OXFORD

JNCI J Natl Cancer Inst (2015) 107(9): djv222

doi:10.1093/jnci/djv222
First published online August 18, 2015
Commentary

75TH ANNIVERSARY COMMENTARY

The Role of Multidrug Resistance Efflux Pumps in Cancer: Revisiting a *JNCI* Publication Exploring Expression of the MDR1 (P-glycoprotein) Gene

Michael M. Gottesman, Ira H. Pastan

Affiliations of authors: Laboratory of Cell Biology (MMG) and Laboratory of Molecular Biology (IHP), Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Correspondence to: Michael M. Gottesman, MD, Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Bethesda, MD 20892–4256 (e-mail: mgottesman@nih.gov).

The *Journal of the National Cancer Institute* published our paper entitled "Expression of a Multidrug Resistance Gene in Human Cancers" (1) 26 years ago. It was the first systematic determination of whether the energy-dependent multidrug efflux pump P-glycoprotein (P-gp), the product of the ABCB1 (MDR1) gene, was expressed in human cancers and has been cited over 1200 times. Four hundred cancers were studied, and the conclusion was that expression was widespread both in intrinsically drug-resistant cancers such as colon, pancreatic, liver, adrenocortical, and kidney cancers, and in some cancers that acquired resistance, such as leukemias, lymphomas, breast cancer, and neuroblastoma. Other cancers at the time of initial presentation, such as lung cancers (except for neuroendocrine tumors), ovarian cancer, esophageal cancer, and mesothelioma expressed little or no P-glycoprotein. The major conclusions of this paper, which have withstood the test of time (see below) were: 1) that P-gp was expressed at levels sufficient to confer drug resistance in many different difficult-to-treat cancers, 2) that its expression appeared during the acquisition of resistance for some cancers, indicating a possible role for P-gp in acquired resistance, 3) that many cancers did not appear to express P-gp mRNA at detectable levels and therefore efforts to inhibit P-gp and reverse resistance in these cancers were unlikely to succeed, and 4) that it remained to be proven whether P-gp expression, though perhaps sufficient for drug resistance in cancers, was the main cause of resistance in any cancer. Considerable effort has been devoted to answer this last question: Are P-gp or other multidrug transporters discovered since (see below) useful targets for drug development with the possible outcome of reversing multidrug resistance in cancer?

Limitations of the Initial Study

There were some limitations associated with the original study, most of which were noted in the Discussion to that work. Although most of the data were derived from cancers taken directly from patients, a few of the samples were from cell lines, notably the neuroendocrine lung cancers. We have subsequently shown that there is no correlation between expression of P-gp (or any other gene associated with multidrug resistance) in established cell lines and in cancers taken directly from patients (2,3), so the data on lung cancer neuroendocrine tumors must be interpreted cautiously. As continues to be true for studies done on primary tumors, the cancer itself is an admixture of many different cell types, and although the pathology of the cancers we examined indicates a substantial number of cancer cells in the samples, there are also stromal cells and host-derived macrophages, lymphocytes, and other cell types that could contribute to the relative expression of various genes.

The studies were done using total mRNA samples. Although the mRNA was shown to be of good quality and not degraded, the presence of MDR1 mRNA was determined using a relatively unsophisticated slot blot analysis. Although different mRNA doses were used to confirm a linear assay with the MDR1 signal proportional to the amount of total mRNA applied to the nitrocellulose, both the specificity and the sensitivity of this assay are unknown. We did assure that the levels of MDR1 mRNA we were measuring had biological relevance by comparing the signal to that from selected KB cell lines of known resistance levels.

At the time, we did not know whether expression of MDR1 mRNA correlated with expression of functional P-glycoprotein. We have since shown in correlative studies using the NCI-60 cell lines that the amount of mRNA specific for P-gp, using a more sensitive and sophisticated real-time polymerase chain reaction assay, is a good predictor of the ability of P-gp to protect cells from cytotoxic drugs that are substrates for P-gp (4).

Received: July 15, 2015; **Accepted:** July 20, 2015

Finally, after this work was published, it became clear that there were a total of 48 human ABC transporters, and that up to 13 of them were capable of conferring resistance to one or more anticancer drugs (5,6). Within a few years after this publication, two of the broadest spectrum multidrug resistance (MDR) transporters had been cloned, notably ABCC1 (7) and ABCG2 (8,9). Other members of these families, including ABCB4, ABCB5, and several ABCC family members also had various amounts of broad-spectrum transport activity (5). It was, and remains, unclear whether separate or coincident expression of these transporters with MDR1 contributes to clinical drug resistance in cancer. Future studies will need to dissect more carefully the individual contributions of each of these genes to the MDR phenotype.

Additional Studies Have Confirmed These Results

In short order, our laboratory, in collaboration with clinical investigators, subsequently published many studies on individual cancers, confirming the expression of the MDR1 gene in many of the tumors specified above and others (eg, in urogenital cancers [10,11], in lung cancers [12], in ovarian cancers [13], and in myeloid leukemias [14]). In addition, a great many other papers have appeared confirming the expression of the MDR1 gene in the tumor types specified in the original *Journal of the National Cancer Institute* paper.

With respect to the issue of whether MDR1 gene expression is related to the acquisition of resistance, although MDR1 is not expressed at appreciable levels in ovarian cancers at the time of presentation (2), we have found expression of MDR1 in a subset of ovarian cancers that recur after standard chemotherapy that includes paclitaxel and a platinum compound (13), see above (15,16). Unfortunately, none of these studies reaches statistical significance with respect to the association of P-gp expression with drug resistance because the number of tumors is small, and the percent of P-gp–expressing, chemoresistant tumors is far less than 50%. However, a recent study using whole genome characterization of chemotherapy-resistant ovarian cancer revealed that 8% of chemotherapy-resistant ovarian cancers have fusions of the MDR1 gene to an upstream promoter, presumably enabling expression of MDR1 (17). These results confirm the findings of Huff et al. (18), who found such rearrangements in relapsed childhood acute lymphocytic leukemia. The presence of this kind of genomic alterations argues strongly that expression of MDR1 mediated by these rearrangements confers selective advantage on the cells surviving chemotherapy.

A recent review that summarized data from the Cancer Genome Anatomy Project (19) confirmed that the expression of ABCB1 in many of the intrinsically resistant tumors described in Goldstein et al. (1) was elevated using RNAseq technology. In particular, high levels of expression of ABCB1 were found in cancers of the colon, liver, kidney, and pancreas. Of interest, there was a substantial correlation (r = .659) between expression of ABCB1 and ABCG2 in most of these cancers. These results suggest that multiple ABC transporters may be contributing to the intrinsic resistance of these tumors and provide a ready explanation for why simply inhibiting one of these transporters is not likely to result in circumvention of MDR. Strikingly, studies on acute myelogenous leukemia (AML) before treatment and after development of resistance to chemotherapy also reveal expression of various combinations of multiple ABC transporters (20).

Probably the most compelling experimental data linking ABCB1 gene expression with drug resistance in vivo come from the elegant mouse studies of Borst and colleagues (21). In this work, a mutant mouse carrying p53 and BRCA1 mutations gives rise to murine breast cancers that are susceptible to treatment with docetaxel and doxorubicin. Resistance develops predictably, and many of the resistant tumors express elevated levels of P-glycoprotein. Cancers in which the mouse *mdr* genes have been ablated are hypersensitive to these drugs (22).

Clinical Trials Testing the Role of P-glycoprotein in Multidrug-Resistant Cancers

The correlation of MDR1 mRNA levels with resistance in many cancers led naturally to the development of many inhibitors of MDR1 that might be suitable for testing in clinical trials of drug-resistant cancers. The very first clinical trial in ovarian cancer actually preceded the acquisition of data about the expression of P-gp in that cancer (23). Using verapamil as a relatively nonspecific and nonpotent P-gp inhibitor, no effect on response was seen. Knowing now that only a small subset of recurrent ovarian cancers express P-gp and that doses of verapamil high enough to inhibit P-gp are too toxic to achieve in man, such a result was not unexpected.

Subsequently, there were many trials of "off-the-shelf" P-gp inhibitors (first-generation pharmaceuticals in use for other purposes that were competitive inhibitors of P-gp). Eventually, derivatives of these compounds with lower cytotoxicity (second-generation drugs) and entirely new compounds developed specifically to inhibit P-gp (third-generation drugs) were developed and some were tested. A handful of trials showed marginally statistically significant improvement in response, but most trials were generally not randomized, no effort was made to determine whether the patients' tumors actually expressed P-gp, and because of the inherent cytotoxicity of many of the P-gp inhibitors, doses needed to be lowered below levels that would be expected to inhibit P-gp (24). The largest randomized trial with a third-generation potent P-gp inhibitor (tariquidar) in non–small cell lung cancer had to be closed because of toxicity, but would also not have been expected to show any effect because P-gp is not expressed at significant levels in this cancer (25). It is of interest that the most successful trial to date in AML used cyclosporine A, an inhibitor of all major ABC multidrug transporters (24). More specific agents, such as the potent third-generation inhibitor zosuquidar, failed to show any improvement in response to AML in high-risk older patients who tend to express P-gp (26), consistent either with no significant involvement of P-gp in drug-resistant AML, or, more likely, the need to inhibit other ABC transporters to observe a therapeutic effect.

The overall conclusion from the clinical work is that it is difficult to inhibit P-gp without attendant toxicities, but that a definitive trial in patients known to express P-gp in their tumors using an agent at levels that inhibits P-gp has never been done.

Impact of These Studies

Despite the pessimism about using inhibitors of P-gp to improve the treatment of drug-resistant cancers, knowledge about the expression of P-gp in cancers has led to some significant changes in the process of drug development. The most important contribution of this work has led to the routine screening, by pharmaceutical companies, of new anticancer drugs for whether they are substrates for transport by P-gp and other ABC

transporters. This helps inform the likelihood that they will be successful for treating P-gp–expressing cancers and provides information about how they are handled by the body. For example, oral administration of P-gp–substrate drugs is ineffective because expression of P-gp in the normal intestine prevents its absorption. IV administration is predicted to result in excretion in the bile and urine because of the expression of P-gp in biliary hepatocytes and on the apical surface of epithelial cells of the proximal tubules of the kidney. Furthermore, some drug interactions are also predictable because P-gp substrates are all competitive inhibitors of each other for transport.

Future Prospects

As the practice of medicine becomes more precise and targeted to molecular features of cancers and their hosts, it is inevitable that resistant tumors that express significant levels of P-gp will be found. There have been anecdotal reports of colon cancers expressing high levels of P-gp responding dramatically to doxorubicin in the presence of a P-gp inhibitor. It is expected that as we assemble more and more molecular data on gene expression in cancers, P-gp will once again rear its head as an important target, albeit in perhaps only a subset of cancers that have acquired resistance. Whether or not abrogation of P-gp activity will ever produce significant tumor responses depends on the future efficacy of the P-gp inhibitory drugs we use and a better understanding of the complexity of multidrug resistance in cancer that extends far beyond cell-based resistance mechanisms.

The expression of P-gp and other ABC transporters such as ABCG2 and ABCC1 on capillary endothelial cells of the brain, where they comprise the blood-brain barrier for natural product hydrophobic drugs, raises the prospect of manipulating this barrier to improve delivery of anticancer drugs to the brain. For cancer pharmacology and treatment of primary and metastatic brain tumors, this may prove to be the most important contribution of our knowledge about P-glycoprotein.

Finally, every drug resistance mechanism has a fitness cost for the cells that are deploying these mechanisms. Expression of P-gp makes cancer cells much more sensitive to a whole range of drugs. This collateral sensitivity is a kind of synthetic lethality that makes P-gp–expressing cancers potential targets for killing by agents that do not kill normal cells (27). Several laboratories, including our own, are actively pursuing development of compounds that, in combination with more traditional chemotherapy, might eliminate P-gp–expressing cells.

Funding

This research was supported by the Intramural Research Program of the National Institutes of Health (NIH), National Cancer Institute, Center for Cancer Research.

Notes

We would like to thank the many talented students and trainees who worked with us on the problem of multidrug resistance in cancer, and our many senior colleagues at the NIH and elsewhere who made critical intellectual contributions to this work. We are grateful to Joyce Sharrar and George Leiman for their editorial assistance.

References

1. Goldstein LJ, Galski H, Fojo A, et al. Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst*. 1989;81(2):116–124.
2. Gillet JP, Calcagno AM, Varma S, et al. Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. *Proc Natl Acad Sci U S A*. 2011;108(46):18708–18713.
3. Gillet JP, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. *J Natl Cancer Inst*. 2013;105(7):452–458.
4. Szakács G, Annereau JP, Lababidi S, et al. Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells. *Cancer Cell*. 2004;6(2):129–137.
5. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer*. 2002;2(1):48–58.
6. Keniya MV, Holmes AR, Niimi M, et al. Drug resistance is conferred on the model yeast Saccharomyces cerevisiae by expression of full-length melanoma-associated human ATP-binding cassette transporter ABCB5. *Mol Pharm*. 2014;11(10):3452–3462.
7. Cole SP, Bhardwaj G, Gerlach JH, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science*. 1992;258(5088):1650–1654.
8. Doyle LA, Yang W, Abruzzo LV, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci U S A*. 1998;95(26):15665–15670.
9. Fojo A, Akiyama S, Gottesman MM, Pastan I. Reduced drug accumulation in multiply drug-resistant human KB carcinoma cell lines. *Cancer Res*. 1985;45(7):3002–3007.
10. Kakehi Y, Kanamaru H, Yoshida O, et al. Measurement of multidrug-resistance messenger RNA in urogenital cancers; elevated expression in renal cell carcinoma is associated with intrinsic drug resistance. *J Urol*. 1988;139(4):862–865.
11. Kanamaru H, Kakehi Y, Yoshida O, Nakanishi S, Pastan I, Gottesman MM. MDR1 RNA levels in human renal cell carcinomas: correlation with grade and prediction of reversal of doxorubicin resistance by quinidine in tumor explants. *J Natl Cancer Inst*. 1989;81(11):844–849.
12. Lai SL, Goldstein LJ, Gottesman MM, et al. MDR1 gene expression in lung cancer. *J Natl Cancer Inst*. 1989;81(15):1144–1150.
13. Bourhis J, Goldstein LJ, Riou G, Pastan I, Gottesman MM, Bénard J. Expression of a human multidrug resistance gene in ovarian carcinomas. *Cancer Res*. 1989;49(18):5062–5065.
14. Sato H, Gottesman MM, Goldstein LJ, et al. Expression of the multidrug resistance gene in myeloid leukemias. *Leuk Res*. 1990;14(1):11–21.
15. Gillet JP, Calcagno AM, Varma S, et al. Multidrug resistance-linked gene signature predicts overall survival of patients with primary ovarian serous carcinoma. *Clin Cancer Res*. 2012;18(11):3197–3206.
16. Gillet JP, Wang J, Calcagno AM, et al. Clinical relevance of multidrug resistance gene expression in ovarian serous carcinoma effusions. *Mol Pharm*. 2011;8(6):2080–2088.
17. Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. *Nature*. 2015;521(7553):489–494.
18. Huff LM, Lee JS, Robey RW, Fojo T. Characterization of gene rearrangements leading to activation of MDR-1. *J Biol Chem*. 2006;281(48):36501–36509.
19. Basseville A, Hall M, Chau CH, et al. The ABCG2 multidrug transporter. In: Anthony G, ed. *ABC Transporters - 40 Years On*. New York, NY: Springer; In press.
20. Patel C, Stenke L, Varma S, et al. Multidrug resistance in relapsed acute myeloid leukemia: evidence of biological heterogeneity. *Cancer*. 2013;119(16):3076–3083.
21. Pajic M, Iyer JK, Kersbergen A, et al. Moderate increase in Mdr1a/1b expression causes in vivo resistance to doxorubicin in a mouse model for hereditary breast cancer. *Cancer Res*. 2009;69(16):6396–6404.
22. Rottenberg S, Borst P. Drug resistance in the mouse cancer clinic. *Drug Resist Updat*. 2012;15(1–2):81–89.
23. Ozols RF, Cunnion RE, Klecker RW Jr, et al. Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. *J Clin Oncol*. 1987;5(4):641–647.
24. Shaffer BC, Gillet JP, Patel C, Baer MR, Bates SE, Gottesman MM. Drug resistance: still a daunting challenge to the successful treatment of AML. *Drug Resist Updat*. 2012;15(1–2):62–69.
25. Fox E, Bates SE. Tariquidar (XR9576): a P-glycoprotein drug efflux pump inhibitor. *Expert Rev Anticancer Ther*. 2007;7(4):447–459.
26. Cripe LD, Uno H, Paietta EM, et al. Zosuquidar, a novel modulator of P-glycoprotein, does not improve the outcome of older patients with newly diagnosed acute myeloid leukemia: a randomized, placebo-controlled trial of the Eastern Cooperative Oncology Group 3999. *Blood*. 2010;116(20):4077–4085.
27. Pluchino KM, Hall MD, Goldsborough AS, Callaghan R, Gottesman MM. Collateral sensitivity as a strategy against cancer multidrug resistance. *Drug Resist Updat*. 2012;15(1–2):98–105.