Modulation of cancer cell multidrug ABC transporters

10th French-Belgian ABC Meeting

UCL, Brussels, Belgium

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Cancer cell multidrug resistance and ABC transporters

- Cell growth resistance to multiple drugs,
- Low intracellular accumulation of cytotoxic drugs,
- Due to overexpression of ABC transporters (P-glycoprotein/ABCB1, MRP1/ABCC1 and/or BCRP/ABCG2) within plasma membranes.
- Prevented in vitro by characteristic inhibitors:
  * verapamil/cyclosporine A / P-glycoprotein
  * MK571/probenecid / MRP1
  * FTC/Ko143 / BCRP
3 main multidrug ABC-transporters:

- **class B**: \(\text{ABCB1}\)
- **class C**: \(\text{ABCC1}\)
- **class G**: \(\text{ABCG2} (\text{MXR/BCRP})\)

belong to 3 different classes of the 48 human ABC proteins.
Different strategies to antagonize MDR cancer cells overexpressing ABCB1, ABCC1 or ABCG2

Using nontransported inhibitors:
ABCB1
ABCG2

Using "Collateral Sensitivity" = Achilles' heel
ABCC1

Bypass
Synthesizing nontransported chemotherapeutics (but generally less active ...)

Inhibit
Target
Overlapping patterns for transported substrates

>> inhibition of a single transporter not sufficient to fully abolish cell multidrug resistance

MDR-substrate anticancer agents.
Very few common inhibitors. None for the 3 transporters (even for ABCC1 and ABCG2).

>>> Specific inhibitors may be found
Third-generation inhibitors against P-glycoprotein

S9788

XR9051

MS-209

GF120919 (Elacridar)

VX-710 (Biricodar)

OC144-093

ONT-093 (Ontogen)

LY335979 (Zosuquidar)

**Inhibitors having reached clinical trials:**

XR-9576 (Tariquidar)

R-101933 (Laniquidar)

OC144-093

ONT-093 (Ontogen)

LY335979 (Zosuquidar)

Specific for P-glycoprotein

No

??

??

YES
The half-transporter BCRP / ABCG2 (ABCP / MXR) [discovered in 1998]

- Located within plasma membranes,
- Naturally overexpressed in placenta, liver, small intestine and colon, supporting a role in protection / secretion.
- Physiological transport substrates:
  * pheophorbide a (= chlorophyll catabolite) and porphyrins,
  * urate in kidney proximal tubule cells (Q141K >> gout),
- Identified as a marker of stem cells (“side-population”).
- Overexpressed in many types of tumors,
- Transports mitoxantrone, methotrexate and topotecan (and anthracyclines and rhodamine 123 upon R482 hot-spot mutation).
- Since discovered more recently than ABCB1 >> less inhibitors known.
Inhibition of ABCG2-mediated mitoxantrone efflux by Flavonoids

- Mitoxantrone efflux measured by flow cytometry with ABCG2-transfected HEK-293 cells

- High-affinity inhibition by 6-prenylchrysin and tectochrysin
  >> natural compounds as potent inhibitors

SARs for flavonoid inhibition of wild-type ABCG2

Hydrophobic flavones are specific for ABCG2, versus ABCB1 and ABCC1

Flavone benzopyrane and benzofurane derivatives
Boeravinone derivatives

Acridone derivatives
Pharmacophore molecular modeling

>> good correlation for nearly all compounds

Descriptors:
- Positive roles of the size, polarisability and hydrophobicity
- No clear effect of H-bond acceptor
- Negative effect of H-bond donor

>> different from ABCB1

Specific inhibitors >> likely bind outside the catalytic transport site

The ABCG2-specific inhibitory site overlaps the dual site

- Acridone 4c binding is prevented by GF120918. >> both sites are widely overlapping.

- The specific site of flavonoids is distinct from another specific site related to ATPase inhibition.

- Chromones bind to this FTC/Ko143 site.

>> Polyspecificity for inhibitors and substrates

- ABCG2-specific inhibitory site (acridones 4a-c, Tariq. deriv. 5/6, flavones, rotenoids, OMe trans-stilbenes chalcones)
- Dual inhibitory site (GF120918, Tariquidar, bosutinib)
- Second ABCG2-specific inhibitory site FTC, Ko143, chromones
- Catalytic transport sites for substrates
Chromone derivatives: the best candidates for in-vivo assays


Chromone 6g
high affinity (IC$_{50}$ = 0.11 µM)
complete, non-competitive, inhibition
and low cytotoxicity (IG$_{50}$ > 100 µM)

> very high Therapeutic Ratio (TR) > 1,000

Not transported (only inhibits ATPase activity)

Chemosensitizes resistant cancer cell growth
to mitoxantrone or SN-38 (irinotecan metabolite)

>> suitable for in-vivo assays
Mouse model with ABCG2-expressing human xenografts

**Implantation of ABCG2-positive cells**

**Implantation of control cells**

**Anticancer chemotherapy**

(Irinotecan)

**Drug efficiency on xenograft growth**

ABCG2 allows tumor growth in the presence of irinotecan, by conferring chemoresistance
**In vivo** chemosensitization of tumor growth to irinotecan by either Gefitinib or acridone 4c (MBLI-87)


- no effect of Gefitinib or vector alone on tumor growth
- ABCG2-dependent tumor growth in presence of irinotecan is delayed by Gefitinib

But MBLI-87: low solubility and relatively high cytotoxicity

>> **improve formulation**

>>> use more potent and less toxic compounds (**chromone 6g**).
Targeting the Achilles' heel of Multidrug Resistant Cancer

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SR > 2

**Collateral sensitivity**

**Resistance Ratio**

$$RR = \frac{IC_{50} \text{ (resistant)}}{IC_{50} \text{ (parental)}}$$
Schematic structure of MRP1/ABCC1 [discov. 1992]

- Additional TMD0

- Physiological role in inflammation: efflux of leukotriene LTC4 from leukocytes

- Also transports a number of drugs, such as vincristine
MRP1 is modulated by hydrophobic compounds such as Verapamil


Selective cytotoxicity identified as apoptosis (PS, caspases)

Lauriane DURY, Short Talk, Saturday 10:40

Fast and massive MRP1-mediated GSH efflux induced by Verapamil
Summary of verapamil-induced collateral sensitivity

1) Verapamil binds to MRP1 (competitively to the drug site ? Is it transported ?)
2) It promotes a massive and fast GSH efflux through MRP1
3) Only the S-verapamil enantiomer is active [Perrotton et al. J. Biol. Chem. (2007)]
4) Role of ROS ? amplified effects upon GSH efflux ?
5) This induces a selective apoptosis of MDR cells expressing MRP1 (transfected BHK-21, or SCLC drug-selected H69AR)  >> in vivo experiments on xenografts

>> New potential therapeutic strategy:
   - targeting cancer cells >> limited side effects,
   - new alternative, especially after chemotherapy failure.

Since Verapamil is known to be cardiotoxic
>> HTS of chemical libraries > new classes of apoptogenic compounds,
>> Xanthones and Flavones.
Structure-activity relationships of xanthones to promote GSH efflux and cytotoxicity


Table 1. Structures of the xanthones studied and net GSH efflux induced in BHK-21-MRP1 cells.

<table>
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<th>Compd</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>R⁵</th>
<th>ClogP</th>
<th>Efflux [%][a]</th>
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<td>2</td>
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<tr>
<td>4</td>
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<td>H</td>
<td>Me</td>
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[a] GSH efflux determined at 20 μM.

Table 2. Cytotoxicity of selected xanthones on NCI-H69 (sensitive) and H69AR/MRP1 (resistant) cells.

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<th>Compd</th>
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<td>&gt;100</td>
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<td>51±0.39</td>
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<td>22</td>
<td>54±0.07</td>
</tr>
<tr>
<td>23</td>
<td>&gt;100</td>
</tr>
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</table>

[b] Values represent the mean ± SD of n = 3 experiments.

SR > 9
Brazilian CAPES ("Sandwich PhD")

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