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

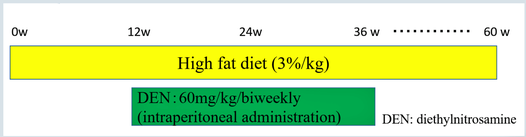
- NAFLD-related liver cancer is increasing worldwide.
- Pathological mechanism regarding NAFLD-related liver cancer remains unclear.
- A useful biomarker for diagnosis of NAFLD-related liver cancer has been expected.

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 establshment)

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



BW: 4kg, 0 week

Diet; 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.

BW: 40kg, 60 weeks

Nakamura et al., *BMC Cancer*, 2019

Results

Multiple liver tumors with NAFLD were observed at 60 weeks.

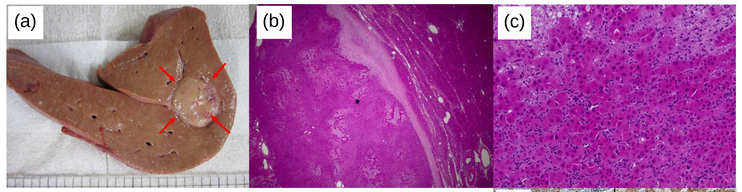


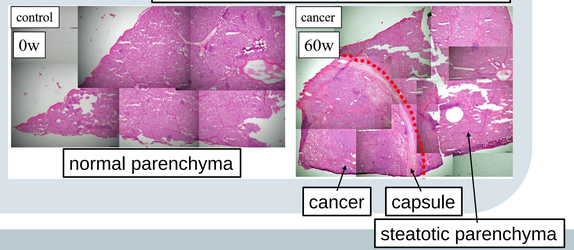
Fig.1 (a) a macroscopic image of the liver tumor (b)(c) HE stainings of the liver; a capsulated tumor with cancer cells that show similar characteristics to human well-differentiated liver cancer (d) Immunostainings of the liver ((left) glutamine synthetase, (right) heat shock protein 70

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

cf. HE stainings after IMS data acquisition



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

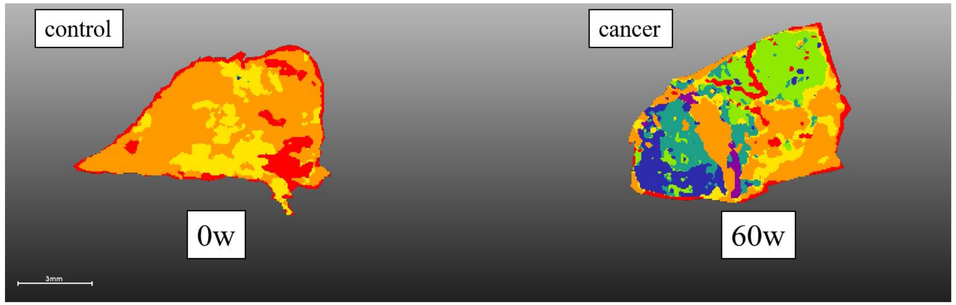
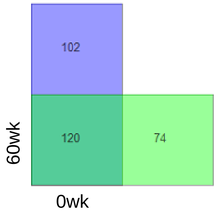


Fig.2 (red); a crack in a tissue slice. (orange and yellow); normal parenchyma. HE stainings failed to discriminate the 2 segments. (yellow-green); a region with unknown significance (blue-green); cancer (marginal region). (blue); cancer (central region).

Shotgun Proteomics

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



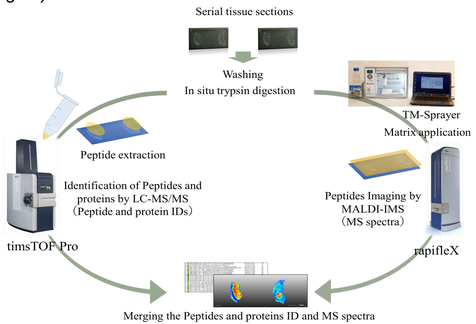


Fig.3 Workflow of MALDI-IMS and Shotgun Proteomics

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.

Imaging MS
Disease markers