Structure and Function of ABC Transporters in Health and Disease

Michael M. Gottesman, M.D.
Chief, Laboratory of Cell Biology
Center for Cancer Research, NCI
National Institutes of Health, DHHS
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Overall Goals

• Define molecular mechanisms of drug resistance in cancer (natural products, platinum compounds)
• Determine the clinical relevance of mechanisms derived from *in vitro* studies
• Develop new approaches to exploit or circumvent clinically significant resistance mechanisms
• To learn more about the cellular pharmacology and pharmacokinetics of drugs
Drug Resistance: Specific Mechanisms

Gottesman et al., in press, Annu Rev Pharm Tox 2015
Cell-based mechanisms of resistance to anti-cancer drugs

- Decreased Uptake-- 100’s of Solute carriers
- Increased Efflux--48 ABC transporters

Reduced apoptosis
- Altered cell cycle checkpoints and/or growth pathways
- Increased metabolism of drugs
- Increased or altered targets
- Increased repair of damage
- Compartmentalization
Why study multidrug transporters?

• Important role in multidrug resistance in cancer and in pathogens
• Important role in drug pharmacokinetics (uptake, distribution, and excretion)
• Important role in drug toxicity
• Key role in development (stem cells, morphogenesis)
• To learn about the biology of all transport systems
ATP-Binding Cassette (ABC) Transporter Superfamily

• One of the largest family of transport proteins known. Currently, more than 2000 members have been identified.

• Transport substrates include-- ions, sugars, glycans, phospholipids, cholesterol, peptides, proteins, toxins, antibiotics, and hydrophobic natural product anticancer drugs.

• Structurally, consist of various combinations of ATP-binding cassettes and segments with 6 trans-membrane domains.
The Eukaryotic ABCome
57 ABC-family genes

From M. Dean
The Clustal W program was used to make the alignment of the NBDs and the tree was built by using the MEGA program -- By Mike Dean, NCI
ABC transporters play excretory and/or protective physiological roles

3 main multildrug transporters:

- ABCB1 (P-gp, P-glycoprotein)
- ABCC1 (MRP1)
- ABCG2 (BCRP, MXR)

Kannan et al., Clin Pharmacol. Ther., 2009
### Human diseases associated with an ABC Transporter

<table>
<thead>
<tr>
<th>Disease</th>
<th>Transporter</th>
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<tbody>
<tr>
<td>Cancer</td>
<td>ABCB1, ABCC1, ABCG2</td>
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<tr>
<td>Cystic fibrosis</td>
<td>ABCC7 (CFTR)</td>
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<tr>
<td>Stargardt disease &amp; AMD</td>
<td>ABCA4 (ABCR)</td>
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<td>Tangier Disease (HDL deficiency)</td>
<td>ABCA1 (ABC1)</td>
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<td>Progressive familial intrahepatic cholestasis</td>
<td>ABCB11(SPGP), ABCB4 (MDR2)</td>
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<td>Dubin-Johnson syndrome</td>
<td>ABCC2 (MRP2)</td>
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<td>Pseudoxanthoma elasticum</td>
<td>ABCC6 (MRP6)</td>
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<td>Persistent hypoglycemia of infancy, neonatal diabetes</td>
<td>ABCC8 (SUR1), ABCC9 (SUR2)</td>
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<td>Sideroblastic anemia and ataxia</td>
<td>ABCB7 (ABC7)</td>
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<td>Adrenoleukodystrophy</td>
<td>ABCD1 (ALD)</td>
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<td>Sitosterolemia</td>
<td>ABCG5, ABCG8</td>
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<tr>
<td>Immune deficiency</td>
<td>ABCB2 (Tap1), ABCB3 (Tap2)</td>
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</tbody>
</table>
ABC transporters that confer MDR: Domain organization

- **ABCB1**: TM Domain, ATP binding, TM Domain, ATP binding
- **ABCC1**: TM Domain
- **ABCG2**:
Overlapping substrate specificity of ABCB1, ABCG2 and ABCC1
## Multiple ABC Transporters Confer Resistance to Anti-Cancer Drugs

<table>
<thead>
<tr>
<th>ABC Transporters</th>
<th>ABC3456</th>
<th>ABC7890</th>
<th>ABC1234</th>
<th>ABC5678</th>
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<tbody>
<tr>
<td>Vinca alkaloids</td>
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<td>Anthracyclines</td>
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<td>Epipodophyllotoxins</td>
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<td>Taxanes</td>
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<td>Kinase inhibitors</td>
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<td>Camptothecins</td>
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<tr>
<td>Thiopurines</td>
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<tr>
<td>Other</td>
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</tbody>
</table>

**Confers resistance**

**Selected**
Hypothetical Model of Human P-glycoprotein

**POINT MUTATIONS**, **PHOTOAFFINITY LABELED**, **REGIONS**, **ATP SITE**, **PHOSPHORYLATION SITES**
P-glycoprotein removes hydrophobic substrates directly from the plasma membrane
Atomic models of the structures of P-gp

Mouse P-gp at 3.8Å (Aller and Chang)  Human P-gp model based on Sav1866 (Xia)
Physiologic Role of P-glycoprotein
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
- Animal models with human cancer xenografts and BRCA1-driven mouse mammary cancers show role for P-gp in MDR (Pajic et al., Cancer Res. 69, 6396-6404, 2009)