Membrane Transport of Chemotherapeutics and Drug Resistance: Beyond the ABC Family of Exporters to the Role of Carrier-mediated Processes

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Chemotherapeutic agents almost invariably require, as a prerequisite for activity, the ability to traverse the tumor cell membrane to interact with, and perturb the activity of, intracellular targets. Transport often precedes the interaction between drugs and intracellular enzymes and may regulate the rate and extent of metabolism to cytotoxic or cytostatic derivatives that achieve the pharmacological effect. Lipophilic drugs readily enter cells by passive diffusion. However, many drugs are hydrophilic, diffuse through lipid membranes very slowly, and can only enter cells by parasitizing a transporter normally used for physiological substrates. Indeed, even transmembrane flows of lipid-soluble compounds, such as cholesterol and long chain fatty acids, can be augmented by specific membrane transporters (1, 2).

Early in the era of modern cancer chemotherapy, transport across cell membranes by carrier-mediated processes was recognized as an important determinant of drug activity and resistance. This was first characterized for the antifolate, methotrexate, transported by the carrier for reduced folates (3) and subsequently implicated in clinical resistance (4–6). Likewise, phenylalanine mustard activity was shown to be dependent on transport mediated by amino acid carriers (7). But over the past 2 decades, the major focus has been on the ABC superfamily of exporters, in particular, the p-glycoproteins responsible for the multidrug resistance phenotype that produces pleiotropic resistance to multiple classes of agents (anthracyclines, Vinca alkaloids, taxanes, etc.) with very different chemical structures and mechanisms of action (8, 9). These transporters couple the hydrolysis of ATP to the transport of drugs, unidirectionally, out of cells. p-Glycoprotein can capture and eject drug molecules within the lipid membrane and in this way also slow drug influx. The MRP family of ABC exporters, currently represented by nine genetically distinct molecules, overlap, in part, in their substrate specificities with p-glycoprotein. MRPs broaden the class of agents that can be pumped out of cells to include cisplatin, topoisomerase I inhibitors, arsenite, methotrexate, nucleosides, and nucleobases. MRPs transport some drugs as conjugates of glutathione, glucuronIDE, or other anions, with the potential for exporting a variety of alkylating and other agents that undergo this detoxification reaction (10, 11). MRPs export some drugs after their entry into cells; therefore, the initial rate of drug uptake is relatively unperturbed, but subsequent accumulation is markedly suppressed.

The article by Mackay et al. in this issue of “Clinical Cancer Research” turns our attention again to the importance of transport carriers, in this case, the nucleoside transporters, members of the Major Facilitator Superfamily, represented in the animal and plant kingdom by hundreds of carriers with extensive homologies across species. Each family of carriers transports specific substrates, such as sugars, amino acids, oligopeptides, organic cations, anions, fatty acids, nucleotides, metals, specific vitamins, or nucleosides (12).

Unlike the MRP and p-glycoprotein families, facilitative carriers achieve the transport of drugs both into and out of cells by processes that are not directly ATP dependent. These carriers can be equilibrating, i.e., facilitate drug uptake without the generation of transmembrane gradients, or they can be concentrative by linking the uphill flow of drug into the cell to the downhill flow of another substrate, usually a sodium ion or proton, via the same carrier.

A variety of transport carriers for nucleosides have now been cloned, many by the group at the University of Alberta in Edmonton, Alberta, Canada. There are two broad categories: the equilibrating and the sodium-dependent, concentrative carriers. Other sodium-dependent nucleoside transporters are present in cells but have not, as yet, been cloned. Many of these carriers are transporters of nucleoside antimitabolites (13, 14). Hence, in any tumor cell, there may be more than one nucleoside transport pathway, and the impact of the loss of one carrier on drug activity will depend on the properties and relative activities of the other routes. It is clear from studies in model systems in vitro that loss of nucleoside antimitabolite transport activity can be an important mechanism of resistance to these agents (15, 16). However, the magnitude of loss of drug transport will be limited by the extent to which the tumor cell can tolerate the concurrent loss of transport of the physiological substrate and, for this and other reasons, the extent to which transport is a major determinant of the clinical utility of these drugs is not known.

With the cloning of facilitative carriers, reagents are now becoming available to quantitate expression in human tumors and to correlate these observations with clinical response to antimitabolites. The article by Mackey et al. (15) describes a sensitive immunohistochemical method based on
a monoclonal antibody to a widely expressed equilibrating nucleoside carrier (hENT1). This reagent makes possible assessment of transporter expression in tumor cells, as distinguished from vascular endothelial or other normal stromal cells present in tumor tissues that express this carrier. The article demonstrates low-carrier expression in 25–30% of the breast cancers studied as compared with normal breast epithelial cells. This was independent of clinical stage, histopathological grade, and estrogen and progesterone receptor status. Hence, resistance by this mechanism may limit drug activity across the spectrum of disease stages. As antibodies to additional nucleoside transporters become available, the potential for developing a more complete understanding of the pharmacological impact of nucleoside carriers on anti-metabolite activity will be enhanced.

There is increasing emphasis on molecular characterization of tumors; in particular, the quantitation of proteins that regulate the cell cycle and apoptosis and the application of high-throughput techniques, such as DNA microarrays, to identify genes that are determinants of response to treatment modalities. It is, however, equally important and now possible to characterize, quantitate, and understand the importance of those critical prerequisites of the drug-cell interaction, such as carrier-mediated transport, that must be achieved before downstream events associated with cell death can occur. This issue now assumes greater importance as new nucleoside antimetabolites enter the clinics, some, like gemcitabine, with activities that extend beyond the hematological malignancies to the more common solid tumors (17). The same is true for new-generation antifolates currently in clinical trials that are direct inhibitors of purine and/or thymidylate synthesis (18). There is now a wealth of new information from laboratory and clinical studies on the molecular basis for antifolate drug resistance and cross-resistance patterns associated with impaired transport mediated by the reduced folate carrier along with the availability of reagents to assess carrier expression (4–6, 19–21). Whereas the focus here is on carrier-mediated processes, it is important to note that there is little information on the importance of MRPs as a resistance mechanism in cellular regimens, and much more work is required to fully understand the role of p-glycoproteins in clinical resistance.

There are other reasons for investigators to be alert to the potential importance of the Major Facilitator Superfamily of transport carriers. New combinatorial chemistry approaches are now widely applied to identify small molecules that inhibit specific protein targets. One critical determinant of the activity of these agents in intact cells will be their ability to penetrate the tumor cell membrane. Molecules that enter cells rapidly, although structurally unique, may nonetheless be substrates for well-characterized or obscure carriers, and the properties of these transporters may turn out to be important determinants of cytotoxicity, selectivity, and resistance. For those molecules that have limited cellular permeability, attempts will be made to modify structures to enhance lipid solubility and passive diffusion into cells. However, another approach can be to couple these agents to substrates of known carriers with relaxed structural requirements. These complexes will then serve as vehicles to achieve transport into cells followed by release of the active agent by endogenous enzymes. This approach may have particular potential for enhancing transepithelial transport, in particular intestinal absorption, to make more chemotherapeutic agents bioavailable by the oral route. Transporters that might be harnessed for this purpose could include, among others, the oligopeptide, anionic, or cationic carriers (22–25).

References


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