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Multidrug resistance in human cancer

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Abstract

Resistance to cytotoxic chemotherapy continues to be a major obstacle to more effective treatment of human cancers. A particular problem in clinical cancer chemotherapy is the phenomenon of simultaneous resistance of cancers to a variety of unrelated cytotoxic agents. Such resistance to multiple drugs is observed much more often than resistance to individual compounds. A similar experimental phenomenon has been termed multidrug resistance or MDR. Much has been learned in recent years about molecular mechanisms which can lead to MDR in cancer cells and a number of studies has been performed to evaluate the clinical relevance of such mechanisms. In particular, P-glycoprotein-associated MDR (MDR1) has received a lot of attention. This review will discuss (i) some principal aspects of drug resistance in cancer with particular emphasis on MDR1; (ii) available data on drug resistance mechanisms in brain tumors; and (iii) our current knowledge on the putative role of P-glycoprotein in the blood-brain barrier.

Principal aspects of chemotherapy resistance

Chemotherapy resistance in cancer can be intrinsic or acquired. Tumors with intrinsic or *de novo* resistance fail to respond to the first chemotherapy given. This continues to be a major problem in various solid tumors, such as non-small cell lung cancers or gastrointestinal carcinomas. In acquired resistance, tumors initially respond to chemotherapy but eventually progress in spite of treatment. In both scenarios, simultaneous resistance to a variety of unrelated cytotoxic agents is much more common than resistance to an individual agent or a particular class of cytotoxic drugs.

When thinking about chemotherapy resistance in cancer, particularly when doing research in this field, it is useful to distinguish between questions related to resistance on the molecular, cellular and clinical level (Table 1). Lack of doing so has often resulted in poor study design and/or misinterpretation of data.

Multidrug resistance

Simultaneous resistance of cancers to multiple agents is a common clinical experience. Thus the experimental phenomenon of MDR has received much attention in recent years. Various molecular mechanisms have been identified which can result in MDR (reviewed in [1–3]). These include overexpression of the MDR1 gene and its protein product P-glycoprotein (Pgp); overexpression of the MRP gene and its protein product, like Pgp a membrane protein belonging to the superfamily of ATP-binding cassette transporter proteins; changes in the glutathione system; and reduced expression and/or activity of topoisomerase II.

A large amount of data has accumulated on the potential role of particular MDR mechanisms in clinical drug resistance. Unfortunately, most of this data must be considered inconclusive. This is partly due to shortcomings in study design and to various problems of the detection methods employed in

Table 1. Chemotherapy resistance in cancer. Some important questions related to the molecular, cellular and clinical levels of drug resistance

Molecular resistance
– Overexpression of drug transport proteins (e.g., P-glycoprotein; MRP p190)?
– Lack of drug transport proteins (e.g., folate transporter)?
– Altered drug target(s) (e.g., topoisomerase II; dihydrofolate reductase)?
– Enhanced drug detoxification (e.g., increased activity of glutathione redox cycle, metallothione or aldehyde dehydrogenase)?
– Enhanced DNA repair (e.g., increased activity of O^6-alkylguanine-DNA alkyltransferase)?
Cellular resistance
– Phenotype (e.g., resistance to single agent or multiple drugs; if multiple drugs, structurally and/or functionally related or unrelated agents)?
– Level of resistance?
– Altered cellular pharmacology of affected drug(s) (e.g., reduced uptake; enhanced efflux)?
– Molecular resistance or resistance due to tumor growth kinetics?
Clinical resistance
– Cellular or pharmacokinetic resistance?
– Impact of cellular/molecular resistance mechanism(s) on treatment outcome?
– Circumvention of resistance feasible?

these studies. However, this is also due to the complexity of clinical MDR. Various cytotoxic agents can be affected by more than one molecular mechanism. For instance, resistance to drugs such as doxorubicin or etoposide can be due to overexpression of Pgp or to reduced activity of topoisomerase II. Furthermore, MDR in human cancers can be multifactorial. Tumors may be composed of subpopulations of cancer cells which use differing resistance mechanisms ('resistance heterogeneity'). Alternatively, various resistance mechanisms may be operating simultaneously in particular cancer cells. Finally, overexpression of 'resistance proteins' such as Pgp might only be an indicator of a particular phenotype of tumor cells which is associated with poor prognosis, e.g., due to overexpression of particular oncogenes and/or loss of function of so-called tumor suppressor genes. Thus unambiguous assessment of the clinical relevance of particular MDR mechanisms with respect to chemotherapy sensitivity will continue to be a difficult task.

P-glycoprotein-associated multidrug resistance

The MDR mechanism most extensively studied in recent years is Pgp-associated MDR, also termed MDR1 or 'classical' MDR [3]. MDR1 is characterized by simultaneous resistance of cancer cells to a variety of structurally and functionally differing cytotoxic drugs, which usually are of natural origin or semisynthetic derivatives of natural product drugs. The clinically most relevant classes of agents affected by MDR1 are listed in Table 2, which also shows various cytotoxic drugs not involved in MDR1. These include platinum compounds, alkylating agents such as cyclophosphamide or melphalan, antimetabolites such as methotrexate or 5-fluorouracil, and also the agents most effective in the chemotherapy of brain tumors, i.e., chloroethylnitrosoureas and procarbazine. The pharmacological hallmark of MDR1 is reduced cellular drug accumulation due to enhanced efflux mediated by Pgp, which is believed to function as energy-dependent multidrug efflux pump.

Various methods have been developed to detect overexpression of MDR1 in clinical cancer specimens at the molecular or protein level, e.g., immunostaining and flow cytometry for detection of Pgp, reverse transcriptase polymerase chain reaction (RT-PCR), ribonuclease protection assay and RNA dot or slot blot for detection of MDR1 RNA transcripts. Two issues have to be kept in mind when selecting particular methods for MDR1 detection in clinical tumor samples: (a) MDR1/Pgp is present in

Table 2. Clinically important cytotoxic agents which are affected and not affected by P-glycoprotein-associated multidrug resistance

Affected	Not affected
Antracyclines	Classical alkylating agents
Vinca alkaloids	Chloroethylnitrosoureas
Podophyllotoxins	Procarbazine
Anthracenediones	Platinum compounds
Taxus compounds	Antimetabolites

many normal cells, including liver cells lining the biliary canaliculi, epithelial cells in colon and proximal renal tubuli, adrenal cells, endothelial cells in CNS and testicular capillaries, CD34+ hemopoietic stem cells and various lymphocyte subtypes [4–7]. (b) Low level overexpression of MDR1 has been frequently detected in a variety of tumor types and such low levels might be sufficient to confer clinical drug resistance. Accordingly, an ideal MDR1 detection method would be a so-called *in situ* technique, i.e., a method which allows to discern between normal and malignant cells expressing MDR1/Pgp, which has sufficient sensitivity to detect low level overexpression. Unfortunately, no method is currently available which meets both criteria. In addition to MDR1/Pgp detection methods, assays are available which are capable of analyzing Pgp function in clinical tumor specimens by measuring accumulation and/or efflux of Pgp substrates.

The variety of techniques used to detect MDR1/Pgp expression is one reason for the often discrepant results in studies of MDR1/Pgp expression in clinical tumor samples. In general, MDR1/Pgp overexpression has been more frequently found in cancers which have failed prior chemotherapy than *de novo*. The exception from this rule are cancers arising from tissues which physiologically express Pgp, such as colon, kidneys or pancreas, where MDR1/Pgp can often be detected prior to any cytotoxic treatment. Studies in acute leukemias and various childhood cancers have shown a significant correlation between MDR1/Pgp-positivity of tumors and poor treatment outcome, i.e., response to chemotherapy, disease-free as well as overall survival [8–10]. However, the question remains whether the poor treatment results in Pgp-positive patients were indeed the result of Pgp-mediated chemotherapy resistance or Pgp was only an indicator of a more malignant phenotype (see above).

A number of agents from various pharmacological classes of drugs has been shown to be capable of reversing MDR1 *in vitro* and in animal models [11]. Various examples of these so-called chemosensitizers (CS) are shown in Table 3. The major mechanism through which CS appear to function appears to be competitive inhibition of the hydrophobic binding of MDR1 drugs to Pgp. As a result, Pgp-mediated efflux of the cytotoxic agents is inhibited which leads to increased intracellular accumulation and thus cytotoxicity.

Many CS have been used in clinical MDR reversal studies (reviewed in [12–15]). Thus far, clinical effectiveness of CS has been low in patients with solid tumors. However, in various hematologic neoplasms including multiple myeloma and acute myeloid leukemia the addition of CS to chemotherapy in previously drug-refractory patients has been able to re-induce remissions in a significant portion of patients. Thus the concept of MDR1 reversal not only appears to function in experimental systems but also in patients. A number of problems have been observed in clinical reversal studies. These include the toxicities of the CS, often preventing dose escalation to achieve plasma levels high enough for effective MDR1 reversal, and pharmacokinetic interaction between CS and MDR1 drugs. For instance, addition of cyclosporin A to etoposide has been shown to result in an increase in etoposide AUC (area under the plasma disappearance curve) by more than 50% due to reduced renal and nonrenal elimination of the drug [16]. This increase was associated with significantly enhanced bone marrow toxicity. It has long been a concern that effective inhibition of physiologic Pgp function might increase chemotherapy toxicity, as Pgp might be in-

Table 3. Agents capable of reversing P-glycoprotein-associated multidrug resistance

Classes of drugs	Examples
Calcium channel blockers	Verapamil, bepridil, nifedipine, diltiazem, *dexverapamil, dexniguldipine, Ro 11-2933*
Calmodulin inhibitors	Trifluoperazine, thioridazine, chlorpromazine, clomipramine
Lysosomotropic agents	Quinine, quinidine, chloroqine, quinacrine
Steroids	Progesterone
Antiestrogens	Tamoxifen, toremifene
Cyclic peptide antibiotics	Cyclosporin A, *PSC 833, SDZ 280–446*
Miscellaneous	Dipyridamole, amiodarone, cefoperazone, ceftriaxone, erythromycin, *S 9788*

Investigational agents.

volved in the elimination of drugs, the protection of tissues such as the CNS, and the protection of particular normal cells such as early bone marrow progenitor cells.

Chemotherapy resistance in brain tumors

Efficacy of chemotherapy in adult brain tumors such as glioblastoma multiforme or high-grade astrocytomas has remained poor [17]. Although the majority of cancers initially respond to chemotherapy, responses tend to be short and impact of chemotherapy on survival seems minimal at most. Table 4 highlights some critical questions to be asked with respect to the clinical, cellular and molecular levels of chemotherapy resistance in brain tumors. In controlled trials, only chloroethylnitrosoureas and procarbazine have demonstrated unequivocal activity in brain tumors in adults. Accordingly, brain tumors are *de novo* resistant to the vast majority of cytotoxic agents. Such resistance might be of cellular/molecular and/or pharmacokinetic origin.

Table 4. Chemotherapy resistance in brain tumors. Some important questions related to the clinical, cellular and molecular levels of drug resistance

Clinical resistance
– *Cellular or pharmacokinetic resistance?*
– *Which drugs have proven activity in brain tumors?*
– *Which are the molecular mechanisms which can confer resistance to these particular agents?*
– *Which are the mechanisms that can confer resistance to cytotoxic agents with no activity in brain tumors (e.g., lack of access due to blood-brain/tumor barrier; molecular mechanisms)?*
– *Clinical relevance of molecular resistance mechanisms present in brain tumors?*
– *Circumvention of resistance feasible?*
Cellular resistance
– See Table 2
Molecular resistance
– Increased DNA repair (e.g., increased activity of O6-alkylguanine-DNA alkyltransferase)?
– Enhanced drug detoxification (e.g., increased activity of the glutathione redox cycle)?
– Overexpression of drug transporter proteins (e.g., MDR1/Pgp, MRP/p190)?

Permeability of the blood-brain barrier is limited for most cytotoxic agents. Accordingly, even for such highly chemotherapy-sensitive cancers as acute leukemias or non-Hodgkin's lymphomas the CNS can function as 'sanctuary' from the effects of systemic chemotherapy. There is some data to suggest permeability in brain tumor capillaries to be increased as compared to normal vessels. Nonetheless, most cytotoxic agents seem to be unable to cross the blood-tumor barrier. Furthermore, distances between capillaries and cancer cells are often substantial in brain tumors and passive diffusion capacity of many cytotoxic agents is limited.

Little data is available to date on molecular resistance mechanisms operative in brain tumors and the clinical relevance of such mechanisms is currently unknown [18]. Various studies have found Pgp expression in brain tumor cells at differing frequencies [19, 20]. No data has been reported on MDR mechanisms other than MDR1/Pgp in clinical brain tumor specimens or on the role of DNA repair enzymes such as O_6-alkylguanine-DNA-alkyltransferase (ATase) in brain tumor resistance. Enhanced ATase activity can confer resistance to drugs such as chloroethylnitrosoureas and procarbazine, and in nitrosourea-resistant glioma-derived tumor cell lines increased ATase activity has been documented [21].

MDR1/Pgp is known to be strongly expressed in endothelial cells of CNS capillaries [7]. In cultured human brain capillary endothelial cells, Pgp has been shown to transport various known Pgp substrates such as *Vinca* alkaloids or rhodamine 123 [22]. CS such as verapamil or cyclosporin A were able to block this transport. Accordingly, MDR1/Pgp is believed to play a role in the blood-brain barrier [23]. Systemic administration of cytotoxic agents pumped by Pgp does not result in significant CNS concentrations, and it seems reasonable to suggest Pgp to be one mechanism preventing these drugs from entering the CNS. Thus, the inactivity of such agents in brain tumors might be due to limited access to the target. Thus one might speculate that effective blockage of Pgp function by CS could render drugs such as anthracyclines active in the treatment of brain tumors. On the other hand, in clinical MDR reversal studies Pgp inhibition might lead to

CNS toxicities by such agents. Both possibilities are of major clinical interest and studies are clearly needed which evaluate CS effects on uptake of MDR1 drugs in normal brain as well as in brain tumors. Various positron emitting radionuclides have been used with success for anthracycline labeling [24]. Thus PET might prove to be a useful technique for studying those issues.

Obviously, much remains to be learned about the mechanisms underlying chemotherapy resistance in brain tumors. Such data appears important for designing therapeutic strategies which can lead to better treatment of patients suffering from such cancers.

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