Viral oncogenesis and hepatocellular cancer

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Viruses and HCC: a Pathologist’s View

- Virus
  - Changes in cellular environment:
    - Modulation of apoptotic and cell signalling pathways
    - Epigenetic dysregulation
    - Transcriptional reprogramming
    - Chronic inflammation
    - Tumour Promoters
    - Tumour Suppressors
    - Oxidative Stress
    - Immune reactions
  - Druggable targets

- Patient
  - Development of HCC
HPV and cancer of the cervix

Role of HPV E6

Role of HPV E7

Apoptosis  S-phase Arrest  Degraded p53

Ubiquitin-mediated proteolysis

Inactive E2F

Degradation

Cellular Proliferation
Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets

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Å Exome sequencing tumour vs non-tumour (n = 243)
Å 28,478 somatic mutations
Identified 161 putative driver genes → 11 pathways altered in >5% tumours

HBV related tumours: TP53; HCV: no significant associations

28% patients had alterations targetable by an FDA-approved drug
HCC: a Pathologist's View: What does this tell us?

Virus

Patient

Changes in cellular environment:
- Modulation of apoptotic and cell signalling pathways
- Epigenetic dysregulation
- Transcriptional reprogramming
- Chronic inflammation
- Tumour Promoters
- Tumour Suppressors
- Oxidative Stress
- Immune reactions

Development of HCC

It's complicated!
Viruses and HCC: a Virologist's View

Virus → Patient → STUFF HAPPENS → Development of HCC

Intervention targets
Hepatitis B Virus and HCC

Partially double stranded DNA
3,200 bp
HBV Genome

- Partially double stranded DNA
- Relaxed completely double stranded DNA
- Covalently closed circular DNA - cccDNA
- May integrate into host genome
HBV Replication Cycle

HBV DNA

mRNA + pregenomic copy

Translation

Viral proteins:
- HBsAg
- HBcAg
- HBeAg
- Polymerase

RT activity converts RNA to DNA

Pregenomic RNA and pol encapsidated within core
HBV and HCC

- Accounts for >50% of global HCC
- >80% in highly endemic countries - China
- 350 million chronic infections
- 30-100 fold increased risk HCC
- Liver may NOT be cirrhotic
HBV/HCC Pathogenesis

Å (i) Epigenetic effects of infection
CpG islands
Subject to methylation
Transcriptional silencing
Host antiviral defence
HBV/HCC: Epigenetic field theory

Â HBV infection → induction of DNA methylation transfersases
Â Side effect ī silencing of cellular suppressor genes
Â Malignant cells ī contain hypermethylated genes which are hypomethylated in non-tumour adjacent tissue
HBV/HCC Pathogenesis

(i) Epigenetic effects of infection
(ii) HBx, a potential oncoprotein
HBV/HCC: role of HBx
HBx as an oncoprotein?

- Pleiotropic activities
  - Cell cycle regulation
  - Signaling pathways
  - DNA repair

- Direct transforming activity
  - Data are confusing and contradictory
  - Artificial systems with high level expression
    (in hu liver, only low level detectable)
  - Transgenic mice data likewise
HBx as an oncoprotein?

- 184 gene targets directly regulated by HBx via interaction with 144 TFs
- Experimental data very difficult to interpret e.g. HBx → block or induce apoptosis
HBx and trHBx

- HBV DNA integration - viral breakpoints are often at 3' end HBx gene → C-terminal truncated HBx (trHBx)
- Cellular localisation differs
  - wtHBx: Cytoplasmic, near to mitochondria
  - trHBx: Nuclear
**wtHBx and trHBx are different**

<table>
<thead>
<tr>
<th>Effect/property</th>
<th>Protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wtHBx</td>
<td>trHBx</td>
</tr>
<tr>
<td>Stimulation of HBV replication</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Inhibition of cell growth</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Transactivation</td>
<td>Yes</td>
<td>No (?)</td>
</tr>
<tr>
<td>Inhibition of cell transformation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Predominant localization</td>
<td>Cytoplasm</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Expression in hepatocellular carcinoma cells</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Expression in nontumorous tissues</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Enhancement of invasiveness and metastasis formation</td>
<td>Yes</td>
<td>Yes</td>
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HBx as an oncoprotein: hypothesis

- Wt HBx is pro-apoptotic
- Wt HBx proto-oncogene activated by 3' deletion to v-onc tr HBx
  - Anti-apoptotic
  - Stimulates cell proliferation
  - Facilitates HCC
HBV/HCC Pathogenesis

(i) Epigenetic effects of infection
(ii) HBx, a potential oncoprotein
(iii) integration of HBV genome
HBV/HCC: Integration of the HBV genome

- Early in chronic infection, in >80% HCCs
- Described on nearly all Hu chromosomes
- Probably not random
- Targeted to active transcription sites
- Gene families involved in cell survival, proliferation and immortalisation
  - hTERT, PDGF R, MLL, 60s Ribo genes
- Near Long Interspersed Nuclear Elements
Integration of HBV genome: consequences

- Multiple mutagenic events: deletions, translocations
- Enhanced expression of nearby genes
- Disruption of individual protein production
- Generation of hybrid HBV-human transcripts
HBV-Human hybrid transcripts

- HBx-LINE1
  - Viral promoter and part of HBx coding sequence
  - Human LINE1 element downstream
- Found in >33% HCCs
- Associated with poor patient survival
- Promotes cell invasion and migration in vitro
- Doesn’t appear to encode a functional protein; activity not abolished by Stop codon downstream of HBx start
Hepatitis B virus-human chimeric transcript HBx-LINE1 promotes hepatic injury via sequestering cellular microRNA-122

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miR-122

- miR-122 KO mice develop spontaneous HCC
- miR-122 acts as an anti-inflammatory tumour suppressor in liver
Data

- Bioinformatics search → 6 miR-122 sites within HBx-LINE1
- Within tumours, -ve relation between HBx-LINE1 levels and miR-122 abundance
- Pro-proliferative effects of Wt-HBx-LINE1 (e.g. $\beta$-catenin signalling, epithelial-mesenchymal transition, enhance cell migration) abolished by mutations in miR-122 binding sites
Hypothesis

- HBx-LINE1 promotes HCC progression by sequestering cellular miRNA-122

- ? How do de-repressed miR-122 targets drive HCC

- ? What level of HBx-LINE1 is needed?

- ? Might this be a way of monitoring risk

- ? And act as novel antiviral target
HBV and HCC: what matters

• HBV infection is preventable
  • And vaccination reduces HCC development:
    • HBV vaccination programme introduced in Taiwan 1984

• Annual incidence of HCC in children 6-14 years of age:
  • 1981-1986 0.70 per 100,000
  • 1986-1990 0.57 per 100,000
  • 1990-1994 0.36 per 100,000

HBV and HCC: what matters

- HBV infection is preventable
- Chronic HBV infection is treatable e.g. tenofovir, entecavir
  - Drugs with high potency
  - Drugs with high barriers to resistance
- Risk of HCC is directly related to HBV DNA levels
HCV and HCC

Å +ve ss RNA genome
Å No DNA intermediate
Å HCC almost always arises in cirrhotic liver
  ï 1-7% per year
  ï ? Suggests carcinogenesis is a more indirect consequence of chronic inflammation
HCV/HCC: The Virus

H77 genome (accession NC_004102)

- IRES; miRNA 122
- NS2 protease
- NS4a cofactor for NS3
- NS5b RdRp
- NS5a multifunctional
- NS3 protease and helicase
- NS4b membranous web
- P7 ion channel
miR-122 KO mice develop spontaneous HCC
miR-122 anti-inflammatory tumour suppressor in liver

miR-122 is an essential co-factor for HCV replication

? Sequestration of miR-122 by HCV → HCC?
Oncogenic effects of HCV proteins

- Data generated in artificial over-expression of individual proteins in experimental cell culture or animal models
- Relevance to in vivo infection is uncertain
Interactions of HCV with cellular components in cirrhotic tissue microenvironment that promote hepatocarcinogenesis

Hoshida et al Journal of Hepatology 2014; 61: S79-S90
Host Factors

- HCV cirrhosis risk score
  - Panel of 7 SNPs predict risk of cirrhosis
- HCV-HCC - >100 SNPs in the literature conferring ↑risk of HCC in HCV patients
- IL-28B SNPs - relation to HCC hard to unpick
- Many others eg cytokine genes, fibrosis related genes, CYP enzymes
HCV and HCC: what matters

- Prevention of infection
  - No vaccine available
- HCV is treatable
Different classes of direct acting antiviral agents (DAA)

Asselah et al. Liver Int. 2012
ION-2

Overall prior treatment experienced: With and without cirrhosis

REFERENCES:
1. HARVONI Summary of Product Characteristics, November 2014

ION-2 Study Design: a randomised, open-label trial evaluating 12 and 24 weeks of treatment with HARVONI with or without RBV in GT 1 HCV-infected subjects who failed prior therapy

RELAPSE:
- 7 relapses in the 12-week LDV/SOF group
- 4 relapses in the 12-week LDV/SOF + RBV group
- 0 relapses in the 24-week LDV/SOF group
- 0 relapses in the 24-week LDV/SOF + RBV group
How good are these drugs?

- Very!
- All oral, extremely well tolerated
- HIV co-infection - no problem
- >95% SVR rates in almost all populations
- Decreased (e.g. 80% to 90%) if
  - Cirrhosis
  - Genotype 3
Public Health Scandals (1)

• We are missing almost half of the epidemic!

• Risk groups
  - PWID
  - Ethnic minorities
  - Ex-IDU (hidden)
Public Health Scandals (2)

We are ABJECTLY failing patients through allowing NHS England to ration use of DAAs.

DAAs are NICE approved but NHSE rationing contradicts the NICE process.
HCV and HCC: what matters

- Prevention of infection
  - No vaccine available
- HCV is curable
  - But SVR does not reduce HCC risk to zero
**Increased risk in HCV patients with SVR**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Patients</th>
<th>Follow-up Months (range)</th>
<th>HCC Risk Factors</th>
<th>HCC Incidence SVR+</th>
<th>SVR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshida et al., 2004 [2]</td>
<td>2392 (7)</td>
<td>45 (6–93)</td>
<td><strong>Advanced fibrosis; age &gt; 60 male sex</strong></td>
<td>0.4%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Shiratori et al., 2005 [17]</td>
<td>271 (7)</td>
<td>100.8 (76.8–136.8)</td>
<td>Age &gt; 60 albumin &lt; 4 mg/dL</td>
<td>2.4%</td>
<td>5%</td>
</tr>
<tr>
<td>Bruno et al., 2007 [4]</td>
<td>920 (8)</td>
<td>96 (6–169)</td>
<td>Cirrhosis; age &gt; 54 Male sex; platelets &lt; 109,000</td>
<td>0.7%</td>
<td>2%</td>
</tr>
<tr>
<td>Cardoso et al., 2010 [16]</td>
<td>307 (18)</td>
<td>42 (12–216)</td>
<td>Cirrhosis; bilirubin &gt; 0.9 mg/dL albumin &lt; 4 g/dL platelets &lt; 150,000</td>
<td>0.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Yu et al., 2006 [3]</td>
<td>1619 (16)</td>
<td>70 (12–180)</td>
<td>Genotype 1; age</td>
<td>0.76%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Veldt et al., 2007 [5]</td>
<td>479 (4.9)</td>
<td>25.2 (9.6–58.8)</td>
<td>No features found</td>
<td>0.4%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Van der Meer, 2012 [7]</td>
<td>530 (11.4)</td>
<td>100 (77–144)</td>
<td>Male sex; age &gt; 49 diabetes genotype 3; alcohol abuse</td>
<td>0.3%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Mallet et al., 2008 [6]</td>
<td>96 (11.5)</td>
<td>118 (86–138)</td>
<td>* Histological cirrhosis persistence; anti-Hbc +</td>
<td>0.3%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Fattovich et al., 1997 [15]</td>
<td>329 (12.7)</td>
<td>60</td>
<td>Bilirubin &gt; 1 mg/dL age &gt; 57</td>
<td>1%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

* SVR patients; **non-SVR and untreated patients.

Annual incidence rate of HCC in the general population is around 0.0014%
HCV and HCC: what matters

- Clinical consequence: how to manage patients with HCV-associated cirrhosis?
- Surveillance:
  - Not particularly effective
  - Needs to be targeted
- STOP-HCV: MRC Stratified Medicine
  - Non-invasive biomarkers
  - Host genetics
  - Viral genomics
## Summary

### HBV
- Ccc DNA and integration
- HCC may occur in non-cirrhotic liver
- Viral factors incl HBx
- ?miR-122 sequestration
- Preventable
- Controllable
- Who should be monitored?

### HCV
- No DNA intermediate
- Cirrhosis is major contributor to HCC
- Viral factors
- ?miR-122 sequestration
- Not preventable
- Curable
- Who should be monitored?