The role of Hepatitis C Virus in hepatocarcinogenesis

Laura Beretta
Fred Hutchinson Cancer Research Center

Incidence and mortality of the five most common cancers worldwide, 2000

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Mortality</th>
</tr>
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<tbody>
<tr>
<td>Lung</td>
<td>Lung</td>
</tr>
<tr>
<td>Breast</td>
<td>Stomach</td>
</tr>
<tr>
<td>Colon</td>
<td>Liver</td>
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<tr>
<td>Stomach</td>
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<tr>
<td>Liver</td>
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600,000 new cases per year
Variations in the mortality rates of HCC
(the rates are reported per 100,000 persons)

El-Serag and Rudolph, Gastroenterology 2007

Risk Factors for HCC Worldwide, by area, 2000

Persistent HBV or HCV infection account for over 80% of HCC cases
the causative agents of HCC are quite well established.
Trends in US Cancer Mortality Rates

Liver cancer has the fastest growing death rate in the US

El-Serag and Rudolph, Gastroenterology 2007

Trends in the age distribution of HCC in the US

Incidence rate per 100,000 PY

El-Serag and Rudolph, Gastroenterology 2007
Hepatitis C Virus-associated Hepatocellular Carcinoma

Adjusted incidence rate per 100,000

Japan  USA
21.4    7.2

Estimated Incidence of Acute HCV Infection
United States, 1960-2001

Source: Hepatology 2000;31:777-82; Hepatology 1997;26:S2-S5; CDC, unpublished data
Hepatitis C estimated prevalence

<table>
<thead>
<tr>
<th>WHO Region</th>
<th>Total Population (Millions)</th>
<th>Hepatitis C prevalence Rate %</th>
<th>Infected Population (Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>602</td>
<td>5.3</td>
<td>31.9</td>
</tr>
<tr>
<td>Americas</td>
<td>785</td>
<td>1.7</td>
<td>13.1</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>466</td>
<td>4.6</td>
<td>21.3</td>
</tr>
<tr>
<td>Europe</td>
<td>858</td>
<td>1.03</td>
<td>8.9</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>1 500</td>
<td>2.15</td>
<td>32.3</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>1 600</td>
<td>3.9</td>
<td>62.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5 811</strong></td>
<td><strong>3.1</strong></td>
<td><strong>169.7</strong></td>
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</tbody>
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The connection between virus and cancer

Natural History of HCV Infection

- HCV infection
- Chronic hepatitis
- Cirrhosis
- HCC

3%-8% / year

<table>
<thead>
<tr>
<th>HCV chronic infection</th>
<th>Noncirrhotic HCV individuals: &lt;0.1%/year</th>
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<tbody>
<tr>
<td>Cirrhotic patients:</td>
<td>3%-8%/year</td>
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</table>
it remains a challenge to provide a clear and consistent picture of the connection between virus and cancer whether the virus plays a direct or indirect role
Iberetta, 3/30/2008

regional difference
Iberetta, 3/31/2008
Association with co-factors

Environmental or genetic background underlying the incidence of HCC development reported in Japanese and Western HCV cohorts

Excessive alcohol consumption

HBV co-infection

Overweight, Diabetes, Insulin resistance

Hepatic steatosis which involves an accumulation of intracytoplasmic lipid droplets

Indirect involvement of the virus in HCC

Chronic hepatic inflammation and cirrhosis are thought to play an important role in tumor initiation: necrosis and regeneration enhance mutagenesis in host cells, the accumulation of which culminates in HCC.
extremely rare occurrence of HCC in patients with autoimmune hepatitis in whom severe inflammation in the liver persists indefinitely

absence of a complete set of genetic and epigenetic aberrations required for carcinogenesis.

HCV induces genome instability

Iberetta, 3/31/2008
Hepatitis C Virus
Enveloped, single-stranded, positive-sense RNA virus

Mice with transgenic expression of viral proteins
(summary of 18 models)

Core protein:  Development of steatosis and HCC later in life
Inhibition of microsomal triglyceride transfer protein activity
which is essential for hepatic lipoprotein assembly and secretion
Increase the concentration of monosaturated fatty acids
Activation of RXR-alpha and PPAR-alpha
Induction of Insulin resistance
Synergy of HCV core and alcohol in inducing oxidative stress

Structural proteins Core-E1-E2:  Similar results

E1-E2, E2, NS3-NS4A or NS5A:  No major histological changes to the liver

<table>
<thead>
<tr>
<th>Slide 15</th>
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<tbody>
<tr>
<td>I2</td>
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<td>I6</td>
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**Relevance - Recent reports on HCV patients**

Chronic hepatitis C can result in fatty changes in the liver and hepatic steatosis is associated with increased frequency of HCC.

Correlation between HCV infection and insulin resistance

Involvement of the liver microsomal triglyceride transfer protein in HCV induced liver steatosis

Increased core genetic variability in tumors compared to adjacent cirrhotic liver, supporting the importance of core genetic variability in hepatocellular carcinogenesis.

HCV core variants isolated from liver tumor but not from adjacent non-tumor tissue interact with Smad3 and inhibit the TGF-beta pathway.


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**These studies strongly support a direct role for core protein in liver pathogenesis. Core may predispose subjects to HCC development through its contribution to the onset of steatosis and insulin resistance.**
even if we consider the variability of morphological observations and the diversity of biochemical data.

these data are particularly relevant considering recent reports on HCV patients.
Recent development of cell culture models of HCV infection and replication

Protein changes upon HCV replication

Petra Mannova  Romain Parent  Sufen Shang  Neha Lohia
HCV Infection of Huh7.5 cells

Huh7.5 Culture Supernatant and Intracellular HCV RNA LEVELS

Transcriptomic

Proteomic

Activation of RXRA and PPARA
Activation of the PI3K/Akt/mTOR pathway

Mannova et al. MCP 2006

N-Ras/PI3K/Akt/mTOR pathway and HCV replication

Increased N-Ras levels in sub-cellular sites of HCV replication and stimulation of the PI3K-Akt-mTOR pathway by HCV contribute to the maintenance of steady-state levels of HCV replication.
The PI3K/Akt/mTOR pathway and liver progenitor cells

Liver progenitor cells may be important in carcinogenesis resulting from chronic liver diseases. These cells may function as tumor progenitors.

Romain Parent
The human bipotent liver progenitor HepaRG cell line

Established from the peritumoral region of an HCV-induced Edmondson grade I HCC

Share several markers with the regenerative neoductules of the same area studied by IHC

Share some features with liver progenitor cells

Parent et al. Gastroenterology 2004

Progressively acquire a polarized hepatocytic phenotype

Day 2

Day 4

Day 15

Day 42
Translational control plays a prominent role in the hepatocytic differentiation of HepaRG liver progenitor cells

Over-expression of an activated mTOR mutant (ΔTOR) lacking the R (repressor) domain
Impaired hepatocytic differentiation in ΔTOR-expressing HepaRG cells

Reversion by rapamycin of the altered phenotype
Loss of responsiveness to TGF-β in ΔTOR-expressing HepaRG cells.

Activated mTOR specifically targets:

- Cell death-related network: TNF superfamily members, interferons and caspases
- Lipid homeostasis-related network: PPARA, PPARD, RXRA and RXRB

Parent et al Cancer Res 2007
Parent and Beretta Genome Biology 2008
Nude mice transplanted with Δ TOR cells expressing Firefly Luciferase
week 2

Liver specific PTEN knock-out results in HCC in mice

Derek Masse    Garrett Booth

Horie et al J Clin Invest 2004
Collection of liver tissue at 3-months intervals

<table>
<thead>
<tr>
<th>Wild type</th>
<th>PTEN mutant</th>
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<tr>
<td>6 months</td>
<td>6 months</td>
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A

B

C

D

E

F

Hepatomegaly and liver steatosis
US/Histology Show Early HCC at 9 months

All mice develop tumors by 12 months

Natural History of HCV Infection

3%-8% / year

15% (10-30%)

90% (30-65%)

100%
an increasing body of evidence suggest a direct involvement in cellular metabolic disturbance