Phenotypical characterization of intrahepatic cholangiocarcinomas stroma: the more fibrous, the less pejorative.
INSERM UMR1149 Beaujon Hospital, Clichy, France

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is a rare liver tumor showing a growing incidence (2). Its prognosis is poor due to its late diagnosis and the lack of therapeutic options available. The 5 years survival rate does not exceed 10%. The surgery which is the only curative option, is feasible in 20% of the cases (3). Morphologically, ICC is characterized by an abundant fibrous stroma, composed of extracellular matrix (ECM) proteins and different types of cells: myofibroblasts, endothelial and immune cells.

The cancer-associated fibroblasts (CAFs) are mostly derived from activated hepatic stellate cells, and produce ECM components. These cells interact with tumoral cells through many paracrine ways. Stroma is becoming a major focus in cancer studies. Its prognostic value is still low and can be considered either as a defense from the host (4) or a tumoral supportive tissue (5). As stroma is considered as a niche contributing to tumoral progression, targeting it is an attractive therapeutic approach (6).

AIM

We aim to characterize stromal pattern of a set of ICC with complementary approaches in order to assess its prognostic value, and define new potential therapeutic targets.

MATERIAL & METHODS

Fifty cases of ICC, free of neo-adjuvant therapy, resected in Beaujon Hospital between April 2002 and September 2013, were studied. Immunohistochemistry (IHC) was performed on representative tissue sections from formalin fixed, and paraffin embedded (PPE) samples, using anti-pan-cytokeratin, c-SMA, α-SMA, αSMA, antibodies, and Microphthalmic staining for stroma quantification. All slides were scanned and automatically quantified using dedicated algorithms (Aperio® software).

To better characterize the collagen component, 26 cases were studied using multiphotonic microscopy (Histofinder® analytical software). Assessment of quantity and quality of collagen was obtained by existing tissue at 650m wavelength. This analysis was made on tissue microarray (TMA) from PPE samples, built for the purpose of the study, by selecting 5 to 7 spots / tumor (Fig. 2). A total of 326 tumoral spots were analyzed, as well as 3 non tumoral spots used for controls. Statistical analysis was done on Excel®, Minitab® and Prism®.

RESULTS

Fibrosis amount

Surface of collagen in tumor samples was quantified by 2 morphometric approaches: picorivirus staining [%] and multiphotonic microscopy (MMP) [COLlagen Area Ratio]. Results were similar with the 2 methods. Median collagen value was 40.6% (9.7-76.6%) and 32.4% (6.8-93.1%) for P5 staining and MMP, respectively.

Fibrosis (PP) staining assessment: Percentage of surface of collagen

Fibrosis Cellular composition

Myofibroblastic cells produce ECM components including collagen. They are characterized by cytoplasmic α-EMA marker (Fig. 6). In the overall series, amount of c-SMA-positive cells was not correlated with fibrosis median value 6.7% (range 1.2-18.8%), whatever the quantitative approach used (CAR, Fig. 7).

Fibrosis 3D organization

Reticulation of collagen was evaluated on TMA samples by scoring number of branchpoints in collagen fibers skeleton (Histofinder analysis). Our data showed that the Collagen Reticulation Index (CRI) median value was 2.78 (2.41-3.28). The CRI was not correlated with the amount of fibrosis (Fig. 8), nor myofibroblastic cells (Fig. 9). No correlation was observed with survival nor recurrence (Fig. 10).

Fibrosis on TMA

Table 1: Clinical and pathological data of the 50 cases of ICC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62.88</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td>T classification</td>
<td>TX</td>
</tr>
<tr>
<td>T stage</td>
<td>TX</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Histological grade</td>
<td>2 (15)</td>
</tr>
<tr>
<td>Histological grade</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Histological grade</td>
<td>4 (5)</td>
</tr>
<tr>
<td>TNM</td>
<td>TX/TC</td>
</tr>
<tr>
<td>Lymph vessels invasion</td>
<td>No</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>No</td>
</tr>
<tr>
<td>Nodules</td>
<td>No</td>
</tr>
<tr>
<td>MVD</td>
<td>3.57</td>
</tr>
<tr>
<td>Median tumor size [mm] [range]</td>
<td>93.96 (25.11-203.98)</td>
</tr>
</tbody>
</table>

Fibrosis: A prognostic factor

Survival data demonstrated that patients with more fibrous tumors (rich in basement membrane) have longer time to recurrence compared to patients with less fibrous tumors (intermediate and poor) assessed by picorivirus staining. (Fig. 5).

Figure 5: Time to Recurrence (TTR) according to (A) a set of 50 cases, p<0.04 and (B) clustering analysis, data of 25 cases, p<0.015.

DISCUSSION & CONCLUSION

Based on a series of 50 ICC, this morphological study shows that extent of tumor stroma is not associated with amount of myofibroblastic cells, and, compared to other malignancies, is associated with a better prognosis. These results support the hypothesis that stroma may be considered as a barrier against tumoral spread and suggest that targeting myofibroblasts would not represent a relevant therapeutic strategy.

Quantitative and qualitative analysis of tumor stroma may be accurately performed on FFPE tissues, including TMA allowing the study of a large number of cases. Picorivirus staining and multiphoton microscopy are reliable procedures and provide concordant results.

Although collagen 3D organization could have been an obstacle to vascularization and drugs delivery, collagen reticulation was not a prognostic factor in this series.

ACKNOWLEDGEMENTS

Thanks to S. Leu for the multiphotonic microscopy techniques. Special thanks to technicians from the pathology department, Beaujon Hospital and Team 9 INSERM UMR1149 Beaujon Hospital, Clichy.

REFERENCES


DISCLOSURES

The authors have no disclosures to report.