MULTIFACTORIAL NATURE OF TUMOR DRUG RESISTANCE

G.I. Solyanik

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine, Kyiv, Ukraine

Tumor drug resistance (TDR) remains a major obstacle for successful treatment of cancer. Till nineteenth years of past century, resistance of tumors to anticancer drugs was most often ascribed to gene mutations, gene amplification, or epigenetic changes that influence the uptake, metabolism or export of drugs from single cells. Meanwhile it became apparent that TDR was formed at the different level of tumor biological structure: in addition to intracellular mechanisms, interactions of cancer cells (multicellular mechanisms) as well as solid tumor microenvironment (including tumor vascularization, components of extracellular matrix and connective tissue) played an important role in protecting cancer cells from initial drug exposure. The limited ability of cancer drugs to penetrate tumor tissue and to reach tumor cells in a potentially lethal concentration makes a significant contribution to low efficacy of cancer therapy and is often resumed as an occurrence of TDR. Failure to recognize such clinical drug resistance cannot be explained entirely by mechanisms operative at the level of the single cell may lead to disappointing results in clinical trials. Presented data demonstrate a multifactorial nature of TDR. Pharmacokinetics and pharmacodynamics aspects of TDR mechanisms are analyzed. The methods to overcome TDR and to increase the efficacy of cancer therapy are discussed. Key Words: tumor drug resistance, cancer drugs, pharmacokinetics.

Genes are not proteins, proteins are not cells, cells are not tissues, tissues are not organs, organs are not mice, mice are not patients. With each increase in biological complexity comes increased potential for resistance (K.D. Miller, 2003)

An experience of use of the conventional methods of treatment of malignant tumors, such as surgical treatment, radiation therapy, and chemotherapy, has demonstrated their limited possibilities and comparatively low efficacy against locally advanced and disseminated forms of malignant neoplasia. Analysis of patterns of anticancer chemotherapy (ACT) which are related to low specificity of antitumor action and high toxicity toward normal organs and tissues shows that it’s necessary to find a way to elevate ACT efficacy. In clinical practice low selectivity of ACT action is manifested in a low difference between the dose of anticancer drug (required to achieve a desired anticancer effect) and the dose causing significant damage of normal organs and tissues (life-treating dose).

Selection of optimal strategy and tactics of treatment is similar to a search of a path on a ridge in a fog where a step to left (toward the decrease of anticancer drug dose) — may result in lethal outcome owing to tumor process manifestation, while a step to right (toward dose elevation) — may lead to patient’s death caused by the development of irreversible side effects.

Among multiple factors that determine tumor response on ACT, tumor drug resistance is considered as the main factor that limits an efficacy of the treatment.

The terms «resistance» and «sensitivity» are similar; moreover, the differences between them have quantitative but not qualitative character. If 100% of tumor cells survive after the action of cytotoxic agent (at definite concentration) then one could say about complete resistance. If less than 100% of cells survive, then partial resistance (or partial sensitivity) could be stated.

In reality, cancer cells that demonstrate resistance at one dose or concentration of anticancer agent may reveal expressed growth suppression at higher doses of the same preparation.

In clinical practice complete tumor resistance to cancer chemotherapy by single agents could be regarded in the case when an anticancer agent administered at maximal tolerated dose (MTD) is not capable to stop tumor growth.

Two forms of tumor drug resistance (TDR) are recognized. If administration of the drug (at MTD) at a first line of cancer chemotherapy is unable to inhibit tumor growth, then it is said about natural or preexisting tumor resistance to this preparation. Non small cell lung cancer may serve as an example of preexisting resistance. Meanwhile, there is often observed a situation when the preparation initially effective against certain tumor, losses its efficacy during the process of therapy. Such situation is considered as an acquired drug resistance. For example, an efficacy of treatment of patients with small cell lung cancer is very high in more than 90% of cases, however in many patients just in 6–9 months after the treatment, recurrence of tumors with extremely resistant phenotype is observed.

Phenomenon of TDR has been firstly found and then received its scientific development in the studies of antibacterial therapy but nor anticancer one. The history of these studies began from 1943, after the publication of the work of S. Luria and M. Delbrück [1], where it was shown that resistance of bacteria to phagocytosis resulted from the genetic mutations. In 1979, Canadian scientists J. Goldie and A. Coldman [2] whose research was based on clonal theory of tumor origin [3, 4] and who adapted the results of studies of prokaryotic cell resistance, created the basis of clonal-selection concept of drug resistance formation of tumor cells.

In the frame of this concept the high frequency of genetic mutations in tumor cells leads to an appearance of different tumor clones possessing various sensitivities to the action of damaging agents. Selection factors such as cytotoxic agents cause the death of the cells that are sensitive to their action but exert no cytotoxic affect on resistant
Invasion
Metastasis

Hydrophobic, heterocyclic antineoplastic drugs such as consisting of 49 transporters) that facilitates the efflux of MDR1 protein is a member of a family of transporters (Figure) [5]. That results in the considerable changes of tumor cellular composition towards domination of resistant subpopulations and alterations of tumor growth kinetics and sensitivity to cancer chemotherapy [6, 7].

Figure. Clonal-selection concept of malignant tumor progression.

Such prehistory-selection study in oncology has strictly determined the direction of these researches: about a quarter of the century all the efforts of scientists have been mainly concentrated on the study of intracellular mechanisms of drug resistance. Meanwhile, in 90th, it became clear that the phenomenon of drug resistance of malignant tumors is not limited by an assessment of separate cells and in many respects is different from a resistance to antibacterial and antiviral therapy.

Drug resistance of malignant neoplasia is formed at three levels of biological organization of tumor — the level of single cells (intracellular mechanisms); the level of multicellular formations (contact/adhesive mechanisms); at the level of whole tumor possessing organ structure with an intrinsic components of extracellular matrix, connective tissue and vasculature. The basis of tumor tolerance to performed therapy is formed by pharmacokinetic or pharmacodynamic mechanisms or by their combination.

Due to intense in vitro experiments, intracellular mechanisms of drug resistance formation are studied more complete at functional as well as molecular-biological levels.

**Intracellular mechanisms of TDR.** Intracellular mechanisms form defensive reaction of cells in three main directions.

1. Decrease of drug accumulation in the cell:
   - Enhanced elimination;
   - Decreased uptake;
   - Inactivation (intracellular metabolism).
2. Activation of reparative processes (DNA)
3. Alteration/modification of targets of drug action.

As far as the majority of antineoplastic drugs exert their tumoricidal effects against intracellular targets on cellular molecules (DNA for instance), their intracellular distribution plays an important role in TDR formation. That’s why, the mechanisms related to the decrease of intracellular accumulation of anticancer drugs (so called pharmacokinetic mechanisms) due to overexpression of P-glycoprotein or multidrug resistance-1 (MDR1) were among the first ones that have been studied in details [8–11]. It is known that MDR1 protein is a member of a family ATP-dependent transmembrane transporter proteins (consisting of 49 transporters) that facilitates the efflux of hydrophobic, heterocyclic antineoplastic drugs such as doxorubicin, vincristine, taxol, etoposide etc. Such form of resistance was named multidrug resistance (MDR) [12].

The attribute of MDR is that the tumor with an emergence of MDR to the action of a single cytotoxic agent, acquires cross-resistance to many other drugs which can also be exported by ATP-dependent transporters. The progress in molecular-genetic methods allowed to isolate and to characterize a great number of ATP-dependent transporters and to estimate their cytostatic substrates (Table).

**Table.** Cytotoxic drugs and their ATP dependent transporters

<table>
<thead>
<tr>
<th>Cytotoxic drugs</th>
<th>ATP dependent transporters</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin-D; daunorubicin; docetaxel; doxorubicin; etoposide; mitoxantrone; paclitaxel; teniposide; topotecan; vinblastine; vincristine; vinorelbine paclitaxel; vinblastine doxorubicin; epirubicin; etoposide; methotrexate; vincristine; vinorelbine cisplatin; doxorubicin; etoposide; methotrexate; mitoxantrone; vincristine; doxorubicin; mitoxantrone; topotecan</td>
<td>MDR1 (P-gp) multidrug resistance protein 1 (P-glycoprotein)</td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone; paclitaxel; vinblastine; vincristine; vinorelbine; cyclophosphamide; cisplatin; doxorubicin; etoposide; mitoxantrone; daunorubicin; doxorubicin; etoposide; methotrexate; mitoxantrone; vincristine; doxorubicin; mitoxantrone; topotecan</td>
<td>MDR2 multidrug resistance protein 2 multidrug resistance protein 1</td>
<td></td>
</tr>
<tr>
<td>CMOAT canalicular multi-organic anion transporter</td>
<td>MRP2 multidrug resistance protein 2</td>
<td></td>
</tr>
<tr>
<td>MXR mitoxantrone resistance protein</td>
<td>BCRP breast cancer resistance protein</td>
<td></td>
</tr>
</tbody>
</table>

As far as P-gp transports the largest number of cytostatics, the possibility of its inhibition aimed on overcoming drug-resistance was under the study for more than a quarter of a century [13, 14]. The mechanism of tumor sensitization with the modulators of the the first-generation (verapamil, cyclosporin, dextrerapamil) was based on their competitive binding with the transporter, thus impeding an elimination of anticancer drug. The main disadvantage of these modulators was their high toxicity at the doses required for tumor sensitization [15, 16]. The efforts to modify their action and to decrease the toxicity did not achieve the desired result. The modulators of the second-generation did not reveal significant toxicity, but significantly affect the pharmacokinetics of anticancer preparations. At present time the new generation of MDR modulators based on direct inhibition of the transporter (tarquidar, cyclopropidibenzosuberane, zosuquidar) has been developed [17, 18]. These modulators do not demonstrate an expressed toxicity and do not interact with cytochrome P450, i.e. do not influence anticancer drug pharmacokinetics. Presently, clinical trials of third generation modulators are on the way, and the preliminary results are promising, showing that partly the problem of MDR will be solved.

The other mechanism that helps tumor cell to avoid death caused by some cytotoxic agents (alkylating antineoplastic drugs) — is an activation of reparative processes in cells, at first hand — repairation of DNA damage that in the majority of cases is realized with participation of the DNA repair protein, O6-alkylguanine-DNA alkyltransferase (AGAT) [19]. Tumor cells can be classified into two groups depending on the relative level of AGAT. Cells with low or absent AGAT activity are quite sensitive to alkylating agents. Cells containing relatively high levels of AGAT activity are consequently resistant to these drugs. AGAT inhibitors (O6-benzylguanine) allow potentiation cytotoxic action of alkylating agents, but only against tumor cells with high levels of AGAT activity [20].

A significant role in DR formation is played by pharmacodynamic mechanisms related to alteration or modifica-
tion of targets of action of anticancer drugs. During the last decade these mechanisms were in the spite of interest due to the development of a new class of anticancer preparations, so-called targeted cancer therapeutics. Targeted therapies are designed to selectively inhibit a target that is abnormal in malignant compared with normal tissues; these drugs often affect events in signalling pathways that drive abnormal growth [21]. Protein kinases have emerged as one of the most frequently targeted families of proteins in drug discovery. Currently, there are dozens of drugs (small molecules or monoclonal antibody) approved for clinical use that target protein kinases and/or the receptors that activate them.

As it has been supposed, that the process of the formation of cancer cell resistance to the action of targeted therapies will be either slowed, or decreased. Amazing success of imatinib against chronic myelogenous leukemia (as well as trastuzumab in management of both early and advanced breast cancer) has revolutionized the whole area of targeted cancer therapeutics. However, enthusiasm for their striking efficacy has been tempered by the development of clinical resistance [22, 23]. In many cases, resistance results from kinase domain mutations and/or overexpression of the coding gene and/or loss of critical tumor suppressors [24–26].

To overcome resistance to imatinib, several novel BCR-ABL inhibitors have been developed (Nilotinib, Sunitinib etc.) and are in clinical trials, though it is inevitable that resistance to second-generation inhibitors will occur as well [27]. Nonetheless, kinases represent an attractive target for therapeutic intervention and, at present, about 20 different kinase inhibitors are in clinical trials.

Other mechanisms of resistance to targeted agents are connected with the development of alternative pathways of cancer cell survival and disease progression. For example, progression of chronic myelogenous leukemia to more advanced stages under the inhibition of BCR-ABL by imatinib may result from the activation of SRC family tyrosine kinases LYN and HCK [28]. One of the mechanisms of TDR Bevacizumab (monoclonal antibody to vascular endothelial growth factor — main proangiogenic factor) is an induction of tumor angiogenesis by other endogenous mediators of neovascularization (FGF-2, TGF-β, PDGF etc.). Activation of alternative pathways is one of the mechanisms of resistance to Trastuzumab (monoclonal antibody to erbB-2 — human epidermal growth factor receptor 2).

Taking into account the multiplicity of alternative metabolic pathways in a cell, providing its survival upon the action of antineoplastic agents, combination of targeted drugs (polychemotherapy analog) with different mechanisms of action could provide benefits in cancer therapy.

**Multicellular mechanisms of TDR.** During long time the limitations of clinical chemotherapy have been ascribed to mechanisms that mediate drug resistance at the cellular level. Functional gene mutations or other changes that affect the expression of genes encoding proteins that influence the uptake, metabolism, and export of drugs from a single cell are important determinants of drug resistance, as are epigenetic changes that can lead to transient drug resistance. However, substantial evidence suggests that mechanisms that involve the tumor microenvironment also mediate resistance of solid tumors to chemotherapy.

More than a decade ago Teicher et al. [29] established that drug selection in vivo differed from selection in vitro. They showed that EMT-6 murine mammary tumors selected for resistance to *cis*-diaminedichloroplatinum, carboplatin, cyclophosphamide, or thiopeta *in vivo* for 6 months lost the drug-resistant phenotype when cultured *in vitro*. Moreover the response of spheroids to drugs differs from that of cells in monolayer culture not only because of limited drug penetration and differences in hypoxia and proliferation status, but also because of the cell adhesion effect. This feature is common to other multicellular cultures and to tumors *in vivo*. This form of acquired tumor drug resistance was named as multicellular drug resistance (MCR) [30–33].

Numerous studies showed an important role of integrin-specific adhesion of cells to fibronectin (FN) (not E-cadherin-mediated adhesion) that provides an initial survival advantage to FN-adherent cells under cancer drug treatment. An increased expression and/or high affinity state of α4β1 (and/or α5β1) integrin expression and FN adhesion were shown to be associated with tolerance of cancer cells to doxorubicin, etoposide, mitoxantrone, melphalan etc. [34–36].

Multicellular mechanisms of drug resistance associated with enhanced cell adhesion, represent the form of collective defense from the action of damaging agents. Such defense results in:

- decreased bioavailability of anticancer agents,
- decreased proliferative activity of tumor cells,
- support of cell survival via contact interaction.

Anticancer drugs and nutrient substrates enter tumor cells from blood stream by diffusion in tumor tissue. The diffusion rate is quite low. For instance, the rate of oxygen diffusion is the highest one, and its penetration depth is rarely higher than 5–7 tumor cell layers. For molecules with higher molecular weights the penetration depth is lower. An increase of intercellular contacts results in a decrease of both a diffusion rate and penetration depth of nutrient substrates and anticancer agents inside the tumor. As a result, a decreased level of accumulation of cancer drugs and an appearance of large hypoxic areas and deficiency of other substrates required for cancer cell activity are an attribute of malignant tumors. On the one hand the deficiency of nutrient conditions on significant deceleration of cellular aggregates due to transition of cells from proliferating to resting state [37, 38]. On the other hand the expression of hundreds of genes in cancer cells (including MDR one’s) is altered under hypoxic conditions. For instance MDR1 expression has been shown to be induced by hypoxia [39]. A conserved hypoxic response is mediated by the transcription factor known as hypoxia-inducible factor (HIF) [40, 41].

The decrease of proliferative activity as a result of the enhancement of cell adhesion represents a classic mechanism of regulation of cell division, first of all for a normal cell. This mechanism is preserved in tumor cell as well, however it could be realized through the biochemical pathways other than that in the normal cells.

Croix et al. [42] showed that spheroid culture of EMT-6 mammary tumor cells inhibited 4-HC- and γ-irradiation-mediated cell death when compared with cells cultured as a monolayer. This intercellular adhesion-mediated resistance correlated with a 15-fold increase in the expression
of cyclin-dependent kinase p27Kip1 and decreased proliferation. Critical role of p27Kip1 in intracellular contact-mediated resistance to a lot of different cancer drugs has been revealed. The methods of inhibition of this kinase aimed on overcoming MCR are presently in active development.

**Tissue mechanisms of DR.** The study of molecular mechanisms of intracellular and multicellular types of TDR allowed revealing the key molecular markers that, from one side, were used as the targets for therapeutic intervention directed on tumor sensitization, and, from other side, could be considered as challenging markers for evaluation of a degree of tumor resistance to the action of antineoplastic agent. For instance, P-gp hyperexpression is considered as a marker of tumor resistance to anthracyclines; elevated level of glutathione-S-transferase — as a marker of resistance to platinum-containing preparations; amplification of dehydrofolate reductase — as a marker of resistance to methotrexate; decreased level of topoisomerase 1 — as a marker of resistance to camptothecin analogs; decreased level of topoisomerase II is associated with the resistance to etoposide, tenoposide, antracyclines, and metaxantrone [43, 44].

Meanwhile, often molecular markers are not predictive of response to single-agent chemotherapy in cancer patients. An absence of correlation between expression of «resistance» markers and clinically observed sensitivity of the tumors to the action of anticancer preparations may be caused by tumor heterogeneity with spatially nonuniform distribution of cellular subpopulations with different sensitivity [45, 46]. That's why an evaluation of the degree of tumor resistance (or sensitivity) carried out with the use of a part of tumor material (biopsy or surgical one), may contain a significant error. The possibility to obtain a false result elevates markedly in a case if the sampling of tumor material is performed after any type of treatment (for example, neoadjuvant chemotherapy).

Significant influence on the efficacy of ACT has the peculiarities of cancer drug biodistribution as well as tumor microenvironment — so-called tissue mechanisms of resistance.

Solid tumors are not aggregates of cancer cells — they are organ-like heterogeneous and complex structures. They consist of cancer cells and stromal cells that are embedded in an extracellular matrix and nourished by a vascular network. Each of these components may vary from one location to another in the same tumor and influence the outcome of cancer therapy.

Upon high sensitivity of tumor cells to ACT its low efficacy may be determined by the next events:
- after administration, the preparation does not reach tumor tissue or penetrate into it in a form of inactive metabolites (the decrease of tumor bioavailability) [47, 48];
- tumor forms an adaptive reaction at tissue level using extracellular matrix components, connective tissue and vascular network.

It is known that cancer patients are characterized by the significantly decreased life-time of pharmacological agents (including anticancer ones) in a body due to impaired liver and kidney function and hypoalbuminemia with altered drug binding [49]. Apart from this, imbalance between production and regulation of proangiogenic and antiangiogenic factors in tumor cells (shifted to dominance of proangiogenic stimuli) leads to disorganization of tumor vascular network. Blood vessels in tumors are often dilated and convoluted and compared with normal tissues have branching patterns that show excessive loops and arterio-venous shunts. Such abnormal vasculature worsens transport function of blood and decreases diffusive flux of anticancer drugs into tumor tissue.

Interaction of tumor cells with the components of extracellular matrix, cytokines, and growth factors produced by microenvironment contributes into formation of drug resistance. Among them, the special place is occupied by endogenous mediators of angiogenesis that promotes survival and progression of tumor cells under cytotoxic chemotherapy not only via induction of angiogenesis, but also due to activation of intracellular signal cascades providing apoptosis inhibition and activation of migratory and invasive properties of tumor cells [50]. Regulation of tumor cell activity by proangiogenic factors is performed by paracrine and autocrine mechanisms. Moreover it was shown that the production of proangiogenic factors by tumor cells depended on the degree of their resistance and might significantly rise upon its elevation [51, 52].

**CONCLUSIONS**

TDR phenomenon represents multidimensional and multifactorial problem. The efforts to overcome this problem by it resolution into simple components and to solve it just in one platitude did not give, are not giving and, the most likely, will never give the possibility to achieve a wishful results. This is related to the development of the methods of evaluation of tumor sensitivity, as well as to the development of drug resistance modifiers. As it follows from multifactorial nature of TDR phenomenon and significant variability of physiological and pathophysiological characteristics of cancer patients, the way to overcome tumor drug resistance and to increase an efficacy of anticancer therapy may be find through complex scientific studies and individualization of anticancer chemotherapy.

**REFERENCES**


