Translating Molecular Virology into Novel Therapies for Hepatitis

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6/22/11

Disclosures: Eiger BioPharmaceuticals Inc., Roche, Romark
Hepatitis viruses:

Important world wide causes of liver disease

Unfortunately, current therapies are inadequate for many

Fortunately, molecular virology

→ new targets

→ novel therapies
Examples of such “bench-to-bedside” efforts

clinical trial

IND-enabling studies

target to lead studies
Hepatitis delta virus (HDV)

Worst form of human viral hepatitis
~ 15 million world-wide; ~ 70K in U.S.
No effective Rx

HDV
ss circular RNA
delta antigen
HBV surface Ag

HBV
ds DNA
HBV core Ag
HBV surface Ag

Presented at the 6th Int. Workshop on Clin. Pharmacology of Hepatitis Therapy, 22 – 23 July 2011, Cambridge, USA
Double rolling circle model of HDV genome replication
RNA editing generates two types of delta antigen

delta antigen isoform: small

195 a.a.

196 UAG

required for replication

delta antigen isoform: large

195 + 19 a.a.

196 UGG

inhibits replication; required for packaging with HBsAg

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Prenylation of large delta antigen is required for HDV assembly

Prenylation site = “CXXX box”

Prenylation -- site-specific lipid modification of proteins

mevalonate  →  isoprenoids (prenyl lipids)
- farnesyl (C15)
- geranylgeranyl (C20)
Hypothesis:

*Pharmacologic* inhibition of large delta antigen prenylation can prevent HDV particle formation
large antigen:  

Ser 211 large antigen:  
(Cys-->Ser)

farnesyl transferase
In vivo treatment of HDV viremia

(Bordier et al. 2003 JCI)
Attractive features of prenylation inhibition antiviral therapy:

• target host enzyme
different paradigm: deprive virus access to host function
  ?more difficult to develop resistance?

• well tolerated

• orally available compounds--ready for clinical trials
  HDV = prototype (orphan designation)---> IND approved
  multiple viruses --> broaden indication
EBP921
HDV Proof of Concept Trial

- N=16
- Primary Inclusion: Confirmed HDV infection
- Primary Exclusions: HIV, HCV, advanced cirrhosis
- Open Label Rx: 2 dosing regimens
- Duration: 28 days of dosing, option to extend
- Primary Endpoint: \( \Delta \) in HDV-RNA from baseline
Conclusions

1) HDV--important, fascinating biology, inadequate Rx

2) Prenylation is key to HDV morphogenesis (pharmacologic target)

3) Farnesyl transferase inhibitors abrogate assembly in in vitro and in vivo models

4) These types of compounds represent a new class of potential antiviral agents (HDV, HAV, HSV, others) --clinical trials
Hepatitis C virus (HCV)

-- important cause of chronic liver disease
(~170 million; 4-5 in US)
#1 cause of HCC, liver transplantation in US

Only 2-3% of HCV patients in the developed world are treated
Hepatitis C virus (HCV)

- **C**: Core
- **E1**: Envelope
- **E2**: Envelope
- **NS2**: Non-structural (NS)
- **NS3**: Non-structural (NS)
- **NS4B**: Non-structural (NS)
- **NS5A**: Non-structural (NS)
- **NS5B**: Non-structural (NS)

**Arrows:**
- Core → Envelope
- Protease
- Helicase
- ?
- Polymerase

**Regions:**
- Structural
- Non-structural (NS)
NS4B

--27 kD
--nucleotide binding motif; GTPase
--cellular transformation
--intracellular membrane rearrangements (membranous web)
--amphipathic helix (AH1) assembles NS proteins
NS4B

-- NS4B has a second amphipathic helix (4BAH2)

-- 4BAH2 is highly conserved across HCV isolates

Hypotheses:

-- Role in formation of membranous web

-- Essential for genome replication

-- Represents candidate new anti-HCV target
4BAH2 promotes lipid vesicle aggregation

(C) POPC

(D) POPC + 4BAH2

(E) POPC + 4BAH1

(Cho et al. 2010, Science Translational Medicine)
An intact amphipathic helix is required for vesicle aggregation

(Cho et al. 2010, Science Translational Medicine)
An intact 4BAH2 is required for HCV genome replication

(Cho et al. 2010, Science Translational Medicine)
Screen for small molecule inhibitors of 4BAH2 function

- Identified small molecule inhibitors with nM activity against HCV replication
- Inhibitors display synergy with clemizole; IND enabling studies

(Cho et al. 2010, Science Translational Medicine)
Conclusions

1) HCV NS4B has a second N-terminal amphipathic helix (4BAH2)

2) Conserved across natural HCV isolates

3) 4BAH2 is essential for HCV RNA replication

4) 4BAH2 promotes lipid vesicle aggregation

   relevant to formation of membranous web basis for HTS

5) Small molecule inhibitors of 4BAH2 function inhibit HCV

    genome replication and can synergize with clemizole

6) 4BAH2 inhibitors represent new potential class of anti-HCV agents; potential for combination with other emerging anti-HCV drugs (a derivative of C4 → IND-enabling studies)
Phosphoinositides—classical role in signaling
Also recognized by, and regulate function of, proteins
(e.g. involved in intracellular vesicular membrane trafficking)
NS5A amphipathic helix (AH):
-- mediates membrane association
-- essential for RNA replication
-- interacts with intracellular membrane trafficking machinery

Hypothesis: PIP2 modulates NS5A function
NS5A AH binds PIP2
The NS5A amphipathic helix specifically binds lipid vesicles containing PIP-2
PIP-2 binding is mediated by a pair of conserved positively-charged amino acids.

Basic Amino Acid PIP2 Pincer (BAAPP) domain

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PIP-2 binding is mediated by a pair of conserved positively-charged amino acids.
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(C)

**Graphs showing the relationship between time and dissipation (x10^-6) for different target peptides.**

- **Left graph:**
  - X-axis: Time (Sec)
  - Y-axis: Dissipation (x10^-6)
  - Legends: NH2C, NH2C, NH, NH26

- **Right graph:**
  - X-axis: Time (Sec)
  - Y-axis: Dissipation (x10^-6)
  - Legends: NH2C, NH2C, NH, NH26

**Legend:**
- NH2C
- NH2C
- NH
- NH26

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NS5A colocalizes with PIP-2 in the context of the HCV replication complex
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PIP-2 mediates HCV RNA genome replication

1. Bart79I WT
2. Bart79I K20AK26A
3. Bart79I Pol -
PIP-2 mediates HCV RNA genome replication
PIP-2 mediates HCV RNA genome replication
Potential new anti-HCV strategies:

-- Targeting interaction between PIP2 and NS5A BAAPP domain (e.g. neomycin)

-- Targeting enzymes that regulate PIP2 at HCV replication sites (e.g.
Development of small molecule inhibitors of PI-4 kinases

Rationale:
PIP2 binding important for HCV replication

Targeting PIP2 production (e.g. siRNA knockdown of PI4-kinase) inhibits HCV replication, but well tolerated by host cells.

Have initiated collaboration with Shokat lab:
Non-specific inhibitor → removed toxicity → increased specificity → potent inhibitors of HCV replication

Ongoing optimization, pharmacokinetics, metabolite analysis
Valuable probes of host cell biology
Applicability to other targets
Conclusions

1) NS5A specifically binds PIP2
2) HCV--first example of PIP2-dependent viral genome replication
3) HCV NS5A AH--first example of BAAPP domain mediating PIP2 binding
4) Novel MOA; likely widespread among viruses
5) HTS to identify novel inhibitors of PIP2-BAAPP motif interaction
6) Specific PI-kinase inhibitors--> anti-HCV and broad-spectrum antivirals
7) NS proteins (e.g. NS4B, NS5A) represent rich source of novel targets, basis of future cocktail components