Metabolic Profiling of Cancer-associated Fibroblasts (CAFs) Isolated from Cholangiocarcinoma Patients

แบบแผนสารเมแทบอไลท์ของไฟโบรบลาสต์ที่สัมพันธ์กับมะเร็งที่แยกได้จากผู้ป่วยมะเร็งท่อน้ำดี

Saikhim Sringan (สายขิม ศรีงาน)* Dr.Nisana Namwat (ดร.นิษณา นามวาท)**
Dr.Watcharin Loilome (ดร.วัชรินทร์ ลอยลม)** Dr.Jutarop Phetcharaburanin (ดร.จุฑารพ เพชระบูรณิน)***

ABSTRACT

Cancer-associated fibroblasts (CAFs) play a role in promoting carcinogenesis and tumor progression. There are few studies about metabolic profiling in CAFs and their metabolites affect cholangiocarcinoma (CCA) progression. This study aims to elucidate the metabolic profiles in CAFs compared to normal fibroblasts (NF) using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis by metabolomic approach. The OPLS-DA multivariate analysis was used for determining the candidate metabolites in CAFs, compared with NF. The results showed that five metabolites including phenylpyruvic acid, hypoxanthine, oleamide, phosphatidylcholines (PC; 18:1(9Z)/16:0), and sphinganine trended to increase in CAFs were validated using OPLS-DA methods. This result provides a basic knowledge in CAFs' metabolism to understand the tumor biology of CCA.

บทคัดย่อ

เซลล์ไฟโบรบลาสต์ที่สัมพันธ์กับมะเร็งเซลล์ไฟโบรบลาสต์ที่สัมพันธ์กับมะเร็ง (cancer-associated fibroblasts, CAFs) มีบทบาทในการส่งเสริมการก่อมะเร็งและการลุกลามของเนื้องอก มีการศึกษาไม่มากนักเกี่ยวกับแบบแผนเมแทบอลิซึมและสารเมตาโบไลท์ใน CAFs ที่มีผลต่อการลุกลามของมะเร็งท่อน้ำดี ในการศึกษานี้มีวัตถุประสงค์เพื่อหาความแตกต่างระหว่างแบบแผนเมแทบอลิซึมของเซลล์ไฟโบรบลาสต์ปกติกับไฟโบรบลาสต์ที่สัมพันธ์กับมะเร็ง โดยใช้เทคนิคอัลตราไฮเพอร์ฟอร์แมนซ์ลิควิดโครมาโทกราฟี-แทนเดมแมสสเปกโทรเมตรี การวิเคราะห์พหุตัวแปรด้วยโมเดล O-PLS-DA ถูกใช้เพื่อค้นหาสารเมตาโบไลท์หลักในไฟโบรบลาสต์ที่สัมพันธ์กับมะเร็ง ผลการศึกษาแสดงว่าไฟโบรบลาสต์จากมะเร็ง พบว่าสาร phenylpyruvic acid, oleamide, hypoxanthine, phosphatidylcholines (PC; 18:1(9Z)/16:0) และ sphinganine มีแนวโน้มสูงขึ้นกว่าเซลล์ไฟโบรบลาสต์ปกติอย่างมีนัยสำคัญ ซึ่งผลครั้งนี้สามารถได้รับความรู้พื้นฐานบางอย่างเกี่ยวกับเมแทบอลิซึมของ CAF เพื่อให้เข้าใจชีววิทยาโรคมะเร็งของมะเร็งท่อน้ำดี

Keywords: Cancer-associated fibroblasts (CAFs), Metabolomics, Cholangiocarcinoma (CCA)
คำสำคัญ: เซลล์ไฟโบรบลาสต์ที่สัมพันธ์กับมะเร็ง เมแทบอโลมิกส์ มะเร็งท่อน้ำดี

*Student, Master of Science Program in Medical Biochemistry and Molecular Biology, Department of Biochemistry, Faculty of Medicine, Khon Kaen University
**Associate Professor, Department of Biochemistry, Faculty of Medicine, Khon Kaen University
***Lecturer, Department of Biochemistry, Faculty of Medicine, Khon Kaen University

Introduction

Cancer-associated fibroblasts (CAFs) are a heterogeneous population of activated fibroblasts which are the most abundant and the most important cell type of tumor microenvironment (TME) (Kwa *et al.*, 2019). CAFs basically function to maintain the homeostasis of stroma and define the structural integrity and mechanical properties. Heterogeneous origins of CAFs include adipocytes, epithelial/endothelial cells, hematopoietic stem cells and resident tissue fibroblasts (Shiga *et al.*, 2015). Some factors secreted from cancer cells and stromal cells (including fibroblasts) can stimulate and encourage the proliferation of fibroblasts and correlates with cancer progression (D. A. Bronzert, 1987). CAFs also contribute in modulation of functions and behavior of the surrounding cells during tumor progression (Santi *et al.*, 2018). Moreover, there are studies suggested that CAFs can encourage tumorigenicity by promoting of tumor initiation, remodeling the extracellular matrix (ECM), involving in immune and TME modulation, angiogenesis and also facilitating in cancer migration and metastasis (Ohlund *et al.*, 2014). CAFs interact with cancer cells through various molecules, including symbiotic nutrients and signaling metabolites. Metabolites cross-feeding between CAFs and cancer cells support bioenergetics and biosynthesis previously investigated in prostate, breast and pancreatic cancer cells. Moreover, there is evidence regarding metabolites act as a stabilizer of signaling proteins for hepatoma cell growth (Lyssiotis and Kimmelman, 2017). Therefore, elucidating the metabolic profiles of CAFs which may have abilities to encourage cancer progression using a metabolomic approach. Metabolomics is the study of metabolome within biological samples, cells, biofluids, tissues, or organisms to comprehensively identify and quantify all endogenous and exogenous small molecules/metabolites (<1 kDa) in a biological system in a high-throughput manner (Nalbantoglu, 2019). Liquid chromatography mass spectrometry (LC-MS) is one of common instruments used for identification and quantification in the metabolomic work.

Cholangiocarcinoma (CCA) is one of the most common cancers and a major public health problem in the Northeast Thailand. Recently, the study about TME as a therapeutic target in cancer has been interesting meanwhile CAFs is one of cell types associated with cancer progression. There are evidence regarding the effect of CAFs on CCA progression that provides poor clinical outcomes. The α-SMA, a CAF marker, is well correlated with clinicopathological parameters of patients with intrahepatic CCA. Results of overall survival rates and recurrence-free survival of patients with α-SMA- positive CAFs are much worse than those of patients with α-SMA- negative CAFs (Sha *et al.*, 2018). However, there are few studies about the metabolic profiling of CAFs in CCA. The aim of this study was to elucidate the metabolic profiles of CAFs compared with normal fibroblasts using statistical analyses including unsupervised method (principal component analysis; PCA) and supervised method (Orthogonal partial least squares discriminant analysis; O-PLS-DA). Significant metabolites that can distinguish between CAFs and normal fibroblasts (NF) may be useful for explaining the mechanism of cancer progression.

Objectives of the study

The aim of this study was to reveal the different metabolites between CAFs and normal fibroblasts using the metabolomic approach.

Methodology

Primary CAFs isolation

Tissues collected from patients with diagnosed CCA, which undergo surgical resection at Srinagarind Hospital, Khon Kaen University, are in part of fibrosis close to tumor or adjacent areas. Small, chopped tissues digestion using type IV collagenase at 37°C for 30 min. The cell precipitations were suspended with the culture media with Dulbecco's Modified Eagle Medium (DMEM) (Gibco™, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco™, USA) and 100 IU/ml of penicillin-streptomycin (Gibco™, USA) and filtrated through the cell strainer. The separated cells and retained tissue pieces were cultured in each flask containing culture medium, at 37°C in a humidified atmosphere containing 5% CO_2.

Normal fibroblasts (NF)

Normal Adult Human Primary Dermal Fibroblasts (ATCC®, USA) was activated and cultured in Fibroblast Basal Media (ATCC®, USA) supplemented with Fibroblast Growth Kit components (ATCC®, USA). Subsequently, we switched to culture and expand with Dulbecco's Modified Eagle Medium (DMEM) (Gibco™, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco™, USA) and 100 IU/ml of penicillin-streptomycin (Gibco™, USA).

Western blot analysis

For protein extraction, samples were resuspended with 100 μl of RIPA lysis buffer containing protease inhibitor followed by centrifugation at 14,000 x g, 4°C for 10 min, and supernatants were collected. Then sample was determined for the protein content using the Pierce BCA™ Protein Assay kit (Pierce Biotechnology). Protein extracts were solubilized in the sample buffer containing sodium dodecyl sulfate (SDS) and β-mercaptoethanol and boiled at 95°C for 5 min. Sample solution was loaded and separated with 10% (w/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred from gel to a PVDF membrane. Membranes were blocked with 5% (w/v) skimmed milk in Tris buffered saline (TBS) and probed with anti-α-SMA monoclonal antibody (ab7817, Abcam, USA) for CAF identification. Primary antibodies were incubated at 4°C for overnight followed by secondary antibodies for 1 h at room temperature and exposed with ECL™ Prime Western Blotting Detection Reagent for chemiluminescent detection.

Sample preparation

Samples were collected as one million cells of each sample which an appropriated sample size of cultured cells for the UHPLC-MS/MS platform (referred from Center for Innovative Technology) for 5-dependent replicates (n=5) for each cell culture including 3 primary-isolated CAFs (#J132, K088, and K112) and normal fibroblasts. Cell pellets were washed with 1X phosphate-buffered saline (PBS) pH 7.4 and stored at -80 °C. Cell pellets were simultaneously extracted and resuspended with cold methanol and freeze-thaw lysis

with liquid nitrogen to release intracellular metabolites. Cell lysates were sonicated in chilling ice for 3 cycles of pulse on 30 sec and pulse off 5 sec. For phase extraction, cold HPLC-grade water and chloroform were added with ratio of solvents, methanol: water: chloroform is 1:1:3. All sample solutions were frequently mixed and centrifuged at 4,000 x g, 4℃ for 20 minutes. Eventually, solutions were separated into 3 layers then aqueous phase was transferred to new microtube and solvent was removed by vacuum concentrator at 40℃. Samples were reconstituted by buffer along with a reconstitution blank, subsequently centrifuged at 13,000 x g, 4℃ for 10 min and transferred to insert vials. Remaining solution samples were divided as for quality control (QC) collection and 1:2, 1:4, 1:8, 1:16 of QC dilutions. All samples and QCs were analyzed on UHPLC-ESI-Q-TOF-MS/MS (Bruker, Germany) with the reverse-phase platform, Bruker intensity solo HPLC C18 2.1 x 100 mm, 2 µm column (Bruker, Germany) for initial separation and 100% water with 0.1% formic acid (FA) was used as mobile phase A, and 100% acetonitrile with 0.1% FA as mobile phase B.

Data processing and metabolite identification

MS data analysis was performed using the Data Analysis software package (version 4.1. Build 4.1.362.7, Bruker Daltonics) and MetaboScape® version 4.0 software. The data was defined as type group, constructed bucket table, then annotated using *Spectral library* (*e.g.,* Bruker HMDB metabolite library), and *Smartformula.* Eventually, we gained the spectrum data including retention time, mass to charge ratio, chemical formula and accuracy mass and some known metabolites. Likewise, unknown metabolites were identified *via* METLIN webpage (https://metlin.scripps.edu/) and Human Metabolome Database (HMDB) (https://hmdb.ca/).

Data analysis

After data processing, the data was exported in excel files (.exe) or csv files (.csv) presenting parameters including mass to charge ratio (m/z), retention time, group of samples and metabolite composition. Statistical analysis of metabolite profiles was determined between CAFs and NF groups using Metaboanalyst webpage and multivariate Data Analysis Software (Umetrics SIMCA®). Statistical analyses including unsupervised method (principal component analysis; PCA) and supervised method (Orthogonal partial least squares discriminant analysis; O-PLS-DA) were used for identifying a significant difference of metabolite(s) between groups. Model validation was analyzed by cross-validated analysis of variance (CV-ANOVA) with significant at $P < 0.05$. Metabolite intensities were determined as a significant difference through the unpaired t-test ($p < 0.05$) on GraphPad Prism 8 software (California, US).

Results

To investigate the difference between normal fibroblasts (NF) and CAFs, alpha-smooth muscle actin (α-SMA) protein expression which is one of marker protein of CAFs was examined using western blot analysis. Our result showed that the α-SMA protein expression was presented in 3 primary-isolated CAFs of patient id including #J132, #K088, and #K112, while it was not detected in NF (Figure 1). We explored the metabolic profiles in CAFs and NF using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Multivariate analysis was conducted via SIMCA® software,

PCA, PLS-DA, and OPLS-DA were used for evaluating metabolites. PCA analysis revealing overview of all samples; groupings of samples (clusters), trends and outliers was shown in Figure 2. The PCA score plots of the model between these groups demonstrated a separation along the second principal component (Q2=0.421 R2; PC1=33.7%, PC2=56.6%), indicating that there was apparent clustering between NF and CAFs. Using supervise analysis, PLS-DA and OPLS-DA, the score plots showed good visual separation among grouping of both cell types and showed significantly validated model with $p < 0.05$ (p=8.61E-13 and 1.55E-13, respectively). Table 1 showed the parameter of each model including R2X, R2Y, Q2Y and CV-ANOVA ($p < 0.05$). Loading plot of pairwise O-PLS-DA model showed the significant metabolites were selected based on a variable importance in projection (VIP) value more than 1.0 and p (corr) cutoff value of 0.6. Five known metabolites in CAF group were identified and matched databases including phenylpyruvic acid, hypoxanthine, oleamide, phosphatidylcholines (PC; 18:1(9Z)/16:0), and sphinganine that were found significantly higher than in NF (Table 2). Moreover, we compared each CAF sample from three patients with NF using the statistical analysis. The results showed that all three cases had valid models (Figure2A-2C). Using a t-test analysis, we found that all 5 metabolites trended to be increased in CAFs compared to NF. It was noted that hypoxanthine was significantly increased in all cases of CAFs (#J132, #K088, and #K112) compared with NF (Figure 3).

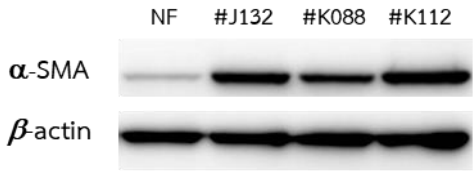

Figure 1 Western blot analysis of α-SMA protein expression in CAFs isolated from patient tissues with CCA (#J132, #K088, and #K112) and NF.

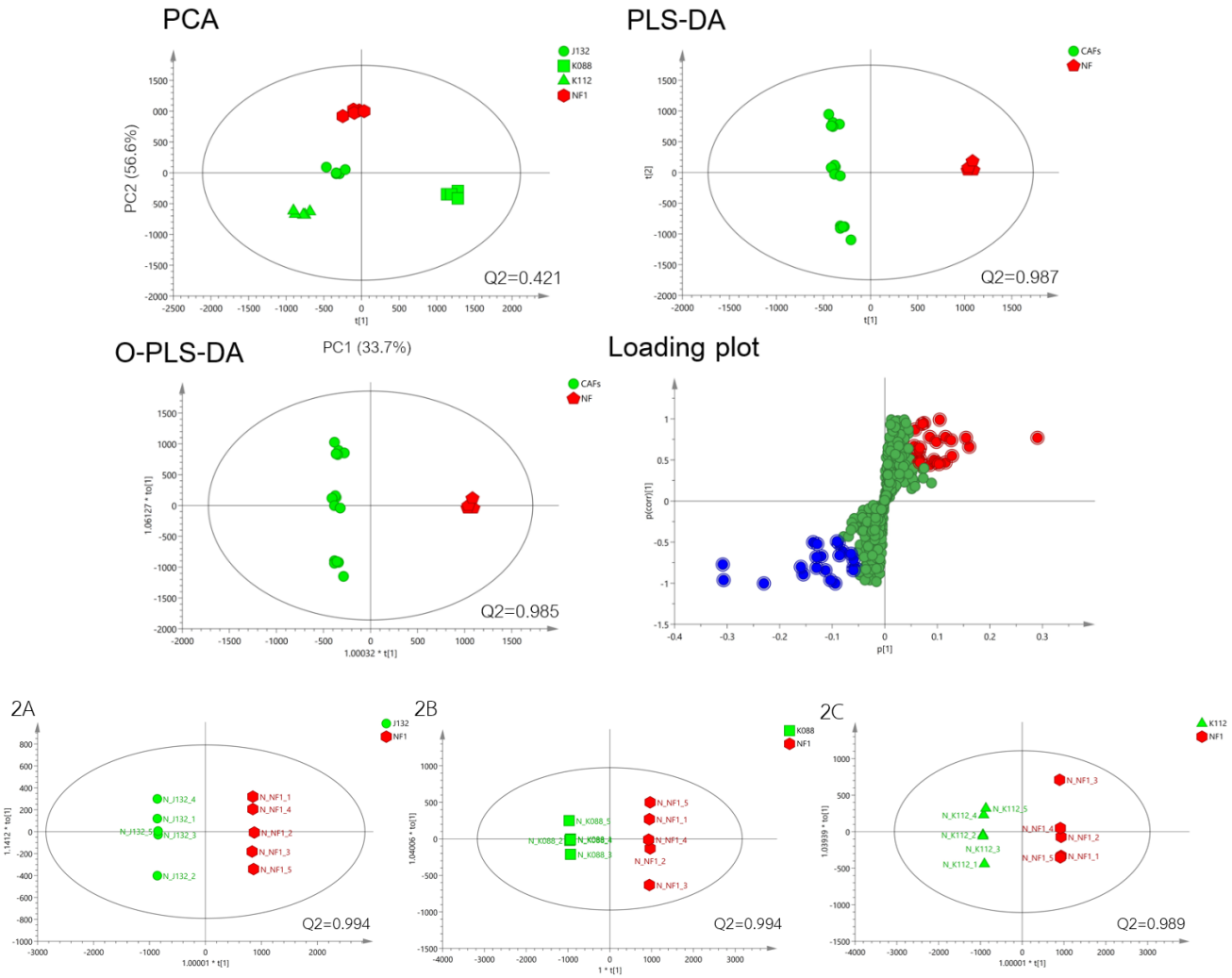

Figure 2 PCA, PLS-DA, and OPLS-DA score plots between CAFs and NF. Loading plot of OPLS-DA (PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; OPLS-DA, orthogonal partial least squares discriminant analysis), loading plot (S-plot) of p(1) and p(corr)(1) were construct for the OPLS-DA models using Pareto scaling.

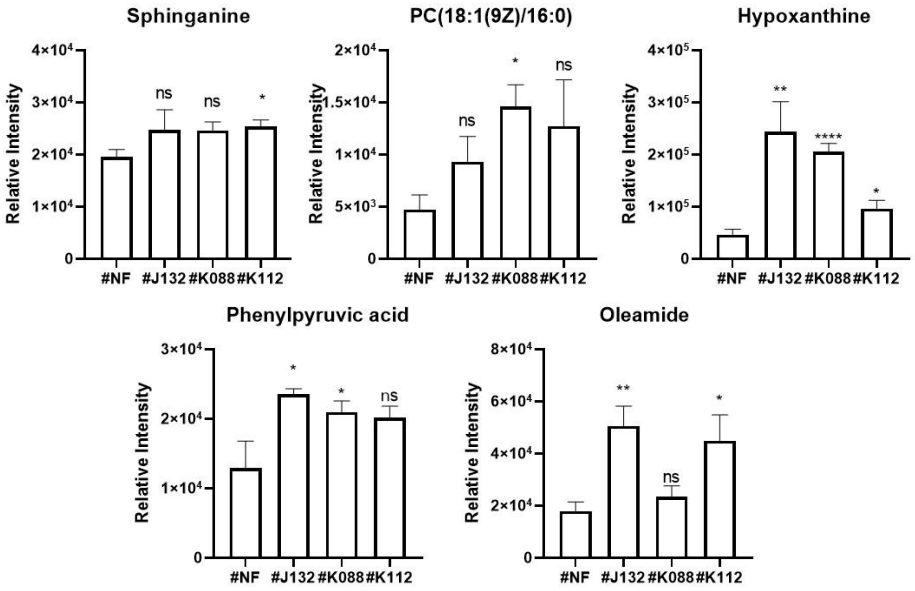

Figure 3 Relative intensity of significant metabolites in comparison of CAF and NF. Statistical significance was determined as *p < 0.05, **p < 0.01 and ****p < 0.0001, respectively. ns: no significant

Table 1 Model parameters for PCA, PLS-DA, and OPLS-DA. CV-ANOVA showed lower P values, indicating that the separation between the groups is significant.

Model	Model parameter	CAFs vs NF	#J132 vs NF	#K088 vs NF	#K112 vs NF
PCA	R_X^2 (cum)	0.566	0.727	0.747	0.730
PLS-DA	R_Y^2 (cum)	0.485	0.680	0.737	0.726
	Q^2 (cum)	0.987	0.990	0.996	0.994
	p value	8.61E-13	2.90E-05	1.20E-05	4.75E-05
O-PLS-DA	R_Y^2 (cum)	0.485	0.680	0.737	0.726
	Q^2 (cum)	0.985	0.994	0.994	0.989
	p value	1.55E-13	8.70E-06	9.62E-06	4.44E-05

R^2: a measure of model fit to the original data

Q^2: a measure predictive ability statistic of model

Table 2 Significant metabolites of CAF group compared with normal fibroblasts though multivariate analysis (O-PLS-DA model)

No.	Significant metabolites	Formula	Adduct	m/z	RT	p(corr)	Delta (ppm)
1	Sphinganine	$C_{17}H_{37}NO_2$	M+H	288.29	12.33	0.81	1
2	PC(18:1(9Z)/16:0)	$C_{42}H_{82}NO_8P$	M+H	760.59	15.50	0.81	3
3	Hypoxanthine	$C_5H_4N_4O$	M+H	137.04	1.35	0.77	1
4	Oleamide	$C_{18}H_{35}NO$	M+H	282.28	17.80	0.69	0
5	Phenylpyruvic acid	$C_9H_8O_3$	M+H	165.05	12.11	0.67	2
6	Unknown 1	$C_{11}H_{12}O$	M+H	161.10	12.12	0.99	-
7	Unknown 2	$C_{13}H_{14}O_2$	M+H	203.11	12.12	0.99	-
8	Unknown 3	$C_{17}H_{29}N_2O_3P$	M+H	341.20	12.40	0.97	-
9	Unknown 4	$C_{24}H_{27}N_3$	M+H	358.23	11.89	0.96	-
10	Unknown 5	$C_{17}H_{24}O_3$	M+H	277.18	12.12	0.90	-
11	Unknown 6	$C_{11}H_{15}NO_2$	M+H	194.12	1.35	0.84	-
12	Unknown 7	$C_{12}H_{14}O$	M+H	175.11	12.11	0.84	-
13	Unknown 8	$C_{16}H_{33}NO$	M+H	256.26	17.69	0.82	-
14	Unknown 9	$C_{17}H_{26}O_4$	M+H	295.19	12.98	0.80	-
15	Unknown 10	$C_{10}H_{18}N_6O$	M+H	239.16	12.97	0.71	-
16	Unknown 11	$C_{14}H_{20}O_3$	M+H	237.15	15.62	0.68	-

m/z, observed mass-to-charge ratio; RT, retention time (min)

Statistical significance was determined as *p(corr) $\leq$ 0.6.

Discussion and Conclusions

Several steps of cancer progression require the contribution of tumor microenvironment to support the continuous tumor growth, metastatic distribution and colonization (Chen and Song, 2019). Though, there are some studies showing normal fibroblast capabilities to inhibit cancer cell proliferation and motility *in vitro* (Stoker M. G., 1966) (Alkasalias *et al.*, 2014). However, cancer cells can stimulate and turn them into activated fibroblasts which significant in the progression *via* cross-feeding of numerous factors. Our study emphases the possibility of CAF metabolite production may enhance cancerous extension and existence. The results of metabolomics using multivariate analysis; PCA, PLS-DA, and O-PLS-DA revealed that phenylpyruvic acid, hypoxanthine, oleamide, phosphatidylcholines (PC; 18:1(9Z)/16:0), and sphinganine were increased in CAFs. Interestingly, sphinganine is precursor of sphingosine and sphingosine 1-phosphate (S1P) (Bertea *et al.*, 2010) which is a bioactive sphingolipid involved in many cellular processes, including cell proliferation, differentiation, motility (Maceyka *et al.*, 2012), and well-established properties on angiogenesis, carcinogenesis and immunity. S1P contributes in

a variety of signaling pathways such as several cytokines, growth factors, hormones and their receptors (Zhang *et al.*, 2013). Phosphatidylcholine (PC), the major phospholipid component of eukaryotic membranes, contributes to oncogenic transformation, cell proliferation and survival (Ridgway, 2013). Accumulating evidences revealed that PC(16:0/18:1) was localized in tumor regions of breast tumor tissues (Chughtai *et al.*, 2013), colorectal tissue (Mirnezami *et al.*, 2014), and gastric cancer tissues (Takashi Uehara and Mitsutoshi Setou, 2015) that were increased PC(16:0/18:1) concentrations in cancer tissue samples relatively to healthy tissues. Phenylpyruvic acid is a keto-acid that is an intermediate or catabolic byproduct of phenylalanine metabolism. In primary ovarian cancer found significant changes by increasing phenylpyruvate and phenyllactate in epithelial ovarian cancer compared to normal but phenylpyruvate decreased in metastatic compared to epithelial ovarian cancer. (Fong *et al.*, 2011). Oleamide is another lipid which have been considered as a new class of biological signaling molecule because of its role in cancer (Ruiying *et al.*, 2020) but the mechanism is unclear. Four metabolites including phenylpyruvic acid, oleamide, phosphatidylcholines (PC; 18:1(9Z)/16:0), and sphinganine were verified individually using unpaired t-test method, it was found that 4 metabolites were clearly increased in CAFs, however they showed significant levels in some cases. The reason is possibly due to each culture has different passages that reflect their metabolism. Remarkably, hypoxanthine showed its significant level in all cases of CAFs suggesting for the preferent metabolism in stromal cells. Hypoxanthine is a metabolite produced from the purine metabolic pathway. Hypoxanthine can be converted to inosine monophosphate (IMP) and adenosine 5'-monophosphate (AMP) and guanosine 5'-monophosphate (GMP) which are major purine nucleotides and plays a significant role in cellular metabolic processes as it is a precursor to adenosine-5'-triphosphate (ATP), energy metabolite. *De novo* purine biosynthetic pathway can improve in cancer cells because purine nucleotides are fundamental and necessary for cancer cell proliferation (Yin *et al.*, 2018). In summary, these metabolites that were significantly increased in CAFs showed a positive effect in cancer cell progression. CAF metabolites may secret and cross-feeding with cancer cells and implicate in hallmark of cancers. Therefore, CAF metabolism may be targeted for precise cancer therapy that could provide better survival outcomes.

Acknowledgements

This study was supported by Cholangiocarcinoma Research Institute (CARI), Faculty of Medicine, Khon Kaen University, Thailand.

References

Alkasalias T., Flaberg E., Kashuba V., Alexeyenko A., Pavlova T., Savchenko A., Szekely L., Klein G. and Guven H. Inhibition of tumor cell proliferation and motility by fibroblasts is both contact and soluble factor dependent. Proc Natl Acad Sci U S A 2014; 111(48): 17188-17193.

Bertea M., Rutti M.F., Othman A., Marti-Jaun J., Hersberger M., von Eckardstein A. and Hornemann T. Deoxysphingoid bases as plasma markers in diabetes mellitus. Lipids Health Dis 2010; 9: 84.

Chen X. and Song E. Turning foes to friends: targeting cancer-associated fibroblasts. Nat Rev Drug Discov 2019; 18(2): 99-115.

Chughtai K., Jiang L., Greenwood T.R., Glunde K. and Heeren R.M. Mass spectrometry images acylcarnitines, phosphatidylcholines, and sphingomyelin in MDA-MB-231 breast tumor models. J Lipid Res 2013; 54(2): 333-344.

D. A. Bronzert P.P., H. N. Antoniadest, A. Kasid, N. Davidson, R. B. Dickson, and M. E. Lippman. Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines. Cell Biology 1987; 84: 5763-5767.

Fong M.Y., McDunn J. and Kakar S.S. Identification of metabolites in the normal ovary and their transformation in primary and metastatic ovarian cancer. PLoS One 2011; 6(5): e19963.

Kwa M.Q., Herum K.M. and Brakebusch C. Cancer-associated fibroblasts: how do they contribute to metastasis? Clin Exp Metastasis 2019; 36(2): 71-86.

Lyssiotis C.A. and Kimmelman A.C. Metabolic Interactions in the Tumor Microenvironment. Trends Cell Biol 2017; 27(11): 863-875.

Maceyka M., Harikumar K.B., Milstien S. and Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012; 22(1): 50-60.

Mirnezami R., Spagou K., Vorkas P.A., Lewis M.R., Kinross J., Want E., Shion H., Goldin R.D., Darzi A., Takats Z., Holmes E., Cloarec O. and Nicholson J.K. Chemical mapping of the colorectal cancer microenvironment via MALDI imaging mass spectrometry (MALDI-MSI) reveals novel cancer-associated field effects. Mol Oncol 2014; 8(1): 39-49.

Nalbantoglu, S., 2019, 'Metabolomics: Basic Principles and Strategies', in S. Nalbantoglu, H. Amri (eds.), Molecular Medicine, IntechOpen, London. 10.5772/intechopen.88563.

Ohlund D., Elyada E. and Tuveson D. Fibroblast heterogeneity in the cancer wound. J Exp Med 2014; 211(8): 1503-1523.

Ridgway N.D. The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. Crit Rev Biochem Mol Biol 2013; 48(1): 20-38.

Ruiying C., Zeyun L., Yongliang Y., Zijia Z., Ji Z., Xin T. and Xiaojian Z. A comprehensive analysis of metabolomics and transcriptomics in non-small cell lung cancer. PLoS One 2020; 15(5): e0232272.

Santi A., Kugeratski F.G. and Zanivan S. Cancer Associated Fibroblasts: The Architects of Stroma Remodeling. Proteomics 2018; 18(5-6): e1700167.

Sha M., Jeong S., Qiu B.J., Tong Y., Xia L., Xu N., Zhang J.J. and Xia Q. Isolation of cancer-associated fibroblasts and its promotion to the progression of intrahepatic cholangiocarcinoma. Cancer Med 2018; 7(9): 4665-4677.

Shiga K., Hara M., Nagasaki T., Sato T., Takahashi H. and Takeyama H. Cancer-Associated Fibroblasts: Their Characteristics and Their Roles in Tumor Growth. Cancers (Basel) 2015; 7(4): 2443-2458.

Stoker M. G. S.M., O'Neill C. Growth inhibition of polyoma- transformed cells by contact with static normal fibroblasts. J. Cell Sci 1966; 1: 297-310.

Takashi Uehara H.K., Shinichiro Miyazaki, Ichirota Iino, Tomohiko Setoguchi, Yoshihiro Hiramatsu, Manabu Ohta, Kinji Kamiya, Yoshifumi Morita, Hiroki Tanaka, Satoshi Baba, Takahiro Hayasaka, and Mitsutoshi Setou a.H.K. Overexpression of lysophosphatidylcholine acyltransferase 1 and concomitant lipid alterations in gastric cancer. Ann Surg Oncol 2015.

Yin J., Ren W., Huang X., Deng J., Li T. and Yin Y. Potential Mechanisms Connecting Purine Metabolism and Cancer Therapy. Front Immunol 2018; 9: 1697.

Zhang L., Urtz N., Gaertner F., Legate K.R., Petzold T., Lorenz M., Mazharian A., Watson S.P. and Massberg S. Sphingosine kinase 2 (Sphk2) regulates platelet biogenesis by providing intracellular sphingosine 1-phosphate (S1P). Blood 2013; 122(5): 791-802.