A gene expression signature of microvascular invasion in hepatocellular carcinoma in formalin fixed-paraffin embedded biopsies

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Vascular invasion in HCC

• **Vascular invasion**: Major prognostic factor associated with mortality and tumor recurrence
  • Macroscopic or microscopic (mVI)

• **Definition of mVI**: presence of clusters of tumor cells in vessels located in the tumor capsule and/or in the surrounding liver parenchyma

• Presence (or absence) of mVI = **not assessable before surgery**
  - Only detectable by microscopic examination of the surgical specimen
  - Main limit for optimal management of patients

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Biomarkers of mVI

• Several gene expression signatures of mVI, obtained from surgical samples of HCC described in the literature
  none of these signatures have been validated so far in formalin-fixed and paraffin-embedded (FFPE) HCC biopsies


• Several proteins biomarkers of mVI also published
  Ex: PIVKA-II, H4K20, H4K16


  Limited performance (Identification of 40% of HCC with mVI in FFPE biopsies (Se: 43%, Sp:95%)

  35-gene signature, AUC: 0.60 (Se=0.70, Sp=0.51)
Nanostring Technology

• Transcriptomic technology based on direct measurement of transcript abundance (maximum 800 different transcripts), by using multiplexed, color-coded probe pairs

  No step of reverse transcription of mRNA or cDNA amplification

• More sensitive than microArray analysis → good relevance in FFPE tissue specimens

• Requirement of a small amount of RNA → good relevance in biopsy sample

• Validated in breast cancer and in mantle cell lymphoma in FFPE biopsies

Aim of the study

Define a gene expression signature associated with mVI in HCC, applicable on FFPE biopsies using a Nanostring approach.
Study design

Test series (N=69)

HCC Surgical specimens (N=39)  HCC Biopsies (N=30)

Definition of the mVI signature

VALIDATION series
HCC Biopsies (N=39)

Validation of the mVI signature
Methods

• **Constitution of two series** from archived FFPE tissue samples of HCC (Department of Pathology, Beaujon Hospital, 1994-2017):

  For all FFPE biopsies of HCC included, the corresponding surgical specimen was available

• **Clinicopathological data** were collected for each case.

• **Pathological review**: Definition of two groups of tumors according to the presence (mVI+) or the absence (mVI-) of mVI on the surgical specimen.

• **Nanostring technology**: use of signature of 200 genes established with literature and RNA sequencing data.
Results
<table>
<thead>
<tr>
<th></th>
<th>Test series (N=69) (%)</th>
<th>Validation series (N=39)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Age (mean)</strong></td>
<td>61</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td><strong>Macroscopic examination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 nodule</td>
<td>58 (84)</td>
<td>36 (92)</td>
<td>0.22</td>
</tr>
<tr>
<td>Size (mean, cm)</td>
<td>6.4</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Satellite nodule</td>
<td>18 (26)</td>
<td>11 (28)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Microscopic examination</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>26 (38)</td>
<td>8 (21)</td>
<td>0.065</td>
</tr>
<tr>
<td>Medium</td>
<td>37 (54)</td>
<td>27 (69)</td>
<td>0.11</td>
</tr>
<tr>
<td>Weak</td>
<td>6 (9)</td>
<td>4 (10)</td>
<td>0.79</td>
</tr>
<tr>
<td>mVI</td>
<td>36 (52)</td>
<td>26 (67)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Etiologies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VHC</td>
<td>20 (29)</td>
<td>8 (21)</td>
<td>0.33</td>
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<tr>
<td>NASH</td>
<td>16 (23)</td>
<td>11 (28)</td>
<td>0.56</td>
</tr>
<tr>
<td>Alcohol</td>
<td>10 (14)</td>
<td>2 (5)</td>
<td>0.14</td>
</tr>
<tr>
<td>VHB</td>
<td>14 (20)</td>
<td>8 (21)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Fibrosis in non tumoral liver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>10 (14)</td>
<td>7 (18)</td>
<td>0.64</td>
</tr>
<tr>
<td>F1</td>
<td>8 (12)</td>
<td>4 (10)</td>
<td>0.63</td>
</tr>
<tr>
<td>F2</td>
<td>11 (16)</td>
<td>4 (10)</td>
<td>0.41</td>
</tr>
<tr>
<td>F3</td>
<td>15 (22)</td>
<td>10 (26)</td>
<td>0.64</td>
</tr>
<tr>
<td>F4</td>
<td>25 (36)</td>
<td>14 (36)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Similar clinico-pathological data between the two cohorts
Excellent correlation between HCC biopsies and corresponding surgical samples

Mean correlation index: 0.97 [0.87-0.95]
Definition of a mVI transcriptomic signature: test series

Unsupervised analysis (200 gene signature)

Differential gene expression analysis
39 differentially expressed genes
(logFC ≥ |0.5| & Adj Pvalue < 0.1)

Up-regulated

Down-regulated
Definition of a mVI transcriptomic signature

10-gene signature

<table>
<thead>
<tr>
<th>Gene</th>
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<tbody>
<tr>
<td>PIR</td>
</tr>
<tr>
<td>BUB1</td>
</tr>
<tr>
<td>TAF9</td>
</tr>
<tr>
<td>NRCAM</td>
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<td>UGT2B7</td>
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<tr>
<td>NARF</td>
</tr>
<tr>
<td>TM6SF2</td>
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<tr>
<td>FGFR4</td>
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<tr>
<td>PGLS</td>
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<tr>
<td>PGC</td>
</tr>
</tbody>
</table>

Prediction algorithm: k Nearest Neighbor analysis
Performance of 10-gene mVI signature: validation series

- Sensitivity: 0.92
- Specificity: 0.62
- Accuracy: 0.82
Discussion

• Excellent transcriptomic correlation between biopsies and corresponding surgical specimens
• Successful transcriptomic analysis on FFPE samples and particularly on biopsies

Transcriptomic analysis is achievable on HCC routine biopsies

• **Good performance of the mVI signature** obtained compared to the literature
  
  Se: 92%, Sp: 62 % vs
  
  Se: 70%, Sp: 51% (Mínguez et al, J Hepatol. 2011;55(6):1325-31)

• Signature limited to 10 genes → best relevance for a routine use
Conclusion

Resectable HCC

Biopsy

Transcriptomic mVI signature

HCC without mVI

Surgical resection

Adaptation of therapeutic strategy

HCC with mVI

TACE before surgery