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Review Article

MECHANISMS OF DRUG RESISTANCE IN CANCER CELLS

*M. Perwaiz Iqbal**

SUMMARY

Development of drug resistance is a common problem in cancer chemotherapy. For the past several years, investigators have been striving hard to unravel mechanisms of drug resistance in cancer cells. Using different experimental models of cancer, some of the major mechanisms of drug resistance identified in mammalian cells include: (a) Altered transport of the drug [decreased influx of the drug; increased efflux of the drug (role of P-glycoprotein; role of polyglutamation; role of multiple drug resistance associated protein)], (b) Increase in total amount of target enzyme/protein (gene amplification), (c) Alteration in the target enzyme/protein (low affinity enzyme), (d) Elevation of cellular glutathione, (e) Inhibition of drug-induced apoptosis (mutation in *p*53 tumor suppressor gene; increased expression of bcl-xL gene).

Other novel mechanisms in various types of cancer cells include: Over-expression of cytochrome P450 protein, ATP-binding cassette transporter BCRP, sodium channel protein, S-adenosylmethionine synthetase, and loss of functional retinoblastoma protein.

An understanding of these mechanisms provides us the basis for the development of drugs which can specifically interact with the cause of resistance and restore the sensitivity of the tumor cell. This reversal of drug resistance has a significant role in modern day cancer chemotherapy.

KEY WORDS: Mechanism, drug resistance, cancer cells, methotrexate.

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MECHANISMS OF DRUG RESISTANCE

Development of drug resistance is a common problem in cancer chemotherapy. There is plenty of information on "how microorganisms develop resistance to various drugs", but our knowledge regarding the mechanisms underlying drug resistance in mammalian cell is still poor.

With recent development of techniques in the field of cell and molecular biology, it has been possible to unravel some of the molecular mechanisms of resistance to anticancer drugs in mammalian cells.

So far the following 5 major mechanisms of drug resistance in cancer cells have been identified:

1. Altered transport of the drug
   i. decreased influx
   ii. increased efflux
2. Increase in total amount of target enzyme/protein (gene amplification).
3. Alteration in the target enzyme/protein (low-affinity enzyme).
4. Elevation of cellular glutathione.
5. Inhibition of drug-induced apoptosis.

* M. Perwaiz Iqbal, Ph.D.
Professor of Biochemistry
Department of Biological & Biomedical Sciences,
The Aga Khan University,
Stadium Road, P.O. Box-3500
Karachi 74800, Pakistan.

Correspondence:

Dr. Mohammad Perwaiz Iqbal
E-mail: perwaiz.iqbal@aku.edu

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The discovery of the first three mechanisms of drug resistance was mainly because of the extensive research work carried out on anticancer drug methotrexate (MTX). This drug has been in use since 1951, and has achieved the prominence of being the most widely used anticancer drug[1]. It is a folate antagonist which kills the proliferating cells by inhibiting the enzyme dihydrofolate reductase (DHFR), thereby blocking the pathway of *de novo* DNA synthesis[2-4]. This drug has produced excellent results in controlling choriocarcinoma, Burkitt's lymphoma, acute leukemia and psoriasis, but continued administration to patients often results in emergence of drug resistance, hence prompting many studies to unravel the underlying mechanism.

1. ALTERED TRANSPORT OF THE DRUG

When there is a change in one of the transport proteins of a particular drug, then the influx of the drug in cancer cell or its efflux might get affected, resulting into decreased quantity of the drug inside the cancer cell.

i. Decreased influx of the drug

An alteration resulting from a mutation in surface membrane protein that is involved in the transport of the drug inside the cell or its decreased expression might lead to reduced uptake of the drug and, hence, the processes inside the cell would not be inhibited. Figure 1 is the diagrammatic representation of this phenomenon with regards to this mechanism of resistance. A number of studies have revealed decreased influx of MTX due to low level or nonfunctioning of folate carrier protein[4-6]. This phenotypic change arises because of either decreased transcription or mutation in the gene for folate carrier.

ii. Increased efflux of the drug

Increased efflux of the drug from the cancer cells would be because of any of the following:

a. Decreased polyglutamate formation.
b. Increased expression of P-glycoprotein.
c. Increased expression of multiple drug resistance protein.

a. Decreased polyglutamate formation

Retention of folate analogues, such as, MTX, inside the cell is dependent upon their conversion into polyglutamate form. Polyglutamation is a process by which multiple glutamic acid residues are added enzymatically to such drugs. Decreased level of activity of folylpolyglutamate synthase, possibly because of mutations in its gene, would lead to decreased polyglutamation inside the cell and, hence, rapid efflux of the drug. Increased efflux would leave little amount of MTX inside the cell to inhibit DHFR. This phenomenon is diagrammatically shown in Figure 2. Michael Kuehl and his associates have even shown that the retention of MTX in CCRF-CEM T-lymphoblast cells increased as the number of glutamic acid residues in the polyglutamate of MTX increased[7]. Cowan and Jolivet have shown that the resistance to MTX exhibited by a human

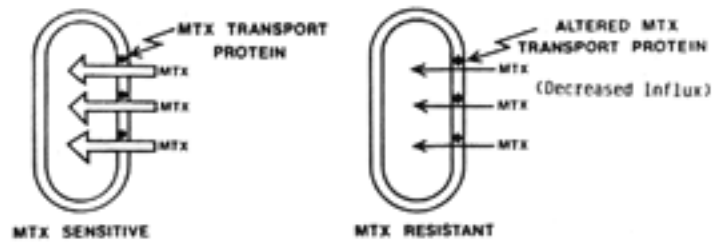

Figure 1: Altered transport of methotrexate (MTX) across the cell membrane of MTX-sensitive and MTX-resistant cells due to alteration in a transport protein for MTX.

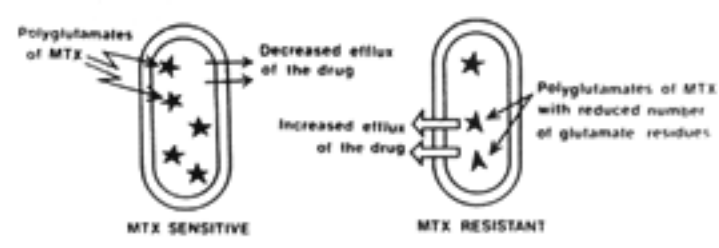

Figure 2: Polyglutamates of MTX and efflux of this drug from MTX-sensitive and MTX-resistant cells.

breast cancer cell line was due to decreased formation of MTX polyglutamates in these cells[8]. Similar results have been reported by Pizzorno *et al.* in CCRF-CEM cells after short-term, high dose treatment with MTX[9].

A decreased accumulation of MTX polyglutamates in cells can also be due to increased breakdown due to increased activity of the lysosomal enzyme folylglutamyl hydrolase[10, 11].

This process is achieved by the active transport of MTX polyglutamate into lysosomes followed by hydrolysis by folylpolyglutamyl hydrolase[12]. This leads to increased efflux of monoglutamate form of MTX from the lysosomes and cells.

b. *Increased expression of P-glycoprotein*

Multidrug resistance (MDR) describes a complex phenotype whose predominant feature is resistance to a wide range of structurally unrelated cytotoxic agents, many of which are anticancer drugs[13, 14].

A wide variety of biochemical changes have been detected in MDR cell lines[14]. The most consistent change is the increased expression of P-glycoprotein, a plasma membrane glycoprotein of molecular weight 170,000. The level of P-glycoprotein expression correlates with degree of drug resistance[15, 16]. This protein mediates energy-dependent export of a wide variety of drugs involved in MDR. Figure 3 is the diagrammatic representation of this process.

P-glycoprotein has been found to be present in biopsy specimens from patients with ovarian and sarcoma tumors and in leukemic cells from patients with acute myelocytic leukemia[17, 18].

c. *Multidrug Resistance Associated Protein*

Few years ago, another MDR-associated protein (MRP) has been identified to be present in most human tissues and overexpressed in several tumor types. It is a glycoprotein of molecular weight 190,000 and is associated with energy-dependent efflux of various drugs[19, 20].

2. INCREASE IN TOTAL AMOUNT OF TARGET ENZYME/PROTEIN (GENE AMPLIFICATION)

The first evidence of this phenomenon in mammalian cells was provided by Schimke *et al.* who demonstrated significant increase in level of DHFR in an MTX-resistant cell line[21, 22]. The sensitivity of the enzyme towards the drug remains the same, however, there would be an excess of enzyme relative to the concentration of the drug inside the cell. Thus, the pathways or biochemical processes which were to be inhibited by the drug would continue and the cell would escape inhibition by the drug. Figure 4 illustrates this phenomenon in a diagrammatic manner.

A number of laboratories, including ours have shown this process in a number of types of cancer cells[21-33].

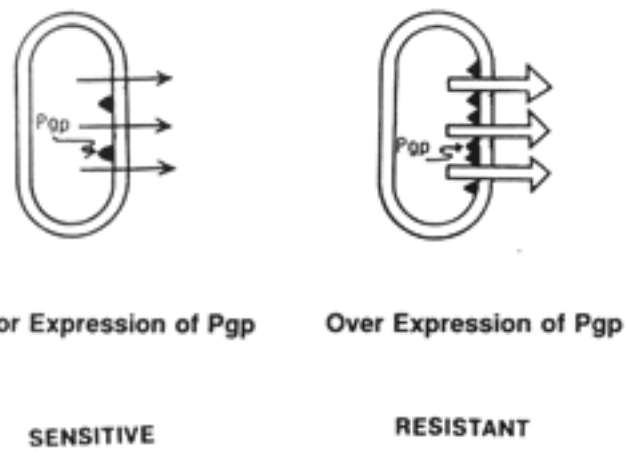

Figure 3: Expression of P-glycoprotein and efflux of drug from MTX-sensitive and MTX-resistant cells.

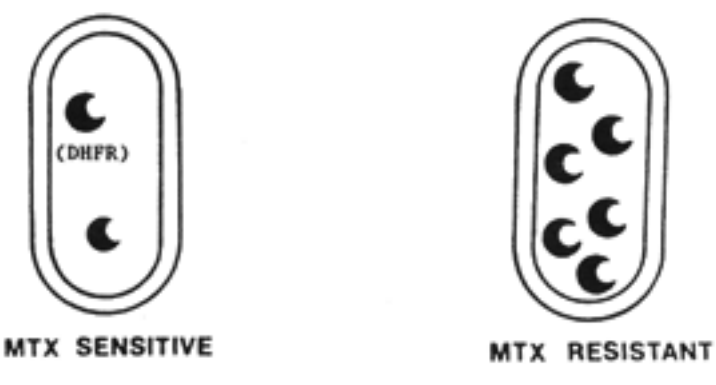

Figure 4: Total amount of dihydrofolate reductase (DHFR) in MTX-sensitive and MTX-resistant cells.

Figure 5 shows the concentration values of DHFR in MTX-sensitive and MTX-resistant cell lines of L1210 leukemia. There is nearly 17 times more enzyme in MTX-resistant L1210 leukemia cells compared to MTX-sensitive cells.

Schimke, Bertino and their associates have shown that although the increased level of the enzyme, DHFR, can be due to decreased catabolism of the enzyme due to its stabilization as a result of binding to the inhibitor, yet enzyme induction is the major cause of this increase in the enzyme level[21]. This enzyme induction takes place as a result of gene amplification, the process whereby a small part of the genome, representing one or more genes, is duplicated locally within a chromosome[21, 22]. Such an amplification of genes is stable if it is localized in a specific region of a chromosome or unstable if localized in the nucleus as extrachromosomal DNA[22, 34].

Figure 6 is the diagrammatic representation of an amplification of DHFR gene in chromosome number 2 of Chinese hamster ovary cells made resistant to MTX. Compared to chromosome number 2 of the drug-sensitive cell, there is an expanded region in the long arm of this chromosome in the drug-resistant cell. Using nuclear hybridization technique, it was found that the drug-resistant cell has 150 copies of DHFR gene[22].

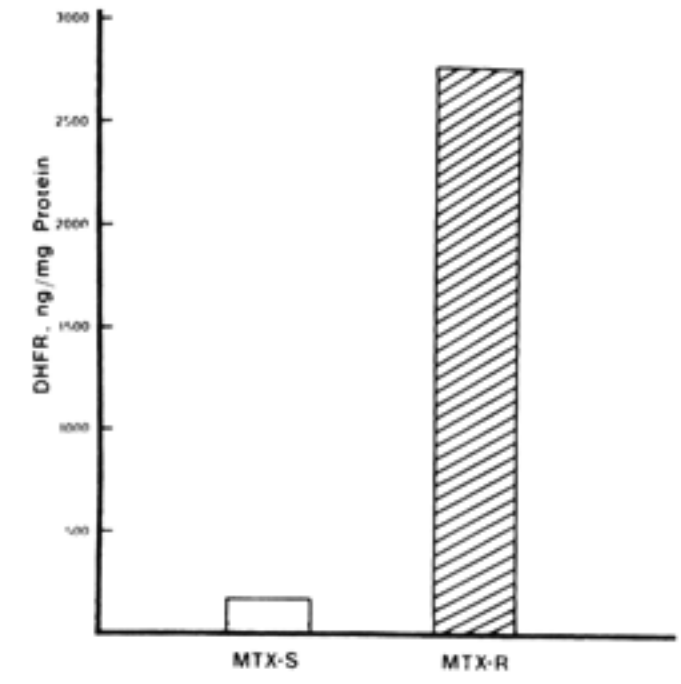

Figure 5: Concentration of dihydrofolate reductase in leukemia cell lines L1210 MTX-sensitive (MTX-S) and L1210 MTX-resistant (MTX-R).

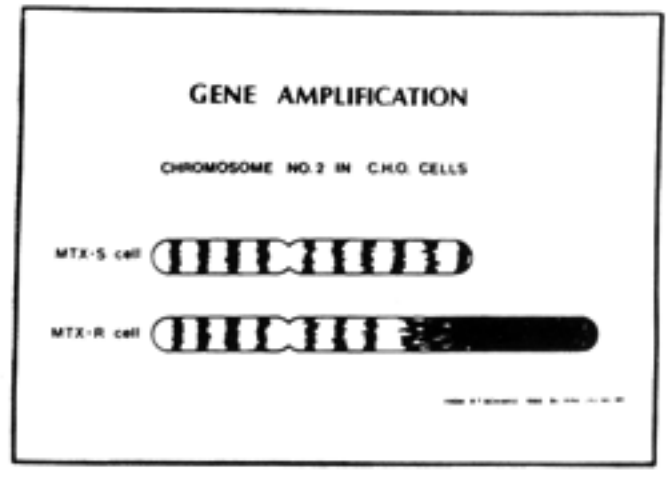

Figure 6: Chromosome number 2 in MTX-sensitive and MTX-resistant Chinese hamster ovary cells [Reference no. 22].

3. *ALTERATION IN THE TARGET ENZYME/ PROTEIN (LOW AFFINITY ENZYME)*

Presumably because of mutation, there is a structural change in the target enzyme such that the normal high affinity for the drug is lost. When this happens, the enzyme would no longer be inhibited by the drug at least at the conventional doses. Figure 7 illustrates this phenomenon diagrammatically.

Mutations in DHFR leading to a decreased binding of MTX have been reported in a number of tumors[35, 36]. These mutations with one exception, have involved hydrophobic amino acids in the folate binding region of the enzyme[37]. Evidence in support of this

Figure 7: Alteration in the active site of dihydrofolate reductase in MTX-resistant cell compared to MTX-sensitive cell leading to loss of high affinity of enzyme for MTX.

mechanism has been provided by a number of laboratories[38-44].

Albrecht and his associates by a multi-step selection procedure have isolated an MTX-resistant Chinese hamster cell line which contained structurally altered DHFR[40]. The mutant enzyme had single pH optimum for the reduction of dihydrofolate as compared to double pH optima observed in the wild-type enzyme. The mutant enzyme was also found to have a dramatically altered affinity for MTX. It was shown that to achieve a 50% inhibition of the enzyme from the mutant cells, a 10-fold excess of MTX was required when compared to the amount of drug required to achieve a 50% inhibition of enzyme activity in parental cells. We have also shown this low affinity form of DHFR in leukemia cells many of them resistant to MTX therapy[45-47].

Perhaps, the success of high-dose MTX therapy in many tumors is also based partly on the ability of the large doses of MTX to inhibit even low affinity form of DHFR and, thereby, blocking the DNA synthesis.

4. ELEVATION OF CELLULAR GLUTATHIONE

Glutathione is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) present virtually in all mammalian cells. It offers protection to cells by the destruction of reactive oxygen compounds, free radicals, and other toxic compounds of endogenous and exogenous origin. Because of this property it has an important role in drug detoxification.

There is considerable evidence of suggest that the development of resistance to alkylating agents and possibly, cisplatin is associated with increased intracellular glutathione (GSH) levels[48-50].

Drug-resistant tumor cells have been shown to contain levels of GSH several orders of magnitude higher than those measured in wild-type cells. GSH may reduce cytotoxicity by facilitating the metabolism of drugs to less active compounds or by detoxification of the free radicals[51, 52]. Additionally, GSH may enhance the repair of drug-induced injury, primarily at the DNA level. There is also considerable evidence that sensitivity to alkylating agents can be restored by depletion of intracellular GSH[53-57].

Buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, has been demonstrated to lower GSH levels in human ovarian cancer cell lines, resulting in an increase in melphalan and carboplatin cytotoxicity[51,58,59]. Similarly, in animal studies, BSO decreases GSH levels in the tumor cells resulting in increased melphalan cytotoxicity and improved survival[60]. Hence, GSH is involved in the development of drug resistance, and its depletion may restore or enhance the cytotoxic activity of several drugs.

Ifosfamide (IFEX) is an analog of cyclophosphamide. It is increasingly used in a wide variety of cancers. It has been shown to lower intracellular GSH levels, thereby increasing the sensitivity of the cells to cytotoxic drugs[61, 62].

We have also observed a similar inhibitory effect of IFEX on intracellular GSH levels in peripheral blood lymphocytes obtained from patients with ovarian carcinoma[63]. This decline in GSH levels by IFEX resulted in better response of these patients to cisplatin treatment. This provided another evidence that an increase in intracellular GSH levels in cancer cells is one of the major mechanisms of drug resistance. Depletion of GSH overcomes this drug resistance and restores the chemosensitivity of malignant cells.

5. INHIBITION OF DRUG-INDUCED APOPTOSIS

Cell death caused by a cytotoxic drug, such as, MTX is also dependent on the presence of factors that inhibit apoptosis, i.e., mutant *p*53 gene product, absence of retinoblastoma gene product, or increase in the expression of the bcl-2 gene[64-67]. Apoptosis is the "programmed-cell death".

Any factor inhibiting this programmed cell death might lead to development of drug resistance in the tumor.

i. *Role of p53 tumor suppressor gene*
Mutations in *p*53 gene or its deletion by cytotoxic drugs could lead to defective

apoptotic pathway resulting into drug-resistant tumors. Scott Lowe and his associates at Massachusetts Institute of Technology, Boston have shown that tumors in which *p*53 gene is fully expressed, there is a high proportion of apoptoic cells. Such tumors regressed after treatment with adriamycin[64]. In contrast, *p*53 deficient tumors (*p*53-/-) treated the same way with adriamycin continued to grow and contained very few apoptotic cells. It was suggested that inactivation or absence of *p*53 gene product would make apoptosis defective in tumors leading to development of drug resistance in such tumors. Reintroduction of normal *p*53 function in these tumors would enhance apoptosis after chemotherapy.
Fujiwara *et al.* have successfully used this approach in enhancing response of a lung carcinoma cell line to cisplatin[68]. All of these lines of evidence show, the critical role played by *p*53 gene in tumor regression and its absence or defect leading to drug resistance.

ii. *Role of Bcl-2 protein*
A gene involved frequently in non-Hodgkin's lymphomas, called *bcl*-2 (for B-cell lymphoma-2) codes for a protein which blocks programmed cell death. Studies of Bcl-2 protein function using gene transfer approaches in mammalian cells, for example, have demonstrated that overproduction of this oncoprotein can render cells relatively more resistant to induction of drug-induced apoptosis[69] .
Although the precise mechanism by which Bcl-2 exerts its effect is not known, but unusual intracellular location of Bcl-2 (outer mitochondrial membrane) suggests that it may function in an antioxidant pathway. Keeping in view of the fact that mitochondrial and ER membranes are major sites of free-radical generation in cells, the association of Bcl-2 with outer mitochondrial membrane and its ability to prevent accumulation of lipid peroxides lend support to the notion that Bcl-2 inhibits drug-induced apoptosis through an anti-oxidant mechanism.

6. OTHER NOVEL MECHANISMS

In addition to the above mentioned major mechanisms of drug resistance in cancer cells, some other novel mechanisms have also been suggested. These include:

i. *Overproduction of cytochrome P450 protein*[70]
A number of cytochrome P450 enzymes are known to metabolise a wide variety of anticancer drugs. McFadyen *et al.* have recently shown that overexpression of human cytochrome P450 CYP1B1 in Chinese hamster ovary cells decreases the sensitivity of these cells to the anticancer drug docetaxel[70].

ii. *Overexpression of ATP-binding cassette transporter breast cancer resistant protein (BCRP)*
Overexpression of an ATP-binding cassette transporter BCRP was found to be associated with increased efflux of certain topoisomerase inhibitors from human colon cancer cells[71].

iii. *Overexpression of a sodium channel protein*
Increased expression of alpha subunit of the amiloride-sensitive sodium channel in an MCF-7 human breast cancer cell line resistant to a number of drugs was found to be associated with increased efflux of these drugs without increase in MDR-1 or MRP expression[72].

iv. *Overexpression of S-adensoylmethionine synthetase*
S-Adenosylmethionine synthetase which catalyzes the synthesis of adenosylmethionine from methionine and ATP, is the major donor for transmethylation reactions. Overexpression of this enzyme in murine neuroblastoma (MNB) cells was associated with increased resistance to

a nucleoside analogue. This cellular adaptation allowed sufficient adenosylmethionine to be synthesized, so that the viability of the MNB cell could be maintained even in the presence of high concentrations of adenosyl homocysteine[73].

v. *Loss of functional retinoblastoma protein* [67]. Loss of functional retinoblastoma protein may contribute to antimetabolite resistance, because cells lacking this protein may have increased levels of enzymes associated with proliferation (e.g., DHFR and thymidylate synthase) as a consequence of increased levels of free E2F-1, a transcription factor that, in a heterodimeric complex with another protein, DP-1, is normally inactive, because it is bound to hypophosphorylated retinoblastoma protein[67]. When cells progress from the G_1 to the S phase, retinoblastoma protein gets hyperphosphorylated and releases the bound E2F-1-DP-1 heterodimer, which then activates the transcription of genes involved in DNA synthesis. A human osteosarcoma cell line that lacks retinoblastoma protein, SaOs2, was found to be intrinsically resistant to MTX, unlike cells with retinoblastoma protein. When a cDNA encoding retinoblastoma protein was introduced into SaOs2 cells, their sensitivity to MTX was restored, in association with decreased levels of DHFR mRNA and protein[74]. Low levels of retinoblastoma protein were found in 18 percent of patients with acute lymphocytic leukemia and in 19 percent of those with acute myelocytic leukemia[75, 76]. However, the effect of the lack of retinoblastoma protein on the sensitivity of cells to MTX and other drugs has not been determined.

CONCLUSION

An understanding of the above mentioned mechanisms provides us the basis for the development of drugs which can specifically interact with the cause of resistance and restore the sensitivity of the tumor cell. This reversal of drug resistance has a significant role in modern day cancer chemotherapy.

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