PREDICTING SUSCEPTIBILITY AND RESISTANCE FOR HEPATITIS B VIRUS CAPSID ASSEMBLY EFFECTORS

BRYAN D. COX, PH.D.
EMORY UNIVERSITY, DEPARTMENT OF PEDIATRICS
LABORATORY OF BIOCHEMICAL PHARMACOLOGY
EMORY CENTER FOR AIDS RESEARCH
Central hypothesis that resistant mutations (accessible by single-nucleotide substitution) decrease inhibitor binding while minimally perturbing native function.
Simulating Mutations and Analysis of Energy Calculations

- Sample Mutant Low Energy Rotamers
- Prune by Sterics and Minimize Energy

Energy of Inhibitor Complex

- Native F2L
- Inhibitor F2L
- Resistant F2L

Energy of Functioning Complex

Recapturing Known Viral Resistance Mutations to Nucleoside Analogs

HIV-RT Resistance Against (-)-FTC

- 114 ALA
- 160 PHE
- 65 LYS
- 74 LEU
- 151 GLN
- 72 ARG
- 115 TYR
- 184 MET

M184V

M184I

Hepatitis C Virus Resistance against 2’-C-Me-U

- 48 ARG
- 141 LYS
- 158 ARG
- 224 PHE
- 282 SER
- 287 THR
- 291 ASN

S282T

S282V
Central role of HBV Capsid Protein in Viral Replication Cycle

HBV capsid is essential for viral replication
- Viral entry and nuclear trafficking
- Uncoating and release of rcDNA
- Packaging and assembly of pgRNA
- Scaffold for viral genomic processing
- Budding and release of viral particles
- Establishing and maintaining cccDNA

HBV capsid assembly effectors inhibit replication
- Capsid is conserved across HBV serotypes
- Effective against nucleoside-resistant strains
- Potential synergy with existing therapies
- Diminish cccDNA levels

HBV Capsid Assembly Effectors (CAEs) as Next-Generation Antiviral Agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>HBV DNA</th>
<th>Cytotoxicity (IC&lt;sub&gt;50&lt;/sub&gt;, µM)</th>
<th>Therapeutic Index (HepG2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>EC&lt;sub&gt;90&lt;/sub&gt; (µM)</td>
<td>HepG2</td>
</tr>
<tr>
<td>GLP-26</td>
<td>0.003</td>
<td>0.03</td>
<td>&gt;100</td>
</tr>
<tr>
<td>GLS4</td>
<td>0.08</td>
<td>0.28</td>
<td>86.3</td>
</tr>
<tr>
<td>3TC</td>
<td>0.04</td>
<td>0.30</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

What mutations confer resistance to GLS4 and GLP-26? Which mutations are cross-resistant? Resistant to only one inhibitor?
HBV CAEs Disrupt Pre-Formed Capsids: GLP-26 is a Sulfamoyl-Benzamide-Like Agent

1. Induce assembly

2. Incubate with agent

Image with EM

Vehicle

Regular Spheres
~40 nm Diameter

GLS4 – Class I

“Cracked Egg Shells”
>50 nm

GLP-26 – Class II

Firm assemblies,
“Hard-Boiled Eggs”
<40 nm
HBV Capsid Mutation Profile
Unbound vs. GLS4 Complex

Identified 18 SNSs Predicted to Confer Resistance to GLS4

V124W
V124F
T33Q
T109I
Y118F
T33N

Correctly Recaptured 4/6 (66%) of Reported Mutants that Confer Resistance
HBV Capsid Mutation Profile
Unbound vs. GLP-26 Complex

Model of GLP-26 Bound HBV Capsid (PDBID 5T2P)

Identified 13 SNSs Predicted to Confer Resistance to GLP-26
Comparing SNP Resistant Mutations for GLS4 vs. GLP-26

GLS4 Resistant
- F23L
- I105F
- S106I
- T109I
- F110Y
- V124L

GLP-26 Resistant
- F23Y
- L30F
- T33I
- L37F
- V124F
- T128I
- Y132N,Q,H,F
- L140I,V,F

Cross-Resistant
- T109I
- F23L
- L105F
- S106I
- F110Y
- V124L
- L30F
- T33I
- L37F
- V124F
- T128I
- Y132N,Q,H,F
- L140I,V,F
T109I Is a Naturally Occurring HBV Mutant that is Slightly Resistant to GLS4 but Susceptible to GLP-26

<table>
<thead>
<tr>
<th>Compound</th>
<th>WILD-TYPE HBeAg Secretion EC$_{50}$ (µM)</th>
<th>T109I MUTANT HBeAg Secretion EC$_{50}$ (µM)</th>
<th>Fold-Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLS4</td>
<td>1.7</td>
<td>4.1</td>
<td>2.4</td>
</tr>
<tr>
<td>GLP-26</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Interaction with ethyl-ester
NO EQUIVALENT IN GLP-26
Conclusions

Developed computational methods to simulate effects of mutations for the purpose of identifying resistance

Compared predicted resistant mutations for GLS4 vs. GLP-26.
  ◦ Simulated 1080 mutations in unbound and CAE-bound complexes.
  ◦ Fewer SNSs confer resistance to GLP-26 (12) than GLS4 (18).
  ◦ T109I decreases activity of GLS4 but remains susceptible to GLP-26.
    ◦ Confirmed susceptibility experimentally

Continuing to apply computational approach to other systems of interest.
  ◦ HIV, HCV, Norovirus, Zika virus, Dengue Fever virus and other Flaviviruses.
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