

Lipid MALDI MS Profiles of Gastric Cancer

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Abstract: Tissue matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) may identify lipids differentially expressed between cancer and adjacent normal tissue. To identify lipidomic profiles for gastric cancer, 24 gastric cancers were profiled for lipid by the histology-directed, tissue MALDI MS technology. Lipid profiles differed between gastric cancer and adjacent normal tissue samples. At $P < 0.05$, median class prediction accuracy in 100 random training-to-test partitions was 83.3% (5/6) for all classifiers tested. A peak at m/z 741.6 (sphingomyelin 34:1 K) was overexpressed, and a peak at m/z 782.6 (phosphatidylcholine 34:1 Na) was underexpressed in gastric cancers compared with normal tissue. Thus, lipid MALDI MS analysis may capture a global alteration in lipid profile of gastric cancer tissue, distinguishing cancerous epithelium from normal epithelium.

Keywords: Gastric cancer, lipid, MALDI, profile.

INTRODUCTION

Gastric cancer is the second most common cause of cancer death, and the incidence of diffuse-type gastric cancer has not been decreasing in Western countries [1]. There are no tissue-specific biomarkers that can be used for immunohistochemical diagnosis of gastric cancers. Tumor markers, such as CEA, CA19-9, and CA72-4, are not useful for the gastric cancer screening because of low sensitivity and specificity [2]. Clearly, there is an unmet need for sensitive and specific diagnostic markers for gastric cancer, especially early-onset gastric cancers [3].

Protein and lipid profiles obtained from histology-directed, tissue matrix-assisted laser desorption/ionization mass spectrometry (MALDIMS) are demonstrated to accurately differentiate cancer tissue from adjacent normal tissue [4]. Using 2,5-dihydroxybenzoic acid (DHB) and α -cyano-4-hydroxycinnamic acid (CHCA) matrix, our group identified lipid profiles for several common cancers [5, 6]. We initiated a study to evaluate whether histology-directed, lipid MALDI MS analysis may assist with the diagnosis of gastric cancers.

MATERIALS AND METHODS

Acquisition and Processing of MALDI MS Data

This study was performed on surgical samples collected from young gastric cancer patients undergoing surgery at several participating hospitals in Korea from 2008 to 2012. All patients signed informed consents. Basically, histology-directed MALDI MS analysis methods, pioneered by Dr. Caprioli, were used for this study [reviewed in [7]]. 10 μ m-thick cryosection slides were obtained from each frozen tissue. One section slide was stained with hematoxylin and eosin (H&E). Next tissue sections were thaw-mounted to indium tin oxide (ITO) slides (HST Inc., Newark, NJ) and dried in vacuum. 2,5-dihydroxybenzoic acid (DHB) and α -cyano-4-hydroxycinnamic acid (CHCA) was dissolved in 70% methanol plus 0.1% trifluoroacetic acid and 1% piperidine [5]. This matrix solution was deposited on cryosection slides by the Chip-1000 instrument (Shimadzu, Kyoto, Japan) [8]. Interval between matrix spots was 400 μ m. Mass spectra were acquired using UltrafleXtreme (Bruker Daltonics, Bremen, Germany) at a laser frequency of 1,000 Hz. Calibration standards were as follows; m/z 674-834 (positive ion mode) and m/z 564-906 (negative ion mode). Mass spectra originating from tumor (or glandular epithelium)-rich spots only were acquired for subsequent processing (Fig 1A). ClinProTools (v 2.2, Bruker Daltonics) was used for peak processing.

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Statistical Analysis

Average-normalized datasets of each of positive- and negative-ion modes were combined. Bioinformatic analyses were performed using BRB-ArrayTools (version 4.1, Biometrics Branch, US National Cancer Institute) [9]. To assess the predictive power of discriminatory peaks, we performed class prediction analyses by randomly dividing the whole dataset into training and test subsets with 1-to-1 ratio, using statistical analysis methods previously described [10]. For each random dataset, predictors identified in each training set were used to predict the class label of samples in the test set. To assign the molecular identify of informative peaks, MALDI MS/MS analysis was performed on

cryosections, and the data were mapped to public lipid databases (www.lipidmaps.org).

RESULTS

Histology-directed, lipid MALDI MS analyses were performed for 24 retrospective, frozen surgical tissue samples (12 pairs of cancer and adjacent normal tissue samples) (Table 1). There were 7 females (58.3%) and median age was 35 years [3]. Six patients (50%) had signet ring cell carcinomas. The average MALDI MS spectra were composed of median 5 individual measurements for cancer samples and median 4 individual measurements for normal samples. The average mass spectra for gastric cancers and

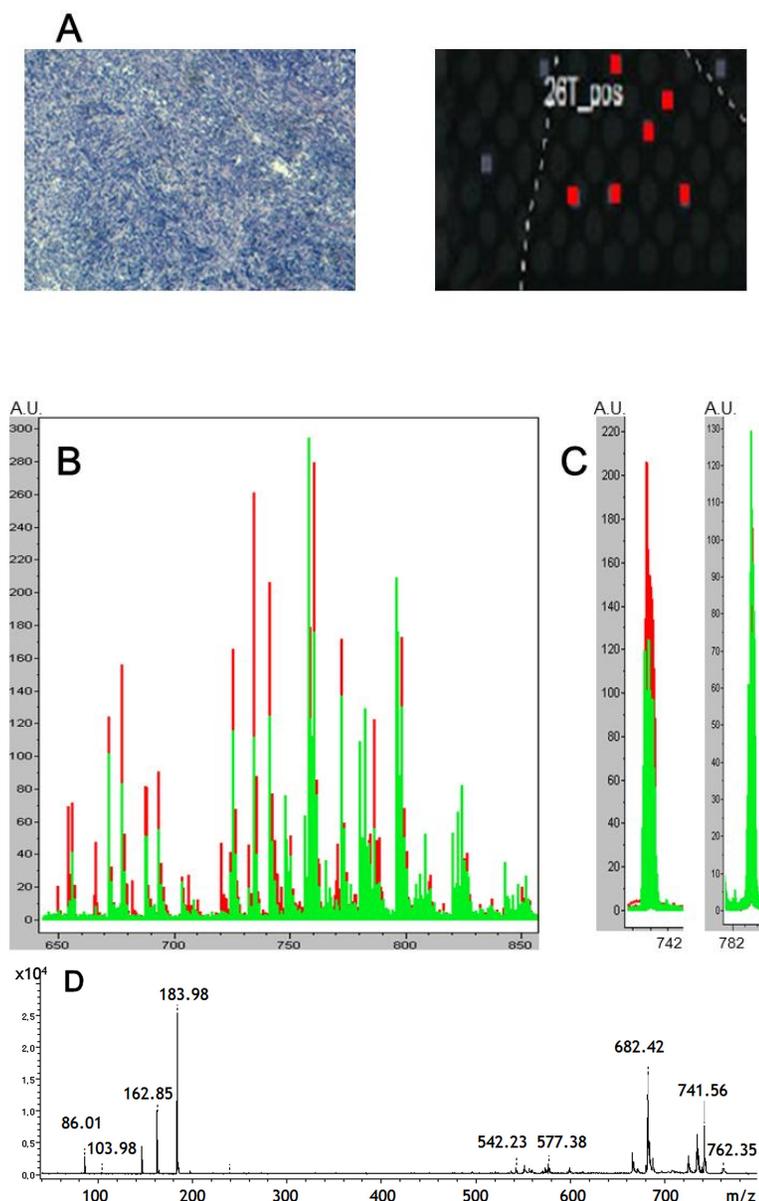


Fig. (1). A. An optical image of matrix-loaded spots on an ITO cryosection slide, and a corresponding H&E section for a representative gastric cancer sample (x 40 magnification). Mass spectra originating from tumor-rich spots (red) only were processed for subsequent statistical analyses. B. Overlays of average mass spectra obtained from gastric cancer samples (red) and adjacent normal tissue samples (green) in the positive ion mode [10]. C. Intensity profiles of SM 34:1 (m/z 741.6, left) and PC 34:1 (m/z 782.6, right) in gastric cancers (red) and normal tissue samples (green). D. The MS/MS profile for a peak at m/z 741.6, which was assigned as SM 34:1 [M+K]+.

Table 1. Peaks Differentially Expressed Between Gastric Cancers and Normal Samples at Feature Selection $p < 0.05$.

Increased in Cancer			
m/z	P	Ratio ¹	Assignment
P741.6	0.002	1.3	SM 34:1 [M+K] ⁺
P693.62	0.030	1.2	
P786.64	0.036	1.2	PC 33:0 [M+K] ⁺
Decreased in Cancer			
m/z	p	Ratio	Assignment
P782.6	<0.001	0.7	PC 34:1 [M+Na] ⁺
P636.0	<0.001	0.7	
P804.6	0.006	0.8	

Ratio¹, tumor/normal; P, positive ion mode; SM, sphingomyelin; PC, phosphatidylcholine

adjacent normal tissue are shown in the left panel of Fig. (1B). Finally, 74 processed peaks (28 and 46 for positive and negative modes, respectively) were analyzed.

Twelve pairs of gastric cancer and adjacent normal tissue samples were compared using paired *t*-test. Table 1 lists 6 peaks were differentially expressed between cancer and normal samples at feature selection $p < 0.05$ (Fig 1C). Permutation *P* value for cross-validated misclassification rate was at borderline significance, when all of the 12 pairs were used as a training set (the compound covariate predictor: 0.03, diagonal linear discriminant analysis: 0.01, 1-and 3-nearest neighbors: 0.08 and 0.1, nearest centroid: 0.1, and the support vector machine: 0.04). These results suggest that cancer and normal samples are different in lipid profiles. To assess the predictive power of discriminatory peaks, we performed class prediction analyses by randomly dividing the whole dataset into training and test subsets with 1-to-1 ratio [10]. At $P < 0.05$, median class prediction accuracy in 100 random training-to-test partitions was 83.3% (5/6) for all classifiers tested. Thus, lipid profiles of gastric cancers are different from those of adjacent normal tissue.

Using MS/MS analysis, peaks overexpressed in gastric cancers were identified for the exact molecular assignment (Table 1). Peaks at *m/z* 741.6, *m/z* 786.6, and *m/z* 782.6 in the positive ion mode were identified as SM 34:1 [M+K]⁺, phosphatidylcholine (PC) 33:0 [M+K]⁺, and PC 34:1 [M+Na]⁺, respectively (Fig. 1D).

DISCUSSION

This study compares lipid profiles between gastric cancers and adjacent normal tissue. There is an urgent, unmet need for novel markers for this aggressive disease [3]. Advantages of histology-directed MALDI MS technology include rapid procedure time, low reagent cost, and small amount of tissue required. According to the MALDI MS analyses of gastric cancer samples, sphingomyelin 34:1 was identified to be the most significantly overexpressed lipid. In addition, PC 34:1 [M+Na]⁺ (*m/z* 782.6), which is over expressed in lung cancers and cholangiocarcinomas [5, 6], was underexpressed in gastric cancer patients, while PC 33:0

[M+K]⁺ was overexpressed. Future larger-scale studies will be needed to validate our findings.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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ABBREVIATIONS

MALDI MS	=	Matrix-assisted laser desorption and ionization mass spectrometry
DHB	=	2,5-dihydroxybenzoic acid
CHCA	=	α -cyano-4-hydroxycinnamic acid
ITO	=	Indium tin oxide
SM	=	Sphingomyelin
PC	=	Phosphatidylcholine

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