Unraveling pathogenesis of dilated cardiomyopathy (DCM) on J2N-k Hamster model Using MALDI-Imaging Mass Spectrometry in combination with shotgun proteomics

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Introduction

Dilated Cardiomyopathy (DCM), a group of disorders characterized by cardiac dilation and reduced left ventricular ejection fraction, has an extremely poor prognosis. To investigate the pathogenesis of DCM, we performed global proteomic analysis of myocardial tissues from J2N-k cardiomyopathic hamsters. This model exhibits symptoms similar to those of human DCM, owing to the deletion of the δ-sarcoglycan gene. J2N-n hamsters are also available as a non - cardiomyopathic control. In this study, we tried in situ proteomics for cardiac tissues from hamsters by integrating Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) and Trapped ion mobility spectrometry (TIMS), which implements online parallel accumulation-serial fragmentation (PASEF).

Methods

◎**Animals;** J2N-k and J2N-n hamsters at 4- to 13-weeks of age.
◎**Sample preparation;** 10 μm frozen sections of hearts were cut on a cryostat and transferred to conductive Indium-Tin-Oxide (ITO) coated glass slides.
◎**MALDI-IMS (no digestion);** Sinapic Acid (SA) 10 mg/ml in 50% Acetonitrile was uniformly deposited on the slide by using TM-Sprayer (HTX Imaging). Then extracted peptides and proteins are measured by using rapifleX (Bruker Daltonik GmbH) with a spatial resolution of 50 μm. Lons were detected in mass range of m/z 2,000-20,000.
◎**MALDI-IMS with shotgun proteomics (Fig. 1);** α-cyano-4-hydroxycinnamic acid (CHCA) 10 mg/ml in 70% Acetonitrile was uniformly deposited on the slide by using TM-Sprayer. Then extracted peptides and proteins are measured by using rapifleX with a spatial resolution of 50 μm. Lons were detected in mass range of m/z 800-4,000. On-tissue digestion with trypsin was performed with TM-Sprayer. By using tims TOF Pro with nanoElute (Bruker Daltonik GmbH), shotgun proteomics was performed with the same tissue sample as well as laser micro-dissected samples. Column used was 25 cm × 75 μm, C18 column.
◎**Data Analysis;** Obtained mass spectra as well as annotated proteins and peptides were visualized with flexAnalysis, flexImaging5.0 and SCiLS Lab 2018b/2019b software (Bruker Daltonik GmbH). About 2,000 proteins were successfully annotated with ProteinScape4.0 (Bruker Daltonik GmbH), and database was Swiss-prot.

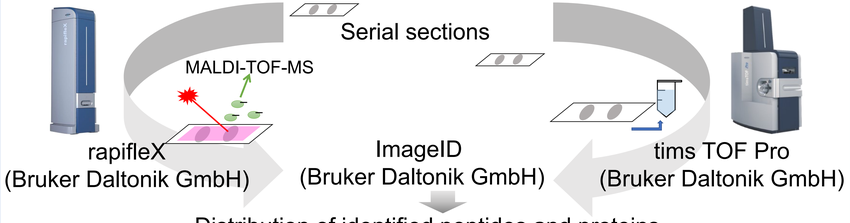

Fig. 1 Workflow image of combined analytical method

Results

①The Analysis of native proteins by MALDI-IMS (no digestion)

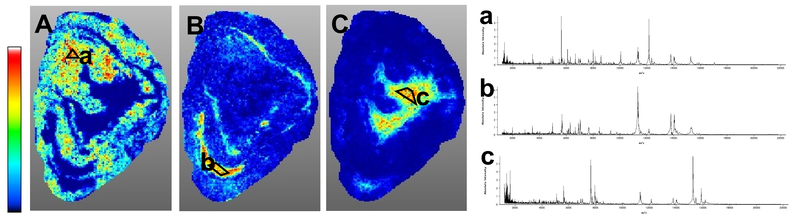

Fig. 2 Typical images of 8-week-old J2N-k by pLSA.
A; Non pathological myocyte area, B; Inflammatory region, C; Heart chamber
a – c; Mean spectrum of ROI in A-C

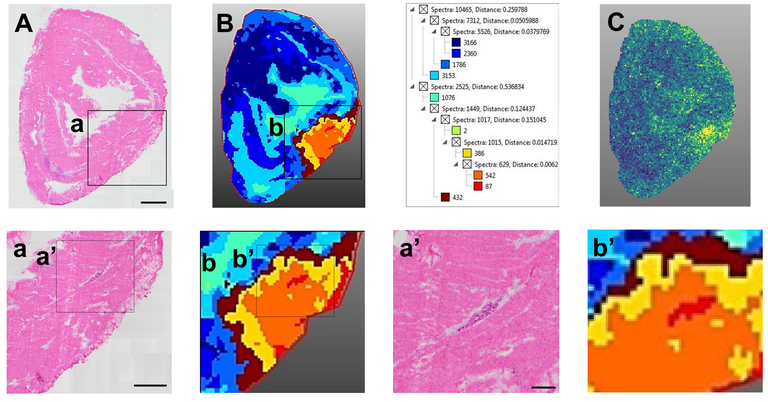

Fig. 3 *In-situ* proteome by segmentation analysis
A, a, a' ; HE staining of 8-week-old J2N-k heart after IMS (bars; A = 1 mm, a = 2 mm, a' = 200 μm)
B, b, b' ; Segmentation map (SCiLS Lab 2018b.)
C ; Single peak image (SCiLS Lab 2019b.)

②MALDI-IMS with shotgun proteomics

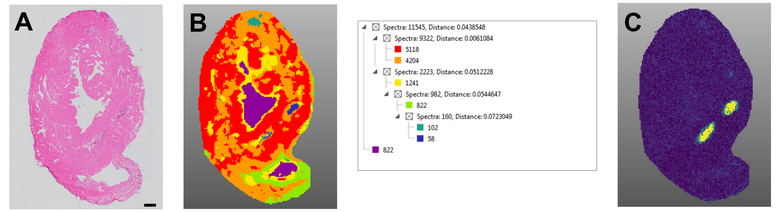

Fig. 4 *In-situ* proteome with on-tissue digestion by segmentation analysis
A; HE staining of 8-week-old J2N-k heart after IMS (bar = 500 μm)
B; Segmentation map, C; Single peak image (SCiLS Lab 2019b.)

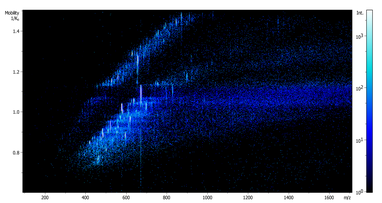

Fig. 5 Heatmap of 8-week-old J2N-k heart by Shotgun proteomics About 2,000 proteins were identified.

③Immunohistochemistry

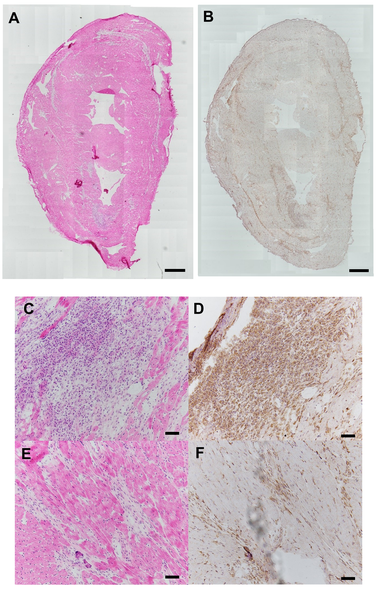

Fig. 6
A; HE staining of J2N-k heart
B; Immunohistochemistry for calreticulin. This protein is assumed to coincide with specific lesion of J2N-k hamster.
(bars = 2 mm)
C and E; enlarged view from A.
D and F; enlarged view from B.
This protein was annotated and validated its specific localization to cell infiltration.
(bars = 500 μm)

Conclusions

- We have established protocol of MALDI-IMS for heart tissues of J2N-k and J2N-n hamsters at proteomic level.
- We have succeeded in obtaining proteomic profiles of J2N-k hearts, which is coincided to histological features such as inflammatory cell infiltrations.
- We will further identify proteins specific to each lesions of J2N-k hearts through an integrated protocol of MALDI-IMS and shotgun proteomics.