

Lipidomics จากพลาสมาของผู้ป่วยมะเร็งท่อน้ำดีชนิดภายนอกตับโดย MALDI-TOF Lipidomics from Plasma of Extrahepatic Cholangiocarcinoma Patients by MALDI-TOF

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บทคัดย่อ

มะเร็งท่อน้ำดีอาจมาด้วยอาการท่อน้ำดีอุดตัน ซึ่งต้องจำแนกจากภาวะที่ไม่ใช่มะเร็งชนิดอื่นๆ การใช้ตัวตรวจวัดในซีรัมในปัจจุบันนั้นยังไม่ได้เพียงพอเนื่องจากความไม่จำเพาะของตัวตรวจวัด การศึกษา Lipidomics อาจช่วยให้พบตัวตรวจวัดชนิดใหม่ๆ ในน้ำเลือดของผู้ป่วยมะเร็งท่อน้ำดีได้ การศึกษา Lipidomics จากน้ำเลือดของผู้ป่วยมะเร็งท่อน้ำดีโดยใช้เทคนิค MALDI-TOF นั้นแสดงรูปแบบของลิพิดที่แตกต่างระหว่างผู้ป่วยมะเร็งท่อน้ำดีกับคนปกติ ลิพิดที่ตำแหน่ง 808.05 แสดงให้เห็นความแตกต่างซึ่งอาจนำไปใช้ในการวินิจฉัยมะเร็งท่อน้ำดีต่อไปได้

ABSTRACT

Cholangiocarcinoma can present as obstructive jaundice that should be discriminated from other benign conditions. The using of serum marker for diagnosis of cholangiocarcinoma is not suitable in clinical practice due to the non-specific of present markers. Lipidomics might be an approach for determination of new plasma biomarkers for cholangiocarcinoma. Lipidomics from plasma by MALDI-TOF showed peak patterns different between cholangiocarcinomas and controls. The identification of specific peak difference indicated that m/z 808.05 peak might be used for identify the specific lipids as marker for diagnosis of cholangiocarcinoma.

คำสำคัญ: Lipidomics, มะเร็งท่อน้ำดี, MALDI-TOF

Keywords: Lipidomics, cholangiocarcinoma, MALDI-TOF

INTRODUCTION

Cholangiocarcinoma (CCA) can be divided into extrahepatic CCA (ECC) and intrahepatic CCA (ICC). ECC can cause biliary obstruction which sometimes difficult to discriminate from other benign disease. Nowadays, serum markers for CCA are not appropriated for precise diagnosis (Gatto M *et al.*, 2010, Cardinale V *et al.*, 2010). Lipidomics might be an approach for investigation of new plasma biomarkers for ECC patients, in addition to conventional approaches (Meikle P., 2009). Therefore, Matrix-assisted Laser Desorption Ionization/time of flight (MALDI-TOF) was used to investigate distinguish plasma lipid peak patterns from extrahepatic cholangiocarcinoma (ECC) compared to healthy controls.

MATERIALS AND METHODS:

1. Plasma samples

EDTA bloods from ECC patients in Siriraj Hospital were taken by Dr. Warayu Prachayakul, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University. Informed consent was obtained from each patient. The Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, approved the research protocols (Si521/2010). Plasma was taken after low-speed centrifugation.

2. Lipid extraction and MALDI-TOF

Plasma lipids were extracted by Bligh and Dyer method (Touchstone J.C., 1995) from plasma of 3 ECC patients and 3 healthy control. After that, lipid concentrations were determined by phospho-vanillin reaction (Knight J.A., 1972). Lipidomics were investigated both linear mode and reflectron mode by MALDI-TOF (ultraflex III TOF/TOF, Bruker Daltonics, Billerica, MS, USA). Equal amount of lipid solutions were loaded with appropriated matrix (2,5-dihydrobenzoic acid (DHBA) and trifluoroacetic acid (TFA) in ratio 1:4). Flex analysis[®] software (Bruker Daltonics) was used for baseline adjustment and subtraction and spectral recalibration. ClinProTools[®] software version 2.2 (Bruker Daltonics) was used for statistical analysis.

RESULTS AND DISCUSSION

Three plasma from ECC patients and 3 from healthy controls were analyzed to identify cholangiocarcinoma-specific lipid profile using MALDI-TOF analysis. Mass spectra were acquired on individual spots for each person from both linear and reflectron modes. The linear mode spectra were averaged together after pre-processing to create one average spectrum per category. Principal component analysis (PCA) plot graphically demonstrated that ECC and healthy were separately clustered in an unsupervised analysis (Figure 1). The peak with mass per charge ratio (m/z) 808.05 was determined as peak which was down expressed in ECC and healthy (Figure 2). The reflectron mode spectra didn't show significant difference (data not shown).

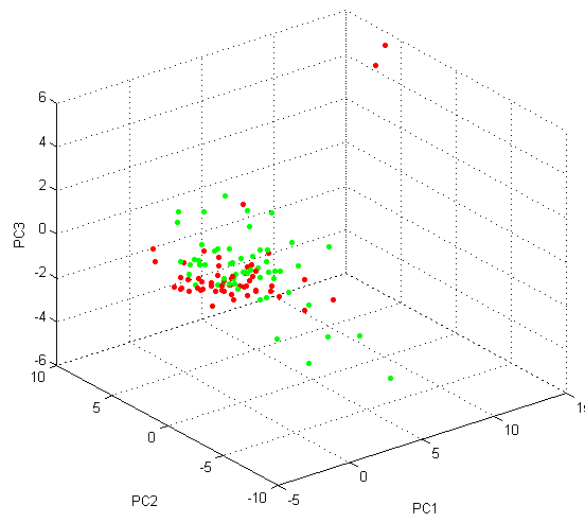


Figure 1 Principal component analysis plot for 3 ECC (shown in red) and 3 healthy (shown in green), which graphically represents 1-correlation distances among category.

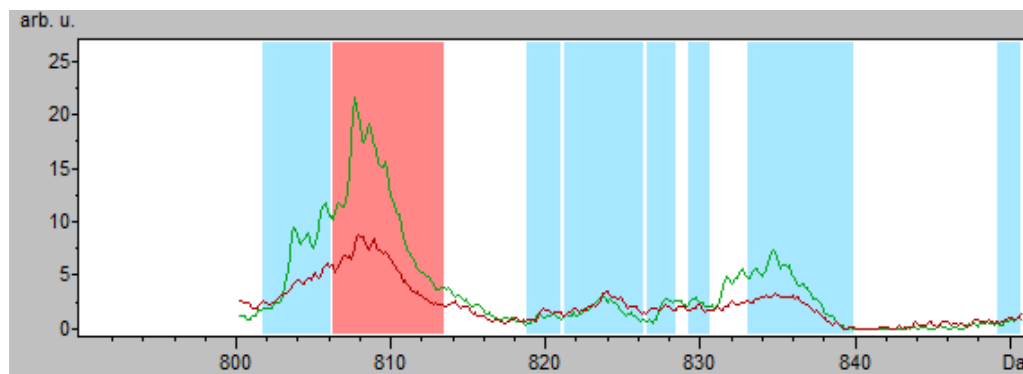


Figure 2 Intensity profile for representative peak showed down-expression in ECC samples at m/z 808.05.

The result showed that the principal component analysis pattern of lipidomics of ECC patients was different from that of healthy persons when analyzed with ClinProTools[®] software. However, the individual analysis did not show characteristic pattern of each categories. The determination of specific peak difference indicated m/z 808.05 peak that down-regulated in ECC samples compared to control samples with P value < 0.00001 . So, the identification of specific peak difference might be used for discrimination of the individual as disease or normal control.

CONCLUSION

Lipidomics from plasma by MALDI-TOF showed peak patterns different among individuals, the integration of results from the same group showed the significant difference but unsuccessfully compared between individuals. The identification of specific peak difference indicated that m/z 808.05 peak had down-regulation in ECC samples compared to control samples with P value < 0.00001 . This might be used for identify the specific lipids as marker for diagnosis of ECC. Further study, we would like to investigate consistency of these peaks among higher size of patients for identification of specific abnormal lipids.

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REFERENCES

- Cardinale V, Semeraro R, Torrice A, Gatto M, Napoli C, Consiglia M, *et al.* Intra-hepatic and extra-hepatic cholangiocarcinoma: Newinsight into epidemiology and risk factors. *World J GastrointestOncol.* 2010; 2(11): 407-416
- Gatto M, Alvaro D. New insights on cholangiocarcinoma. *World J Gastro intest Oncol.*2010; 2(3): 136-145
- Knight J.A, Anderson S, and Rawle J.M. Chemical Basis of the Sulfo-phospho-vanillinReaction for EstimatingTotal Serum Lipids. *Clin Chemistry.* 1972; 18 (3): 199-202
- Meikle P, Barlow C and Weir J. Lipidomics and Lipid Biomarker Discovery: *Australian Biochemist.* 2009; 40(3): 12-16
- Touchstone J.C. Thin-Layer Chromatographic Procedures for Lipid Separation: *Journal of Chromatography.* 1995; 671: 169-195
- Zografos G.N, Farfaras A, Zagouri F. Cholangiocarcinoma: principles and current trends. *Hepatobiliary Pancreat Dis Int.* 2011; 10(1): 10-20