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IS THE MULTIDRUG RESISTANCE AN ATP CHANNEL?

Abraham EH, Prat AG, Gerweck L, Seneveratne T, Arceci RJ, Kramer R, Guidotti G, Cantiello HF. The multidrug resistance *(mdr1)* gene product functions as an ATP channel. Proc Natl Acad Sci USA 1993;90: 312-316.

ABSTRACT

The multidrug resistance *(mdr1)* gene product, P-glycoprotein, is responsible for the ATP-dependent extrusion of a variety of compounds, including chemotherapeutic drugs, from cells. The data presented here show that cells with increased levels of the P-glycoprotein release ATP to the medium in proportion to the concentration of the protein in their plasma membrane. Furthermore, measurements of whole-cell and single-channel currents with patch-clamp electrodes indicate that the P-glycoprotein serves as an ATP-conducting channel in the plasma membrane. These findings suggest an unusual role for the P-glycoprotein.

COMMENTS

The multidrug resistance (MDR) phenomenon exemplifies how molecular biology can identify genes and protein sequences before the specific protein has been purified and sequenced. The MDR phenomenon involves resistance of cancer cells to a group of anticancer agents that do not, superficially at least, share common structure. The phenotype results from overexpression of a family of transmembrane proteins of approximately 170 kD, which were initially identified after labeling by affinity probes of anticancer drugs. Victor Ling of the Ontario Cancer Center discovered MDR proteins and presciently called them "P-glycoprotein," on the basis of the hypothesis that altered cell permeability to anticancer drugs was responsible (1). Subsequent studies revealed that MDR gene products code for 170-kD membrane glycoproteins, which by hydropathy analysis, span the plasma membrane 12 times and have 2 nucleotide-binding sites and were postulated to function as ATP-dependent efflux pumps (2). Cells that overexpress MDR gene products pump hydrophobic cationic drugs, such as vinblastine, daunomycin and others, out of the cell presumably faster than the compounds can interact with vital cell machinery and exert their anticancer effects. Much support exists for this hypothesis: (a) MDR-expressing cancer cells are drug resistant and rapidly efflux anticancer drugs; (b) transfection of the MDR1 gene into naive cells produces an MDR phenotype and expression of a 170-kD membrane P-glycoprotein; and (c) kinetic studies of drug-sensitive and MDR-related resistant cell lines indicate that the former pump anticancer drugs out faster than do the latter.

Nevertheless, uncertainties have surfaced about how P-glycoproteins function. Why should a cell use ATP to efflux relatively hydrophobic molecules that can readily return to the cell by nonionic diffusion? P-glycoprotein substrates are neither chemically modified nor rendered hydrophilic, and no downstream binding molecule exists to retain them extracellularly. Their detection in the apical domain of several polarized normal cells prompts inquiry into a potential natural substrate or substrates. One hypothesis is that cells use ATP to prevent formation of potentially transforming or damaging DNA adducts from naturally occurring hydrophobic pesticides. We tend to think that pesticides were invented by the chemical industry and neglect the fact that every plant protects itself by a host of such molecules.

If P-glycoproteins function as specific efflux pumps, a direct relation should be found between the amount of P-glycoprotein in a cell and the initial rate of substrate efflux. Rigorous testing of this type has not been performed. Many studies reveal that cells that overexpress P-glycoprotein transport more substrate than do control cells; however, one recent study found no relation between the initial efflux rate and P-glycoprotein content (3). Cells that expressed the highest levels of P-glycoprotein had increased intracellular pH, which suggests that drug efflux may be caused by proton movement! This association was not observed in another study but requires further investigation.

Clinical interest in reversing anticancer drug resistance has prompted study of many potential drugs and chemicals that may block the MDR phenotype. Verapamil, cyclosporine and a few other compounds appear to be competitive inhibitors; however, it is not known in most studies whether the potential reversing agent is a substrate for P-glycoprotein. Hydrophobic bile acids, hormones and drugs block the MDR phenotype noncompetitively and are not substrates for the P-glycoprotein transport function. Whether these compounds will prove to have clinical value in treating drug-resistant cancer patients remains to be determined.

At the structural and molecular level, other considerations pertain regarding how P-glycoprotein functions as a transporter. Comparison of the derived sequence of P-glycoproteins with sequence data banks revealed similarities with a wide variety of membrane trans-

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porters in yeast, bacteria, plasmodia and other parasites, as well as cystic fibrosis transport receptor (CFTR), the cystic fibrosis gene product. Major homology and in some cases identity were demonstrated in the two nucleotide-binding domains and overall topology of the 12 membrane-spanning domains. Recent studies indicate that P-glycoprotein also functions as a chloride channel (3). Cellular hypotonicity activates the chloride channel function. Transportable drugs prevent channel activation but not preactivation. The transport and channel functions of P-glycoprotein were separated by mutations in the nucleotide-binding domains of the protein (4). Thus P-glycoprotein appears to be a bifunctional protein with both ion channel and transport activities. *In situ* hybridization studies with probes for CFTR and P-glycoprotein reveal that the two genes have complementary patterns of expression; this finding suggests that CFTR and P-glycoprotein may serve similar roles in epithelial cells and provides additional evidence that P-glycoprotein may regulate cell volume (5).

Abraham et al. now provide electrophysiological evidence that P-glycoprotein may function as an ATP channel. They emphasize that the mechanism of transport is not known for any member of the ATP-binding cassette superfamily. Using patch-clamp technique, they demonstrated that cells release ATP in direct relation to the relative level of P-glycoprotein. These provocative studies do not demonstrate that P-glycoprotein transports ATP, but the association suggests this as a likely hypothesis. In an abstract presented at the annual meeting of the Society of General Physiologists, these investigators also report that expression of CFTR results in appearance of an ATP channel (6). Perhaps all ATP-binding cassette transporters primarily transport ATP, and the other substrates (i.e., hydrophobic drugs and chloride) are secondary. Further studies are required to demonstrate a direct relation between ATP secretion and P-glycoprotein including reconstitution studies. However, the hypothesis that these studies generate is provocative. ATP has long been considered to be restricted to the intracellular domain. Studies in many cell types indicate that ATP can be extracellular and also a neurotransmitter. Purinergic receptors of several types have been identified on neural and epithelial cells and function in signal transduction.

Many epithelial and endothelial cells contain abundant calcium-magnesium ecto-ATPase and AMPase activities that hydrolyze ATP to ADP, AMP and eventually to adenosine (7). Sodium-dependent adenosine transporters have been demonstrated in the apical domain of hepatocytes, small intestinal mucosal and proximal tubular cells. *In vitro,* right-side-out canalicular membrane vesicle preparations rapidly hydrolyze ATP, and the resulting adenosine is transported into the vesicle (8). Do ATP-binding cassette transporters function in a similar manner? Are they related to naturally occurring homologs that secrete ATP and form adenosine that is then conserved? Not all cells can make adenosine, which is critical for many cellular functions. We are not used to thinking of ATP as a secretory modality; traditional concepts concern the energy expended in making ATP and utilization of the stored energy to drive metabolic processes and ion pumps. The studies of Abraham et al. raise provocative and fundamental questions.

In normal liver, P-glycoproteins are restricted to the bile canalicular domain of the plasma membrane (9). The major gene product results from mdr2, which does not confer multidrug resistance after transfection (10). The substrate for mdr2 is not known. Other newly described canalicular ATP-dependent transport systems involve bile acids and non–bile acid organic anions (11, 12). The relation, if any, between these systems and ATP transport remains to be determined.

The multidrug-resistant genes and their products provide an exciting arena for the interface of biology, transformation, drug-resistance and transport. The novel hypothesis regarding their mechanism and function provides a challenge for experimental study. Much remains to be learned and applied to human physiology and disease.

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