SHIMADZU

Application News

MALDI-TOF Mass Spectrometry No.B18

Identification of Proteins by LC-MALDI System (1) [Analysis of Samples Obtained from Frozen Tissue Sections]

We conducted proteome analysis using a two-dimensional gel electrophoresis system and frozen tissue sections obtained by laser microdissection (LMD).
Two-dimensional gel electrophoresis is a technique that is recognized for its excellent reproducibility and its ability to quantitate. Therefore, it is still the most widespread analytical method in proteomics.
Its ability to identify several thousand proteins from a single electrophoretic run and allow the results to be registered in a database, as well as the low initial cost investment represent just a few of its many benefits. In addition, thanks to powerful database functionality and advancements in mass spectrometry, protein identification has become incredibly simplified.
LMD refers to the technique in which a laser beam is used to selectively cut out target cells present on a tissue section under an optical microscope. Up to now, conducting proteomic analysis of cancerous tissue has been limited to the use of techniques that assume the cancerous tissue contains only cancer cells or techniques that involve the separation of cancerous tissue from non-cancerous tissue under the naked eye. With experiments burdened with this level of analysis accuracy, some margin of error can be expected in the analytical results due to the inclusion of proteins from non-target cells, and reduced contrast of protein expression.
The use of LMD permits isolation of a uniform cell cluster from a mixed tissue sample, and offers the added benefit of enabling the analysis of gene- and protein-expression specific to those cells. Despite the difficulty in collecting a large number of cells, it was shown that two-dimensional gel electrophoresis could be conducted from a very small quantity of cells using fluorescent dye (SYPRO Ruby).
Furthermore, identification of the phosphorylation site of protein was demonstrated.
Here, the sample consisted of oral squamous cell carcinoma isolated from normal mucosal epithelium of human tongue tissue by LMD.

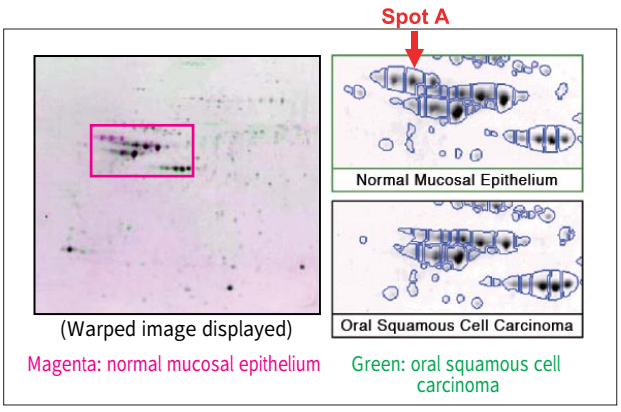


Fig. 1 2DE Image Analysis Using Progenesis PG200

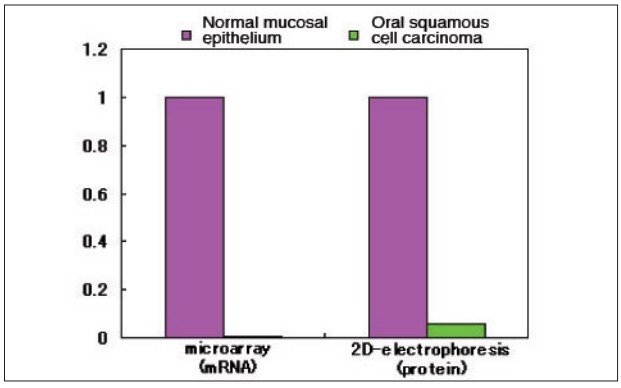


Fig. 2 Variation in Keratin13

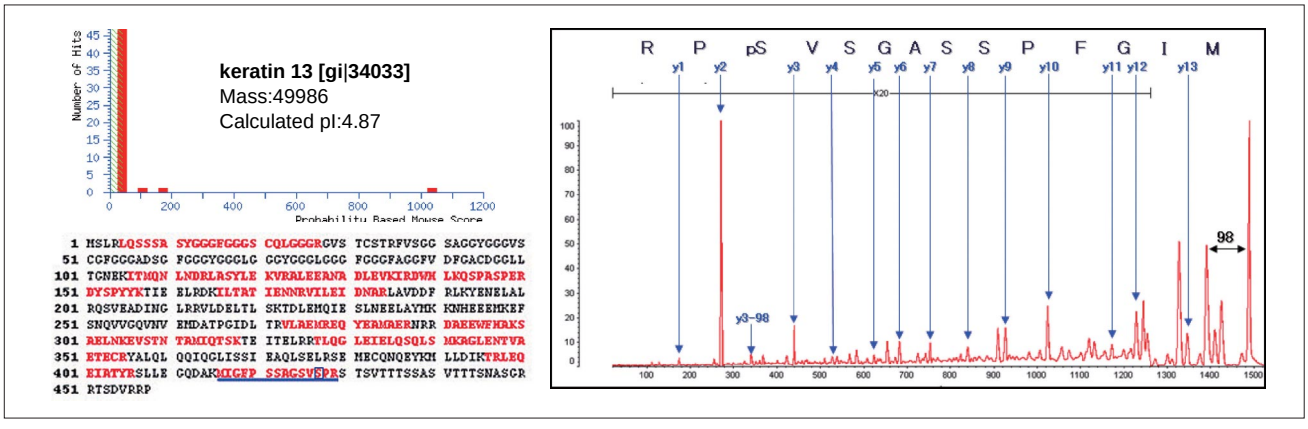


Fig. 3 (Left) Identification of Keratin13 by LC-MALDI System
(Right) MS/MS Spectrum of *m/z* 1488.58 (MIGFPSSAGSVS*PR: S*, phosphoserine)

We conducted analysis using the Progenesis PG200 imaging software following two-dimensional gel electrophoresis (Fig. 1). A comparison of the two images confirms that in the oral squamous cell carcinoma image, the horizontally-arranged spot group is greatly reduced. The results of identification using the AXIMA Performance revealed that this spot group is keratin13. Here, microarray analysis results confirmed a change equivalent to a 0.00036-fold reduction in keratin13 at the gene level. From the two-dimensional gel electrophoresis results, a 0.0588-fold reduction in keratin13 was confirmed as the amount of change at the protein level (Fig. 2).
In addition, after excising and conducting in-gel digestion of Spot A of Fig. 1, we performed automated MS/MS measurements by LC-MALDI (Fig. 3). The results not only confirmed the identification of keratin13 as the protein, but also that the 427th serine was phosphorylated (report is available).
These results demonstrate the usefulness of combining two-dimensional gel electrophoresis of micro-samples obtained using LMD with application of the AXIMA Performance for protein and post-translational modification identification.

[Sample Preparation]

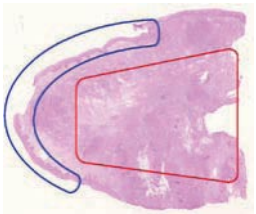
1. First we excised each of the sites from a frozen 7 µmm-thick tissue section that was mounted on film. (Three tissue sections were collected.)
2. After adding 100 µL of lysis buffer to the collected samples, extraction was conducted for 30 minutes using an ultrasonic cleaner.
3. Following centrifugal separation, the supernatant was used as the sample.

(Lysis Buffer)
6 M urea, 2 M thiourea, 3 % CHAPS, 1 % Triton X-100

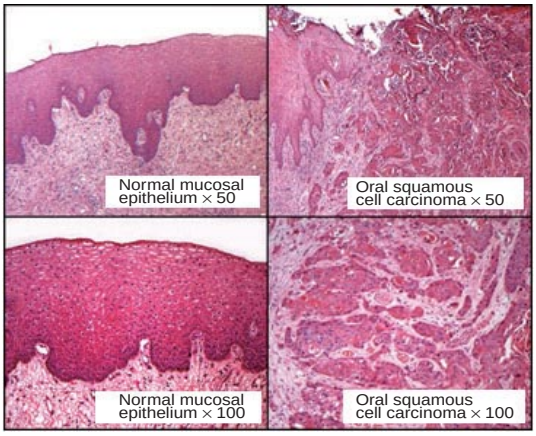
[Analysis Flow]

Two-dimensional gel electrophoresis
CoolPhoreStar® (Anatech Corp.)
↓
Imaging analysis
Progenesis PG200
↓
Gel excision
FluoroPhoreStar3000® (Anatech Corp.)
↓
In-gel digestion
↓
MS/MS automatic measurement by LC-MALDI
AXIMA® Performance

Provided by Professor Gou Yamamoto, Department of Oral Pathology, School of Dentistry, Showa University



Site: human mouth
Tissue: squamous cell carcinoma
(highly differentiated)



Portion within blue-outlined area: normal mucosal epithelium
Portion within red-outlined area: oral squamous cell carcinoma

(Reference) Transverse section of analyzed sample

[References]
T. Kondo et al., Proteomics 2003, 3, 1758_1766
The data presented here was acquired through joint research with Professor Gou Yamamoto, School of Dentistry, Showa University.

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