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

Okohta Iguchi1,2, Mayuka Kosugi2, Naohiko Nakamura2, Takashi Nirasawa4, Ryo Kajita4, Etsuro Hatano2, Shugo Ueda1, Hiroaki Terajima1, Shinji Uemoto2, Masaya Ikegawa3

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

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

Methods (model establishment)
Animal: A 3 months-old male Micmipig (BW: 4kg) was purchased from Fujimura Inc. (Shizuoka, Japan).

Diets: (a) An originally modified high-fat diet (D13091201) was purchased from Research Diets Inc. (NJ, USA).
Liver biopsy: Under general anesthesia, open liver biopsy was performed before (0 week) and 60 weeks after the experiment.

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 o-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.

Conclusions
- Proteomic MALDI imaging succeeded in classifying normal and diseased livers.
- It also reflected intratumoral heterogeneity and structures which could not be classified on HE stainings.

Hierarchical clustering analysis discriminated and visualized 5 regions on MALDI-IMS.

Shotgun Proteomics

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).

Fig. 3. Workflow of MALDI-IMS and Shotgun Proteomics

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

<table>
<thead>
<tr>
<th>Control</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>152</td>
</tr>
<tr>
<td>91</td>
<td>74</td>
</tr>
<tr>
<td>60w</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. (a) A macroscopic image of the liver tumor (b)(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 synthase, (right) heat shock protein 70