Molecular characterization of NAFLD-related liver cancer in pig using MALDI imaging mass spectrometry and shotgun proteomics

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Overview
NAFLD (nonalcoholic fatty liver disease), Liver cancer, MALDI-IMS, Shotgun proteomics

Introduction
• NAFLD-related liver cancer is increasing worldwide.
• Pathological mechanism regarding NAFLD-related liver cancer remains unclear.
• A useful biomarker for diagnosis of NAFLD-related liver cancer has been expected.

Aim
• To establish a pig model which develops NAFLD-related liver cancer
• To elucidate an on-tissue-based biomarker for NAFLD-related liver cancer

Methods (model establishment)

Animal: A 3 months-old male Microminipig (BW: 4kg) was purchased from Fujio Inc. (Shizuoka, Japan).

Methods (MS data acquisition)
MALDI imaging: The MALDI measurement were carried out on a rapifleX (Bruker) and data analysis was performed using SCILS Lab 2019 software. MALDI measurements were done in a positive mode using α-cyano-4-hydroxycinnamic acid as a matrix with a mass range of 800-4000 Da. The lateral resolution for the MALDI imaging was set to 50 μm.

Shotgun proteomics: Shotgun proteomics from serial sections of MALDI-IMS with 10 μm thickness were carried out using timsTOF Pro (Bruker) with nanoElute system.

Results
Multiple liver tumors with NAFLD were observed at 60 weeks.

Fig. 1 (a) a macroscopic image of the liver tumor (bi)(c) HE stainings of the liver; a capsulated tumor with cancer cells that show similar characteristics to human well-differentiated liver cancer (d) Immunostainings of the liver (left) glutamine synthetase, (right) heat shock protein 70

Fig. 2 (red): a crack in a tissue slice. (orange and yellow): normal parenchyma. HE stainings failed to discriminate the 2 segments. (yellow-green): a region with unknown significance (blue-green): cancer (marginal region), (blue): cancer (central region).

Table 1 Number of proteins identified with timsTOF LC-MS/MS

<table>
<thead>
<tr>
<th>Protein</th>
<th>MS/MS spectra</th>
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<tbody>
<tr>
<td>Protein 1</td>
<td>150</td>
</tr>
<tr>
<td>Protein 2</td>
<td>130</td>
</tr>
<tr>
<td>Protein 3</td>
<td>74</td>
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</tbody>
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Conclusions
• Protemal MALDI imaging succeeded in classifying normal and diseased livers.
• It also reflected intratumoral heterogeneity and structures which could not be classified on HE stainings.