

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

ASMS 2020, TP214

OKohta Iguchi^{1,2}, Mayuka Kosugi³, Naohiko Nakamura², Takashi Nirasawa⁴, Ryo Kajita⁴, Etsuro Hatano², Shugo Ueda¹, Hiroaki Terajima¹, Shinji Uemoto², Masaya Ikegawa³

1. Department of Gastroenterological Surgery and Oncology, Tazuke Kofukai Medical Research Institute, Kitano Hospital, Osaka, Japan
2. Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
3. Department of Life and Medical Systems, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan
4. Bruker Japan K.K., Yokohama, Kanagawa, Japan

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

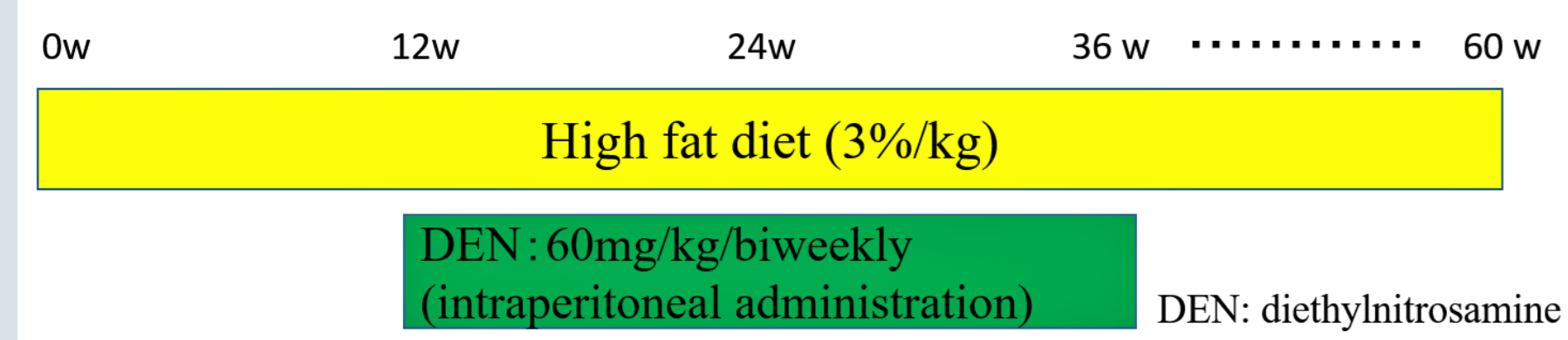
- To establish a pig model which develops NAFLD-related liver cancer
- To elucidate an on tissue-based biomarker for NAFLD-related liver cancer

Methods (model establishment)

Animal: A 3 months-old male Microminipig (BW: 4kg) was purchased from 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.

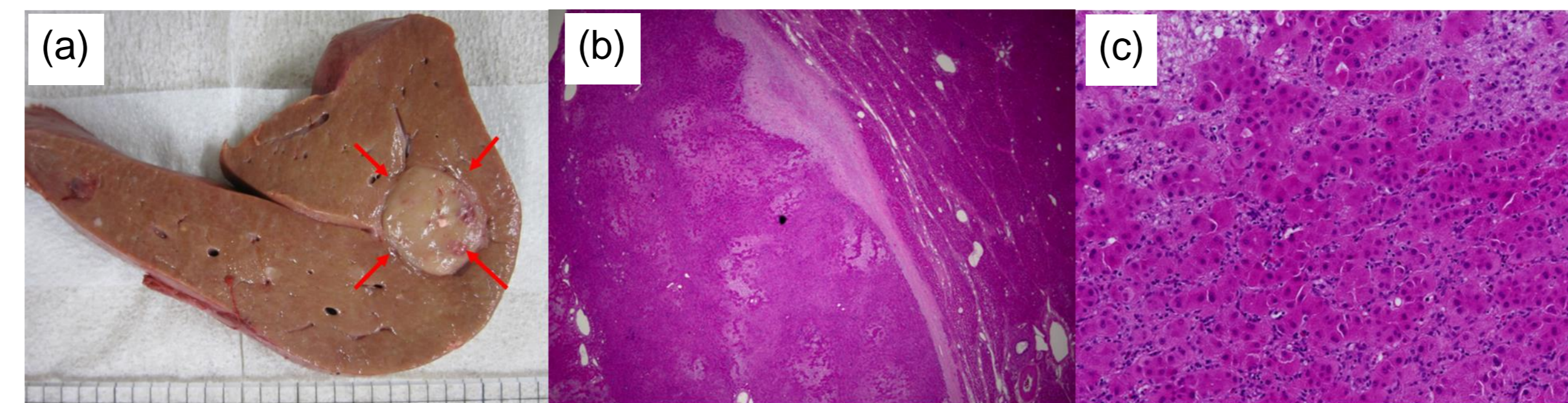
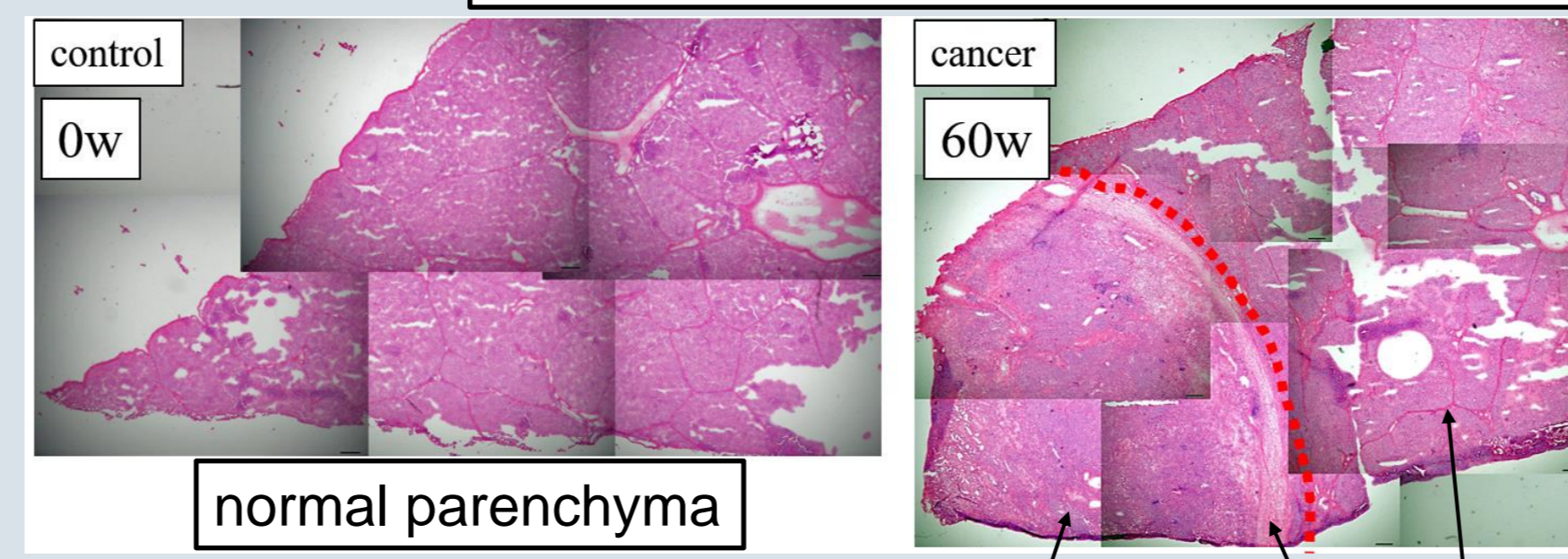


Fig.1 (a) a macroscopic image of the liver tumor (b)(c) HE stainings of the liver; a capsulated tumor with cancer cells that show similar characteristics to human well-differentiated liver cancer (d) Immunostainings of the liver ((left) glutamine synthetase, (right) heat shock protein 70

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 μ m.

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



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

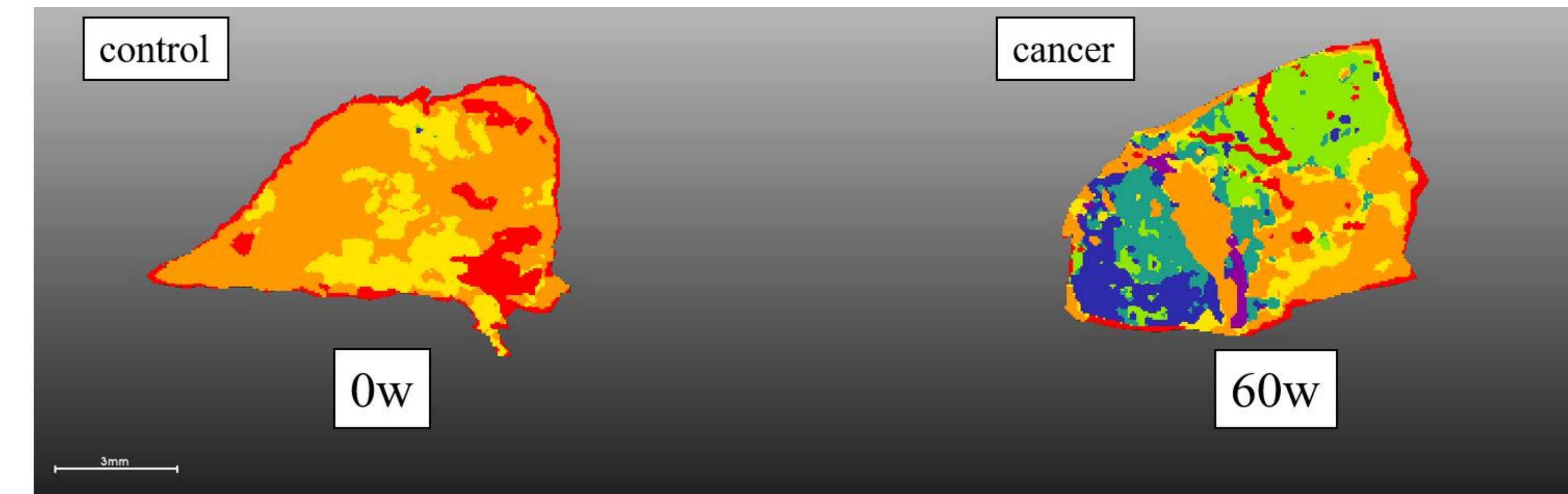


Fig.2 (red); a crack in a tissue slice. (orange and yellow); normal parenchyma. HE stainings failed to discriminate the 2 segments. (yellow-green); a region with unknown significance (blue-green); cancer (marginal region). (blue); cancer (central region).

Shotgun Proteomics

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

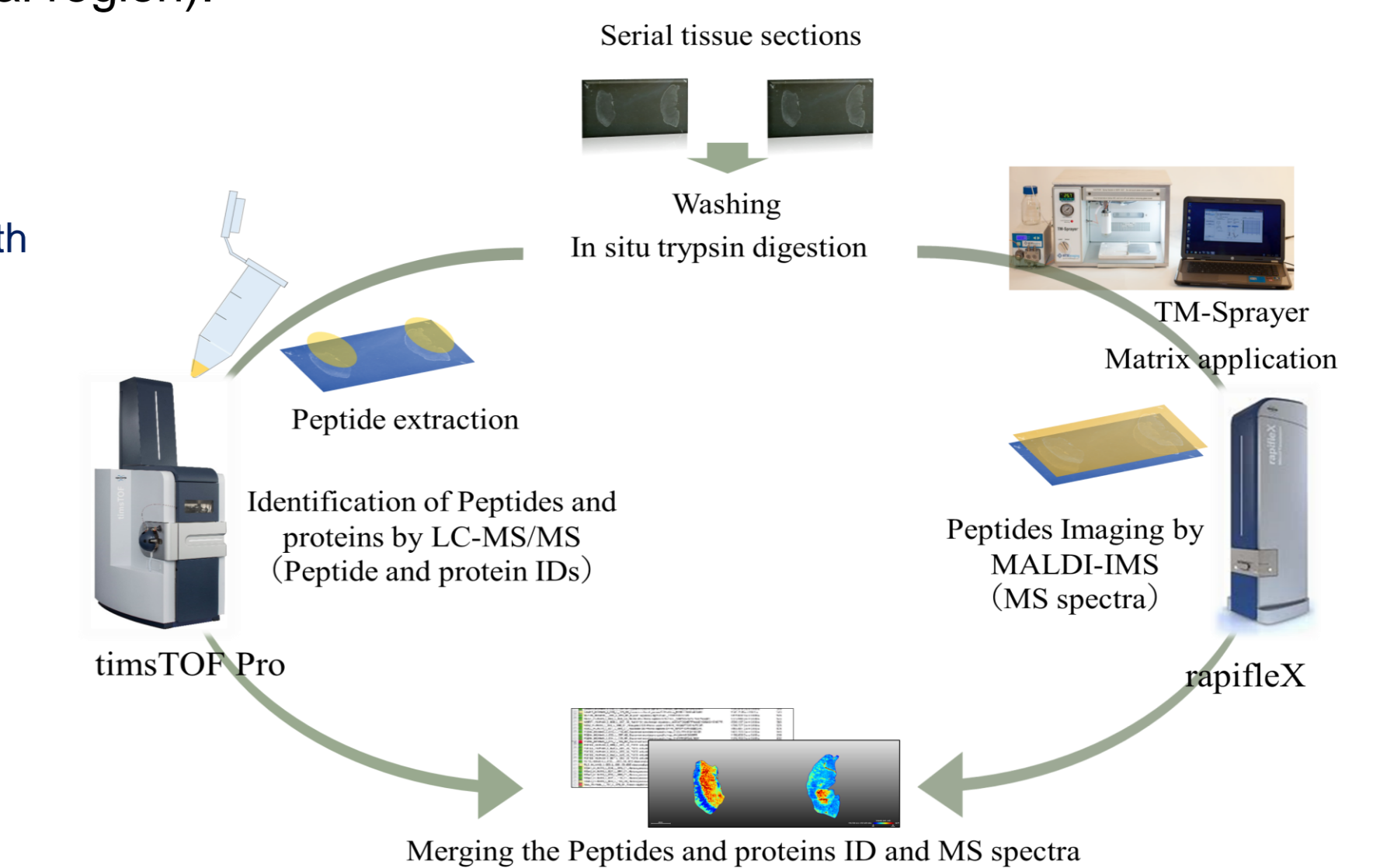
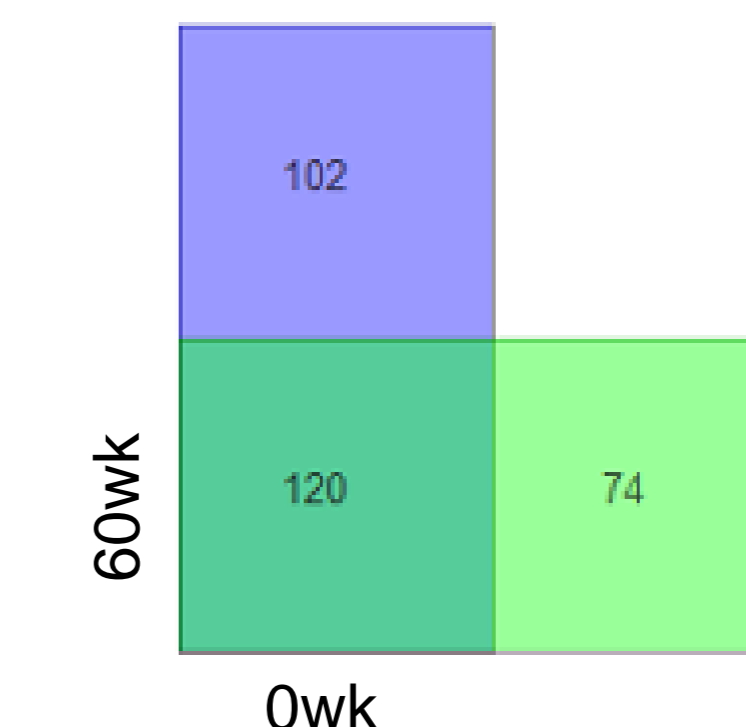


Fig.3 Workflow of MALDI-IMS and Shotgun Proteomics

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