Prognostic value of desmoplastic stroma in intra-hepatic cholangiocarcinomas

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Introduction: intra-hepatic cholangiocarcinoma (ICC)

- Rare tumors
  - 3% of cancers of the digestive tract

- Aggressive tumors
  - Diagnosis at a late stage
  - High metastatic potential
  - 5 year Overall survival
    - 5–10%

- Curative treatment
  - Surgery
    - Relapse+++  
  - Low efficiency of chemotherapy

- Identify new therapeutic targets
Introduction : tumor stroma

- ICC are characterized by the presence of a desmoplastic stroma
- Role in initiation and tumor progression
- Potential therapeutic target
  - Anti-angiogenic
  - Immunotherapy
  - Hyaluronidase

- Role of stroma in ICC still questioned
The aim of the present study was to characterize the stromal compartment in ICC through a multiparametric morphological analysis.

In order to identify new therapeutic targets.
Materials and Methods: patients and samples

- 49 patients with ICC surgically resected
- Representative tumor paraffin block for each case was selected
  - Sirius red staining
  - Immunohistochemistry
    - Alpha smooth muscle actin (cancer associated fibroblasts)
    - CD8, CD68, CD163 (immune markers)
    - CD31 (microvessel density)
- Tissue microarray (n=26)
  - Second harmonic generation and two photon excitation fluorescence microscopy (HistolIndex®)
    - Qualitative aspect of collagen fiber
    - 16 parameters
      - CRI: collagen reticulation index
Each sirius red slide was scanned and the proportionated stromal area (PSA) was automatically quantified.

Each immunohistochemistry slide was scanned and automatically quantified using a specific software.
For all cases, we evaluated ASI by calculating the ratio between the area of smooth muscle actin positive cells and the area of collagen stained by sirius red staining.
# Results: patients

<table>
<thead>
<tr>
<th>variable</th>
<th>Value (%)</th>
<th>n=49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (45%)</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>27 (55%)</td>
<td></td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>20 (50%)</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>37 (79%)</td>
<td></td>
</tr>
<tr>
<td>Positive lymph node</td>
<td>13 (35%)</td>
<td></td>
</tr>
<tr>
<td>R0 resection</td>
<td>12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Differentiation grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>18 (37%)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>23 (47%)</td>
<td></td>
</tr>
<tr>
<td>poor</td>
<td>8 (16%)</td>
<td></td>
</tr>
<tr>
<td>Satellite nodules</td>
<td>26 (53%)</td>
<td></td>
</tr>
<tr>
<td>Size &gt; 80mm</td>
<td>26 (53%)</td>
<td></td>
</tr>
</tbody>
</table>
Results: stroma

- **Proportionated stroma area (PSA)**
  - Mean: 44% [10–78]
  - Median: 44.64%

<table>
<thead>
<tr>
<th></th>
<th>Low PSA n=24, (%)</th>
<th>High PSA n=25, (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular invasion</td>
<td>22 (95.7)</td>
<td>15 (62.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>Grade differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (12.5)</td>
<td>15 (60)</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>17 (16.7)</td>
<td>6 (24)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (16)</td>
<td>4 (16)</td>
<td></td>
</tr>
</tbody>
</table>

- **Activated Stroma Index (ASI)**
  - Mean: 17.8 [2–58]
  - Median: 14.12

- **Collagen Reticulation Index (CRI)**
  - Mean: 2.73 [2.35–3.10]
  - Median: 2.76
Results: Disease Free Survival (PSA)

- High
  PSA > median
- Low
  PSA < median
- \( p = 0.077 \)
Results: Disease Free Survival (ASI)

- High ASI
- Low ASI
- $P=0.05$
Results: Overall Survival (CRI)

- High CRI
- Low CRI
- $P=0.026$
Conclusions

- Desmoplastic stroma features in ICC (poorly cellular and poorly reticulated) are correlated with a better phenotype
- Challenge the Interest to target stroma in ICC
  - Supported by data
    - Pancreatic cancer: desmoplasia was also demonstrated to be a protective factor
    - Murine model of melanoma: inducing inhibition of desmoplastic response led to an increase of spontaneous metastasis

- Potential effective drugs for modulating reticulation properties
  - Target Lysil Oxidase: enzyme which regulates collagen reticulation