Benign hepatocellular tumors: an update, from molecular biology to pathologic diagnosis

Haeryoung Kim, M.D., Ph.D.
Department of Pathology
Seoul National University Bundang Hospital
Seoul National University College of Medicine

15th May, 2014 / KSP Spring meeting short course
Benign hepatocellular tumors

- Benign proliferation of hepatocytes with a mass-forming presentation
- Focal nodular hyperplasia (FNH)
- Hepatocellular adenoma (HCA)

Common features of FNH and HCA
- Female predominance
  - Female/male ratio: FNH – 8:1, HCA – 9:1
- Proliferation of mature hepatocytes
- Background liver: normal

Nault et al. Gastroenterology 2013
**FNH**
- Most common benign hepatocellular tumor
  - Estimated prevalence: 0.4~3 / 100
  - Second most common benign liver nodule (after hemangioma)

**HCA**
- Much lower than FNH: 3 / 100,000
- More common in Western countries:
  - lower use of oral contraceptives in Asia?
- **New risk factors:**
  - obesity, alcohol → inflammatory HCA
  - recent 3rd generation of oral contraceptives with low dose of estrogen

*Nault et al. Gastroenterology 2013*
This morning….

- Focal nodular hyperplasia

- Hepatocellular adenoma
  - Inflammatory HCA (I-HCA)
  - HNF1α-mutated HCA (H-HCA)
  - β-catenin-mutated HCA (B-HCA)
  - Unclassified HCA
Focal nodular hyperplasia

- **Definition**
  - Normal-like hepatocytes with 1- to 2-cell-thick plates
  - **Plurinodular pattern** separated by fibrous bands
  - **Central stellate scar** with radiating fibrous cords
  - **Dystrophic arterial vessel**
    - Intimal thickening
  - Absent portal vein
  - Ductular reaction at fibrous bands/parenchyma interface
Gross findings

- Well-demarcated but non-encapsulated
- Solitary in 2/3 cases
- Lighter than surrounding liver
- Central gray-white, depressed stellate scar with radiating fibrous septa
Pathogenesis of FNH

• Reactive polyclonal proliferating hepatocytes
  – Local vascular malformation → increased blood flow →
    abnormal hepatocyte proliferation

• Considered a benign hepatocellular “tumor” due to
  mass-forming presentation…. *but* is a non-neoplastic
  hyperplastic lesion
• Driving event in FNH formation: vascular abnormalities

• Gene expression studies:
  – Angiogenesis: increased ANGPT1/ANGPT2 mRNA expression ratio
  – TGFβ pathway-related genes overexpressed: central fibrous scar
  – Wnt/β-catenin target gene overexpression: GLUL (→ encodes GS)
The role of glutamine synthetase (GS) in FNH diagnosis

- GS (encoded by \textit{GLUL}) is a target of $\beta$-catenin
- GS overexpression: $\beta$-catenin mutation or pathway activation

- Normal livers:
  - $\beta$-catenin and GS expressed in immediate perivenous areas of normal livers
  - (2~3 layers of hepatocytes around central vein)

- FNH:
  - Abnormal arterial blood flow
    - $\beta$-catenin activation extends further upstream towards inflow area
    - map-like GS staining pattern
  - No $\beta$-catenin mutation
• “Map-like” GS pattern is characteristic of FNH
  – Very specific and unique
  – 5~10 layers of hepatocytes around veins & no staining around fibrous scar → map-like pattern
  – Now used as a routine diagnostic tool
• Application of glutamine synthetase on needle biopsies
Glutamine synthetase

β-catenin: membranous
• Histologically and radiologically similar to FNH
• Background liver: *cirrhotic*

• Abnormal blood flow in cirrhosis

• Gene expression profile different from typical FNH
  – No β-catenin activation
  – Downregulated *GLUL* → parenchymal GS staining −/ ±
  – Different pathophysiological processes for FNH and FNH-like nodules?
Cirrhotic background liver

FNH-like nodule
Fibrous septa with abnormal arterial structures
Inflammatory cell infiltration
Mallory-Denk bodies
Mildly increased hepatocyte plate thickness
Hepatocellular adenoma

• Monoclonal proliferation of mature hepatocytes
• Absence of portal triad and interlobular bile ducts
• Background liver: normal

• Clinical significance of HCA
  – Risk of bleeding (20-25%)
  – Malignant transformation (up to 7%)

• HCA has recently become a heterogeneous entity
  – Genotype/phenotype classification in 2006 (Bioulac-Sage, Zucman-Rossi et al, Bordeaux, France)
Morphology

- Pale, yellow-tan nodules
- May be bile-stained, necrotic or hemorrhagic
- Often subcapsular, large
- Well demarcated but non-encapsulated, solitary / multiple (adenomatosis)
- Sheets and cords of cells resembling normal hepatocytes or hepatocytes with hydropic or eosinophilic cytoplasm
- Absence of portal tracts and intralobular bile ducts
- Thin-walled vascular channels and small arteries
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Molecular</th>
<th>Clinical</th>
<th>Microscopy</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNF1A-mutated HCA (30-40%)</td>
<td>Biallelic HNF1A inactivation</td>
<td>Germline mutation: younger, MODY3, adenomatosis</td>
<td>Marked steatosis</td>
<td>LFABP loss</td>
</tr>
<tr>
<td>β-catenin-mutated HCA (5-10%)</td>
<td>β-catenin gene mutation</td>
<td>Androgen intake, glycogenosis type I, familial adenomatosis polyposis</td>
<td>Cytologic atypia, pseudoglandular pattern</td>
<td>Aberrant β-catenin expression Strong, diffuse GS</td>
</tr>
<tr>
<td>Inflammatory HCA (40-50%)</td>
<td>In-frame deletions of gp130 (60%)</td>
<td>Obesity, alcohol, inflammatory syndrome</td>
<td>Inflammatory infiltrates, sinusoidal dilatation</td>
<td>SAA, CRP positive</td>
</tr>
<tr>
<td>Unclassified HCA (10%)</td>
<td>-</td>
<td>-</td>
<td>Negative for steatosis, cytologic abnormalities, inflammatory infiltrates</td>
<td>Normal LFABP positivity, no other specific findings</td>
</tr>
</tbody>
</table>
HNF1A-mutated HCA (H-HCA)

- 30~40% of HCA
- *HNF1A* mutations (Hepatocyte nuclear factor 1α)

- Biallelic somatic *HNF1A* mutations in tumor cells (90%)
- Germline inherited *HNF1A* mutation in 1 allele (10%):
  - HCA in patient with “maturity-onset diabetes type 3” (MODY3)
  - “Adenomatosis”: >10 HCAs

- No additional genetic alterations identified
  - No *CTNNB1* (β-catenin), *IL6ST*, *GNAS*, *STAT3* mutations

- Lower risk of malignant transformation compared to other types
  - Typical small H-HCA on MRI can be followed up
- HNF1A controls hepatocyte differentiation, glucose and lipid metabolism
- HNF1A mutation
  - Aberrant fatty acid synthesis activation
  - Marked steatosis
    • Typical, but not specific for H-HCA
    • 35% of IHCA and UHCA
- Liver fatty acid-binding protein (LFABP)
  - Abundant in normal hepatocytes
  - Involved in cytoplasmic trafficking of fatty acid
  - Specifically down-regulated in H-HCA
• F/29
• Ovarian cyst found during work up
• Oral contraceptive

• Liver imaging (CT/ MRI)
  – 2.1cm fat containing lesion, most likely hepatic adenoma, probably HNF1A-mutated subtype
  – At least 9 other similar-looking subcentimeter-sized lesions in both lobes of liver

• Needle biopsy was performed
Diffuse steatosis
No significant inflammation
No significant nuclear atypia
Loss of LFABP expression in *HNF1A*-mutated HCA
β-catenin-mutated HCA (B-HCA)

- 10-15% of HCAs (B-HCA + B-IHCA)

- Exclusive of *HNF1A* mutations, but can be combined with gp130 or *GNAS* mutations

- **Males**, androgen administration, glycogenosis, FAP
- Cholestasis
- Cytologic atypia, architectural atypia (small cell change, pseudoglandular structures)
- Often difficult to differentiate from WD HCC
- High risk of malignant transformation to HCC

- **Nuclear** localization of β-catenin
- Glutamine synthetase: homogenous strong expression
• **M/65**
• **History of lung cancer & renal pelvis TCC**
• **Liver imaging:**
  – 1.5cm-sized enhancing nodular lesion in right lobe with diffusion restriction, slightly hyperintense on T2WI
  – Increasing size on serial CT review
  – Cannot exclude possibility of malignant lesion (metastasis)

• **Liver needle biopsy with radiofrequency ablation**
- Slightly increased trabecular thickness
- Mild nuclear atypia

Pseudoglandular structures

Cholestasis

Loss of reticulin framework
<table>
<thead>
<tr>
<th>Normal liver: Membranous</th>
<th>β-catenin-mutated HCA: Nuclear / cytoplasmic expression</th>
</tr>
</thead>
</table>

Glutamine synthetase: Diffuse homogeneous strong expression in β-catenin-mutated HCA (vs. perivenular staining in normal liver)
β-catenin mutated HCA

Architectural atypia (frequent pseudoglandular structures) : should exclude possibility of well differentiated HCC!

Glypican-3, heat shock protein 70…

Nuclear beta-catenin
• β-catenin activation in “atypical HCA-like neoplasms”
  – Cytogenetic alterations similar to HCC
  – Recurrence/metastasis in 2 cases, transition to HCC in 1 case
    → represent “extremely well-differentiated HCC?”

• Criteria for AHN
  – Atypical age/sex
    • Men of any age / Female >50y or <15y
  – Atypical morphology
    • Focal cytologic or architectural atypia in <5% of tumor
      (small cell change, pseudogland formation, nuclear atypia)
“Well-differentiated hepatocellular neoplasm of uncertain malignant potential (HUMP)”

- “HUMP would be the best term to use”: convey necessity for close follow up

<table>
<thead>
<tr>
<th>Table</th>
<th>Proposed entities considered to represent well differentiated HUMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Lesions with features of hepatocellular adenoma morphologically, but:</td>
</tr>
<tr>
<td></td>
<td>A. Focally histologically atypical</td>
</tr>
<tr>
<td></td>
<td>• Focal reticulin loss</td>
</tr>
<tr>
<td></td>
<td>• Focal cytological atypia (small cell change, nuclear atypia) in &lt;5% of tumor (1)(^a)</td>
</tr>
<tr>
<td></td>
<td>• Focal architectural atypia (pseudogland formation) in &lt;5% of tumor (1)(^a)</td>
</tr>
<tr>
<td></td>
<td>B. Genetically atypical</td>
</tr>
<tr>
<td></td>
<td>• (\beta)-Catenin activated tumors(^b)</td>
</tr>
<tr>
<td></td>
<td>C. Clinically atypical</td>
</tr>
<tr>
<td></td>
<td>• Female &gt;50y or &lt;15y(^a)</td>
</tr>
<tr>
<td></td>
<td>• Male</td>
</tr>
<tr>
<td></td>
<td>2. Lesions with features of hepatocellular carcinoma morphologically that can regress with treatment of underlying disease:</td>
</tr>
<tr>
<td></td>
<td>A. Anabolic steroid-induced tumors</td>
</tr>
</tbody>
</table>

\(^a\) The precise degree of atypia and the age cut-offs are currently not known with certainty and require further study.

\(^b\) Nuclear/cytoplasmic positivity for \(\beta\)-catenin without other features of atypia is of unknown significance at this time.
Inflammatory HCA (IHCA and B-IHCA)

- 40-50% of HCA
- Activation of JAK/STAT pathway
- Mutations in *IL6ST* (gp130), *STAT3*, *GNAS*
- β-catenin mutation in 10% IHCA (B-IHCA)
  - HCC risk↑

Risk factors
- Obesity (high BMI), alcohol
- Diabetes mellitus, fatty liver…
Histologic findings of IHCA

- Sinusoidal dilatation
- Hemorrhage
- Dystrophic vessels, naked arterioles
- Inflammation:
  - polymorphic infiltrates
- Remnant portal tract-like structures
- Ductular reaction
- Steatosis (not as extensive as H-HCA)

- Histologic overlap with FNH → DDx may be difficult

Immunohistochemistry

- Overexpression of acute phase reactants:
  - Serum amyloid A (SAA)
  - C-reactive protein (CRP): more sensitive than SAA, low specificity
- Glutamine synthetase, β-catenin
Inflammation-associated proteins: serum amyloid A, C-reactive protein
Pitfalls of SAA and CRP interpretation on liver biopsies

• Serum amyloid A
  – Expressed in peritumoral hepatocytes in 15%
  – Focal positivity in viral hepatitis
  – Normal liver: 3%

  – SAA+, presence of interlobular bile ducts, absence of diffuse CD34 staining
    : favor non-neoplastic liver

• C-reactive protein
  – Higher sensitivity than SAA
    • Helpful for SAA-negative IHCAs
  – Relatively low specificity:
    • Non-neoplastic liver: hemorrhage/necrosis, inflammatory syndrome, portal branch embolization
    • 78% of FNH
• F/34
• 건강검진상 우연히 발견된 liver mass

• Liver MRI:
  – 3.7cm size mass at S6, no definite gross fat
  – Impression: Hepatocellular adenoma > HCC W/D > FNH
Hemorrhage and telangiectasia

Inflammatory foci
Inflammatory cell infiltration
Sinusoidal dilatation, telangiectasia
Unclassified HCA (HCA, not otherwise specified)

- <10% of HCA
- No HNF1A or β-catenin mutations
- No expression of inflammatory proteins (SAA, CRP)
- Unclassifiable due to near-total necrosis or hemorrhage…
Molecular classification of HCA
Composite algorithm for diagnosis and treatment of FNH and HCAs

Suspicion of benign hepatocellular tumors
Liver tumor, normal tumor markers, absence of cirrhosis, no past history of cancer, no typical features of angioma at MRI

Contrast-enhanced MRI, CT scan or ultrasonography

---

Diagnosis of FNH at MRI
1. Isosignal or faint hyposignal in T1
2. Diffuse enhancement at the arterial phase
3. Central scar in hyper signal in T2 and enhancement during late phase

Suspicion of FNH
at imaging but absence of all typical criteria using at least two radiological exams

Tumor biopsy

Histological diagnosis and immunohistochemistry
- Normal like hepatocytes in 1 to 2 cell thick plates
- Fibrous central scar with dystrophic arterial vessels
- Map like pattern of glutamine synthase staining

Other liver tumors

Firm diagnosis of FNH

---

Suspicion of HCA
(absence of FNA criteria at imaging)

Female
Male

---

Suspicion of HCA
HNF1A mutated HCA (MRI features)
Inflammatory HCA (MRI features)
Undetermined HCA or doubt with HCC

Radiological diagnosis
No biopsy

< 5cm
Biopsy
> 5cm
Surgery

Histological diagnosis of HCA
IHC: LFABP1, CRP SAA, β-catenin, glutamine synthase
Search for β-catenin mutations

Other liver tumors

---

Loss of LFABP
HNF1A mutated HCA

50% of the β-catenin mutated HCA are also inflammatory

Overexpression of GS and β-catenin nuclear accumulation or β-catenin mutations

Other liver tumors

---

Stop OC
Clinical and contrast-enhanced MRI/ultrasonography or contrast enhanced ultrasonography
Follow up

Stop OC
Surgery

Stop OC

---
HCA versus FNH?

- Histologic findings + clinico-radiologic correlation

- Immunohistochemistry
  - Glutamine synthetase (GS): pattern
    - Map-like stain vs diffuse homogeneous stain vs neg.
  - Serum amyloid A (SAA)
    - Positive in most IHCA
    - Usually negative in FNH (positivity: 15~17%)
  - C-reactive protein (CRP)
    - Positive in IHCA (~100%), but low specificity (FNH: 78%)
  - Liver fatty acid binding protein (LFABP)
    - Loss in HNF1A-mutated HCA, high specificity

Joseph et al., 2014; Bellamy et al., 2013; Bioulac-Sage et al., 2012
# Summary of IHC markers in benign hepatocellular tumors

<table>
<thead>
<tr>
<th>Dx</th>
<th>GS</th>
<th>β-catenin</th>
<th>LFABP</th>
<th>SAA/CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNH</td>
<td>+ (map-like)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>H-HCA</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>B-HCA</td>
<td>+</td>
<td>+/-*</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IHCA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B-IHCA</td>
<td>+</td>
<td>+/-*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>UHCA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Non-tumoral liver</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Absence of β-catenin-labeled nuclei cannot be considered as a criterion to rule out b-HCA

*Bioulac-Sage et al. AJSP 2012 (modified)*
<table>
<thead>
<tr>
<th>Perivascular GS pattern</th>
<th>Patchy and perivascular GS pattern</th>
<th>Pseudomembrane GS pattern</th>
<th>H&amp;E with nodular architecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
<td>c</td>
<td>i</td>
</tr>
<tr>
<td>d</td>
<td>e</td>
<td>f</td>
<td>j</td>
</tr>
<tr>
<td>g</td>
<td>h</td>
<td>i</td>
<td>l</td>
</tr>
</tbody>
</table>

Joseph et al., 2014
Standard histology

HCA
- Certain
- Probable
- Doubtful

FNF
- Certain
- Probable
- Doubtful

IHC HCA subtyping

GS (pivotal role)

Normal
- LFABB T-/NT+
- CRP/SAA T+/NT-
- ALL-

H-HCA
IHCA
UHCA

Abnormal

Diffuse/heterogeneous (strong/moderate) (β-HCA)

β-cat.

β-IHCA
β-HCA

T+/NT-

β-IHCA
β-HCA

T-/NT-

β-IHCA
β-HCA

See Figure 1b

Stop*

Stop**

Stop

Stop

Stop#

Stop#

Bioulac-Sage, 2012; Balabaud, 2013
HCA versus well-differentiated HCC?

- **Histologic findings**
  - Cytologic / architectural atypia
  - Stromal invasion
    - Helpful (if present)
  - Reticulin loss:
    - HCA typically retains reticulin framework, but often difficult to interpret
    - Steatotic HCA and non-neoplastic fatty liver may show reticulin loss

- **Immunohistochemistry**
  - Glypican-3
  - Heat shock protein 70 (hsp70)
  - Glutamine synthetase
    - Helpful for HCC vs dysplastic nodule
    - Not helpful for HCC vs HCA
  - CD34: sinusoidal capillarization in HCC > HCA (spectrum)
Treatment of FNH and HCA

- **Focal nodular hyperplasia**
  - No treatment or follow-up necessary for definite FNH
  - Surgery considered for huge or symptomatic FNH

- **Hepatocellular adenoma**
  - Large tumors (>5cm): surgery
  - β-catenin mutation present: surgery (risk of malignant transformation)
Take home messages

- HCA should be differentiated from FNH
  - Risk of bleeding
  - Malignant transformation

- Immunohistochemistry is helpful in subclassifying HCA

- The risk of malignant transformation is the highest in β-catenin-mutated HCA