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 Micro pig (BW: 4kg) was purchased from Fuji Mira Inc. (Shizuoka, Japan).

High fat diet (7%) (MEN: 36g/kg directly intermixed administration)

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.

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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

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).

Methods (MS data acquisition)
MALDI imaging: The MALDI measurement were carried out on a rapifleX (Bruker) and data analysis was performed using Scilab 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 timeTOF Pro (Bruker) with nanoElute system.

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