A Fresh Angle on P–Glycoprotein to Overcome Tumor Chemoresistance

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XXIX Symposium on Bioinformatics and Computer–Aided Drug Discovery
MDR – MultiDrug Resistance

ATP-binding cassette (ABC) transporter family

Human genome contains 49 ABC genes organized into 7 subfamilies (ABCA–ABCG)

Clinically relevant ABC transporter for anticancer therapy:

- **MDR1** (Multidrug resistance protein 1; also known as P–glycoprotein or P–gp) encoded by the *ABCB1* gene;
- **MRP1** (Multidrug resistance–associated protein 1) encoded by the *ABCC1* gene;
- **BCRP** (Breast cancer resistance protein) encoded by the *ABCG2* gene.

Current strategy to overcome tumor resistance

Combined treatment with anticancer drugs and P–gp inhibitors

+ = drug

= inhibitor
## Efficiency of P-gp inhibitors in patients

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier</th>
<th>Drug</th>
<th>Study completion</th>
<th>Phase</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>NCT00001302</td>
<td>Valspodar + Vinblastine</td>
<td>2002</td>
<td>I</td>
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<td>NCT00001383</td>
<td>Valspodar + Paclitaxel</td>
<td>2001</td>
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<td>NCT0001944</td>
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<tr>
<td>NCT00011414</td>
<td>Tariquidar + Doxorubicin, Vinorelbine, or Docetaxel</td>
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<tr>
<td>NCT00048633</td>
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<tr>
<td>NCT04603066</td>
<td>Tariquidar + 5-HT3R antagonist ondansetron, in patients with neuropathic pain</td>
<td></td>
<td>I/II</td>
<td>RECRUITING</td>
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</table>
PROTAC concept

PROTAC = PROteolysis TAgeting Chimera

Ligand of protein of interest (POI)  Linker  Ligand of E3 ubiquitin ligase

Warhead

Ligand of protein of interest (POI)

E2

UB

E3 ligase

POI

Recycle

Degrade the entire target

Proteasome

Sun et al. *Signal Transduct. Target Ther.*, 2019, 4, 64.
**PROTAC for membrane protein**

Why not?!

- some evidence that the stability of the MDR1 gene product (P–gp) could be regulated by ubiquitination;
  

- some evidence that increased ubiquitination level of P–gp is accompanied by decreased P–gp protein expression;
  

- some evidence that ubiquitination of transmembrane proteins promotes release of the protein from the membrane.

Ubiquitination of proteins


TMDs membrane

68 lysine residues
Used P–gp models

PDB id 7A65

inward-facing conformation

PDB id 6C0V

outward-facing conformation
Choice of E3 ligase

GPS–Uber

(ubiquitin–protein ligase enzymes–substrate relationship prediction)

- CRBN sites (not found);
- VHL sites (yellow);
- ...
- Mdm2 (green).

gpsuber.biocuckoo.cn/
Protein–protein docking

Mdm2 complex with isoindolinone inhibitor (PDB id 7BMG)

7A65

6C0V
Search for “PROTAC” site

Commonly used for search and development of P–gp inhibitors
Working within TMD: transported substance or inhibitor?
Groundwork

Move towards the nucleotide binding domain (NBD):
Talk by Aleksandra Sagaidak

Library of ATP (AMP) mimetics
Docking of compound library

TMDs

membrane

TMD

NBD

??
Docking results

<table>
<thead>
<tr>
<th>Compound</th>
<th>NBD</th>
<th>new site</th>
<th>TMD</th>
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<tr>
<td>AICAR</td>
<td>-5.3</td>
<td>-5.3</td>
<td>-5.6</td>
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<tr>
<td>Cladribin</td>
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<td>-6.0</td>
<td>-6.2</td>
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<tr>
<td>CompC</td>
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<td>-3.9</td>
<td>-5.4</td>
</tr>
<tr>
<td>CompC del</td>
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<td>-2.5</td>
<td>-4.8</td>
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<tr>
<td>DKPP</td>
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<td>-4.2</td>
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<tr>
<td>FM04</td>
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<td>-4.7</td>
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<tr>
<td>PheCH₃Cl R</td>
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<tr>
<td>ZMP</td>
<td>-5.4</td>
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</table>

Library of its modifications
Independent confirmation of our results

Identification of Binding Sites in the Nucleotide-Binding Domain of P-Glycoprotein for a Potent and Nontoxic Modulator, the Amine-Containing Monomeric Flavonoid FM04

Abstract

We have previously discovered an amine-containing flavonoid monomer FM04 as a potent P-glycoprotein (P-gp) inhibitor (EC_{50} = 83 nM). Here, a series of photoactive FM04 analogues were synthesized and used together with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify the FM04-binding sites on P-gp. Point mutations around the photo-crosslinked sites were made for verification. Together with the results from mutational studies, molecular docking, and molecular dynamics simulations, it was found that FM04 can interact with Q1193 and I1115 in the nucleotide-binding domain 2 (NBD2) of human P-gp. It was proposed that FM04 can inhibit P-gp in 2 novel mechanisms. FM04 can either bind to (1) Q1193, followed by interacting with the functionally critical residues H1195 and T1226 or (2) I1115 (a functionally critical residue itself), disrupting the R262-Q1081-Q1118 interaction pocket and uncoupling ICL2-NBD2 interaction and thereby inhibiting P-gp. Q1118 would subsequently be pushed to the ATP-binding site and stimulate ATPase.

It is the right way to go on!
Conclusions

• We simulated the formation of P–gp complex with Mdm2;
• We identified P–gp site suitable for binding a chimeric molecule;
• We generated primary library of structures with high affinity to the site of interest.
This work was supported by the Russian Science Foundation (project no. 23–13–00344)

Thank you for attention!

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