeting the cancer stem cell while avoiding damage to the normal tissue stem cell.

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