Discovery of Biomarkers for Endometrial Carcinoma by Direct Profiling of Proteins in Tissue Sections Using Selective MALDI-Spotting and MALDI MS

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Overview

Purpose: Discovering biomarkers for endometrial carcinoma rapidly by direct profiling of proteins in tissue sections.

Methods: nano-scale MALDI-Spotting and MALDI QqTOF mass spectrometry.

Results: Five up-regulated protein markers for endometrial carcinoma were discovered with the new tool.

Introduction

In recent years, various technologies have been used to acquire protein profiles (or maps) for biomarker discovery. Many technologies (e.g. 2D PAGE etc.) require multiple steps of sample handling and separation. These steps are time-consuming and may lead to protein losses. Direct profiling of proteins in tissue sections provides an alternative and rapid way for biomarker discovery. The challenge is to acquire protein profiles from a user-defined group of cells (e.g. cancer). Here we report our recent work in discovering biomarkers for endometrial carcinoma by direct profiling proteins in tissue sections using a nano-scale MALDI spotter and MS.

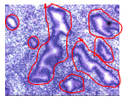
Methods

1. Materials and Instrument:
Normal endometrium and endometrial cancer tissues were snap-frozen in liquid nitrogen within 15-20 minutes of surgery. Normal and cancer tissue sections were cut with a cryostat at -15 °C and mounted on conductive carbon membrane or glass slides. Consecutive sections were cut and stained with Cresyl Violet (CV) or Methylene Blue (MB) to confirm pathological classification. Images from the paired stained tissues were taken under microscope and/or high resolution camera and used to locate cells of interest for profiling. A nano-scale MALDI spotter (LEAP Technologies, Inc.) was used to spot nano liters of sinapinic acid solution onto selected cells on the sections. Protein profiles were acquired using a Qstar XL MALDI-Qq-TOF mass spectrometer (Applied Biosystems). Peak intensities were normalized with internal standards and then subjected to analysis for biomarker discovery.

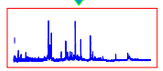
2. Experimental Work Flow

Cut tissue sections

Thaw sections on MALDI Plate



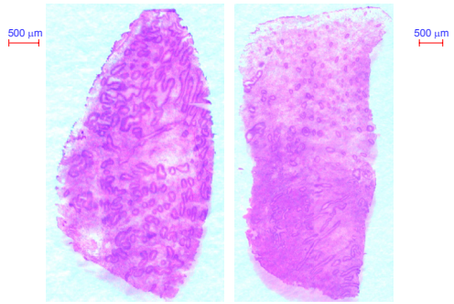
Spotting Matrix solution on selected cells using MALDI Spotter (LEAP Inc.)



Profiling proteins on QSTAR XL MALDI –QqTOF MS (Applied Biosystems)

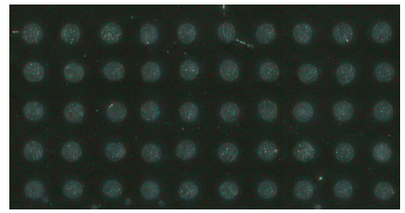
Results

Tissue Images Acquired with MALDI-Spotter Carmera



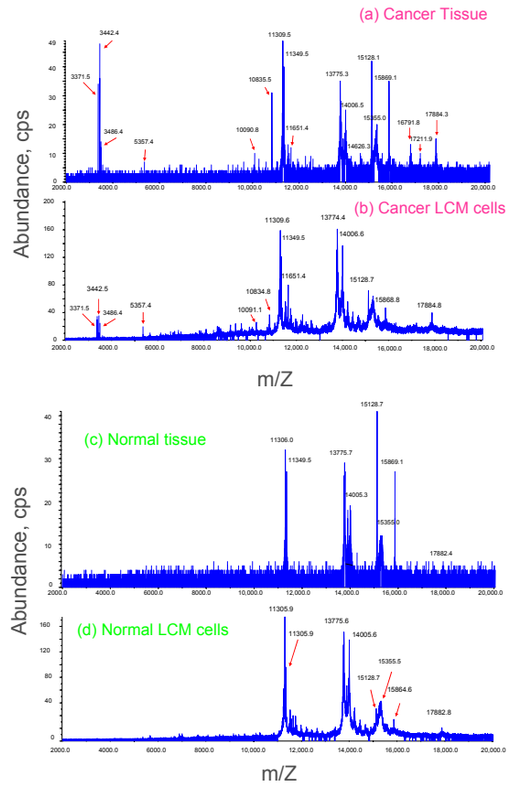
Endometrial tissue consists of glands, lumens, and supporting stroma. The cancer sites are typically the epithelial cells that lined the glands. The gland size is around 500 µm in a cancer tissue and 100 to 400 µm in a normal tissue. The image on the left shows abnormal, crowded glands within the endometrium. Definitive histopathologic examination of this case confirmed the pathologic diagnosis of adenocarcinoma. The image on the right shows normal glands within the endometrium.

Matrix Solution drops spotted by MALDI-Spotter



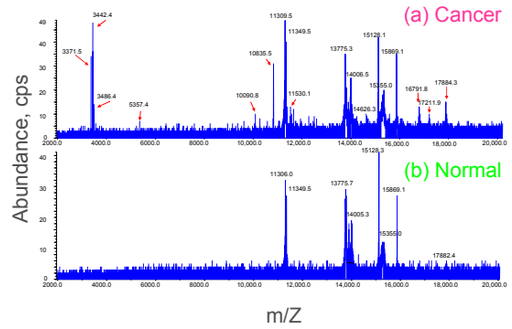
With MALDI-Spotter, 3-10 nL of sinapinic acid solution can be spotted onto one gland comprising cancerous epithelial cells.

Protein Profiles from tissue sections and LCM cells



The protein profiles of tissue sections are similar to those from Laser Capture Microdissected tissue samples. This suggests that the selective MALDI-Spotting technology is successful.

Tissue Protein Profiles Acquired by MALDI-MS



Five proteins were found up-regulated consistently by comparing the protein profiles obtained using MALDI-MS for 8 cancer and 8 normal tissue sections. The masses of these distinct peaks are 3372, 3443, 3487, 10835 and 10843 Th. All five proteins were found up-regulated by MS analysis of laser capture microdissected endometrial samples in previous work. Two of these proteins at 10834 and 10842 Da likely correspond to calgranulin A and chaperonin 10, which have been identified in endometrioid adenocarcinoma in whole tissue homogenate mass spectrometric analysis.

Conclusions

- The combination of nano-scale MALDI spotter technology and MS provide a new tool for biomarker discovery.
- Five up-regulated protein markers for endometrial carcinoma were discovered with the developed new tool.
- Reducing the matrix solution drop size might help to improve the selectivity further.

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