Pathological Analysis of Small Hepatocellular Carcinoma with Poor Prognosis

Haeryoung Kim, M.D., Ph.D.
Department of Pathology
Seoul National University Bundang Hospital
Small HCC

• Definition: HCC < 2cm
• Majority of small HCCs are composed of well-differentiated cancerous tissues
Gross classification of small HCC

- Vaguely nodular type
- Distinctly nodular type
Small HCC of vaguely nodular type

- Majority detected by US during periodic F/U study of high-risk population
  - Distinct hypoechoic or hyperechoic nodular lesion
- More frequent among smaller tumors
  - Occasionally >2.0cm, rarely > 3.0cm
- AFP and PIVKA-II not elevated
- Majority diagnosed only by US-guided fine-needle biopsy
Small HCC of vaguely nodular type

- Gross: indistinct margins
  - Sometimes difficult to distinguish from surrounding cirrhotic liver
  - Uniformly distributed WD tumor with replacing growth pattern at tumor boundary

- Preserved basic architecture of background liver
  - A few portal tracts retained within tumor
  - Deformed due to “stromal invasion”
  - Stromal invasion: most helpful clue to distinguish small WD HCC from high-grade DN
Small HCC of vaguely nodular type

• “nodule-in-nodule” appearance
  – Morphologic expression of dedifferentiation process in early-stage WD HCC

• Fatty change
  – 1.1~1.5cm: 42.4%

• Vascular invasion, intrahepatic micrometastasis: absent
  – “carcinoma in situ”, “early HCC”
F/51, HCV+

1.7cm vaguely nodular HCC
M/55, HBV+

1.8cm vaguely nodular HCC
Small HCC of distinctly nodular type

- Often diagnosed by various imaging modalities without biopsy

Already advanced cancer despite small size

<table>
<thead>
<tr>
<th></th>
<th>Vaguely nodular (n=37)</th>
<th>Distinctly nodular (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (mm)</td>
<td>11.9±3.3</td>
<td>16.0±3.3</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>93%</td>
<td>64%</td>
</tr>
<tr>
<td>Capsule</td>
<td>0%</td>
<td>53%</td>
</tr>
<tr>
<td>Well-differentiated</td>
<td>85%</td>
<td>15%</td>
</tr>
<tr>
<td>Moderately diff</td>
<td>0%</td>
<td>58%</td>
</tr>
<tr>
<td>Portal vein invasion</td>
<td>5%</td>
<td>27%</td>
</tr>
<tr>
<td>Intrahepatic mets</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>Angiography</td>
<td>Hypovascular</td>
<td>Hypervascular</td>
</tr>
</tbody>
</table>

Kojiro M. Liver Transpl 2004
Differences in clinical outcomes

Vaguely nodular small HCC

• Best prognosis
• Although recurrence due to multicentric occurrence of the tumor cannot be prevented, it is important to detect and treat while vaguely nodular because of the significantly higher 5-YSR with this type
Differences in clinical outcomes

Distinctly nodular small HCC

- ↑Size → ↑extranodular tumor growth & intrahepatic metastasis
  - <1.5cm: 5.3%
  - 1.6-2.0cm: 16.7%
  - 2.1-3.0cm: 23.6%

- Relatively high risk of local recurrence after local ablation therapies
Small HCC with aggressive behavior in SNUBH

• Small HCC without preoperative Tx, F/U data available
• Early recurrence (<2 years)
  – n=6 (vaguely nodular: 0)
• Late recurrence (>2 years)
  – n=2 (vaguely nodular:1)
Histological prognostic factors

• Edmondson’s grade
• Vascular invasion
• Intrahepatic metastasis
Histological prognostic factors

- Size (<2cm) & vascular invasion: independent predictors of survival in small HCC patients

*Kikuchi L et al. J Clin Gastroenterol 2009*

---

**TABLE 3. Univariate Analysis of Predictive Factors of Survival in 74 Cirrhotic Patients With Small HCC**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 58/ &gt; 58</td>
<td>36/38</td>
<td>0.74</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>53/21</td>
<td>0.41</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes/no</td>
<td>48/26</td>
<td>0.801</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes/no</td>
<td>16/58</td>
<td>0.50</td>
</tr>
<tr>
<td>Child-Pugh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B and C</td>
<td>45/29</td>
<td>0.007</td>
</tr>
<tr>
<td>MELD score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 11/ &gt; 11</td>
<td>42/32</td>
<td>0.016</td>
</tr>
<tr>
<td>Portal hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes/no</td>
<td>59/15</td>
<td>0.22</td>
</tr>
<tr>
<td>α-fetoprotein &gt; 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 100/ &gt; 100</td>
<td>53/21</td>
<td>0.006</td>
</tr>
<tr>
<td>Echogenicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoechogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes/no</td>
<td>47/27</td>
<td>0.417</td>
</tr>
<tr>
<td>HCC diameter (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 20/ &gt; 20</td>
<td>35/39</td>
<td>0.009</td>
</tr>
<tr>
<td>No. nodules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/ &gt; 1</td>
<td>53/21</td>
<td>0.041</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes/no</td>
<td>3/71</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

HCC indicates hepatocellular carcinoma; MELD, model for endstage liver disease.
Histological prognostic factors

• Predictors of survival after resection of early HCC (<5cm)
  – Size >2cm, multifocality, vascular invasion

Tumor differentiation
• Mitosis
• Multipolar mitosis:
  - indicator of chromosomal instability
  - high MM associated with poor prognosis
Histological prognostic factors

• Microvascular invasion
Infiltrating lymphocytes in HCC

• Prominent TIL in 11/163 (6.7%) previously untreated small HCC (<3cm)
• CD8+ cytotoxic lymphocytes: immunosuppressive effect on tumor cells?
• Low recurrence rate, increased survival rate

Wada Y et al. Hepatology 1998
M/59, HBV+

2cm distinctly nodular HCC

Current status: 14 months postop
No evidence of disease recurrence
Progenitor cell features in HCC

- Hepatic progenitor cells: ductules and canals of Hering
- 10-20% of HCC express CK19 → progenitor cell origin?
  - hepatocellular carcinoma with progenitor cell features
  - combined hepatocellular-cholangiocarcinoma
  - intermediate carcinoma
- Clinically similar to ordinary HCC
  - frequent association with cirrhosis
  - positive for viral markers
  - elevated AFP
- Poor prognosis, increased recurrence
- Resistance to chemotherapy and radiotherapy
Canals of Hering and ductules

CK19
Prognostic value of biliary marker expression in HCC

• Significantly shorter survival in CK19+ HCC patients without any treatment

• CK19 expression is an independent predictor of postop recurrence

• CK19+ HCCs showed higher recurrence after transplantation compared to CK19- HCCs
  - Durnez A et al. Histopathology 2006
Prognostic value of biliary marker expression in HCC

- Mucin-producing cancer cells in HCC indicate aggressive tumor behavior
  - 35/953 small HCC (<3cm) with CK7 expression
  - CK19+/mucin+ cases showed a poor prognosis

M/64, HCV+

2cm distinctly nodular HCC

1\textsuperscript{st} recurrence: 17 months postoperatively
2\textsuperscript{nd} recurrence: 33 months postoperatively
Currently: alive with disease
Hierarchical cluster analysis

• HB subtype of human HCC (n=14): coclustering with rat hepatoblasts
  → May arise from bipotential hepatic progenitor cells?
• Independently associated with worse overall survival and recurrence

Lee JS et al. Nat Med 2006
Hierarchical cluster analysis

A unique gene expression signature only detectable in subtype HB

- Markers of HPC (KRT7, KRT19, VIM) → **Derivation from HPC?**
- Many direct downstream targets of JUN and FOS are upregulated
  - **MMP1, PLAUR, TIMP1, CD44, VIL2 (EZRIN)**
  - **Invasion and metastasis**
- HPC proliferate and “invade” lobular parenchyma during hepatic injury
Other markers of progenitor cell differentiation

- c-kit (CD117)
- CD133 (prominin-1)
- EpCAM
- MDR1, MRP1, MRP3, BCRP
Non-neoplastic liver

- Histopathologic features of the adjacent non-neoplastic liver may also be related to patient outcome
- *De novo* carcinogenesis rather than local recurrence of primary tumor
Hepatitis activity and fibrosis

- Increase in hepatitis activity and fibrosis stage in HBV/HCV related chronic hepatitis are associated with increased recurrence

- Hepatitis activity was an independent predictor of late recurrence (>2 years) on multivariate analysis

*Imamura et al. J Hepatol 2003*
Hepatitis activity and fibrosis

• Underlying liver fibrosis
  – Associated with worse liver function
  – May indicate pathological and genetic changes throughout the liver leading to a field cancerization effect

  *multicentric hepatocarcinogenesis*

Fatty change in non-neoplastic liver

• Frequently seen in chronic hepatitis C
• Fatty change not only increases the risk of HCC development, but also is associated with a high post-operative recurrence rate

Takuma et al. Liver Int 2007
Liver cell dysplasia

- Microscopic lesions <1mm in diameter
- Do not form circumscribed nodules
- Often found in chronic liver disease

- Two types:
  - Large liver cell change (LLCC) / dysplasia
  - Small liver cell change (SLCC) / dysplasia
Morphological features

Large liver cell change (LLCC)
- Foci of cellular enlargement and nuclear pleomorphism, hyperchromasia, multinucleation
- Frequent in chronic viral hepatitis, cirrhosis
- Recognizable at low power magnification

Small liver cell change (SLCC)
- Foci of crowded small hepatocytes
- High nuclear/cytoplasmic ratio
Large liver cell change & small liver cell change

• There is a general consensus that SLCC is a preneoplastic lesion

• LLCC is still controversial, but there is evidence that LLCC in a background of chronic HBV-related liver disease is closely associated with HCC development and not simply an innocent bystander

  *Kim et al. Hepatology in press*

• “Large cell change”: pathogenetically non-committal term


- p21, p27, p16 checkpoints
- Telomere length
- Senescence associated-β-galactosidase activity
- p53 mutant protein
- γH2AX, micronuclei
- Net cellular gain

Cirrhosis  LLCC  SLCC  HCC
Predictive value of LLCC for HCC development in B-viral hepatitis B

- Chronic hepatitis B patients (n=181)
- F/U after gun biopsy: 115±48 months
- LLCC+ patients at initial biopsy
  - ↑ cumulative probability of HCC development compared to LLCC- patients (p=0.016)
  - approximately 3-fold risk of HCC development
- PPV: 15.9%, NPV: 94.9%

Predictive value of LLCC for HCC development in B-viral hepatitis B

Table 2. Clinicopathological characteristics in relation to the development of hepatocellular carcinoma (HCC)

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Patients with HCC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>mean±SD</td>
<td>n (%)</td>
</tr>
<tr>
<td>Total</td>
<td>181</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 ± 10</td>
<td></td>
<td>45 ± 7</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>148</td>
<td>(81.8)</td>
<td>18</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>(18.2)</td>
<td>0</td>
</tr>
<tr>
<td>LLCD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>82</td>
<td>(45.3)</td>
<td>13</td>
</tr>
<tr>
<td>Absent</td>
<td>99</td>
<td>(54.7)</td>
<td>5</td>
</tr>
<tr>
<td>SLCD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>17</td>
<td>(9.4)</td>
<td>3</td>
</tr>
<tr>
<td>Absent</td>
<td>164</td>
<td>(90.6)</td>
<td>15</td>
</tr>
<tr>
<td>Lobular activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>(5.5)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>(43.6)</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>(44.8)</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>(6.1)</td>
<td>0</td>
</tr>
<tr>
<td>Portoperportal activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>(2.2)</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>(3.3)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>(11.6)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>(22.7)</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>109</td>
<td>(60.2)</td>
<td>14</td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No fibrosis</td>
<td>2</td>
<td>(1.1)</td>
<td>0</td>
</tr>
<tr>
<td>Stage1</td>
<td>25</td>
<td>(13.8)</td>
<td>0</td>
</tr>
<tr>
<td>Stage2</td>
<td>103</td>
<td>(56.9)</td>
<td>10</td>
</tr>
<tr>
<td>Stage3</td>
<td>42</td>
<td>(23.2)</td>
<td>7</td>
</tr>
<tr>
<td>Stage4</td>
<td>9</td>
<td>(5.0)</td>
<td>1</td>
</tr>
</tbody>
</table>

ns: non significant, p>0.05.
Large liver cell change & small liver cell change

- The presence of LLCC and SLCC in the non-neoplastic liver should be noted for further follow up
Molecular prognostic markers

• Ancillary techniques, such as immunohistochemistry, could be performed for further prediction of tumor behavior
• The recent progress in “high-throughput” technology, including DNA microarrays, has lead to the sudden increase in novel prognostic molecular markers
Proliferation index & cell cycle checkpoint proteins

- **Ki-67, PCNA** (proliferating cell nuclear antigen)
  - Increased proliferation index is associated with poor prognosis

- **Cell cycle regulator proteins**
  - p27/Kip1, p57/Kip2: favorable prognosis
  - cyclin A1, cyclin D1 overexpression: associated with poor prognosis
Tumor suppressor gene, p53

• p53 mutation/Tp53 overexpression
  – Poor differentiation
  – High proliferative activity
  – High recurrence rate

– 5 small HCC with Tp53 overexpression (SNUBH)
  • 1 case: LN, omental metastasis, stomach invasion at 4mo
  • 2 cases: local recurrence at 9mo/17mo
  • 2 cases: vascular invasion (+)
Epithelial-mesenchymal transition

- Important step for tumor cell invasion
- Loss of polarity, loss of intercellular adhesion and modification in cytoskeletal structure
- Loss of E-cadherin expression is associated with invasive features, early recurrence and metastasis of HCC
- Other markers: vimentin, beta-catenin, osteopontin, CD44v6
Telomerase activity

- Cancer cell requires telomerase reactivation to maintain telomere length
- **High telomerase activity and increased/maintained telomere length** were associated with poor overall survival in 49 HBV-related HCCs
- High telomerase activity: ↑ stage, mitotic rate, chromosomal instability

*Oh & Kim et al. Lab Invest 2008*
Telomerase activity

- hTERT levels reflect telomerase activity
- HCC with hTERT expression are frequently poorly differentiated
- Potential prognostic marker
Glypican-3, Glutamine synthetase, Heat shock protein 70

• Increasingly used in pathological practice to differentiate between HGDN and WD HCC
• Expression of these markers has also been associated with poor prognosis
  – ↓ survival rate, ↑ recurrence rate
Prognostic value of glypican-3

CONCLUSION

• The gross classification of small HCCs into vaguely nodular and distinctly nodular types has prognostic importance.

• Extranodular growth, vascular invasion and intrahepatic metastasis can occur in distinctly nodular HCC, even if <2cm.

• “Aggressive” histopathological features of the tumor are frequently associated with early recurrence, whereas non-tumor liver pathology probably affects late recurrence.
THANK YOU FOR YOUR ATTENTION!