Disparities in HCV Treatment Response: Molecular Genetics

Kenneth E. Sherman, MD, PhD
Gould Professor of Medicine
Director, Division of Digestive Diseases
Univ. of Cincinnati College of Medicine
VIRAL/HOST FACTORS IN DISEASE PROGRESSION AND RESPONSE

- Virus
  - Viral Load
  - Genotype
  - Quasispecies

- Host
  - Immune Response
  - ?Receptor
  - Polymorphisms
  - ?Lipid Metabolism
  - Iron
Viral Dynamics of Hepatitis C Virus Infection

Rate of production of target cells, \( p_t \)

Cell death \( \delta \)

Uninfected hepatocytes \( T \)

Productively infected hepatocytes \( I \)

Cell death \( \delta \)

Viral load \( V \)

Clearance of virions
HCV RNA levels in Sequential Serum Samples

Gordon et. al., HEPATOLOGY, 1998
Biphasic viral dynamic model

Antiviral therapy

Therapeutic Implications

- When $E < 1$, biphasic: at the same $e$, therapeutic outcome relies on the 2\textsuperscript{nd} decline phase (i.e., Infected cell death rate by individual's immune activity).
- Drug or dosing efficacy is a key parameter in the initial viral decline phase.
- Estimated Time to Clearance is based upon the combination of $E$ and the 2\textsuperscript{nd} Phase Decline slope.
Model predictions on the effect of efficacy of inhibition of viral production

- (A) Effect of efficacy on the kinetics of HCV clearance.
- (B) Effect of efficacy on the time required to reduce viral load to clearance level.
Viral Kinetic Responses

A-A White

Layden-Almer et. al., HEPATOLOGY 2003
WHY???

• African American have altered IRG effect
  – Different Virus
  – Different IRG
HEPATITIS C VIRUS

Genome

- **Structural Proteins**
  - C
  - E1
  - E2/NS1
  - NS2
  - NS3
  - NS4A
  - NS4B
  - NS5A
  - NS5B

- **Non-Structural Proteins**
  - RNA dependent RNA polymerase

- **RNA Binding Site**
- **Envelope Glycoproteins**
- **Signal Peptide (P7)**
- **Serine protease/helicase**

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Internal Ribosomal Entry Site
Phylogenetic Analysis of NS-5 Sequence from 76 HCV Isolates

Gen Virol;1993;74:2391-2399
Geographic Distribution of HCV Genotypes 1-6

Source: Compiled at Chiron Corporation. (Updated 9/15/94)
HCV GENOTYPES BY RACE

Blatt et. al. J VIROL HEP 2000
GENOTYPE AND TREATMENT RESPONSE

- ISDR Mutations (NS5A)
- NS4b Mutations
- Core Mutations
Methods: Analyses

- **N= 32 pairs A-A vs Caucasian**
- **Quasispecies complexity:**
  - Heteroduplex complexity assay (HCA)
  - Cloning and sequence analysis

- **Statistical analyses:**
  - Fisher’s Exact test for differences in proportions
  - Generalized estimating equations model for differences in band counts over time
Amplification of HVR1

136-bp HVR1

Nested PCR

External forward:
5’-GGTGCTCACTGGGGAGTCCT-3’

External reverse:
3’-CATTGCAGTTCAAGGGCCGTGCTA-5’

Internal forward:
5’-TCCATGGTGGGGAAGCTGGGC-3’

Internal reverse:
3’-TGCCAACCTGCGTGGTGTT-5’
Heteroduplex Methodology

1. Homologous strands are present in the PCR product
2. Denaturation/renaturation
3. Formation of homo and heteroduplexes

Gel Electrophoresis
**QUASISPECIES COMPLEXITY**

<table>
<thead>
<tr>
<th>Race</th>
<th>Mean Band Number</th>
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<tbody>
<tr>
<td>AA</td>
<td>2.63 (±1.54)</td>
</tr>
<tr>
<td>CA</td>
<td>2.59 (±1.27)</td>
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</tbody>
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Paired t-test revealed no significant difference (p=0.90)

Keenan et. al., 2004, JID
HVR AMINO ACID SUBSTITUTIONS
In African Americans

- Mean substitutions per site:
  AA 4.2 (±2.4)
  CA 5.1 (±2.7)
- Paired t-test by aa position (p=0.0113)

Keenan et al., JID, 2004
Amino Acid Representation Differences

Keenan et. al., JID 2004
CYTOKINES IN HEALTHY AFRICAN AMERICANS

Kimball et. al., J MED VIROL, 2001
CTL IMMUNE RESPONSE

Ethnicity

- 29 HCV chronically infected males
  - HCV CD4+specific proliferative response
  - IFN gamma ELISPOT
- Better immune response to Ag
- Lower IFN gamma production among AA suggesting dysfunctional T-cells
Conclusion

• HCV-infected African-Americans have both virologic and immunologic differences compared to Caucasians
• Analysis of viral specific sequences that alter IRG pathways is indicated
• New agents will probably be required to reduce treatment disparities