Cancer multidrug resistance

Progress in understanding the molecular basis of drug resistance in cancer promises more effective treatments.

Multidrug resistance, the principal mechanism by which many cancers develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapy. It affects patients with a variety of blood cancers and solid tumors, including breast, ovarian, lung, and lower gastrointestinal tract cancers. Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells. As the tumor begins to grow again, chemotherapy may fail because the remaining tumor cells are now resistant.

Resistance to therapy has been correlated to the presence of at least two molecular "pumps" in tumor-cell membranes that actively expel chemotherapy drugs from the interior. This allows tumor cells to avoid the toxic effects of the drug or molecular processes within the nucleus or the cytoplasm. The two pumps commonly found to confer chemoresistance in cancer are P-glycoprotein and the so-called multidrug resistance–associated protein (MRP). Because of their function and importance, they are the targets of several anticancer efforts.

Historical perspective

That cells have mechanisms to transport a variety of molecules out of the cytoplasm has been known for decades. For example, organic cation transporters were some of the earliest such mechanisms identified, and the kidney's secretory capability in this regard was first demonstrated in 1947[1]. The first specific correlations between cell membrane transporters or pumps and a drug-resistant phenotype were made in Chinese hamster ovary cell lines in the mid-1970s, when it was shown that a glycoprotein of 170 kD, called P-glycoprotein, correlated with the degree of drug resistance in several cell lines[2]. A variety of cells were found that were resistant to colchicine, vinblastine, doxorubicin, vinca alkaloids in general, etoposide, paclitaxel, and other small molecules used in cancer chemotherapy.

P-glycoprotein was purified in 1979[3], and strong evidence in support of its role in pleiotropic drug resistance came in 1982, when it was shown that DNA from resistant cell lines that was transferred to nonresistant cells was able to confer resistance to the latter that correlated with the expression of the protein[4]. The gene for P-glycoprotein, called MDR-1, was cloned in 1985[5], and the protein's putative function as an energy-dependent pump that expels small molecules from inside cells was postulated on the basis of sequence homologies with bacterial hemolysin transport protein and on other studies[6].

Work on a lung cancer cell line that was resistant to doxorubicin and other chemotherapeutic agents showed that this cell line did not overexpress P-glycoprotein, but did express another protein, namely MRP, cloned in 1992[7]. MRP was also found to be a pump, specifically a member of the ATP-binding cassette transmembrane transporter superfamily, and since that time both the MRP and P-glycoprotein have been significant targets for anticancer compounds.

Current state

It was not long before companies responded to the potential that these two multidrug resistance–conferring proteins offered for anticancer drug discovery. Table 1 lists a selection of companies with programs in this area. Isis, for example, is using its antisense technology to develop drugs to block the synthesis of MRP, and its oligonucleotide combinatorial technology to develop drugs that interfere directly with MRP function. Another significant effort is that of Vertex, which is developing two compounds, Incel (biricodar dicitrate, VX-710) and VX-853, to block P-glycoprotein (MDR-1) and MRP. Incel, an intravenous compound, and VX-853, an oral compound, are intended to be administered in combination with cancer chemotherapy agents, since the notion is that they act by preventing cancer cells from physically removing other anticancer drugs from their interior. The Incel multidrug resistance inhibitor is currently being tested in combination with chemotherapy in five phase II clinical trials targeting breast cancer, ovarian cancer, soft tissue sarcoma, small cell lung cancer, and prostate cancer. Another program is that of Cell Therapeutics, which is developing CT-2584. This is a small molecule drug for the treatment of patients with chemorefractory cancers, including prostate cancer and sarcomas.

Table 1. Selected companies with multidrug resistance programs

Company	Program	Status
Aronex (The Woodlands, TX)	Annamycin against chemorefractory cancers	Phase II, 9/2000
Avigen (Alameda, CA)	MDR gene therapy	Patent
Biochem Pharma (Quebec, Canada) / Vertex (Cambridge, MA)	MDR in cancer	Phase II
Cell Therapeutrics (Seattle, WA)	Small molecule inhibitor of MRP for prostate cancer and sarcomas	Phase II
CytRx (Norcross, GA)	MDR in acute leukemia	Patent
Genelabs (Redwood City, CA)	MDR in small cell lung and colorectal cancer	Preclinical
Genetic Therapy (Gaithersburg, MD)	MDR-1 gene for breast cancer	Phase I/II
Genetix Pharmaceuticals (Cambridge, MA)	Bone marrow chemoprotection	Phase I/II
Immunex (Seattle, WA)	Restoration of tumor sensitivity to anticancer drugs	Market
Ingenex (Menlo Park, CA)	MDR gene therapy	Phase i/II
Isis Pharmaceuticals (Carlsbad, CA)	Antisense oligonucleotides for MDR	Phase II/III
Ixsys (San Diego, CA)	MDR monoclonal antibodies	Phase II
SuperGen (San Ramon, CA)	MDR in cancer	Preclinical
Titan Pharmaceuticals (S. San Francisco, CA)	Induction of MDR chemoprotection in stem cells	Phase I
Xenova (Slough, UK)	MDR in cancer	Preclinical

Source: Biovista (www.biovista.com)

It is important to realize that there are two sides to multidrug resistance. On one hand, cancer cells need to lose their chemoprotective features mediated by MRP and MDR-1. On the other, chemotherapy-sensitive noncancerous cells, such as bone marrow stem cells, need to be protected from the effects of chemotherapeutic agents. Bone marrow destruction is the single most important dose-limiting toxicity factor in the treatment of cancer patients. One reason is that recovery of bone marrow requires the removal of the patient from the chemotherapy regime, thus allowing cancer cells to grow again.

There are probably other multidrug resistance inducing molecules in cancer cells that have yet to be characterized, including ones that belong to the MDR-1 and MRP protein superfamilies.

An example of an approach that targets bone marrow is that of Titan Pharmaceuticals. The company is developing a gene-based product it calls MDRx1, which can confer multidrug resistance to blood progenitor or stem cells, thus protecting them against chemotoxicity. MDRx1 involves the insertion of the MDR1 gene ex vivo into stem cells that have been removed from cancer patients. The modified stem cells are then reinfused back into the patients. It is hoped that there they can repopulate the blood system with chemoresistant blood cells. One advantage is that this would potentially allow patients to be given higher doses of anticancer agents than could be given normally.

Industry challenges

As mentioned before, there is a very strong correlation between the expression of the MDR-1 gene in many cell lines or in tumors derived from patients and the multidrug resistance exhibited by these cells. However, multidrug resistance also occurs in cells that do not show this correlation with MDR-18. This, in fact, represents a key challenge to the development of therapies based on the MDR-1 and MRP targets: There are probably other multidrug resistance–inducing molecules in cancer cells that have yet to be characterized, including ones that belong to the MDR-1 and MRP protein superfamilies.

A related challenge is that MRP-1 and MDR have normal chemoprotective functions in cells throughout the body. They are part of the body's defense mechanisms against toxic small molecules, and together with other membrane transporters and pumps, are key participants in the normal function of the liver, kidney, gastrointestal gland, adrenal gland, and blood-brain barrier. Therefore, any approaches that target these molecules must do so in a way that is tissue and/or cancer specific, so as not to affect the normal function of these molecules in healthy cells.

Finally, given the multiplicity of correlations that exist between P-glycoprotein and MRP expression with the expression of other proteins in cancer cells, another challenge is to find good cell-based models that enable the rapid analysis of these relationships in a meaningful manner. Yeast is an excellent model system, and a network of genes has been identified that confers a drug resistance phenotype similar to that of mammalian cells[9]. This system is being used extensively to study expression and functional aspects of resistance-conferring membrane pumps and agents that are effective against them.

Clinical status

Given the importance of MDR in tumor resistance to chemotherapy, it is not surprising that several companies are conducting clinical trials aimed at MDR-1 and/or MRP as targets. Phase II trials investigating the activity of Vertex's Incel in combination with other agents for the treatment of advanced refractory ovarian cancer and small cell lung cancer are currently underway. The compound is being tested in a range of drug-resistant cancers, including breast, ovarian, prostate, and small cell lung cancer. BioChem Pharma is funding the STS trial and will develop and market the product in Canada. MDR-1 and MRP overexpression have been associated with STS that is refractory to chemotherapy.

Preliminary phase I/II data were encouraging, showing that Incel could restore or enhance the activity of the anticancer agent doxorubicin in STS patients who had documented aggressive disease, and who had intrinsic or acquired resistance to doxorubicin. Doxorubicin is the standard chemotherapy for this disease, affecting about 7,000 new patients annually in Canada and the US. Approximately 70% of patients do not respond to initial chemotherapy, relapse is frequent, and the five-year survival rate for patients who are refractory to chemotherapy is a low 10–30%. According to Vertex's data, Incel blocks both MDR-1 and MRP, and thereby restores the sensitivity of tumor cells to treatment by apparently raising the concentration of anticancer agents inside the target cells.

Another ongoing trial is that of Aronex (The Woodlands, TX), using the anthracycline known as annamycin. Annamycin is under development for the treatment of drug-resistant breast cancer, and like Incel, it has the potential to be used in treating a broad range of cancers. One of the potential advantages of this compound is its better safety profile when compared with other anthracyclines used for similar purposes.

Other key issues include, for example, the fact that agents that can reduce MDR in vitro, such as toremifene, do not work in patients, probably because toremifene is bound to serum proteins in blood. However, a recent study shows how short courses of high dose toremifene in combination with vinblastine was generally well tolerated by patients and that it was possible to achieve concentrations of toremifene in vivo that can reverse MDR in vitro, which opens up possibilities to counteract MDR in the clinic using such combination therapies[10]. Finally, a recent report shows how significant MDR can occur in metastases but not in the primary tumor. This is a critical issue impacting the development of agents that are effective on primary as well as metastatic tumors[11].

The future

Although P-glycoprotein and MDR protein are capable of removing a wide variety of anticancer compounds from inside tumor cells, recent work shows that they cannot remove them all. For example, a novel derivative of olivacine, labeled S16020-2, has shown significant antitumor activity both in vitro and in vivo against cells that display resistance mediated by the MDR-1 phenotype. This may be due to the rapid rate of uptake of this compound, which effectively bypasses P-glycoprotein, leading to its higher intracellular accumulation and effectiveness[12]. Other leads with similar activity profiles against multidrug resistant cancers are also likely to resist, and high-throughput screening efforts are being applied to find them (see Lead validation, p. xx–xx).

An important development to watch for in the future of MDR-1 and MRP-mediated multidrug resistance has to do with establishing links between these proteins and other cancer mechanisms, such as mutant p53 expression. Recent work suggests that in some cells, such a non-small cell lung cancer cells, there is a correlation between MRP and mutant p53 expression, which can be used for prognosis[13]. There are many other such correlations, and their potential importance becomes apparent in the context of a pharmacogenomic analysis of cancer multidrug resistance, where patient variability to anticancer agents could be localized conceivably to some of these gene families (see Pharmacogenomics, pp. 40–42).

In addition, recent structural studies are beginning to reveal the actual components of MRP that are necessary for its function[14]. This work will pave the way towards targeting agents specifically to the functional regions of these molecules, thus inactivating them. Such work is already underway. For example, MDR-1 is being extensively analyzed by the US National Cancer Insitute's COMPARE program for the identification of agents in the NCI database that would be predicted to be good substrates and/or inhibitors for the molecule[15].

Cancer defends itself actively by using resistance-mediating transporter mechanisms, and therefore their impairment is likely to have a significant therapeutic benefit.

The future of MDR is also going to see increasing benefit from novel genetic approaches such as transcriptional decoys. For example, a recent report shows how targeting the promoter of the human MDR1 gene with antisense was effective in causing leukemia cells highly resistant to vinblastine to become susceptible to the anticancer agent[16]. Complementing such approaches, synthetic combinatorial chemistry or biology is developing anticancer compounds or peptides that target the regulatory sequences of MDR1 and block its expression[17].

Finally, novel molecules that can inhibit MDR are likely to come from a variety of sources, and the future will continue to see the increasing screening of compounds from a huge variety of settings for this purpose. For example, a recent study describes how the antifungal antibiotic aureobasidin A can be an effective inhibitor of the MDR1 P-glycoprotein[18], and this is likely to become a significant addition to the arsenal of MDR1 inhibitors.

Conclusions

In the fight against cancer, a number of targets are being pursued with equal zeal. The resistance-mediating transporters discussed here represent a significant set of clinically relevant drug targets that have therapeutic as well as diagnostic potential. Cancer defends itself actively by using these mechanisms, and therefore their impairment is likely to have a significant therapeutic benefit. The science in this area is progressing very rapidly, and corporate involvement is likely to follow suit.

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