In situ murine thymic proteomics by using MALDI-Imaging Mass Spectrometry in combination with shotgun LC-MS/MS

Bruker Japan K.K. & Doshisha University

○Haruka Koyama[1], Yudai Tsuji[1], Takashi Nirasawa[2], Yoshiaki Yasumizu[3], Tatsusada Okuno[3], Hiroyuki Shiono[4], Masaya Ikegawa[1]

1: Doshisha University, Kyotanabe, Japan
2: Bruker Japan K.K., Yokohama, Japan
3: Osaka University, Suita, Japan
4: Kindai University Nara Hospital, Ikoma, Japan

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Introduction

Thymus is a lymphoid tissue consisting of an outer cortex and inner medulla, and plays a central role in immunity, including the differentiation and maturation of T cells. Thymus is also thought to be involved in the production of self-antigen of myasthenia gravis, an autoimmune disease. Although steroids are the main choice for the treatment of such a disease, their dosage is determined based on clinical experiments and the mechanism of action remains unresolved, despite their enormous side effects. Here we have tried to obtain *in situ* proteomics in thoracic tissue, including thymus, by using matrix-assisted laser desorption/ionization (MALDI) Imaging Mass Spectrometry (IMS) and LC-MS/MS shotgun proteomics, after the administration of Dexamethasone to mice.

Methods

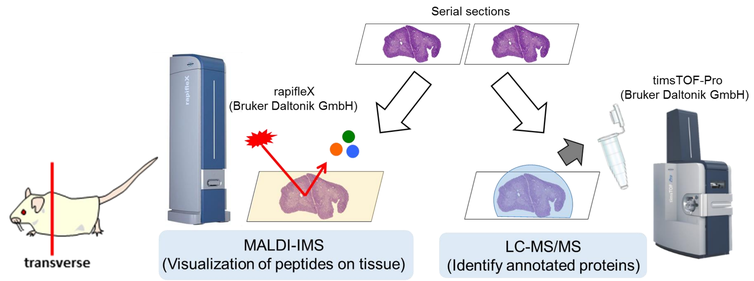
Animals: We used 4 weeks of age, female ICR mice. To induce apoptosis, Dexamethasone (Dex) 5mg/kg and saline as control were intraperitoneally administrated to the mice.

Sample preparation: After 12h administration of Dex, they were quickly frozen with liquid nitrogen and cut on a cryostat at a 12 µm thickness from transverse orientation. The sections were transferred onto conductive Indium-Tin-Oxide (ITO) glass slides and then on tissue digestion with trypsin was performed.

MALDI-Imaging Mass Spectrometry: α-Cyano-4-hydroxycinnamic Acid (CHCA) was uniformly sprayed as a matrix by using TM-sprayer. Spectra was acquired using rapifleX (Bruker Daltonik GmbH), whereas ions were detected with a spatial resolution of 100 µm. MALDI measurements were done with a mass range of m/z=400-4,000.

Shotgun LC-MS/MS: Shotgun proteomics from serial sections from MALDI-IMS were attempted by using timsTOF Pro with nanoElute (Bruker Daltonik GmbH). Column used was 10cm × 75µm, C18 column.

Data analysis: Obtained mass spectra as well as annotated proteins and peptides were visualized with flexiImaging and SCiLS Lab 2022a Software (Bruker Daltonik GmbH). About 1,000 proteins were annotated with ProteinScape4.0 (Bruker Daltonik GmbH).

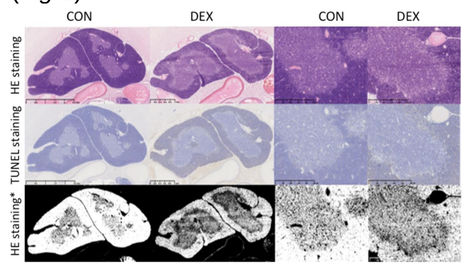


(Fig.1 Workflow images)

Results

Histological Analysis

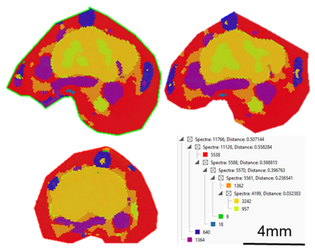
Apoptosis in thymus were visualized by HE staining and TUNEL staining. (*Fig.2*)



(Fig.2, *Binary image of HE staining)

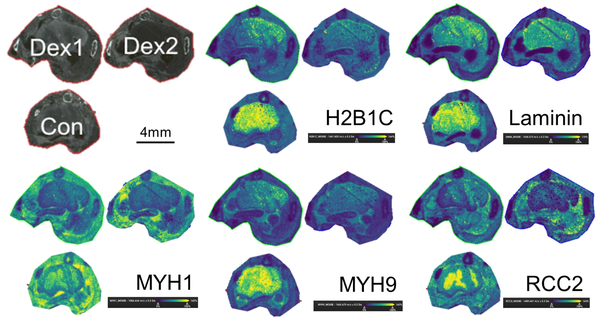
MALDI-IMS

Proteomic segmentation map visualized apoptosis at medulla in Dex treated mouse only. (Fig.3)



(Fig.3, Segmentation Map)

We compared the differences in thymus between two Dex-treated mouse and control one by using ROC analysis. As a result, we identified several single peaks that may reflect proteomic effects of Dex administration.



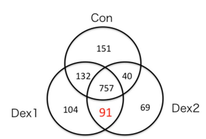
(H2B1C: Histone H2B1-typeC, MYH1: Myosin Heavy chane1, MYH9:Myosin heavy chane9, RCC2: Regulator of chromosome condensation)

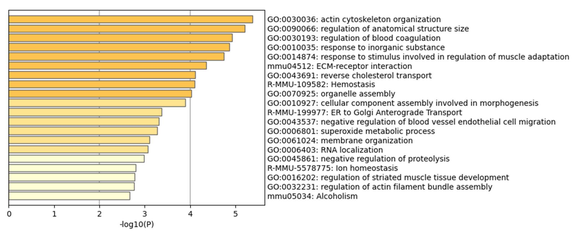
(Fig.4, Single peak analysis of MALDI-IMS on thoracic tissue. Expression pattern of defined proteins that are essential for cell cycle and or cell structure have altered through Dex administration.)

LC-MS/MS

With LC-MS/MS shotgun proteomics, almost 1000 proteins were identified with over 6,000 to 7,000 peptides sequences. (Table1.) Of these we focused on and discussed 91 proteins that were identified in both Dex treated individuals but not in control one.(Fig.5) The gene lists obtained were analyzed for their expression trends using Metascape, a gene ontology tool.(Fig6.)

	Proteins	Peptides
Control	1,080	6,975
Dex1	1,084	7,008
Dex2	957	6,433





(Table.1, The number of proteins and peptides identified by shotgun proteomics, Fig.5, Venn diagram comparing identified proteins between control and Dex treated mouse. Fig.6, protein lists)

Conclusions

The proteomic profile of Dexamethasone administration on thoracic tissue was visualized by using MALDI-IMS and shotgun proteomics.

Defined proteins were annotated reflecting proteomic effects of Dexamethasone administration, especially in thymic medulla.