Ewa Stefanko, Tomasz Wróbel

Mechanisms of Resistance to Cancer Chemotherapy

Mechanizmy oporności w chemioterapii nowotworów

Department of Hematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University, Poland

Abstract

Resistance to chemotherapy is one of the major causes of failure of anticancer treatment. It can occur at every level of the drug action and includes mechanisms such as increased drug efflux and decreased influx, drug inactivation, alterations in the drug target, DNA damage repair, and evasion of apoptosis. In this article some mechanisms of drug resistance in cancer chemotherapy are reviewed (Adv Clin Exp Med 2010, 19, 1, 5–12).

Key words: resistance to chemotherapy, cancer cells, leukemia, lymphomas.

Streszczenie


Słowa kluczowe: oporność na chemioterapię, komórki nowotworowe, białaczka, chloniaki.

Chemotherapy is one of the most important methods of cancer treatment. Despite continuous progress in therapy, disease relapse and resistance to chemotherapy remain challenges for the physician. Neoplastic cells are able to develop some mechanisms that allow them to survive in the presence of a cytotoxic drug. Chemotherapy resistance is one of the factors leading to failure of anticancer treatment. The most important mechanisms of cellular resistance to chemotherapy are disturbances in intracellular transport, drug inactivation, alterations in the drug target, DNA damage repair, and evasion of apoptosis. This article summarize the most important mechanisms of chemotherapy resistance.

Disturbances in Intracellular Transport

In the plasma membrane of many cancer cells are special proteins which mediate the transport of the most commonly used cytotoxic drugs. Disturbances in these transporters may lead to a negative response to therapy by decreased influx or increased efflux of chemotherapeutic agents. There are two main superfamilies of membrane transporter proteins that influence the pharmacokinetics of drugs: ATP-binding cassette (ABC) transporters and solute carrier (SLC) transporters. The ABC proteins are associated with decreased cellular accumulation of cytotoxic drugs. SLCs mediate the uptake of drugs, and lower activity of these transporters may result in resistance to chemotherapy.

Decreased Drug Influx

The major members of the SLC family are SLC22A, SLC19 (reduced folate carrier, RFC), SLC28 and 29 (CNT and ENT nucleoside transporters), SLC7A and 3A (amino-acid transport-
ers), and SLC31A (CTR copper transporter). These proteins are important determinants of sensitivity to many different groups of anticancer drugs.

RFC is a membrane transporter for natural folates and their antagonists such as methotrexate (MTX). The main antiproliferative effect of MTX is inhibition of the activity of dihydrofolate reductase (DHFR), which disturbs the reaction of creating tetrahydrofolate from dihydrofolate and, in the repercussion, the synthesis of DNA. Decreased expression of RFC or inactivating mutations within genes encoding this protein (RFC1 gene) is one of mechanisms of MTX resistance [1]. Polymorphism of the RFC1 gene can influence the effectiveness of MTX therapy. Laverdiere C et al. demonstrated that in children with acute lymphoblastic leukemia (ALL), genotype 80AA was related to considerable resistance to treatment, poor prognosis and shortened overall survival compared with genotype 80GG. In 80AA homozygote patients, significantly higher plasma MTX levels were observed, suggesting decreased drug uptake by cells [2].

The SLC22A family contains three subtypes of facilitated transporters called OCT1 (encoded by SLC22A1 gene), OCT2 (SLC22A2), and OCT3 (SLC2A3). OCT1 mediates the transport and distribution of many cations as well as medicines and toxins. OCT1 is presented on the membrane of hepatocytes, enterocytes, and renal cells. It participates in the transport of cytostatics such as cisplatin, mitoxantrone, and anthracyclines. It also takes part in the transport of imatinib into the cell. Clark et al. showed that patients with CML (chronic myeloid leukemia) with lower expression of OCT1 had poorer cytogenetic and molecular outcome with imatinib treatment. Higher doses of imatinib may overcome the negative impact of low OCT1 activity [3]. However, dasatinib and nilotinib, as the second generation of tyrosine kinase inhibitors, are independent of the expression level of OCT1 and they maintain antileukemic activity irrespective of OCT1 expression [4]. OCT transporters play a role in several types of solid tumors. The expression of OCT3 in kidney carcinoma cell lines increases chemosensitivity to some cytotoxic drugs such as melphalan, irinotecan, and vincristine [5].

**Increased Drug Efflux**

This model of chemoresistance is associated with the presence of special transport proteins in the plasma membrane of tumor cells which resist the penetration of drugs at the cost of energy accumulated in ATP. These proteins belong to ABC (ATP-binding cassette) family proteins and are responsible for multidrug resistance (MDR) [6]. One of the most known proteins of this family is P-glycoprotein (P-gp). P-gp is the product of the mdr1 gene (multidrug resistance protein gene). P-gp is not specific to tumor cells. It occurs in interstitial cells, the cortex of suprarenal glands, kidneys, biliary ducts, lungs, stomach, and hematopoietic system cells. It has a homeostatic role and protects against toxic cell metabolites, external substances, and drugs. Expression of these proteins in the tumor cell membrane leads to resistance to many drugs regardless of their mechanism of action and chemical structure (such as anthracyclines and vincristine). The presence of multidrug-resistant cells does not mean resistance to all cytostatic drugs. Although P-gp is known to decrease the accumulation of cytotoxic drugs in cancer cells, the exact mechanism of how P-gp regulates the intracellular level of drugs is not clear.

Szendrei et al. analyzed response to chemotherapy in the 35 patients treated for chronic lymphocytic leukemia (CLL). The P-gp-positive cases (n = 9) were predominantly non-responders (89%). Most of the P-gp-negative patients (n = 26) responded well (80%) to chemotherapy. Average expected survival rates between P-gp-positive and -negative CLL patients were 84 vs. 203 months [6]. A study by Guillaume et al. found no correlation between multidrug resistance mediated by P-gp overexpression and ZAP-70/CD38 coexpression, two proteins responsible for poor prognosis in B-CLL [7].

Analysis of gene expression in tumor cells suggests correlations between mdr1 expression and resistance to chemotherapy. Studies of blastic cells from patients with acute myeloid leukemia (AML) showed that the expression of mdr1 in CD34+/ CD38- cells was significantly increased in 8 of 10 patients who did not achieve remission after induction chemotherapy. The raised mdr1 expression was not observed in any of the 7 patients who achieved complete remission. Increased mdr1 expression on leukemic cells correlates with resistance to daunorubicin [8]. A clinical study by Kourti et al. found that 18 of 49 patients with ALL had higher expression of mdr1 gene and their prognoses were poorer than those with low expression (event-free survival: 55.56% vs. 86.67%). Mdr1 expression was independent of the initial white blood cell count, immunophenotype, and prednisone response and was significantly higher at relapse than at diagnosis for 4 sample pairs [9].

Polymorphism of the mdr1 gene can also predict outcome to chemotherapy. Wang et al. showed correlation of the mdr1 gene single-nucleotide polymorphisms (SNPs) C1236T, G2677T/A,
and C3435T with the outcome of induction chemotherapy in patients with newly diagnosed AML [10]. Mdr1 G2677T/A genetic polymorphisms were strongly associated with the probability of CR (complete response). There was no correlation between mdr1 C1236T and C3435T and the CR rate. Yang et al. showed that the G571A variant reduced the degree of P-gp-mediated resistance of cancer cells using six cytotoxic drugs: doxorubicin, daunorubicin, vinblastine, vincristine, paclitaxel, and etoposide. There was a minimal effect on doxorubicin and daunorubicin, but mdrl-dependent resistance on vinblastine, vincristine, paclitaxel, and etoposide was reduced by about 5-fold [11].

Another ABC transport protein is MRPI (multidrug resistance-associated protein 1). This protein mediates in the transport of many anions (MOAT, multispecific organic anion transporter) and cytotoxic drugs such as doxorubicin, vincristine, mitoxantrone, and alkylating agents. Some studies have shown a role of MRPI expression in the resistance to cytotoxic drugs such as anthracyclines, vincristine, and epipodophyllotoxins. Clinical studies by Mahjoubi et al. suggest an association between high expression of MRPI and poor clinical outcome in AML patients. The expression of MRPI was particularly high in the M5 subtype [12]. In de novo AML patients, overexpression of MDR1 at diagnosis and co-expression of the other MDR proteins (MRP1, MDR3, breast cancer resistance protein BCRP, and lung resistance protein LRP) together with their functional activity contribute to chemotherapy failure [13]. Candoni et al. showed that the efficacy of liposomal daunorubicin plus Ara-C as re-induction therapy in very poor-risk ALL patients and the response rate seem not to be affected by MRPI expression [14].

Drug Inactivation

The mechanisms that inactivate drugs can diminish the amount of free drug available to bind to its intracellular target. The formation of conjugates between platinum-based compounds such as cisplatin, carboplatin, and oxaliplatin and glutathione (GSH) is a key step in the inactivation of these drugs. Under physiological conditions, GSH is an antioxidant and protects cells against the destruction of DNA and RNA by free radicals. GSH conjugation is catalyzed by glutathione-S-transferase (GST). The resulting complex is a substrate for ABC transport proteins, promoting drug efflux. A high level of intracellular GSH correlates with resistance to platinum drugs. GSH expression has been reported to be increased in some human cancer tissues such as bone marrow, breast, colon, and lungs. Elevated levels of GSH were also observed in squamous cell carcinoma of lung cell lines which were resistant to methotrexate and cisplatin [15].

An increased level of GST-π (a subtype of GST) may lead to resistance to chemotherapy. Significant activity of this enzyme has been found in chronic myeloproliferative disorders as well as acute leukemias. Some data suggest that GST-π is associated with a poor response to treatment [16]. GST-π is a main subtype of GST in lymphoid tissues. Bennaure-Griscelli et al. showed a negative role of GST-π expression in mantle cell lymphoma (MCL) [17]. The GST gene is located on 11q13 and undergoes amplification with the cycline D1 gene (CCND1). The role of CCND1 in the pathogenesis of MCL is well established. Immunohistochemical analysis of GST-π expression was done in 24 patients with MCL (patients with relapse and partial remission after CHOP chemotherapy), 12 with follicular lymphoma (FL), and 69 with diffuse large B-cell lymphoma (DLBCL). High levels of GST-π and CCND1 mRNA were observed in MCL compared with the other lymphomas. Additionally, statistically significant correlation between GST-π level and CCND1 mRNA in MCL was observed. In conclusion, higher expression of CCND1 in MCL is associated with a transcriptional up-regulation of the GST-π gene. This result suggests that the glutathione system may be a factor of drug resistance in MCL.

γ-glutamyltransferase (γ-GT) and γ-glutamylcysteine synthetase (γ-GCS) are other enzymes involved in cellular glutathione homeostasis. γ-GT catalyzes the first step in the degradation of extracellular glutathione. This enzyme is normally found in serum, where it is marker of liver diseases. γ-GT may also play role in regulating platinum drug resistance. γ-GCS catalyzes the first step in the synthesis of GSH. Increased expression of γ-GCS was observed in some cancers, i.e. colon, lung, liver, and others. Asano et al. showed that high levels of GSH and γ-GCS correlated with resistance to doxorubicin in K562/ADR leukemia cells. Indomethacin overcomes doxorubicin resistance by decreasing the intracellular content of GSH and its conjugates with decreasing expression of γ-GCS [18].

Alterations in the Drug Target

Alterations in expression or mutation of a chemotherapeutic drug target can have a major impact on resistance to chemotherapy. Imatinib was introduced in 2001 as the first drug that spe-
cifically inhibited tyrosine kinase activity. A high expression of this enzyme is known to be the main factor of tumor transformation in chronic myeloid leukemia (CML). Higher expression of tyrosine kinase is caused by the generation of a new, pathological BCR-ABL gene resulting from reciprocal translocation between the long arms of chromosomes 9 and 22 (Chromosome Philadelphia-Ph). Imatinib occupies the ATP binding pocket of the Abl kinase domain. This prevents substrate phosphorylation and a lack of signaling. This process inhibits the proliferation and survival of cells. Primary resistance or relapse after imatinib treatment are observed among patients with CML and Ph+ ALL. Point mutations within the ABL tyrosine kinase domain may result in resistance to imatinib and can be assigned to four major groups based on their location in the kinase domain: the ATP binding loop, gatekeeper residue Thr315, the catalytic domain, and the activation loop. Thr315 locates to the periphery of the nucleotide-binding site of ABL and forms a key H-bond interaction with imatinib. The T315I mutation disrupts this H-bond, which impairs imatinib binding, resulting in insensitivity to imatinib. Samples from 23 CML patients receiving imatinib treatment were analyzed by Quyang. In 7 cases, Abl domain point mutations was detected (T315I, Y253H, E253K, F317L, G321W). The incidences of these mutations in the chronic phase (CP), accelerated phase (AP), and blast phase (BP) were 25%, 40%, and 30%, respectively. Six of the 7 patients with mutations were resistant to imatinib [19]. There are several approaches to overcome resistance to imatinib: increasing the dose to 600–800 mg per day or therapy by the novel generation of tyrosine kinase inhibitors. Dasatinib and nilotinib, as second-generation BCR-ABL inhibitors, overcome the majority of mutations, but without impact on the T315I mutation. The identification of mutations within the domain of ABL tyrosine kinase, which can be present in leukemic cells before initiation of imatinib treatment, are valuable prognostic factors in CML. They can also be helpful in the proper selecting of TKI (tyrosine kinase inhibitors) and individualization of treatment.

MTX inhibits the activity of DHFR and disturbs the reaction of creating tetrahydrofolate from dihydrofolate and the synthesis of DNA. Polymorphism and changes in expression of the gene encoding DHFR (DHFR gene) may lead to resistance to MTX. Polymorphism in the promoter of DHFR gene and its high expression correlate with resistance to MTX in ALL patients. Goker et al. observed amplification of DHFR gene and mutation of p53 gene in 38 de novo ALL patients and in 29 patients with relapse after MTX treatment. Thirty-one percent of the patients (9 of the 29 relapsed patients) had a low level of amplification of DHFR gene (2–4 copies), which was associated with a high level of DHFR mRNA. There was correlation between mutations of p53 and amplification of DHFR gene in 7 of 9 relapsed patients [20].

Microtubules (MTs) are the dynamic cytoskeleton of eukaryotic cells. They are composed of α and β tubulins and play an important role in various cellular functions, including cell division and mitosis. Some cytotoxic drugs such as taxanes and Vinca alkaloids inhibit microtubule assembly dynamics, causing cell-cycle arrest and promoting apoptosis. Alterations in the amount of tubulins or changes in tubulin isotype expression have been associated with Vinca alkaloid and taxanes resistance. The main modulators of MTs’ dynamics are MAP (MT-associated protein). These proteins stabilize MTs and lead to their polymerization. Increased expression of MAP4 was observed in vincristine-resistant human leukemia cells [21]. The βII and βIVb tubulin isotypes may serve as predictors of Vinca alkaloid resistance. Gan et al. showed that NCI-H460 lung cancer cells with the βII isotype were less sensitive to vincristine treatment than those with the βIVb isotype [22].

**DNA Damage Repair**

Most cytotoxic drugs exert their action by DNA damage. There are some of recognized DNA repair systems that protect cellular genome from instability. The MMR (mismatch repair) pathway plays an important function in the maintenance of genomic stability. MMR proteins are responsible for correcting insertion or deletion loops and recognize mismatched or unmatched DNA base pairs. Additionally, MMR plays a role in mediating DNA damage-induced cell-cycle arrest and apoptosis. *MLH1* and *MSH2* are important genes in the normal function of the MMR system. Defects or mutations in *MLH1* or *MSH2* were observed in some of cancers such as hereditary nonpolyposis colon cancer (HNPPCC) and colorectal, breast, and ovarian cancer. The consequence of MMR defects is the production of replication errors in the simple repetitive DNA. This leads to a phenomenon known as microsatellite instability (MSI), which was first described in colon cancer. The roles of the DNA mismatch repair pathway and MSI have been observed in hematopoietic malignancies. Mao et al. found that loss of MMR function is associated with refractory and relapsed AML. Eighteen of 53 AML patients had mutations in MMR genes or hypermethylation in the
MLH1 promoter [23]. Miyashita et al. found MSI in 8 tumors from 59 of non-Hodgkin's lymphoma patients [24]. The response to CHOP/VEPA-based therapies had been significantly less effective in those with MSI tumors. MMR defects were also observed in plasma cell dyscrasias. Velangi et al. showed MSI in 15 of 92 patients 7.7% with MGUS/SMM, 20.7% with MM/PCL, and 12.5% with relapsed MM/PCL [25]. Occurrence of MSI was significantly higher in the MM patients than in the MGUS patients. This suggest that MSI can play a role in disease progression.

MMR deficiency seems to confer resistance to some of DNA damage-inducing agents, including platinum drugs. This phenomenon was observed during cisplatin- and carboplatin- but not during oxaliplatin-based therapy. Oxaliplatin is a new platinum analogue and the MMR system does not recognize the adducts formed by this drug, so the repair pathway is not triggered. This new platinum compound has normal cytotoxicity against cells that are resistant to cisplatin and carboplatin. Lin et al. described the role of MMR and p53 protein in the development of cisplatin resistance in human colon carcinoma. Loss of MMR or p53 alone increased the rate of resistance by 1.8- and 2.4-fold; however, loss of both increased the rate to 4.8-fold [26]. The MMR system is a predictive factor of cancer response to 5-fluorouracil. The expressions of two MMR genes, MLH1 and MSH2, were examined in patients with advanced colorectal cancer (CRC) who were treated with irinotecan alone or in combination with 5-fluorouracil and either paclitaxel or docetaxel. In this study, MLH1-deficient cells were resistant to topo-I inhibitors such as camptothecin and topotecan; however, MSH2 cells were not [27].

NER (nucleotide excision repair) is a complex involving at least 17 different proteins. This pathway repairs DNA lesions which alter the helical structure of the DNA molecule and interfere with DNA replication and transcription. Some of the NER genes, including ERCC1, ERCC2 (XPD), and XPB, play a role in anticancer drug resistance in human tumor cells. Barret et al. reported that NER activity was elevated in CLL lymphocytes from treated compared with untreated patients [29]. High levels of ERCC1 have been correlated with poor response to platinum chemotherapeutic agents in non-small-cell lung cancers (NSCLCs). In contrast, Shimizu et al. showed no correlations between mRNA expression of the ERCC1 and ERCC2 and chemosensitivity to cisplatin, carboplatin, and gemcitabine in human lung cancer cell [30]. A cisplatin-based chemotherapy is used in the salvage treatment of diffuse large B-cell lymphomas (DLBCL). In one preliminary study with 7 DLBCL relapsed patients, only one had increased ERCC1 expression, which does not allow predicting response to cisplatin-based treatment [31].

**Evasion of Apoptosis**

Apoptosis (programmed cellular death) is a result of cytotoxic chemotherapy. The onset of apoptosis is regulated by different intra- and extracellular factors. It comes for the amplification of these signals and subsequent activation of the effectors of apoptosis cascades. There are two main pathways for their activation: the intrinsic, regulated by the Bcl-2 family, and the extrinsic, regulated by tumor necrosis factor (TNF). Bax, Bad, and Bak of the Bcl-2 protein family promote apoptosis; others, such as Bcl-XL, Mcl-1, and Bcl-2 itself, are antiapoptotic.

The role of the Bcl-2 family in the regulation of chemotherapy response has been studied in several hematological malignancies. Aqarwal et al. examined the expression of the Bcl-2 family in 116 cases of indolent B-cell NHL. The expressions of Bcl-2 and Bcl-X proteins were increased in most of lymphomas. The expressions of Mcl-1, Bax, and Bak were decreased in most patients with CLL, FL, and marginal-zone B-cell lymphoma (MZBCL) [32]. On the other hand, high activity of proapoptotic proteins such as Bax and Bak is known to be a good prognostic factor in AML, and a high level of Bcl-2 to Bax confers decreased rates of complete remission and overall survival [33].

Another important mechanism of apoptosis regulation is the inhibition of caspase activity by IAP (inhibitors of apoptosis) members such as c-IAP1, c-IAP2, XIAP, NAIP, livin, and survivin. Overexpression of several IAPs has been demonstrated in hematological malignancies, including acute leukemias, myelodysplastic syndrome, chronic myeloid leukemia, chronic lymphocytic leukemia, and lymphomas. A high level of survivin in non-Hodgkin's lymphoma patients was found with more significant expression in aggres-
sive lymphomas than in indolent [34]. Adida et al. detected the expression of survivin in 134 of 222 DLBCL patients. In this group, 5-year overall survival was lower than in the group without expression of this protein (40 vs. 54%) [35]. Troeger et al. analyzed the impact of survivin protein levels on outcome in 66 B-ALL patients (B-cell ALL; acute lymphoblastic leukemia). High expression of survivin was detected in 65% of the leukemic samples. Patients suffering from relapse had higher survivin levels than those with remission [36]. The expression of survivin is an independent risk factor in ALL.

Expression of the IAP family was analyzed in MDS (myelodysplastic syndrome) with comparison to de novo AML and to MDS transforming to overt leukemia. The levels of mRNA survivin, c-IAP1, NAIP, and X-IAP were higher in the MDS than in a control group. Patients with overt leukemia transforming from MDS had decreased expression of mRNA survivin, c-IAP1, and c-IAP2 [37]. GRP78/Bip (glucose-regulated protein/immunoglobulin heavy-chain binding protein) is an antiapoptotic protein. This protein belongs to the hsp70 protein family and promotes tumor proliferation, survival of neoplastic cells, metastasis, and resistance to different types of therapy. Elevated GRP78 level correlates with higher pathologic grade, poor survival, and risk of recurrence in liver, breast, colon, and gastric cancers. In some types of tumor, including lung, bladder, stomach, and breast, GRP78 overexpression leads to resistance to a variety of cytostatics such as topoisomerase inhibitors, cisplatin, and Adriamycin [38, 39]. Expression of GRP78 protein is higher in prostate cancer than in benign prostate tissue. This expression is associated with survival and clinical recurrence [40].

Conclusions

Despite advances in cancer treatment, resistance to chemotherapy is a major obstacle to successful therapy. The mechanisms of chemoresistance can occur at many cellular levels and may exist prior to treatment initiation or be induced by exposure to commonly used chemotherapeutic agents. A better understanding of these mechanisms may lead to the synthesis of new drugs, individualization of treatment, and improved response to anticancer therapy.


Address for correspondence:

Ewa Stefanko
Department of Hematology, Blood Neoplasms, and Bone Marrow Transplantation
Wroclaw Medical University
Pasteura 4
50-367 Wroclaw
Poland
Tel. +48 71 784 25 79
E-mail: ewastefanko@gmail.com

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