Histopathology and molecular classification

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HCC in HGDN, diameter 5 mm

Early-advanced HCC

Hepatocyte

Microscopical level

Macroscopical level

venous-arterial

Dysplastic focus < 1 mm

Dysplastic nodule > 1 mm

Low grade-High grade DN

Genetic alterations

Needle liver biopsies of 115 chronic hepatitis pts; 10 yrs fu

30% LCD
Libbrecht et al Histopathology 2001

22% SCD

Early cancer with a diameter of 10 mm

Hypointense lesion on T2-weighted image of MR

CD34

HCC with a diameter of 20 mm

Enhancing lesion on arterial phase of MR

CD34

Hepatocarcinogenesis is a multistep process
Careinogenic conditions: hepatitis B, hepatitis C, alcohol, NAFLD

Microscopical level

Macroscopical level

venous-arterial

Hepatocyte

Dysplastic focus < 1 mm

Dysplastic nodule > 1 mm

Low grade-High grade DN

Genetic alterations
Mass on surveillance ultrasound in a cirrhotic liver

- Less than 1 cm: Repeat US at 3-4 month intervals
- 1-2 cm: Two dynamic imaging studies
  - Typical vascular pattern with one technique
  - Atypical vascular pattern with both techniques
- Greater than 2 cm:
  - One dynamic imaging technique
  - Atypical vascular pattern
  - Coincidental typical vascular pattern on dynamic imaging
  - Typical vascular pattern on dynamic imaging or AFP > 200 ng/ml

Need for good reproducible and prognostically significant criteria

- DD dysplastic nodules versus early cancer
- Established cancer: prognostically significant classification
  - HCC vs CC
  - Differentiation degree
  - Nuclear atypia

Needle Biopsies

- All diagnostic criteria apply
- Tumoral and neighbouring parenchyma
- In case of ablation, ablation of needle tract

Stromal invasion in early HCC

- Stromal invasion is a helpful clue in the differentiation from high-grade dysplastic nodule.

Stromal invasion

Portal vein invasion

Table 2. Histologic criteria to distinguish hepatocellular nodules

<table>
<thead>
<tr>
<th>Histologic feature</th>
<th>Dysplastic nodules</th>
<th>Occult cancers</th>
<th>Well-differentiated HCC</th>
<th>Moderately differentiated HCC</th>
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<tbody>
<tr>
<td>Nodule histologic grade</td>
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<tr>
<td>Nuclear atypia</td>
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<tr>
<td>Portal vein invasion</td>
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<tr>
<td>Necrosis</td>
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<td>Hemorrhage</td>
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</table>

Need less than portal vein invasion [Kojiro and Roskams, Sem Liver Diseases]
**Glypican-3 IH: sensitivity 77%, specificity 96%**

DD High grade dysplastic nodules vs early carcinoma < 2.5cm

Biopsies included

Panel with HSP70 and GS

Di Tomasso et al Hepatology 07

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**A Molecular Signature to Discriminate Dysplastic Nodules from Early Hepatocellular Carcinoma in HCV-Cirrhosis**


Quantitative real-time PCR analysis of 5 genes
3 up-regulated: TERT, glypican 3, survivin
2 down-regulated: LYVE-1, E-caderin
allows to discriminate between dysplasia and early HCC

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**Need for good reproducible and prognostically significant criteria**

- DD dysplastic nodules versus early cancer
- Established cancer: prognostically significant classification

- HCC vs CC Too Simple!!

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**HPC in Chronic Liver Diseases**

Telomere shortening and replicative senescence of mature hepatocytes (and not of hepatic stellate cells or lymphocytes) is a general feature of the cirrhotic stage of a variety of chronic liver diseases

Wiemann FASEB J 03, Falkowski J Hepatol 03, Rudolph Science 00, Fausto Hepatology 2004

This inhibition of replication is associated with progenitor cell activation in human liver diseases


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**HCCs are derived from a single cell (monoclonal)**

Which cell?
- Longevity
- High self-renewal capacity
Necessary to accumulate mutations

**HEPATOCELLULAR CARCINOMA**

Bile duct cells

Progenitor cells

Hepatocytes

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**Since progenitor cells are activated in chronic liver diseases, they also form a target cell population for carcinogenesis**
"Cancer Stem Cell" was defined 30 years ago (1977) as:
"tumor stem cells are the cell renewal source of a neoplasm and also serve as the seeds of metastatic spread of cancer."

Although they constitute only a small minority population!

Hamburger and Salmon
Primary bioassay of human tumor stem cells

Properties shared by normal stem cells and cancer stem cells

1. capacity for self-renewal
2. ability of multilineage differentiation
3. active telomerase expression
4. activation of anti-apoptotic pathways
5. increased membrane transporter activity
6. ability to migrate and metastasize

Therapeutic implications of Cancer Stem Cells

Most therapies fail to consider the difference in drug sensitivities of cancer stem cells compared to their non-tumorigenic progeny.

Progenitor Cells and Intermediate Hepatocytes

Cancer stem cells and their progeny
15-16% HCC: progenitor cell features/K19+

Durnez A et al Histopathology 2006
Group Llovet ILCA 08: 15%

EXPERIMENTAL SETUP

Laser Microdissection

RNA Isolation

RNA amplification

Gene-expression analysis
- Superarray (real-time PCR 84 genes)

HCC confirmation

Mina Komuta, Bart Spee et al Hepatol 08

Keratin 19 Positive Hepatocellular Carcinoma

Survival

Recurrence


AIM: further investigate the clinicopathological correlates and relevance of KRT19 expression at mRNA level in an extended series of human HCC

Prognostic relevance KRT19 expression

Postoperative Recurrence

Postoperative Survival

KRT19 RNA (continuous variable) correlated significantly with:
- tumor recurrence (p<0.0001, HR: 1.56)
- poor survival (p<0.0001, Hazard Ratio (HR): 1.43)
Accordance with Findings of other Groups

These results are in accordance with previous findings of other groups:

- **Ding et al.**: Overexpression of K19 correlates with HCC metastasis. 


- **Wu et al.**: In univariate analysis, HCC expressing AE1 and K19: significantly shorter survival without any treatment. 

- **Uenishi et al.**: In univariate analysis, HCC expressing K19 and K7: lower tumor-free survival rate after curative resection. 

- **Aishima et al.** (2007): 35 small HCC with biliary differentiation based on morphology, K19, mucin production, compared with 61 ordinary HCC: K19+ more extrahepatic recurrence, worse survival.


Previously identified subtypes of HCC

- **Hepatoblast signature**: Significantly higher KRT7, KRT19, very bad prognosis (survival 11.9mo versus 64.4mo). 

New Prognostically Significant Classification

- **CD15+ (can pattern)**
  - Hepatocyte
  - HCC
  - Mixed hepatobiliary carcinoma
  - Cholangiocyte
  - CC

In future additional markers will be added e.g. microvascular invasion marker, markers for targeted therapy, …


Transcriptome Classification of HCC Is Related to Gene Alterations and to New Therapeutic Targets

- G1 tumors were associated with low copy number of HBV and overexpression of genes expressed in fetal liver and controlled by parental imprinting.
- G2 included HCCs infected with a high copy number of HBV and mutations in *PIK3CA* and *TP53*. G1 and G2 show activation of the AKT pathway.
- G3 tumors have TP53 mutations and overexpress genes controlling the cell cycle.
- G4 heterogeneous: include TCF1-mutated hepatocellular carcinomas.
- G5 and G6 strongly related to beta-catenin mutations and Wnt pathway activation: in particular, G6 tumors have satellite nodules, higher Wnt activation and E-cadherin underexpression.

Conclusion:
- Genetic diversity of human HCC
- Specific identifiers for classifying tumors
- 50% of the tumors were related to WNT or AKT pathway activation
8 genes distinguish between control and cirrhosis
24 genes distinguish between cirrhosis and dysplasia
93 genes distinguish between dysplasia and early HCC
9 genes distinguish between early and advanced HCC.

Cirrhosis
Up-regulation:
- JAG1 (Notch receptor ligand)
- STAT1, CXCL9-11 (Toll-like receptors)

Dysplasia
Up-regulation:
- EPO, EPOR, CISH, STAT3, SOCS3 (Jak/STAT pathway)
- GADD45B, GADD45G, GADD45A (cell cycle control)

Early HCC
Down-regulation
- IFNAR1, TLR4, FOS and CD14 (Toll-like receptors pathway)
- IFNAR1, TLR4, FOS, and CD14 (Toll-like receptors pathway)
- EPO, EPOR, SPRY2, and SOCS2 (Jak/STAT pathway)
- BMPR2, ID2, THBS1, and DCN (TGF-beta pathway)
- FBP1, PCK1, PCK2, GYS2, FOXO1A, SOCS2 (insulin signaling)

Up-regulation
- DKK1, FZD6, FZD7, PCGB1, LEF1 (beta-catenin pathway)
- PTCH (Hedgehog pathway)

Advanced HCC
Up-regulation
- PRIM1, PRIM2 (cell proliferation)
- ASPM, PTTG1, CCNB1, CDKN2C, CDKN2A (cell cycle control)

Conclusions

• Comparison of independent gene expression signatures of human well characterized tumours, can identify novel classes of human HCC that are homogeneous in underlying biology and clinical outcome

• A subgroup of human HCC probably originate from liver progenitor cells. This subtyppe has bad prognosis and can be recognized by keratin 19 immunohistochemical staining

• The basic genetic profile (< cell of origin) of a tumour is present throughout the tumour, although it can look phenotypically very heterogeneous.

• Cancer stem cells should be the target of newly developed therapy. Focus on the human liver progenitor cells and their niche in disease and cancer!

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