Learning Some New Tricks From a Multidrug Transporter

Michael M. Gottesman, M.D.
Chief, Laboratory of Cell Biology
Center for Cancer Research, NCI
National Institutes of Health
January 15, 2009

Estimated New Cancer Cases & Deaths, 2008

Sites	New Cases	Deaths*	%
All Sites	**1,437,180**	**565,650**	**39%**
Prostate	**186,320**	**28,660**	**15%**
Breast	**184,450**	**40,930**	**22%**
Digestive System	**202,720**	**79,090**	**39%**
Pancreas, Liver & Gall Bladder	**68,570**	**56,040**	**82%**
Lung & Bronchus	**215,020**	**161,840**	**75%**
Bladder	**68,810**	**14,100**	**20%**
Kidney & Renal Pelvis	**54,390**	**13,010**	**24%**
Ovary	**21,650**	**15,520**	**72%**
Cervical Cancer	**11,070**	**3,870**	**35%**
Lymphoma & Leukemia	**118,610**	**42,220**	**36%**
Brain & Nervous System	**21,810**	**13,070**	**60%**

***Virtually all deaths are due to chemotherapy resistance CA Cancer J Clin, 2008**

Drug Resistance in Cancer

May affect multiple drugs used simultaneously: known as multidrug resistance (MDR)

Affects all classes of drugs, including newly designed targeted drugs

Just as oncogene targets have been catalogued, we need to enumerate all mechanisms of drug resistance in cancer to solve this problem and circumvent resistance

Mechanisms of resistance to anti-cancer drugs

Decreased Uptake → Reduced apoptosis → Increased efflux
Altered cell cycle checkpoints
Increased metabolism of drugs
Increased or altered targets
Increased repair of damage
Compartmentalization

Ultimate Goals

Molecular analysis of human cancers to predict response to therapy

Use this information to develop novel drugs to treat cancer and new imaging modalities for cancer

To learn more about cellular pharmacology and pharmacokinetics of drugs, including drug uptake, distribution, and excretion

ABC TRANSPORTERS: DOMAIN ORGANIZATION

Graphic illustration of the transmembrane and ATP-binding domains of ABCB1, and ABCC1, and ABCG2 transporters.

Hypothetical Model of Human P-glycoprotein

Graphic illustration of a P-glycoprotein molecule.

Substrates and Reversing Agents of Pgp

Chemical structures for Vinblastine, Colchicine, Actinomycin D, Daunorubicin, Verapamil, and Rapamycin.

P-glycoprotein removes hydrophobic substrates directly from the plasma membrane

Graphic illustration

Homology model of human P-glycoprotein based on the structure of bacterial ABC transporter Sav1866 of *S. aureus*

Graphic illustration

Role of P-glycoprotein in cancer

Approximately 50% of human cancers express P-glycoprotein at levels sufficient to confer MDR

Cancers which acquire expression of P-gp following treatment of the patient include leukemias, myeloma, lymphomas, breast, ovarian cancer; preliminary results with P-gp inhibitors suggest improved response to chemotherapy in some of these patients

Cancers which express P-gp at time of diagnosis include colon, kidney, pancreas, liver; these do not respond to P-gp inhibitors alone and have other mechanisms of resistance

Being able to image P-gp in cancer (and ultimately other transporters that contribute to resistance) could help guide therapy

Physiologic Role of P-glycoprotein

Graphic illustration of P-glycoprotein in intestine, brain and kidney.

ABC transporters determine oral bioavailability, excretion, penetration and protect the organism against airborne xenobiotics

Graphic illustration of P-glycoprotein function in various organs.

Polymorphisms in the human *MDR*1 gene

More than 50 SNPs have been reported in the MDR1 gene.
14 of them are silent polymorphisms.

5 common coding (non-synonymous) polymorphisms have no demonstrable effect on drug transport function.

The synonymous SNP in exon 26 (C3435T) was the first associated with altered MDR1 function and is often part of a haplotype including another synonymous (C1236T) and a nonsynonymous SNP (G2677T).

The C1236T, G2677T, C3435T haplotype has been linked to several different phenotypes

Altered digoxin and fexofenadine pharmacokinetics

Altered toxicity in transplant patients from cyclosporine A, tacrilimus

Altered incidence of Crohn's disease, colon cancer, and Parkinson's disease

*MDR*1 wild-type, SNPs, and haplotypes show similar P-gp total and surface expression

Graphic illustration (gel densitometry).

*MDR*1 wild-type and the haplotype (1236-2677-3435) do not exhibit similar Bodipy-verapamil accumulation

Graph showing counts over fluorescence intensity for WT, X3, and pTM1.

*MDR*1 wild-type and the haplotype exhibit different patterns using rhodamine 123 efflux with cyclosporin A reversing agent

Graph showing Counts over fluorescence intensity for 1236-2677-3435, MDR1 WT, and pTM1 control.

5 mM CsA

*MDR*1 wild-type and haplotype show the same P-gp cell surface expression using MRK16 and 17F9, but not UIC2 - a conformational sensitive antibody

Three graphs are shown, one for17F9, another for MRK16 and the third for UIC2 indicating counts over fluorescence intensity for each.

*MDR*1 wild-type and haplotype show different trypsinization patterns confirming altered conformation

Graphic illustration

Polymorphic forms of P-gp with alleles that don't change amino acid sequence change the conformation of P-gp

In transient transfection experiments, the amount of P-gp mRNA, protein, and protein localization on the cell surface is unchanged.

The conformation of polymorphic P-gp is altered as shown by tryptic peptide analysis and conformation-specific MoAbs.

Translational toeprint experiments show a major delay in translation at the site of the "silent" polymorphism.

Primer extension inhibition (toeprint) assay – to detect presence of ribosome stalling sites

Graphic illustration of mRNA translation (wild type and mutant mRNA + ribosomes).

Reverse transcription products
were resolved by
polyacrylamide gel
electrophoresis

Toeprint analysis of *MDR1* mRNA around the 3435 SNP

Illustration of polyacrylamide gel electroporesis.

	Wild-type	**SNP 3435T**	**SNP 3435A**
Nucleotide	AUC	AUU	AUA
Codon usage	**47%**	**35%**	**18%**

Synonymous SNPs affect P-glycoprotein conformation and function

Graphic illustrations of wild-type Pgp (normal kinetics of translation) and the Pgp Haplotype (rare tRNAs) or altered MRNA structure (altered kinetics of translation).

P-gp expression on cell surface (MRK16) of stably transfected LLC-PK1 cells

Graph showing counts over FL1-Height.

1^0 Antibody: MRK16
2^0 Antibody: Goat anti-mouse FITC

P-gp expression on cell surface (UIC2) of transfected LLC-PK1 cells

Graph indicating counts over FL2-Height

Conformation-sensitive Monoclonal antibody

Vinca-alkaloids

Chemical structure of Vincristine and chemical structure of Vinblastine. Also graphs for Vincristine and Vinblastine indicating cell survival (%) over concentration (M) with WT and variant P-gp.

TAXOL

Two graphs indicating Survival (%) over concentration (M) for Paclitaxel and Docetaxel.

Chemical structure of Vincristine and chemical structure of Vinblastine. Also graphs for Vincristine and Vinblastine indicating cell survival (%) over concentration (M) with WT and variant P-gp.

Anthracyclines

Two graphs, one for Doxorubicin and the other for Daunorubicin, indicating survival (%) over concentration (M)

Chemical structure of Vincristine and chemical structure of Vinblastine. Also graphs for Vincristine and Vinblastine indicating cell survival (%) over concentration (M) with WT and variant P-gp.

Implications

Explains conservation of third position for many codons

Might explain some non-Mendelian inheritance

Might explain linkage of phenotypes to other synonymous polymorphisms

For P-gp, the haplotype could have selective advantage and/or affect drug distribution

For cancers, could affect pattern of MDR and ability to respond to specific inhibitors

ABC Transporters Confer Resistance to Anti-Cancer Drugs

Vinca alkaloids
Antracyclines
Epipodophyllotoxins
Taxanes
Kinase inhibitors
Campthotecins
Thiopurines
Other

Can we discover new drugs that interact with ABC transporter genes?

Use Real Time (RT)-PCR to measure ABC mRNA levels for 48 ABC transporters and 23 solute carrier proteins

Exploit NCI-60 cell line database, with known resistance to 100,000 different drugs, to correlate patterns of drug-resistance and expression of these transporters

Expression of ABC-transporters in the NCI60 panel

Heat map illustration

Search for MDR1-potentiated compounds in DTP's database

Example of NSC 73306.
Graphs of cell survival and sensitivity to NSC 363997.

The cytotoxicity of NSC 73306 is increased in KB-V1 cells

Two graphs, one showing sensitivity (dlogG150) over ABCB1 expression (dCP). The other graph indicates cell survival (%) over concentration (μM)

Chemical structure of NSC73306

Potential Clinical Utility of Discovery of Compounds that Specifically Kill MDR1-Expressing Cells

Can be used in combination with standard chemotherapy to eliminate MDR1-expressing cell populations

Preclinical development of thiosemicarbazones and search for additional compounds with similar properties is underway

Balance of uptake and efflux determines drug accumulation in cancer cells

Graphic illustration

Solute Carriers are plausible uptake transporters for anti-cancer drugs

Graphic illustrations of

SLCO: organic anion transporting polypeptide (OATP) family

SLC22: organic cation and anion transporter (OCT And OAT) families

Summary of SLCO and SLC22 Transporters

Most of the SLCO and SLC22 family members we tested are expressed at some level in cancer cell lines.

By correlating the expression profiles with the growth inhibitory profiles, expression of 3 of the SLCO and SLC22 family members were found to correlate with sensitivity to specific drugs.

Expression of SLC22A4 in KB cells confers sensitivity to mitoxantrone, doxorubicin, carboplatin and cisplatin

Microfluidic technology using TaqMan Low Density Array (TLDA)-based detection to study the mechanisms of multidrug resistance in clinical samples

Goal: To correlate expression of drug-resistance genes with response to chemotherapy in clinical samples

Method: a customized TLDA with probes for 380 drug-resistance genes enabling high-throughput quantitative real-time PCR based on TaqMan chemistry

Graphic illustration

The Anti-Cancer Drug Resistance Transcriptome

Graphic illustration

No ovarian cell line has a drug-resistance gene expression profile similar to any clinical sample

Heat map illustration

Strategies for dealing with MDR1-mediated multidrug resistance

Graphic illustration of Drug structural variation
Dose escalation
Imaging of P-gp in vivo in cancers can enable all of these strategies

Can we use MDR1
Expression as an Achilles
heel to kill MDR cells?

Development of specific inhibitors of P-gp

Poor performance in clinical trials for a number of reasons:

Poor trial design, e.g., cancers don't express MDR1

Side effects due to inhibition of endogenous functions

Acknowledgements

Gergely Szakacs
Jean-Philippe Annereau
Joe Ludwig
Jean-Pierre Gillet
Mitsunori Okabe
Matthew Hall
Chava Kimchi-Sarfaty
Jung-Mi Oh
Andy Fung

Suresh Ambudkar
    Zuben Sauna
    InWha Kim
    Anna Calcagno
Ira Pastan
John Weinstein
Carol Cardarelli
Takaaki Abe
Joe Covey
Henry Fales