Multidrug Resistance (MDR 1) in Leukemia: Is it Time to Test?

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THE USE OF chemotherapeutic drugs in the treatment of leukemia has been plagued by the emergence of resistant cells, either at initial presentation or at the time of relapse. The mechanisms underlying this clinical phenomenon have been studied using in vitro models, leading to the characterization of genes capable of conferring resistance to chemotherapeutic drugs. Among these genes, the P-glycoprotein or multidrug resistance gene (MDR 1) has been of particular interest because its overexpression can lead to resistance to anthracyclines, vinca alkaloids, and podophyllins, drugs that are important in the treatment of leukemia. MDR 1 encodes a membrane protein that acts as an ATP-dependent efflux pump, transporting a number of apparently unrelated organic compounds. The broad spectrum of drugs affected by MDR 1 has made it an attractive candidate to explain the phenomenon of clinical multidrug resistance, whereby a leukemia or tumor becomes refractory to drugs to which it was never exposed. However, proof of the involvement of MDR 1 in clinical drug resistance has been slow to accumulate, primarily because of the difficulty in adapting assays of MDR 1 expression and in planning appropriate clinical trials. Recent clinical studies, including two published in Blood, have attempted to define the clinical significance of MDR 1 overexpression in leukemia at the time of diagnosis and have suggested that it may be appropriate to include elevated MDR 1 expression levels as an adverse prognostic factor in acute myelogenous leukemia (AML). Because a number of drugs have been shown to reverse the action to the MDR 1 protein in vitro, these studies appear to justify the inclusion of such multidrug resistance reversing agents in the induction chemotherapy regimens in cases that have been shown to overexpress MDR 1. These studies raise a number of important questions regarding the normal function of the MDR 1 gene in hematopoietic cells and its ultimate role in the development of resistance to chemotherapeutic drugs.

Much of the difficulty in extrapolating in vitro studies of drug resistance to clinical situations stems from the inherently different selection conditions that are used. Many in vitro studies of drug resistance are performed at concentrations of drug that are considerably higher than those achieved in vivo. While these conditions facilitate molecular studies, they also can lead to distinct genetic mechanisms. In fact, the initial isolation of the MDR 1 gene was made possible by its amplification in drug-resistant cell lines, a phenomenon that has never been reported in clinical specimens. In cultured human cell lines, unlike rodent cells, MDR 1 gene amplification appears to occur only after stepwise selection at high drug concentrations. At lower drug levels, MDR 1 overexpression has been noted in the absence of gene amplification, although the exact mechanism and the underlying genetic alterations have not been identified. In addition to MDR 1 overexpression, at least two other mechanisms of resistance to anthracyclines have been described in vitro: reduced expression or mutation in Topoisomerase II, and altered expression of glutathione transferase. Drug-resistant cultured cells may exhibit a combination of such genetic alterations, but their role in clinical drug resistance is unknown.

MDR 1 itself is part of a multigene family whose members share extensive amino acid homology but appear to have distinct functions. Two mdr genes have been described in humans; three genes are present in mouse and hamster. The genes are closely linked, and are often amplified together in highly resistant cells. However, only MDR 1 and the two rodent genes that are homologous to it have been shown to confer drug resistance in transfection experiments. The second human mdr gene (variably named MDR 2 or MDR 3), along with the third rodent mdr gene, encode proteins that appear to have distinct substrates and are unable to confer resistance to chemotherapy drugs. A number of more distantly related genes, also encoding membrane transport proteins, have recently been discovered, including the cystic fibrosis gene and the HAM genes involved in peptide transport. mdr genes may thus be part of a larger class of membrane transport genes with distinct physiologic roles and endogenous substrates. Much of the information about the normal function of the mdr genes has been derived from studies of their expression patterns. In the mouse, the three mdr genes are differentially expressed in many tissues. In humans, expression of MDR 1 has been noted in organs such as kidney,
colon, placenta, and adrenal gland, and in such specialized structures as the endothelial cells thought to comprise the blood-brain and blood-testis barrier.\textsuperscript{27,30} This finding has led to the suggestion that a physiologic role of MDR 1 is the transport of steroid hormones and the excretion of natural toxins.\textsuperscript{27,31} The expression pattern of human MDR 2/MDR 3 has not been studied as extensively. It appears to be expressed predominantly in the liver,\textsuperscript{15} although expression in prolymphocytic leukemia cells has also been reported.\textsuperscript{32} An interesting observation is that tumors derived from tissues that normally express high levels of MDR 1 are usually resistant to chemotherapy, whereas the initial drug sensitivity of leukemias and lymphomas appears consistent with low MDR 1 expression by normal hematopoietic tissues.\textsuperscript{30}

Implicit in the comparison between malignant cells and their normal counterparts is knowledge of the identity of the transformed stem cell. In leukemia, as in most solid tumors, the precursor cells may constitute a minority of the malignant cell population, with distinct cellular characteristics. A recent provocative study has suggested that hematopoietic stem cells may in fact express detectable levels of MDR 1. Attempts at purifying these cells have often made use of their poor staining with the dye rhodamine, a known substrate for the MDR 1 pump. Chaudhary and Roninson\textsuperscript{30} showed that treatment of these cell populations with verapamil, an mdr reversing agent, led to their increased staining, suggesting that MDR 1 is responsible for the active efflux of rhodamine from these cells. Reversible rhodamine efflux and staining with anti-MDR 1 antibody were shown to be correlated with expression of CD 34, a cell surface marker associated with hematopoietic stem cells. The association between CD34 and MDR 1 expression has also been noted in the bone marrow cells of patients with myelodysplastic syndromes.\textsuperscript{31} These observations lead to the hypothesis that a neoplastic stem cell might be protected from chemotherapy drugs not only by being in a nondividing state, but also by virtue of MDR 1 expression. In addition, the presence of relatively low levels of MDR 1 protein in a small subpopulation of cells, which could not be correlated with MDR 1 messenger RNA (mRNA) content assayed by RNA-polymerase chain reaction (PCR),\textsuperscript{33} shows the importance of technical considerations when screening leukemia samples for MDR 1 expression.

Because no mutation or gene rearrangement has been identified leading to MDR 1 overexpression, screening clinical samples cannot be performed at the DNA level, as is possible for a number of other molecular markers. Instead, differences in expression levels, often relatively modest, must be detected by analysis of MDR 1 mRNA or protein. The ideal assay should be able to distinguish expression of MDR 1 from that of MDR 2/MDR 3, be capable of detecting elevated levels of expression in a small subpopulation of cells, and be reproducible when performed in different laboratories. Few studies published to date have met these criteria. RNA-based assays are limited by the availability of clinical samples that are rapidly frozen so as to prevent RNA degradation. Northern blots, slot blots, and RNase protection analysis have been used, as has RNA-PCR, a highly sensitive technique that can be difficult to quantitate reliably.\textsuperscript{35,36} Perhaps the most informative RNA-based technique is in situ hybridization, which provides both accurate quantitation as well as analysis of individual cells or subpopulations of cells overexpressing MDR 1.\textsuperscript{1} Assays of mdr expression at the protein level have also been used, although most available antibodies do not differentiate between the two mdr proteins. Immunohistochemical staining can be difficult to quantitate, but Dalton et al have made use of a computerized scanning apparatus and have obtained reproducible measurements.\textsuperscript{37} Perhaps more readily available is the fluorescence-activated cell sorter (FACS), which is well suited to studying expression of a membrane protein in hematologic specimens. FACS analysis is both highly sensitive and capable of displaying subpopulations of cells with high MDR 1 expression levels.\textsuperscript{3}

Whichever method is chosen to quantitate MDR 1 expression levels, a critical requirement is the use of consistent standards among different laboratories, both in terms of the drug-resistant cell lines used to calibrate the assays, the percent of positive cells considered meaningful, and the differences in MDR 1 expression that are considered significant. A number of studies have compared measurements of MDR 1 expression by tumor cells with in vitro drug sensitivity assays, so-called clonogenic assays.\textsuperscript{2,38} While a statistical correlation was noted, apparent inconsistencies have pointed to the limitations of both assays. Cases in which cells are resistant to anthracyclines in vitro despite low MDR 1 expression can be explained either by the presence of alternative drug resistance mechanisms, or by the existence of an undetected subpopulation of cells overexpressing MDR 1. On the other hand, in cells with high MDR 1 expression, sensitivity to chemotherapy drugs in vitro may reflect poor viability of MDR 1-expressing cells in culture or doses of drug that overcome the effects of MDR 1 overexpression.

Beyond technical considerations in the measurement of MDR 1 expression, defining the role of this gene in clinical drug resistance has been limited by the absence of large prospective clinical studies. Initial case reports showed the presence of detectable MDR 1 mRNA or protein in some malignant cells from AML,\textsuperscript{39-43} acute lymphoblastic leukemia (ALL),\textsuperscript{44} chronic myelogenous leukemia (CML) in blast crisis,\textsuperscript{45} and multiple myeloma.\textsuperscript{37,46-48} No study has systematically followed-up on patients with drug-sensitive leukemia that became drug resistant at relapse, to determine if a mutation leading to overexpression of the MDR 1 gene can be correlated with the onset of drug resistance. Two clinical studies have recently addressed the significance of MDR 1 overexpression in acute leukemia at the time of diagnosis. A Stanford study, using Slot blot assays, detected elevated MDR 1 mRNA levels in 19% of 31 cases of AML,\textsuperscript{49,50} ALL, and CML in blast crisis at the time of initial diagnosis, and in 50% of 10 different cases at the time of relapse ($P = .06$).\textsuperscript{2} No known prognostic factors could be linked to MDR 1 expression, but the probability of achieving complete remission dropped from 67% to 29% with the presence of elevated MDR 1 expression ($P = .03$). A second study, from Lyon, France, in this issue of Blood,\textsuperscript{6}
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reports measurements of MDR 1 protein by FACS analysis in 150 patients with the initial diagnosis of AML. Of these, 47% were found to have elevated MDR 1 protein in at least 20% of cells analyzed, with a higher fraction of positive cases in patients with a history of myelodysplastic syndromes and a lower percentage in those with promyelocytic leukemia. Elevated levels of MDR 1 protein in leukemia cells correlated with the presence of the CD 34 antigen, although both markers independently conferred a negative prognostic value. Of patients with AML expressing elevated levels of MDR 1 protein, 32% achieved a complete remission, compared with 81% of those without detectable MDR 1 levels (P < 10^-5).

These studies raise interesting questions both about the biology of acute leukemias and about their optimal treatment. It remains possible, and even likely, that some leukemia cells that overexpress MDR 1 do so as a result of a mutation that emerges at the time of initial presentation, or that is selected after an initial course of chemotherapy with anthracyclines. This, however, remains to be documented. An intriguing alternative is that cases overexpress MDR 1 reflect a distinct malignant stem cell, possibly an earlier cell carrying different cell surface markers. If confirmed, this observation would imply a novel classification and prognostic categorization of acute leukemia.

The functional significance of MDR 1 overexpression appears logical and seems to be confirmed by the statistically significant differences in response to chemotherapy. However, caution is called for before concluding that MDR 1 itself is solely responsible for the failure of chemotherapy in leukemias overexpressing this gene, and, therefore, that mdr reversing agents should be incorporated into treatment regimens. MDR 1 expression may itself be a marker for a malignant cell that is drug resistant by a number of different mechanisms. Carefully conducted clinical trials will thus be necessary to determine whether the use of mdr reversing agents is sufficient to reduce the adverse outcome of patients with MDR 1 expressing leukemia. In patients with multiple myeloma expressing MDR 1, early studies have documented transient responses after the addition of verapamil to Adriamycin-containing regimens. It is noteworthy that no untoward toxicity was observed in organs that normally express MDR 1 (ie, kidney, intestines, adrenal) from the combination of verapamil and Adriamycin. However, verapamil itself is difficult to administer because of inherent cardiac toxicity, and major efforts are underway to test less toxic mdr reversing agents, including cyclosporine, phenothiazines, and verapamil isomers. In addition to mdr reversing agents, clinical trials may make use of chemotherapeutic drugs such as high-dose cytotoxic arabino-side and alkylators, which are not substrates for the MDR 1 protein.

The development of in vitro tests to guide the rational use of chemotherapy has long been a goal in the treatment of leukemia. The isolation of the MDR 1 gene has provided a molecular marker, which provides an alternative to clonogenic assays, and offers the possibility of therapeutic interventions based on the results of in vitro testing. However, many uncertainties must be addressed before general screening of AML for MDR 1 overexpression can be recommended: what is the best assay to measure MDR 1 expression and can it be standardized for widespread screening? What is the functional significance of MDR 1 overexpression in leukemia, and can the negative prognosis that it confers be reduced by the use of mdr reversing agents or chemotherapy drugs outside the mdr spectrum? Carefully designed studies are needed to address these issues, which may have significant consequences for the treatment of acute leukemia in the years to come.

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Multidrug resistance (MDR 1) in leukemia: is it time to test? [editorial] [see comments]

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