Energy transfer from adipocytes to cancer cells in breast cancer

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Energy transfer from adipocytes to cancer cells in breast cancer

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The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Medical Science

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December 2016

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December 2016

ACKNOWLEDGEMENTS

I would like to express my special appreciation and thanks to my advisor Professor Ja seung Koo, you have been a mentor for me and always encouraged me to achieve my full potential. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been priceless. It is an honor for me to show my most sincere gratitude to professor Woo-Hee Jung, who continuously paid attention to my subject and advised me to improve my work. I also owe my deep gratitude to professor Yong Bae Kim for the advice to formulate my hypothesis. With all my heart, I appreciate professor Eunah Shin for the continuous encouragement to finish my subject. I wish to express my sincere thanks to professor Sangkyu Park, who helped me to perceive the major concept of my subject.

I would especially like to thank all professors and residents in the Department of Pathology, Yonsei University College of Medicine. All of you have been there to support me when I collected specimens and data for this thesis.

A special thanks to my family. Words cannot express how grateful I am to my parents, sister and parents-in law for all of the sacrifices that you've made on my behalf. At the end I would like express appreciation to my beloved husband and son who were always my emotional support in the moments when there was no one to answer my queries.

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ABSTRACT

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Limitations of current therapeutic approach have raised the need for a novel therapeutic agent in breast cancer. Recently, interest in drugs targeting the tumor microenvironment (TME) had drawn attention in the treatment of breast cancer. Furthermore, recent studies have suggested the role of adipocyte, which consist the TME, in tumor initiation, growth, and metastasis. In this study, we investigated the metabolic interaction between adipocytes and breast cancer cells and its potential as a new therapeutic target in breast cancer. Breast cancer cell lines and human breast cancer tissue samples were evaluated. Compared to cancer cells cultured alone, or the control group, those co-cultured with adipocytes showed lipid transfer from adipocytes to cancer cells and it was different according to the molecular subtype of breast cancer. Breast cancer cells affected the lipolysis of adipocytes and adipocytes affected the ß-oxidation of breast cancer cells. The key molecule of the process was fatty acid binding protein 4 (FABP4), which is combined with free fatty acid (FFA) and supports its migration to cancer cells. When FABP4 was suppressed, lipid transfer between adipocytes and cancer cells, lipolysis of

adipocytes and ß-oxidation of breast cancer cells were reduced. Furthermore, the expression of lipid metabolism-related proteins and lipolysis-related proteins in breast cancer with adipose stroma showed significantly different expression according to the region of breast cancer tissue.

Taken together, we demonstrated the metabolic interaction between adipocytes and breast cancer cells. Breast cancer cells increase the lipolysis in adipocytes and produces fatty acid, and fatty acid enters into cancer cells. Also, adipocytes contribute to the survival and growth of cancer cells through increased mitochondrial β-oxidation by using fatty acid from adipocytes. The key molecule of the process is FABP4 and when FABP4 is suppressed, the metabolic interaction is reduced, suggesting its role as a potential therapeutic target.

Key words: energy transfer, breast cancer, adipocyte, FABP4

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I. INTRODUCTION

Breast cancer is the most common type of cancer in women worldwide. Approximately 1.2 million cases of breast cancer are diagnosed annually. However, due to the large heterogeneity of the disease, no single established treatment yet exists. Even though the introduction of hormonal therapy based on the estrogen receptor (ER) or progesterone receptor (PR) status or target therapy according to the human epidermal growth factor type 2 (HER-2) status lead to an advance in the treatment of breast cancer, up to 20% of patients eventually experience disease progression and even death[1]. Furthermore, triple negative breast cancer (TNBC), which shows negative ER, PR, and HER-2 expression is associated with extremely poor prognosis as effective targeted therapy is not available[2]. Limitations of current therapeutic approach raised the needs for novel therapeutic agent and recently, targeting the TME, not the tumor itself, has evolved as a candidate of therapeutic target. Unlike tumor cells, the TME

includes the peri-tumoral element. In breast cancer, fibrous stroma or adipose stroma surrounds the tumor cells. Normally, volume constituted by adipocytes in the entire breast is 7-56% and are accounted for 3.6-37.6% of the entire breast weight[3], and it account for the major component in breast cancer[4,5]. However, TME studies in breast cancer were mainly performed only in fibrous stroma, especially cancer-associated fibroblast (CAF)[6-9]. Ketone bodies and lactate formed through glycolysis in CAF enters cancer cells and produce adenosine triphosphate (ATP) effectively through oxidative phosphorylation in the mitochondria of cancer cells, which as a whole contributes to their survival and growth[10-13]. However, not only CAF but also the role of adipocyte in tumor initiation, growth, and metastasis has been reported. The role of adipocyte in tumorigenesis is referred as "adiponcosis"[14], and in a study of ovary cancer, lipolysis in adipocytes and mitochondrial β-oxidation in cancer cells by the lipid transfer and the interaction between ovary cancer cells and omental adipocytes were reported[15]. Based on the finding of this study, in vitro and in vivo studies in breast cancer showed that adipocyte enhances tumor growth[16,17] or cancer cell proliferation[17,18]. Furthermore, one study showed by using a breast cancer cell line MCF-7, that insulin like growth factor binding protein 2 secretion by adipocyte stimulates breast cancer invasion[19]. Therefore, in this study, we investigated the metabolic interaction between adipocytes and breast cancer cells and its potential as a new therapeutic target in breast cancer.

II. MATERIALS AND METHODS

1. Culture and treatment of breast cancer cell lines

The general information of breast cancer cell lines used in this study is shown in Table 1. MDA-MB-453, MDA-MB-435S, MDA-MB-231, and MDA-MB-468 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (Hyclone) in a humidified of 5% CO^2 atmosphere. MCF-7 cells were cultured in DMEM with no phenol red (Life Technology, Grand Island, NY, USA) supplemented with 10% FBS, 1% penicillin-streptomycin, and 0.1 mg/ml insulin.

Table 1. General information of used breast cancer cell lines

	MCF-7	MDA-MB-453	MDA-MB-435	MDA-MB-231	MDA-MB-486
Organism	Homo sapiens, human	Homo sapiens, human	Homo sapiens, human	Homo sapiens, human	Homo sapiens, human
Tissue	Mammary gland, breast; derived from metastatic site: pleural effusion	Mammary gland/breast; derived from metastatic site: pericardial effusion	Previously described as: mammary gland/breast; derived from metastatic site: pleural effusion	Mammary gland/breast; derived from metastatic site: pleural effusion	Mammary gland/breast; derived from metastatic site: pleural effusion
Disease	Adeno-carcinoma	Metastatic carcinoma	Previously described as ductal carcinoma	Adeno-carcinoma	Adeno-carcinoma
Cell type	Epithelial		Melanocyte, Melanoma	Epithelial	
Gender	Female	Female	Female	Female	Female
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Black
Molecular subtype	Luminal	HER-2	Basal-like	Claudin-Low	Molecular apocrine

2. Differentiation of 3T3-L1 cells to mature adipocytes

3T3-L1 preadipocytes were purchased from the American Type Culture Collection (ATCC, Chicago, IL; Cat no. CL-173). Cells were maintained in DMEM containing 10% fetal calf serum (Life technology) until adipocyte differentiation. 3T3-L1 preadipocyte cell line was differentiated as previously report[20]. To confirm mature adipocyte differentiation, Oil Red O staining was conducted and immunoblotting was performed with antibodies for adipogeniesis markers. FABP4 inhibitor (BMS309403) was purchased from Cayman chemical and treated in the experiments at 30 μM for the indicated time.

3. Co-culture of breast cancer cells with mature adipocytes

Breast cancer cells and mature adipocytes were co-cultured using Costar Transwell culture vessels (0.4 μm pore size, Corning, NY. USA) for the indicated time. Breast cancer cells were seeded in the upper chamber and co-cultured with or without mature adipocytes at day 10 after induction of differentiation in the lower chamber.

4. Cell proliferation test

To test cell proliferation, breast cancer cell lines were co-cultured with or without mature adipocytes for 48 h, trypsinized, and stained with 0.4% Trypan blue stain solution (Life technology). Viable cells were counted using a homeocytometer by a least three indifferent experiments.

5. Immunocytochemistry and Confocal microscopy

Breast cancer cells were seeded on the coverslips and cultured with or without mature adipocytes. After 2 days of co-culture, cells were fixed with 4% paraformaldehyde for 20 min and permeabilized with 0.2% Triton X-100 for 10

min at room temperature, then washed with PBS. The cells were incubated in blocking solution (5% normal donkey serum in PBS) for 1 h and then incubated with primary antibody in PBS for 1 h 30 min at room temperature. After washing with PBS containing 0.1% Tween-20, the cells were incubated with a second antibody conjugated with a fluorescent dye for 1 h at room temperature. The cells were counterstained with Hoechst 33342 (Invitrogen, Carlsbad, CA) and mounted on slides with mounting medium (Dako, Glostrup, Denmark). Images were acquired under an LSM 700 META confocal laser scanning microscope equipped with epifluorescence and a digital image analyzer (Carl Zeiss, Zena, Germany). Z-stacked images were acquired at 400 x magnification.

6. Quantitative real-time polymerase chain reaction (PCR)

Total RNA of cultured cells was isolated using RNeasy Mini Kit according to the manufacturer's instructions (Quiagen, Valencia, CA). Real-time PCR (RT-PCR) was performed using the One Step SYBR PrimeScriptTM RT-PCR Kit (Takara Shuzo Co, Japan) according to the manufacturer's instructions. The relative mRNA expression levels of target gene were normalized to the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). All primers of target genes were designed using Primer 3 software. Primer sequences are described in Table 2.

Table 2. Primer sequences used for quantitative real-time PCR

Gene	Sequences	Tm
CPT1a	F: TCAGGCCTATTTTGGACGTG R: TGACGTATCCGGGTCTTCAC	60°C
ACOX1	F: TCTTCACTTGGGCATGTTCC R: CCCATCTCTGTCTGGGCATA	60°C
FABP4	F: CATACTGGGCCAGGAATTTG R: TGGTGGTTGATTTTCCATCC	60°C

Abbreviations: CPT1a, carnitine palmitoyltransferase 1a; ACOX1, Acyl-CoA oxidase 1; FABP4, fatty acid binding protein 4.

7. Immunoblotting

The cells were harvested and lysed in RIPA buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, and 1 mM EDTA, 0.1% SDS) containing protease inhibitors. Cellular lysates were centrifuged at 13,000 x g for 15 min at 4°C. The protein concentration of each cell lysate was measured via the BCA assay (Thermo-Scientific), and an equal amount of protein from each sample extract was separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and blotted onto nitrocellulose membranes (Bio-Rad). Western blots were performed as previously described[21], and specific bands were detected using ECL solution kit (GE Healthcare Life Sciences).

8. Human breast cancer tissue selection

We included only those patients diagnosed with invasive breast cancer, and underwent breast cancer surgery at Severance Hospital from January 2002 to December 2006. Patients who received hormonal therapy or chemotherapy prior to surgery were excluded. This study was approved by the Institutional Review Board of Yonsei University Severance Hospital. All slides were retrospectively reviewed by a breast pathologist (Koo JS) and histological evaluation was conducted by hematoxylin and eosin (H&E)–stained slides. The histological grade was assessed using the Nottingham grading system. Clinicopathologic parameters evaluated in each breast cancer included patient age at initial diagnosis, tumor size, lymph node metastasis, tumor recurrence, distant metastasis, and patient's survival.

9. Tissue Microarray

On H&E-stained slides of cancers, a representative area was selected and a corresponding spot was marked on the surface of the paraffin block. Using a biopsy needle, the selected area was punched out and a 3-mm tissue core was placed into a 6 x 5 recipient block. Tissue of invasive cancer was extracted. Two tissue cores were extracted to minimize extraction bias. Each tissue core was assigned with a unique tissue microarray location number that was linked to a database containing other clinicopathologic data.

10. Immunohistochemistry (IHC)

The antibodies used for IHC in this study are shown in Table 3. All IHC staining was conducted using formalin-fixed, paraffin-embedded (FFPE) tissue sections. Briefly, 5-□m-thick sections were obtained with a microtome, transferred into adhesive slides, and dried at 62°C for 30 minutes. After incubation with primary antibodies, immunodetection was performed with biotinylated antimouse immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3′-diaminobenzidine chromogen as substrate. The primary antibody incubation step was omitted in the negative control. Slides were counterstained with Harris hematoxylin.

Table 3. Source, clone, and dilution of used antibodies

Antibody	Clone	Dilution	Company
Molecular subtype-related			
ER	SP1	1:100	Thermo Scientific, CA, USA
PR	PgR	1:50	DAKO, Denmark
HER-2	Polyclonal	1:1500	DAKO, Denmark
Ki-67	MIB-1	1:1000	Abcam, Cambridge, UK

Lipolysis-related			
HSL	Polyclonal	1:100	Abcam, Cambridge, UK
Perillipin A	Polyclonal	1:100	Abcam, Cambridge, UK
FABP4	Polyclonal	1:100	Abcam, Cambridge, UK
CPT-1	8F6AE9	1:200	Abcam, Cambridge, UK
Acyl-CoA oxidase 1	Polyclonal	1:50	Abcam, Cambridge, UK
FAS	Polyclonal	1:200	Abcam, Cambridge, UK

Abbreviations: ER, progesterone receptor; PR, progesterone receptor; HER-2, human epidermal growth factor type 2; HSL, hormone-sensitive lipase; FABP4, fatty acid binding protein 4; CPT-1, carnitine palmitoyltransferase-1; FAS, fatty acid synthase.

11. Interpretation of IHC staining

All IHC markers were accessed by light microscopy. Pathologic parameters such as ER, PR, and HER-2 status were obtained from patients' pathologic report. A cut-off value of 1% or more positively stained nuclei was used to define ER and PR positivity. HER-2 staining was analyzed according to the American Society of Clinical Oncology / College of American Pathologists guidelines using the following categories: 0 = no immunostaining; 1+ = weak incomplete membranous staining, less than 10% of cancer cells; 2+ = complete membranous staining, either uniform or weak in at least 10% of cancer cells; and 3+ = uniform intense membranous staining in at least 30% of cancer cells. HER-2 immunostaining was considered positive when strong (3+) membranous staining was observed whereas cases with 0 to 1+ were regarded as negative. The cases showing 2+ HER-2 expression were evaluated for HER-2 amplification by Fluorescent in situ hybridization.

Acyl-CoA oxidase 1 (ACOX1), carnitine palmitoyltransferase-1 (CPT1), FABP4, hormone-sensitive lipase (HSL), and Perillipin 1 (PLIN1) IHC staining results were determined by the multiplication of the proportion of stained cells and immunostaining intensity. The proportion of stained cells was scored as 0 for negative, 1 for positive with less than 30% of the cells stained, and 2 for positive with greater than or equal to 30% of the cells stained. The immunostaining intensity was scored as 0 for negative, 1 for weak, 2 for moderate, and 3 for strong. A product between 0 and 1 was regarded as negative and a product between 2 and 6 as positive. Ki-67 labeling index (LI) was defined as the percentage of the cells with nuclear expression among cancer cells.

12. Protein extraction from FFPE tissues and Western blot

Protein extractions from FFPE tissues were performed using the Qproteome FFPE tissue kit (Qiagen, Hilden, Germany). Briefly, two or three sections from the same block were deparaffinized in xylene and rehydrated in graded series of alcohol. The tissues were mixed with FFPE extraction buffer containing β-mercaptoethanol, incubated at 100℃ for 20 min, at 80℃ for 2 h with agitation at 750 rpm and then centrifuged for 15 min at 14,000 x g at 4℃. The supernatant containing the extracted proteins were determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA). Equal amount of protein from each sample extract was separated on SDS-PAGE gels and blotted onto nitrocellulose membranes (Bio-Rad). Western blotting was performed with primary antibodies against PLIN1, CPT1A, HSL, FABP4, FAS, and β-actin (Abcam, Cambridge, UK), and specific bands were detected using the enhanced chemiluminescene kit (GE Healthcare Life Sciences, Little Chalfont, UK).

13. Statistical Analysis

Data were processed using SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). Student's t and Fisher's exact tests were used to examine any difference in continuous and categorical variables, respectively. Significance was assumed when $p < 0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor metastasis and time to survival. Multivariate regression analysis was performed using Cox proportional hazards model.

III. RESULTS

1. Selection and induction of differentiation of adipocytes which would be co-cultured with breast cancer cells

The adipocytes which would be co-cultured with breast cancer cells were classified into two groups: primary adipocyte (RM, reduction mammoplasty; TU, tumorectomy) and adipocyte cell line (3T3-L1, 3T3-F442A), and cultured. As a result, 3T3-L1 of adipocyte cell line was selected on account of the relatively long period of subculture and good reproducibility. Three to 14 days after the induction of differentiation by treatment with insulin, dexamethasone and 3- isobutyl-1-methylxanthine to 3T3-L1 cells, the differentiation to mature adipocyte was confirmed with oil red O staining and western blotting. In this study, adipocytes were used 10 days after the induction of differentiation (Figure 1 and 2).

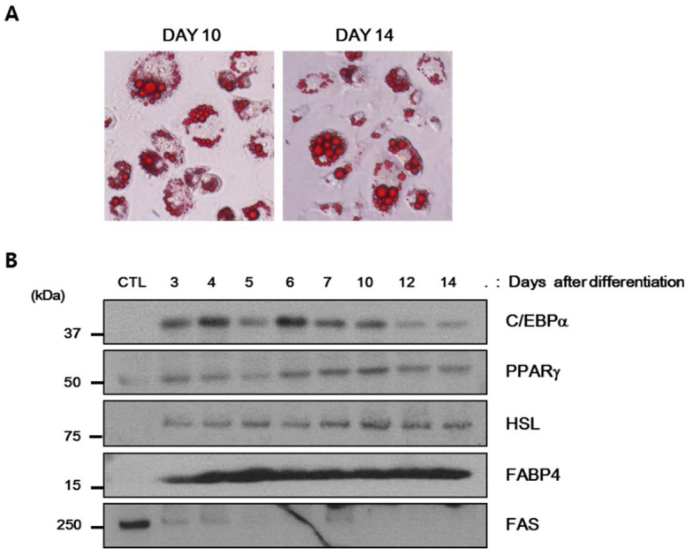


Figure 1. Differentiation of 3T3-L1 cells to the mature adipocytes. (A) At DAY10 and DAY14 after induction of differentiation, mature adipocytes were stained with oil red O and visualized by inverted microscope. (B) After differentiation at the indicated time, cells were lysed and blotted with the

indicated antibodies. Anti-C/EBPa and PPARg antibodies were used as adipogenesis markers.

Figure 2. The schematic representation of co-culture system.

2. Lipid transfer from adipocytes to cancer cells according to the breast cancer molecular subtypes

In this study, breast cancer cells were co-cultured with adipocytes by indirect method using a Transwell. Lipid transfer from adipocytes to cancer cells

according to the molecular subtypes of breast cancer was analyzed by Bodipy 493/503 which stains neutral fat. Compared to cancer cells cultured alone, or the control group, those co-cultured with adipocytes produced more neutral fat (Figure 3A). Compare to the control group, MDA-MB-231 cells about 12 times or more, MDA-MB-435S cells about 4-5 times or more, and MCF-7 and MDA-MB-453 cells about 2-3.6 times or more frequently showed lipid transfer. Therefore, the more frequent lipid transfer takes place in TNBC cells (Figure 3B). Furthermore, cell growth was faster when cancer cells co-cultured with adipocytes than when cultured alone (Figure 3C).

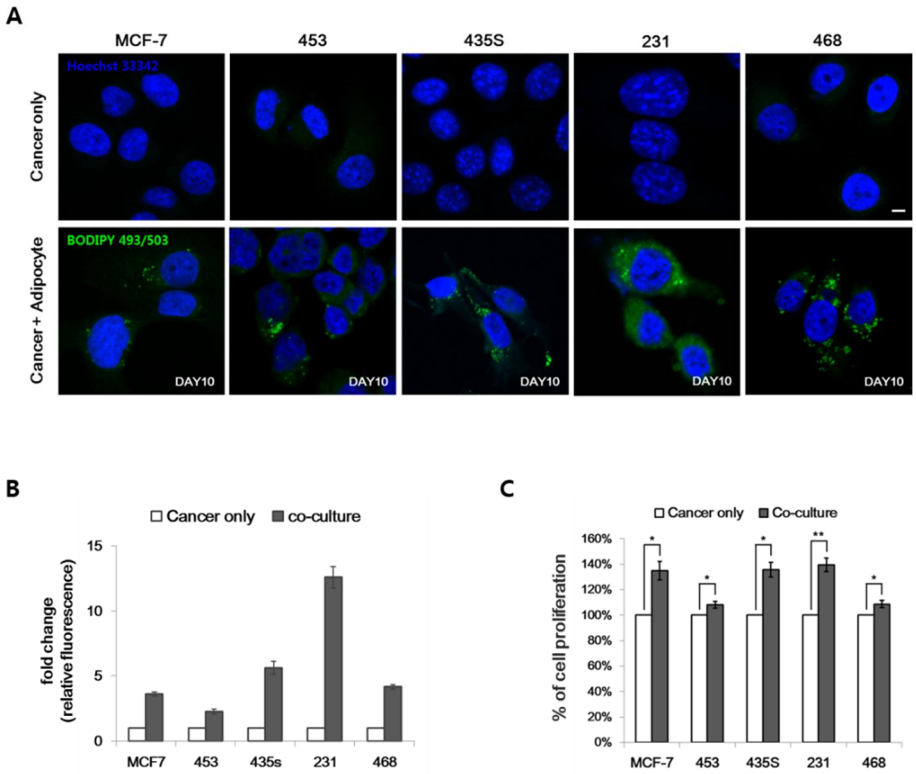
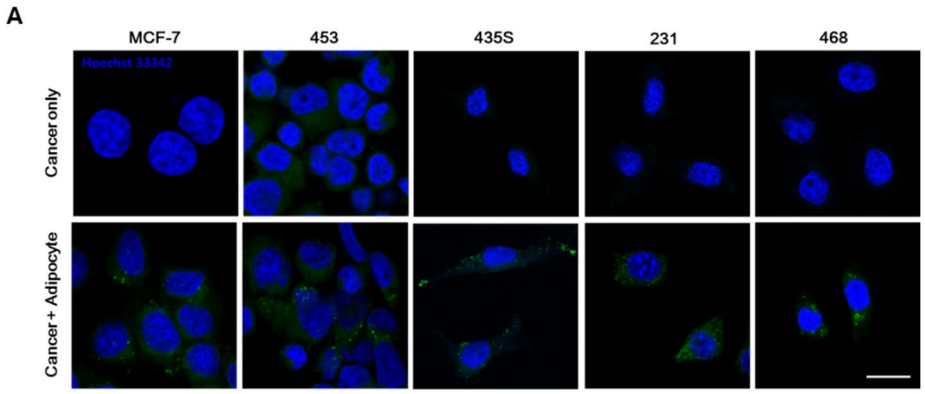


Figure 3. Co-culture of breast cancer cells with mature adipocytes induces lipid transfer into human breast cancer cells. Breast cancer cells were grown on coverslips and co-cultivated with or without mature adipocytes (DAY10) for 48

h. The cells were fixed and stained with BODIPY 493/503 (green). Images were acquired under confocal microscope (A) and relative fluorescence was quantitated by Image J (B). Scale bar, 20mm. (C) Five subtypes of breast cancer cells were co-cultured with mature adipocytes for 48 h and stained with Trypan blue. Viable cells were counted using a hemocytometer. Bars represent the mean ± S.D. of three independent experiments. **p < 0.01; *p < 0.05.

3. The origin of lipid located in breast cancer cells

After the uptake of fluorescent dodecanoic acid analog in adipocytes using Fatty acid uptake assay kit (Molecular Device, CA, USA), adipocytes with fluorescent-labeled lipid were co-cultured with breast cancer cells. We produced the fluorescent image from cancer cells with confocal microscope. As a result, it was confirmed that the fluorescent signal increased in cancer cells (Figure 4A). The signal from 4 cells excluding MDA-MB-453 cells was highly increased about 5-11 times, and that from MDA-MB-231 cells proved the largest augmentation (11.26 times) similar to that in lipid transfer (Figure 4B).



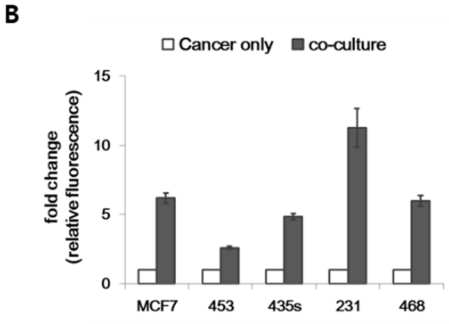


Figure 4. Fatty acid uptake assay. Fluorescence-labeled fatty acids were taken up by mature adipocytes, and the labeled fatty acids were transferred from adipocytes to breast cancer cells. Images were acquired under confocal microscope (A) and relative fluorescence was quantitated by Image J (B). Scale bar, 100mm. Bars represent the mean ± S.D. of three independent experiments.

4. The effect of breast cancer cells on the lipolysis of adipocytes according to the breast cancer molecular subtypes

In results shown above, FFAs derived from adipocytes were increased when breast cancer cells were co-cultured with adipocytes. It suggests the possibility that triglycerides stored in adipocytes were hydrolyzed into FFAs and glycerol. Therefore, we compared the amount of FFAs and glycerol with LIPOLYSIS ASSAY Kit (Zenbio, NC, USA) of two groups: adipocytes cultured alone (the control group) and adipocytes separated after co-culture with breast cancer cells according to the molecular subtypes. As a result, the amount of FFAs and glycerol was greater in the group co-cultured cancer cells with adipocytes than that of each group cultured cancer cells alone or adipocytes alone (Figure 5A and 5B).

For the lipolysis in adipocytes to take place, the activation of ß-adrenergic receptor stimulation and G protein-coupled cascade leading the phosphorylation of HSL and perilipin 1, and consequent hydrolysis of triglyceride is necessary.

Therefore, we analyzed the difference of phosphorylation of HSL between adipocytes cultured alone and of those separated after co-culture with breast cancer cells. Consequently, it was confirmed that significant increase of the phosphorylation of HSL is showed in adipocytes co-cultured with cancer cells from cell lines except MDA-MB-453 cells (Figure 5C and 5D).

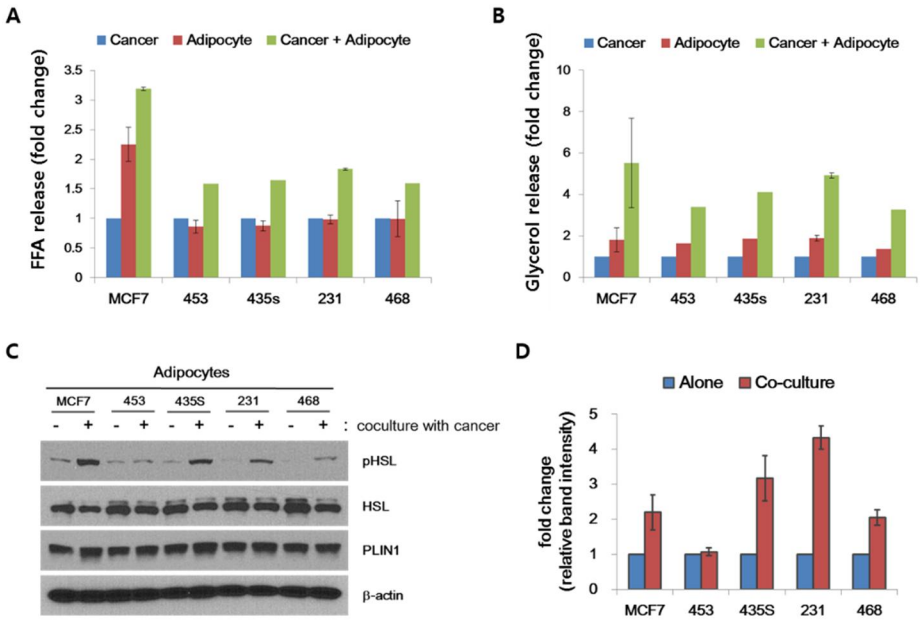


Figure 5. Co-culture of breast cancer cells with mature adipocytes enhances lipolysis in adipocytes. Free fatty acids (A) and glycerol (B) were detected by lipolysis assay in mature adipocytes. (C) Adipocytes were co-cultured with or without breast cancer cells for 48 h and then performed by immunoblotting with the indicated antibodies. (D) Phosphorylated HSL (pHSL, Ser660) was quantitated by Image J. Bars represent the mean ± S.D. of three independent experiments.

5. The effect of adipocytes on the ß-oxidation of breast cancer cells

As lipolysis in adipocytes was increased when co-cultured with breast cancer

cells, we further investigated the metabolic changes in cancer cells. AMP-activated protein kinase (AMPK) is a kinase playing the role as a switch of main metabolism. When AMPK is phosphorylated, the phosphorylation of acetyl-CoA carboxylase as the sub-step of AMPK phosphorylation takes place. As a result, CPT1a transports the fatty acid into the mitochondria, and ß-oxidation will occur. Thus, we compared AMPK phosphorylation of two groups (breast cancer cells cultured alone and breast cancer cells separated after co-culture with adipocytes) to analyze the effect of adipocytes on ß-oxidation of breast cancer cells. It was confirmed that AMPK phosphorylation was significantly increased in cancer cells co-cultured with adipocytes (Figure 6A). In addition, the difference of CPT1a and ACOX1 protein expression level in cancer cells co-cultured with adipocytes was investigated with western blotting. As a result, CPT1a protein expression level was evidently increased in MDA-MB-435S cells. However, the level was not increased in other breast cancer cell lines and ACOX1 protein expression level showed no significant difference in cancer cells co-cultured with adipocytes compared to that in the control group (Figure 6B, left). In consequence, we analyzed CPT1a and ACOX1 mRNA expression level, and identified that CPT1a and ACOX1 mRNA expression level which showed an increase in MDA-MB-435S and MDA-MB-231 cells co-cultured with adipocytes (Figure 6B, right). In MCF-7 cells, the mRNA and protein expression level of CPT1a and ACOX1 was not correlated to the presence of co-cultured adipocytes, but there was a notable different staining pattern with confocal microscopy. When cancer cells cultured alone, CPT1a was distributed over the whole cell. On the other hand, it was accumulated in specific area in the cancer cells co-cultured with adipocytes. Also ACOX1 showed similar pattern with CPT1a and the fluorescence signal was increased in cancer cells co-cultured with adipocytes. In MDA-MB-435S cells, CPT1a was accumulated in specific area within the cells (Figure 6C).

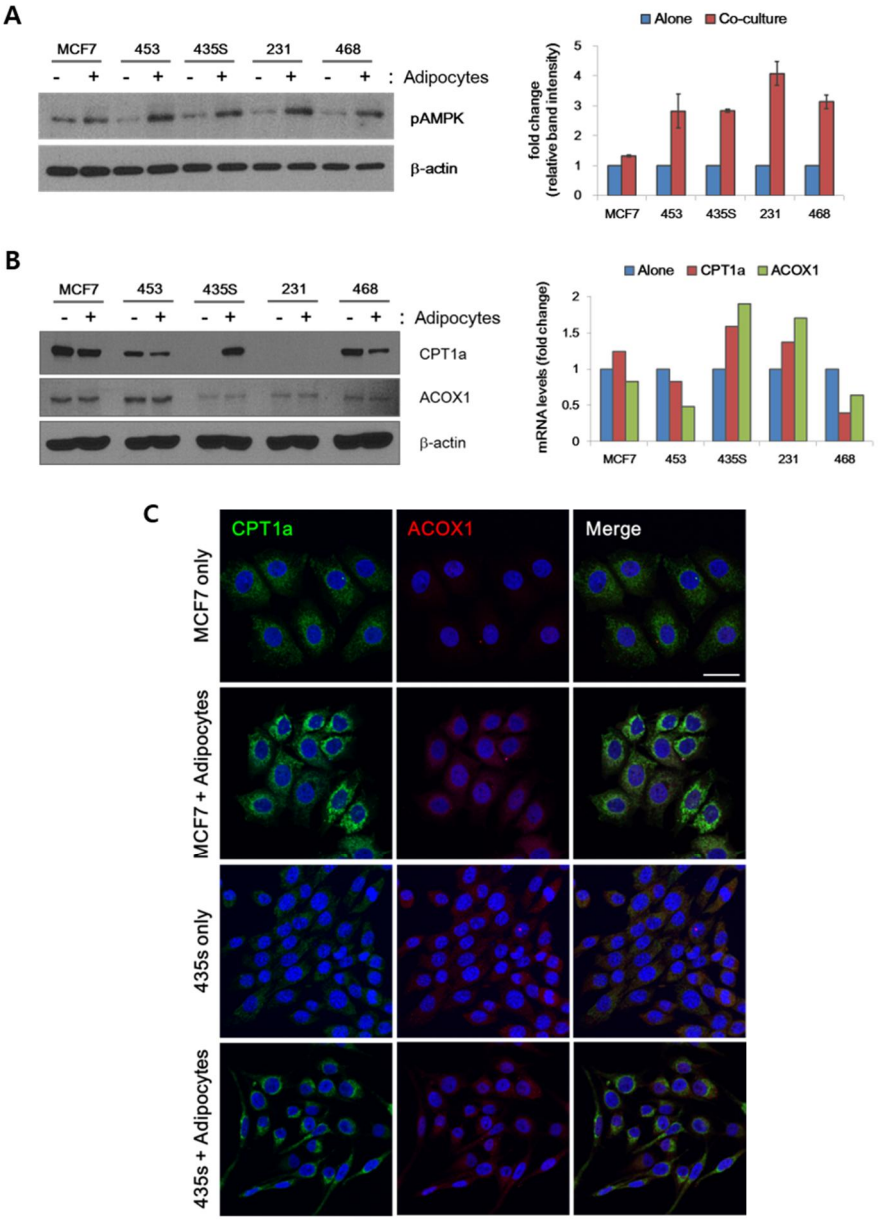


Figure 6. During co-culture, adipocytes activate β-oxidation in cancer cells. (A) Five subtypes of breast cancer cells were co-cultured with mature adipocytes for 48 h. Phosphorylation of AMPK was determined by immunoblotting (left) and

relative band intensity was quantitated by image J (right). (B) The expression of CPT1a and ACOX1 was detected by immunoblotting (left). Relative mRNA expression of CPT1a and ACOX1 was analyzed by real-time PCR (right). (C) Co-culture with adipocytes had concentrated expression of CPT1a and ACOX1 in MCF7, and increased CPT1a expression in MDA-MB-435s cells. Scale bar, 100mm.

6. Co-culture of adipocyte and normal breast cell line

To confirm that these results were specific to breast cancer cell line, we co-cultured a normal breast cell line, MCF10A mammary epithelial cell line with adipocytes. Afterwards, lipid transfer and ß-oxidation was evaluated by isolating MCF10A. Lipid transfer was not noticeable in MCF10A co-cultured with adipocytes as well as MCF10A cultured alone (Figure 7A). In addition, MCF10A showed no changes in the expression of CPT1a, ACOX1, and pAMPK regardless of co-culture with adipocytes (Figure 7B), showing that adipocytes do not affect ß-oxidation of normal breast cell line, MCF10A. These finding suggest that the metabolic interaction between adipocytes and breast cancer cells is specific in breast cancer line, and in further experiments, MCF10A cell line was used as a negative control.

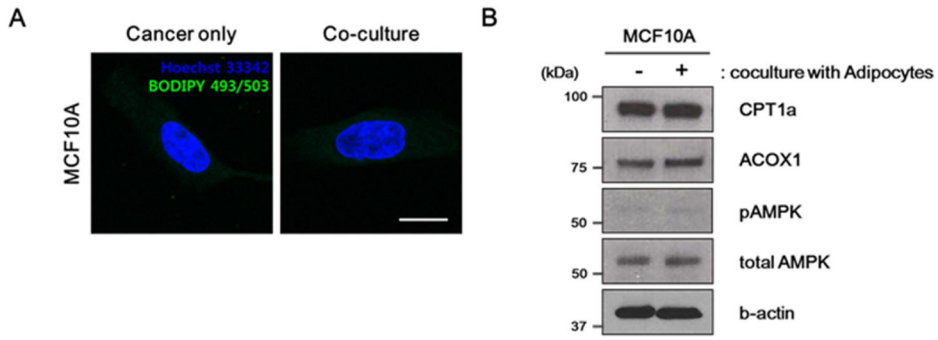


Figure 7. Co-culture with mature adipocytes does not affect both lipid transfer and β-oxidation in MCF10A cells. (A) MCF10A cells were grown on coverslips

and co-cultivated with or without mature adipocytes for 48 h. The cells were fixed and stained with BODIPY 493/503 (green). Images were acquired under confocal microscope. Scale bar, 100 mm. (B) The expression of CPT1a, ACOX1, pAMPK, total AMPK, and β-actin was detected by immunoblotting.

7. The expression of FABP4 in breast cancer cell lines

The expression of FABP4 in various breast cancer cell lines was examined. As a result, all breast cancer cell lines showed higher expression in the case of cancer cells co-cultured with adipocytes than those cultured alone (Figure 8A and 8B). The FABP4 mRNA level was higher in cancer cells co-cultured with adipocytes than those cultured alone in MCF-7, MDA-MB-453, and MDA-MB-468 (Figure 8C).

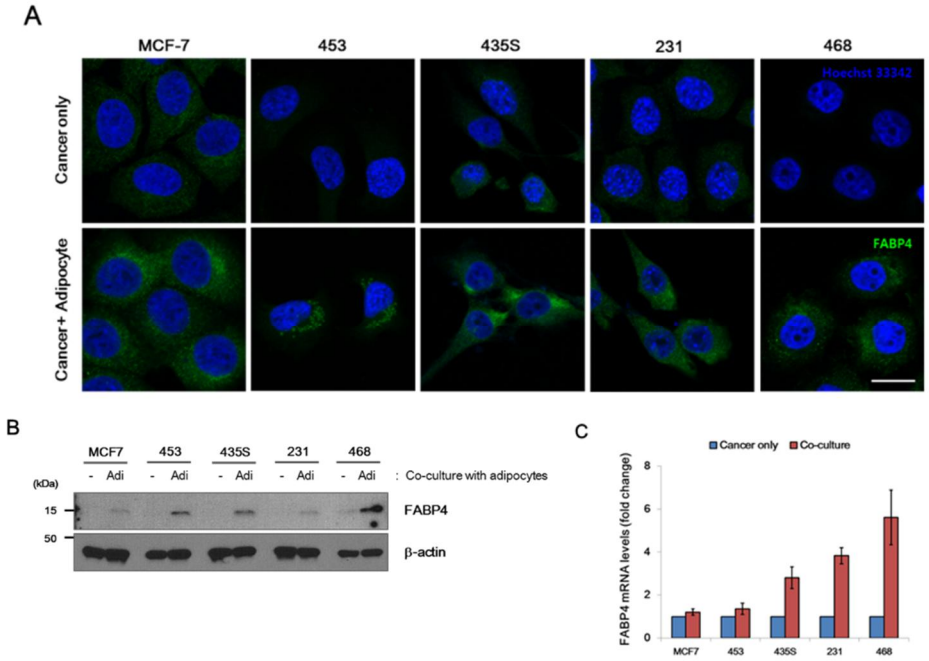
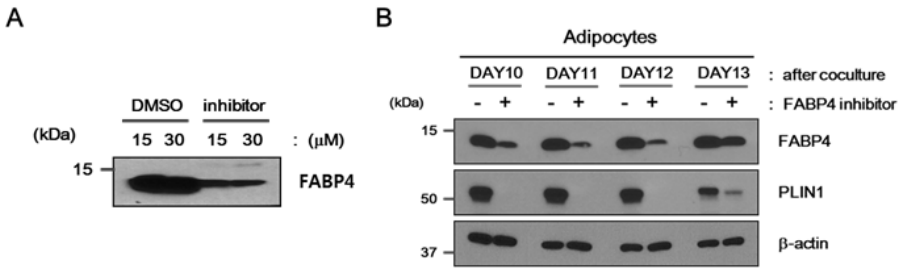


Figure 8. Characterization of FABP4 expression in co-cultured breast cancer cells with mature adipocytes. (A) Five subtypes of breast cancer cells were

grown on coverslips and co-cultured with or without mature adipocytes (DAY10) for 48 h. Breast cancer cells were isolated, fixed, and stained with anti-FABP4 antibody (green). Images were acquired under confocal microscope. Scale bar, 100 mm. (B) The expression of FABP4 was determined by immunoblotting. (C) Relative mRNA expression of FABP4 was analyzed by real-time PCR.

8. Inhibition of FABP expression in adipocytes using FABP4 inhibitor

Incubation of FABP4 inhibitor during 3T3-L1 adipocyte differentiation was performed in different condition (data not shown), and the expression of FABP4 was significantly inhibited when 30mM of FABP4 inhibitor was administered on day 6 (Figure 9A). Adipocyte that was used for co-culture in this study was mature adipocyte which was on day 10 after differentiation. As FABP4 inhibitor which was administered on day 6 should suppress FABP4 expression until day 12, we evaluated the expression of FABP4 on day 10, 11, 12, and 13 after administration of FABP4 on day 6. Our result showed that from day 10 to day 12, FABP4 and PLIN1 expression was reduced (Figure 9B), and oil red O staining also showed that lipid droplet within the adipocyte was significantly reduced (Figure 9C).



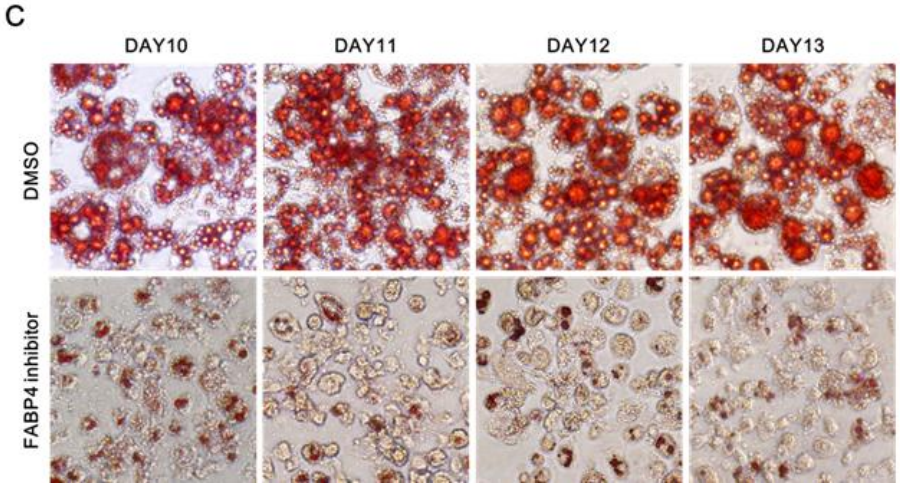


Figure 9. Effect of FABP4 inhibitor on differentiated adipocytes. (A) At DAY6 after induction of differentiation, adipocytes were treated with FABP4 inhibitor or DMSO for 48 h. The cell lysates were subjected to SDS-PAGE and analyzed by immunoblotting. (B and C) FABP4 inhibitor treated adipocytes were maintained for the indicated time. The cells were lysed and blotted with indicated antibodies (B), or stained with oil red O and visualized by inverted bright field microscope (C).

9. The effect of FABP4 inhibition on lipid transfer between adipocytes and breast cancer cells

FABP4 inhibitor (BMS309403) was used to inhibit the expression of FABP4 (Figure 10A). The change of lipid transfer from adipocytes to cancer cells after injection FABP4 inhibitor was studied. As a result, lipid transfer to all breast cancer cells except to normal breast cell was reduced in the case with FABP4 inhibitor injection than in the case without FABP4 inhibitor injection (Figure 10B).

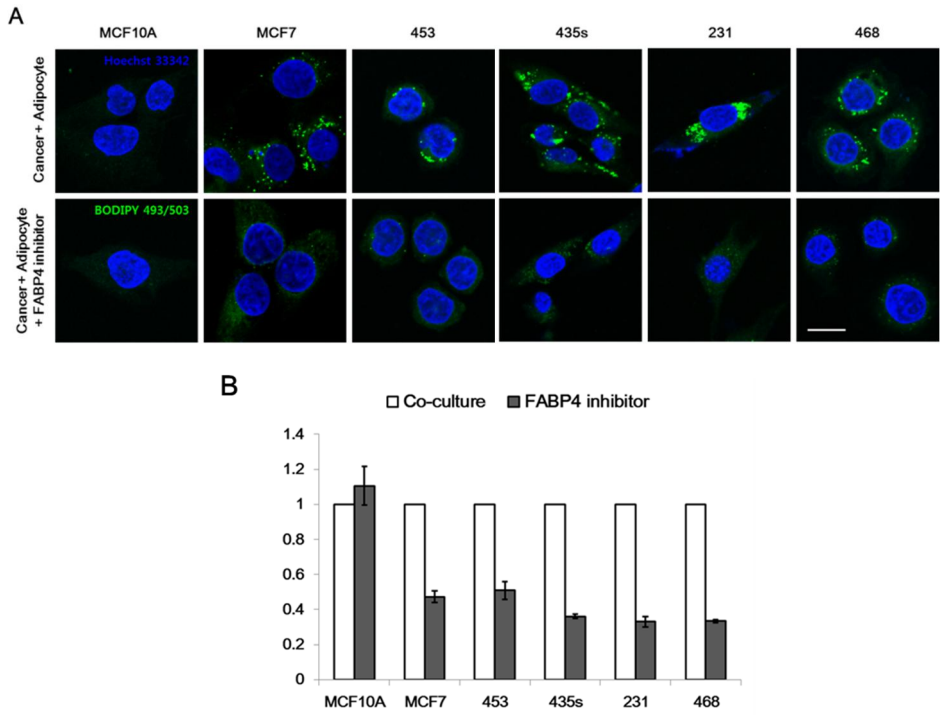


Figure 10. Co-culture of breast cancer cells with FABP4 inhibitor treated adipocytes reduces lipid accumulation in human breast cancer cells. Breast cancer cells were grown on coverslips and co-cultured with mature adipocytes or FABP4 inhibitor treated adipocytes for 48 h. Breast cancer cells were isolated and stained with BODIPY 493/503 (green). Images were acquired under confocal microscope (A) and relative fluorescence was assessed by Image J. (B) Scale bar, 100 mm.

10. The effect of FABP4 inhibition on lipolysis of adipocytes co-cultured with breast cancer cells

The effect of FABP4 inhibition on lipolysis of adipocytes co-cultured with breast cancer cells was examined. As a result, the amount of FFAs and glycerol was reduced in the case with FABP4 inhibitor injection than in the case without FABP4 inhibitor injection. The LIPOLYSIS ASSAY KIT was used for the

measurement (Figure 11A and 11B). The expression of pHSL, HSL, and PLIN1 was reduced in the case with FABP4 inhibitor injection than in the case without FABP4 inhibitor injection in adipocytes co-cultured with breast cancer cells (Figure 11C).

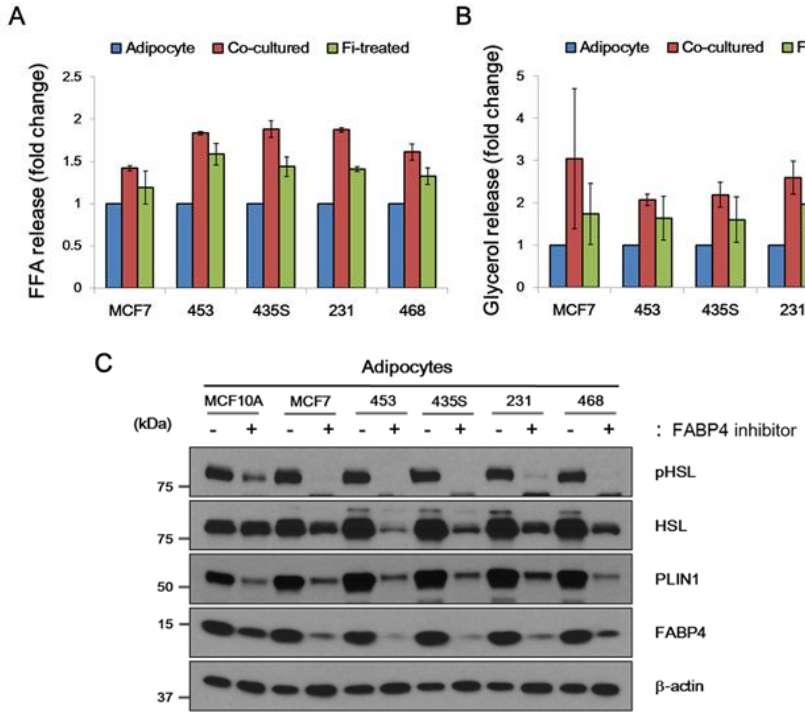
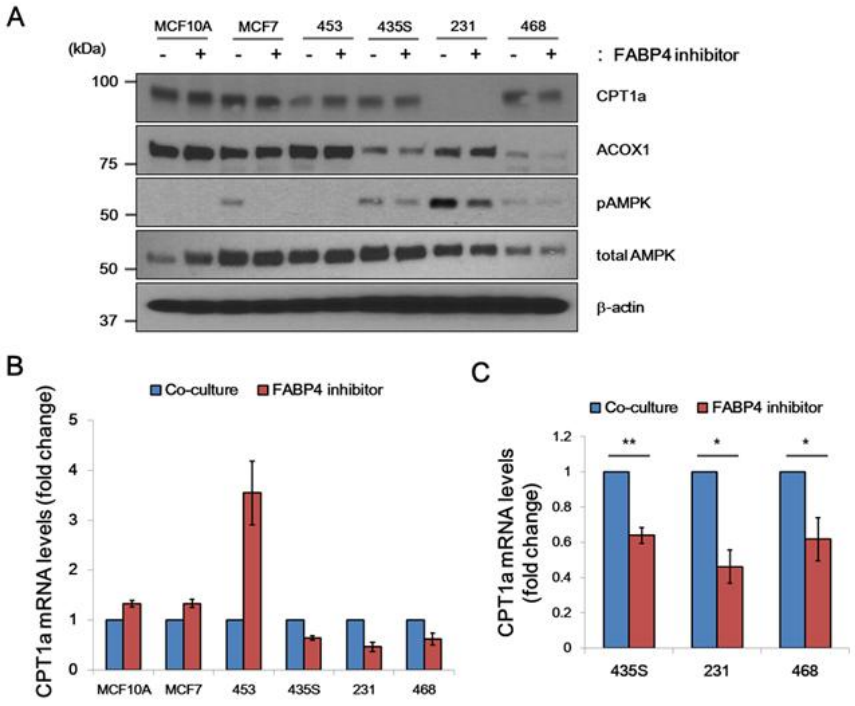


Figure 11. Co-culture of breast cancer cells with FABP4 inhibitor treated adipocytes reduces lipolysis in adipocytes. Free fatty acids (A) and glycerol (B) were detected by lipolysis assay in mature adipocytes. (C) Co-culture of breast cancer cells with mature adipocytes or FABP4 inhibitor treated adipocytes for 48 h, and then adipocytes were isolated and performed by immunoblotting with the indicated antibodies.

11. The effect of FABP4 inhibition on ß-oxidation of breast cancer cells co-cultured with adipocytes

The effect of FABP4 inhibition to the ß-oxidation of breast cancer cells co-cultured with adipocytes was examined. As a result, the expression of pAMPK was reduced in the case with FABP4 inhibitor injection than in the case without FABP4 inhibitor injection in MDA-MB-435S and MDA-MB-231 (Figure 12A). The CPT1a mRNA level was reduced in the case with FABP4 inhibitor injection than in the case without FABP4 inhibitor injection in MDA-MB-435S, MDA-MB-231, and MDA-MB-468 (Figure 12B and 12C). The ACOX1 mRNA level was reduced in the case with FABP4 inhibitor injection than in the case without FABP4 inhibitor injection in MCF-7, MDA-MB-435S, and MDA-MB-468 (Figure 12D and 12E).



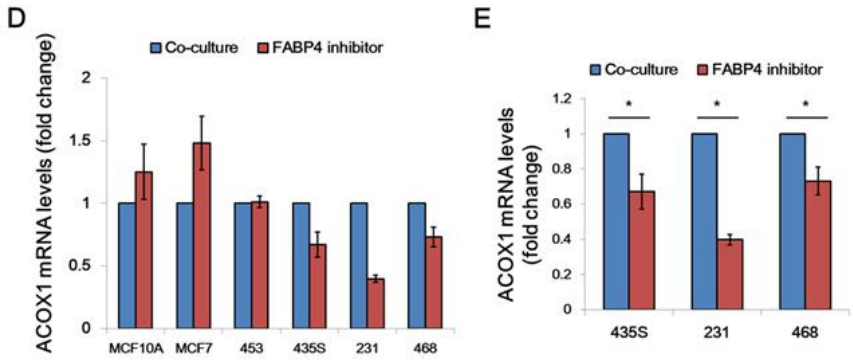


Figure 12. FABP4 plays an important role in the interaction between breast cancer cells and adipocytes. Breast cancer cells were co-cultivated with mature adipocytes or FABP4 inhibitor treated adipocytes for 48 h. (A) The cell lysates were subjected to SDS-PAGE and performed by immunoblotting with the indicated antibodies. (B~D) Relative mRNA levels of CPT1a and ACOX1 were assessed by quantitative real-time PCR. mRNA expression was normalized to GAPDH and presented as fold change. These data were presented as the mean ± SD of three independent experiments; **$p < 0.01$; *$p < 0.05$.

12. The expression of lipid metabolism-related proteins in breast cancer with adipose stroma

The expression of lipid metabolism-related proteins in human breast cancer with adipose stroma was examined. The expression of ACOX1 and CPT1 was higher in cancer cells located within adipose stroma or adjacent to adipose stroma (Figure 13A and 13C) than cancer cells located within fibrous stroma (Figure 13B and 13D). FABP4 was mainly expressed in the adipocytes with different patterns depending on the zone. The expression of FABP4 was higher in the adipocytes located in the interface adjacent to cancer cells (Figure 13E and 13F). HSL was expressed in both cancer cells and adipocytes and the expression of HSL was higher in the adipocytes located in the interface adjacent

to cancer cells like FABP4 (Figure 13G and 13H). PLIN1 was expressed in both cancer cells and adipocytes (Figure 13I and 13J).

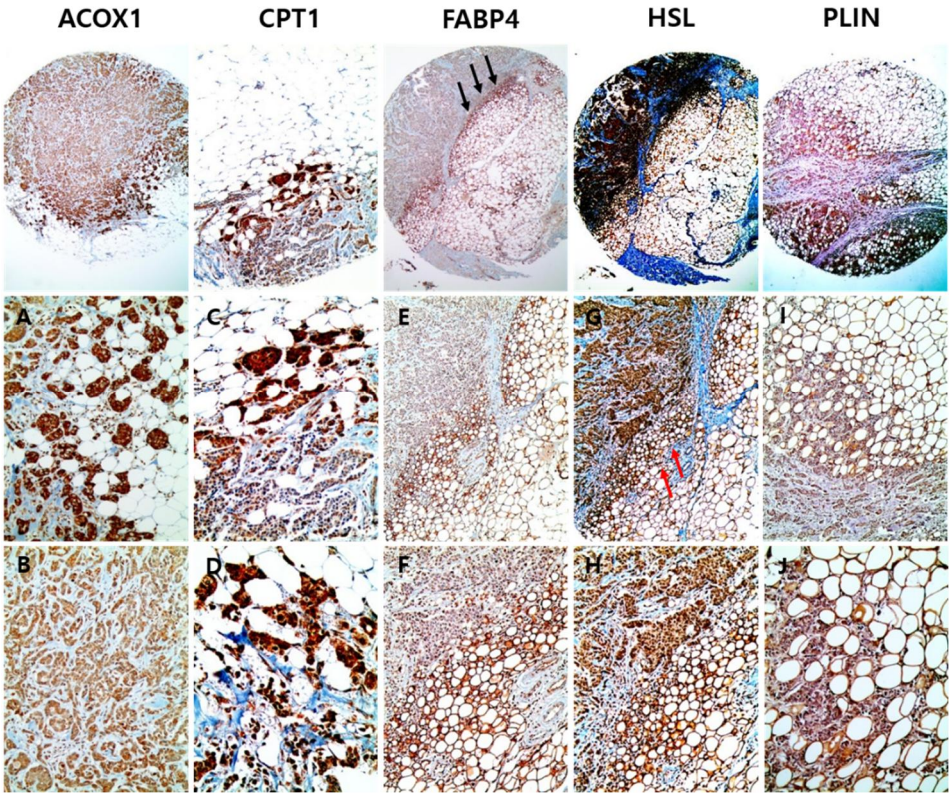


Figure 13. The expression of lipid metabolism-related proteins in breast cancer with adipose stroma. The expression of ACOX1 and CPT1 was higher in cancer cells located within adipose stroma or adjacent to adipose stroma than cancer cells located within fibrous stroma. FABP4 was mainly expressed in the adipocytes with different patterns depending on the zone. The expression of FABP4 was higher in the adipocytes located in the interface adjacent to cancer cells. HSL was expressed in both cancer cells and adipocytes and the expression of HSL was higher in the adipocytes located in the interface adjacent to cancer cells like FABP4. PLIN1 was expressed in both cancer cells and adipocytes.

13. The expression of lipolysis-related protein in the different region of breast cancer tissue

We investigated the expression of lipolysis-related protein in the different region of breast cancer tissue, 1) cancer with fibroblast, 2) cancer with adipocyte and 3) adipocyte nearby cancer (Figure 14A). The expression of PLIN1 and HSL was higher in cancer with adipocyte and adipocyte nearby cancer compared to cancer with fibroblast. The CPT1A expression was highest in cancer with adipocyte. FABP4 was mainly expressed in adipocyte nearby cancer. The expression of FAS was similar in every region (Figure 14B)

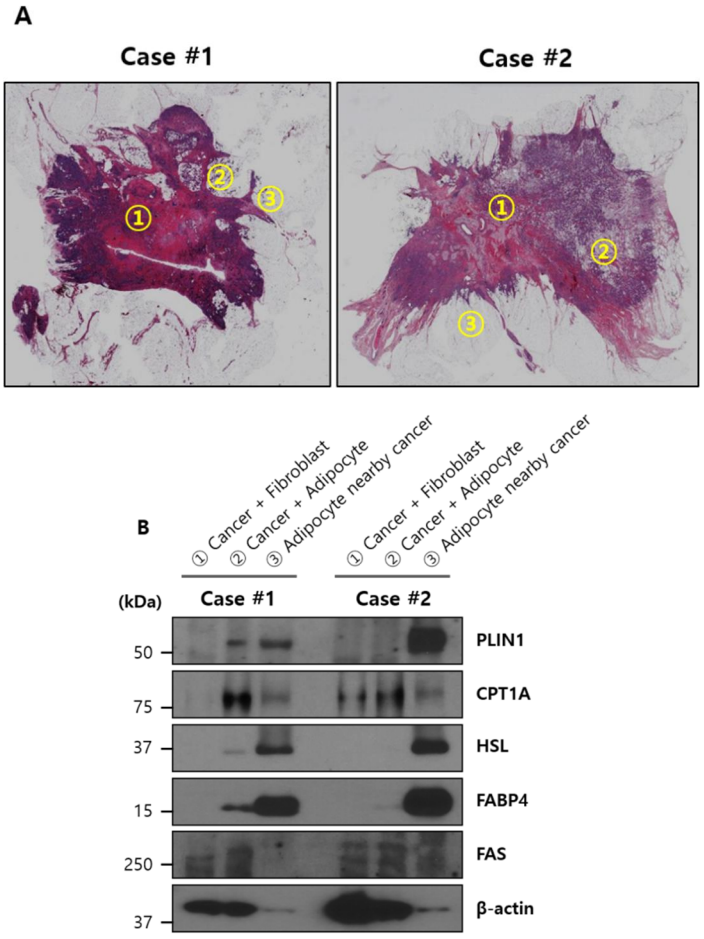


Figure 14. The expression of lipolysis-related protein in the different region of breast cancer tissue. (A) Formalin-fixed, paraffin-embedded breast cancer tissue samples were processed for H&E staining to identify target region. ① Cancer with fibroblast, ② Cancer with adipocyte, ③ Adipocyte nearby cancer from two cases were selected. (H&E, magnification ×12.5) (B) Protein extracts were isolated from FFPE breast cancer tissue samples and western blotting was performed with primary antibodies against PLIN1, CPT1A, HSL, FABP4, FAS, and β-actin.

14. Basal characteristics of patients with breast cancer

This study included 256 cases of breast cancer tissue. According to IHC results, breast cancers were classified into 4 molecular subtypes: luminal A type (126 cases), luminal B type (63 cases), HER-2 type (17 cases), and TNBC type (50 cases). These groups were further divided into TNBC and non-TNBC type and were analyzed in this study. The basal characteristics of the patients are shown in Table 4.

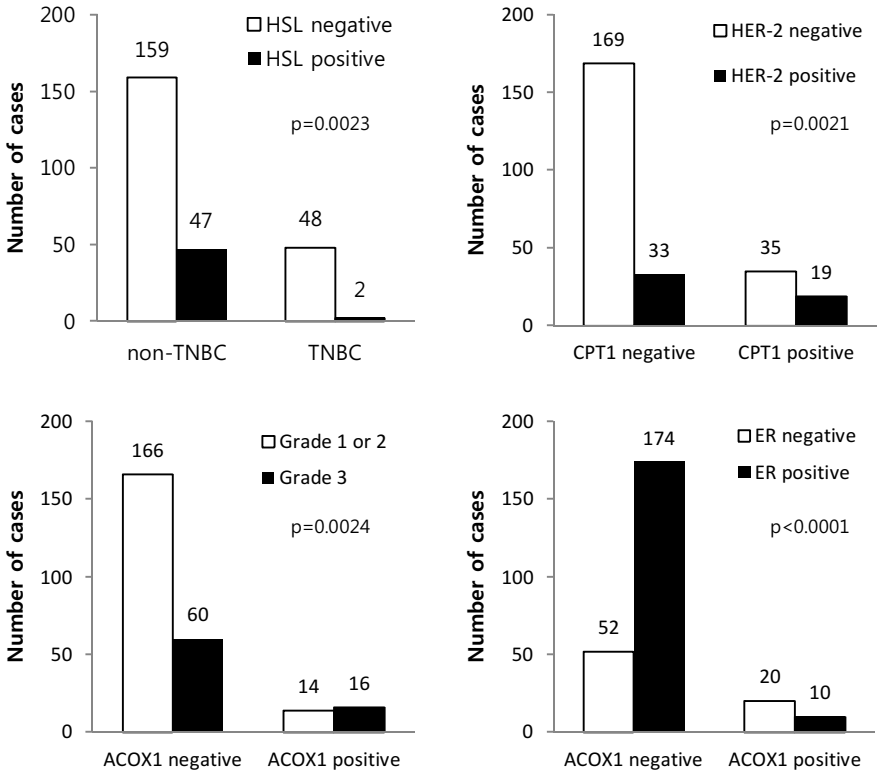
A higher proportion of TNBC had higher histologic grade and larger tumor size ($p < 0.001$ and $p = 0.049$, respectively). Age, lymph node metastasis, stage, and treatment were not associated with molecular subtype.

Table 4. Basal characteristics of patients with breast cancer

Parameters	Total	Molecular subtype		p-value
	N=256 (%)	TNBC	Non-TNBC	
		n= 50 (%)	n= 206 (%)	
Age (years)				0.190
<45	139 (54.3)	23 (46.0)	116 (56.3)	
≥45	117 (45.7)	27 (54.0)	90 (43.7)	
Histologic grade				**<0.001**
1 or 2	180 (70.3)	16 (32.0)	164 (79.6)	
3	76 (29.7)	34 (68.0)	42 (20.4)	
Tumor size (cm)				**0.049**
≤2.0	159 (62.1)	25 (50.0)	134 (65.0)	
>2.0	97 (37.9)	25 (50.0)	72 (35.0)	
Lymph node metastasis				0.215
No	165 (64.5)	36 (72.0)	129 (62.6)	
Yes	91 (35.5)	14 (28.0)	77 (37.4)	
Stage				0.507
I	101 (39.5)	18 (36.0)	83 (40.3)	
II	127 (49.6)	31 (62.0)	96 (46.6)	
III	28 (10.9)	1 (2.0)	27 (13.1)	
Treatment				0.476
No treatment	6 (2.3)	5 (10.0)	1 (0.5)	
Chemotherapy	110 (43.0)	13 (26.0)	97 (47.1)	
Radiotherapy	1 (0.4)	1 (2.0)	0 (0.0)	
Chemotherapy + Radiotherapy	139 (54.3)	31 (62.0)	108 (52.4)	

15. Correlation between the expression of lipolysis-related proteins and clinicopathologic factors

In breast cancer, HSL positivity was associated with non-TNBC subtype (p = 0.0023). CPT1 positivity was associated with HER-2 positivity (p = 0.0021). ACOX1 positivity was associated with higher histologic grade, ER negativity, PR negativity, and HER-2 positivity (p = 0.0024, <0.0001, 0.0002 and <0.0001, respectively). FAS positivity was associated with non-TNBC subtype, lower histologic grade and HER-2 positivity (p = 0.0001, 0.0012, and 0.0039, respectively) (Figure 15).



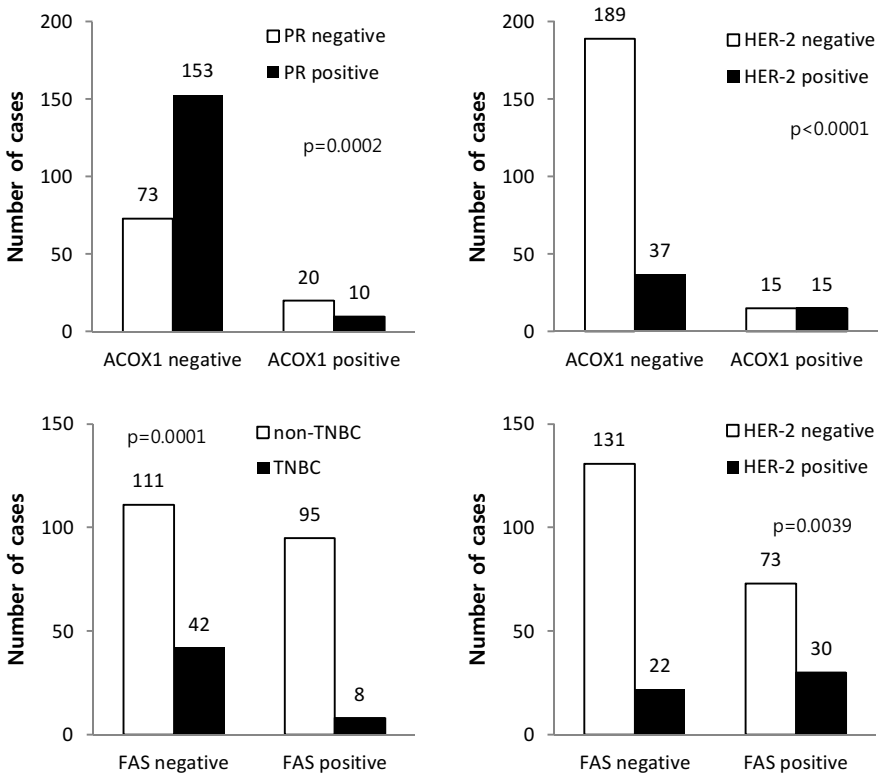


Figure 15. Correlation between the expression of lipolysis-related proteins and clinicopathologic factors in breast cancer. HSL positivity was associated with non-TNBC subtype. CPT1 positivity was associated with HER-2 positivity. ACOX1 positivity was associated with higher histologic grade, ER negativity, PR negativity, and HER-2 positivity. FAS positivity was associated with non-TNBC subtype, lower histologic grade and HER-2 positivity.

16. Expression of lipolysis-related proteins on patient prognosis

We investigated the effect of the expression of lipolysis metabolism–related proteins on prognosis through univariate analysis and multivariate Cox analysis.

In univariate analysis, shorter disease–free survival was associated with FABP4 expression (p = 0.0319) (Table 5). Subgroup analysis which was

performed in TNBC showed that FABP4 expression was associated with shorter disease-free survival ($p = 0.028$) (Table 6). However, in advanced stage breast cancer, disease–free survival and overall survival was not associated with any IHC staining results (Table 7). In multivariate Cox analysis, disease–free survival and overall survival was not associated with any IHC staining results.

Table 5. Univariate analysis of lipolysis–related protein expression in breast cancer on disease free and overall survival by the log–rank test

Parameter	Number of patients/recurrence/death	Disease-free survival		Overall survival	
		Mean survival (95% CI) months	p-value	Mean survival (95% CI) months	p-value
HSL			0.309		0.314
Negative	207/11/14	57.1 (55.4-58.8)		57.6 (56.1-59.2)	
Positive	49/1/5	60.0 (57.6-62.4)		58.1 (56.8-59.4)	
Perlipin			0.208		0.116
Negative	228/12/19	57.2 (55.6-58.8)		57.7 (56.3-59.2)	
Positive	28/0/0	61.1 (59.4-62.7)		61.1 (59.4-62.7)	
FABP4			**0.0319**		0.134
Negative	251/10/16	58.0 (56.6-59.4)		58.5 (57.3-59.7)	
Positive	5/2/3	38.2 (2.7-73.6)		38.4 (3.2-73.5)	
CPT1			0.757		0.272
Negative	202/11/17	57.5 (55.8-59.1)		57.8 (56.3-59.3)	
Positive	54/1/2	58.3 (55.0-61.6)		59.2 (56.4-62.1)	
ACOX1			0.514		0.171
Negative	226/11/16	58.2 (56.7-59.6)		58.5 (57.1-59.9)	
Positive	30/1/3	53.8 (48.2-59.3)		55.1 (50.3-59.9)	
FAS			0.593		0.203
Negative	153/6/8	56.9 (54.9-58.9)		57.5 (55.8-59.3)	
Positive	103/6/11	58.8 (56.7-60.9)		59.0 (56.9-61.0)	

Table 6. Univariate analysis of lipolysis–related protein expression in triple negative breast cancer on disease free and overall survival by the log–rank test

Parameter	Number of patients/recurrence/death	Disease-free survival		Overall survival	
		Mean survival (95% CI) months	p-value	Mean survival (95% CI) months	p-value
HSL			0.970		0.960
Negative	48/4/7	54.2 (49.8-58.6)		55.3 (51.4-59.2)	
Positive	2/0/0	60.5 (28.7-92.2)		60.5 (28.7-92.2)	
Perlipin			0.958		0.962
Negative	46/4/7	53.8 (58.0-62.0)		54.9 (50.8-58.9)	
Positive	4/0/0	62.7 (61.9-63.5)		62.7 (61.9-63.5)	
FABP4			**0.028**		0.077
Negative	48/3/6	55.3 (51.4-59.2)		56.3 (53.0-59.7)	
Positive	2/1/1	35.0 (-308.0-378.0)		35.5 (-301.2-372.2)	
CPT1			0.258		0.590
Negative	46/3/6	54.7 (50.3-59.1)		55.3 (51.2-59.3)	
Positive	4/1/1	52.0 (23.1-80.8)		58.2 (48.8-67.6)	
ACOX1			0.723		0.758
Negative	41/3/6	54.6 (49.8-59.4)		55.3 (50.8-59.8)	
Positive	9/1/1	53.8 (43.2-64.5)		56.3 (50.9-61.7)	
FAS			0.961		0.913
Negative	42/4/6	54.3 (49.5-59.0)		55.5 (51.4-59.6)	
Positive	8/0/1	55.6 (44.1-67.1)		55.6 (44.1-67.1)	

Table 7. Univariate analysis of lipolysis–related protein expression in advanced stage breast cancer on disease free and overall survival by the log–rank test

Parameter	Number of patients/recurrence/death	Disease-free survival		Overall survival	
		Mean survival (95% CI) months	p-value	Mean survival (95% CI) months	p-value
HSL			0.962		0.949
Negative	21/5/4	53.0 (44.6-61.5)		55.4 (48.2-62.7)	
Positive	7/0/0	60.5 (55.8-65.2)		60.5 (55.8-65.2)	
Perlipin			0.956		0.962
Negative	24/5/4	53.6 (46.3-61.0)		55.7 (49.4-62.0)	
Positive	4/0/0	62.7 (55.5-69.9)		62.7 (55.5-69.9)	
FABP4			0.967		0.971
Negative	27/5/4	54.9 (48.2-61.5)		56.7 (51.0-62.4)	
Positive	1/0/0	56.0 (56.0-56.0)		56.0 (56.0-56.0)	
CPT1			0.782		0.479
Negative	19/3/2	54.3 (46.0-62.7)		55.6 (48.0-63.2)	
Positive	9/2/2	56.2 (44.4-67.9)		59.1 (51.3-67.0)	
ACOX1			0.778		0.609
Negative	24/4/3	55.1 (47.9-62.3)		56.5 (50.1-62.8)	
Positive	4/1/1	53.7 (29.9-77.5)		58.2 (47.6-68.8)	
FAS			0.887		0.801
Negative	16/3/2	55.3 (47.2-63.3)		57.3 (51.1-63.4)	
Positive	12/2/2	54.5 (42.5-66.4)		56.0 (44.8-67.1)	

IV. DISCUSSION

In general, breast cancer is one of the tumors with various types of stroma. Classifying breast cancer into two, one is fibrous stroma consisting of extracellular matrix such as fibroblast and collagen and the other is adipose stroma consisting of adipocytes. Adipocytes, especially, compose 70% of the total breast tissue. It is known that cancer cells and stroma interact in diverse ways so the interaction between cancer cells and stroma in the respect of metabolism is well assumed. Reverse Warburg effect is one of the well-known theories explaining the metabolic interaction between cancer cells and fibrous stroma in breast cancer. Briefly, the theory assists that breast cancer cells produce reactive oxygen species such as nitric oxide to place oxidative stress on stromal cells so that HIF-1α and NFκB leads glycolysis, autophagy, and mitochondria dysfunction in stromal cells. Ketone bodies and lactates gained through the glycolysis in stromal cells enter cancer cells to efficiently produce ATP with oxidative phosphorylation in mitochondria, and in the end contribute to the survival and growth of cancer cells. The theory exemplifies such stromal cells with CAF without the expression of caveolin-1[11-13,22].

The metabolic interaction between adipocytes and cancer cells is reported in recent studies. The interaction between metastatic cancer cells and nearby omental adipocytes has been proved in ovary cancer with metastasis to omentum. In short, FFA is released from the nearby omental adipocytes and it is uptaken by metastatic cancer cells to become a substrate of mitochondrial β-oxidation. Compared to Reverse Warburg effect between breast cancer and fibrous stroma, this theory insists that the stromal cells provide another type of energy, FFA in this case, for the growth of cancer cells[15]. Reverse Warburg effect well explains the metabolic interaction between breast cancer and fibrous stroma. However, the interaction between breast cancer and adipose stroma, which is the other important components of breast cancer stroma, is not yet

studied sufficiently. The purpose of this study was to investigate the metabolic interaction between adipocytes and breast cancer cells and to seek for a new therapeutic target in breast cancer.

When cancer cell was co-cultured with adipocyte, the lipid transfer from adipocytes to cancer cells were observed and the amount of lipid transfer was different according to the molecular subtype of breast cancer. Of note, the highest lipid transfer was observed in a TNBC type cell line, MDA-MB-231. As TNBC is characterized as having aggressiveness and rapid proliferation, it could be reasonably speculated that lipid transfer from adipocyte is increased in TNBC. In a similar previous study using a TNBC type cell line MDA-MB-231, adipose microenvironment increased the invasiveness and dissemination of cancer cell by producing chemokine ligand 5[23]. Furthermore, in a previous study using TNBC type cell line MDA-MB-231 and luminal type cell line T47D, co-culture of adipocytes and cancer cells showed cytoplasmic lipid drop accumulation of cancer cells in both cell lines. However, in contrast to our findings, more increased lipid accumulation was observed in T47D than MDA-MB-231[15]. These finding suggest that lipid transfer level could differ regarding cancer cell lines and not always by the difference of molecular subtypes, which should be verified in further studies. Since the proliferation was significantly increased in cancer cells when co-cultured with adipocytes than cultured alone, lipid transfer might play an important role in cancer cell proliferation.

Furthermore, we evaluated the metabolic interaction between adipocytes and cancer cells. As a result, cancer cell increased lipolysis in adipocyte, and adipocyte promoted the β-oxidation in cancer cell via increased AMPK phosphorylation. Co-culture of adipocyte and breast cancer showed that the end product of lipolysis of adipocyte, FFAs and glycerol was increased in every breast cancer cell lines. In a previous publication, adipocytes were shown to

support tumorigenesis, cancer progression and metastasis[5,24]. In addition, considering that obesity is known to be an important risk factor in endometrial cancer, breast cancer, and renal cell cancer[25], it could be speculated that adipocytes play a crucial role in the TME, which is consistent with the finding of this study.

In this study, we have shown that adipocytes contribute to the survival and growth of breast cancer cells through lipid transfer, lipolysis, and β-oxidation. Therefore, inhibiting the metabolic interaction between adipocytes and cancer cells is expected to hamper cancer growth. Among molecules involving the metabolic interaction, we focused on the role of FABP4. FABPs are ~15kDa weighted cytoplasmic proteins which bind to endogenous fatty acids produced by lipolysis of adipocyte and transfer the FFA into the tumor cells[26]. In vitro data from this study showed that co-culture of adipocyte and cancer cell leads to increased expression of FABP4. In a previous study by Nieman KM et al. using a reverse phase protein array, comparison of primary ovary cancer and omental metastatic tissue revealed that FABP4 protein expression was upregulated in omental metastatic tissue and inhibition of FABP4 markedly reduced adipocyte-mediated cancer invasion and lipid accumulation within cancer cells[15]. Furthermore, previous studies also reported that exogenous FABP4 administration induced cancer cell proliferation and progression in breast cancer and prostate cancer cells[27,28]. These finding suggest that FABP is a key molecule involved in the metabolic interaction between adipocytes and cancer cells.

The clinical significance of this study is that we suggested FABP4 inhibition as the potential therapeutic target in breast cancer. FABP4 inhibitor was first introduced as a therapeutic agent for type 2 diabetes[26]. The potency of FABP4 inhibitor was previously verified in several studies[29,30]. We used a well-known FABP4 inhibitor, BMS309403, and it is a cell-permeable and potent inhibitor of

FABP4 which targets the fatty acid-binding pocket[31]. BMS309403 markedly reduced lipid transfer, lipolysis of adipocyte and β-oxidation of cancer cells in this study. Therefore, FABP4 inhibitor can be suggested as a new therapeutic agent to inhibit cancer growth. However, verification in a xenograft model and further clinical trials in breast cancer is required for its clinical application in breast cancer patients.

Furthermore, IHC was conducted in human breast cancer tissues targeting metabolic interaction-related molecules. As a result, the expression of ACOX1, CPT1, FABP4, and HSL showed zonal distribution pattern. FABP4 and HSL showed higher expression in interface adipocytes adjacent to cancer cells. It supports the metabolic interaction between adipocytes and cancer cells observed in our in vitro cell line study. IHC staining of 256 breast cancer cases showed that the expression of lipolysis-related protein was correlated with clinicopathologic parameters. First, CPT1, ACOX1, and FAS expression were correlated with HER-2 positivity, showed similar results with previous studies[21,32-34]. Jin Q et al. has shown that the possible interaction between HER-2 and FAS results in phosphorylation of FAS, increasing its activity and promoting cancer cell proliferation, survival and metastasis[34]. Furthermore, there is other evidence suggesting that lipid metabolism gene differs according to breast cancer subtype. For example, the end product of de novo fatty acid synthesis, palmitate-containing phosphatidylcholine is commonly observed in ER negative and grade 3 breast cancers[35], and the activity of acyl-CoA:cholesterol acyltransferase 1 was reported to be high in ER-negative basal-like breast cancer[36]. Therefore, further study is required regarding lipid metabolism genes and different lipolysis-related protein expression according to breast cancer molecular subtype.

In addition, HSL and FAS expression was related to non-TNBC type, showing that non-TNBC had higher expression of lipolysis-related protein compared to

TNBC. However, TNBC is generally associated with high histologic grade and aggressive clinical behavior[37,38]. Therefore, it is speculated that activity of lipid metabolism, which is an energy source of cancer cell, is increased. These findings were reported in a previous study which showed increased expression of glycolysis-related protein in TNBC and basal-like breast tumor[39,40]. Similar results were observed in our in vitro cell line study, however, IHC results showed controversial results, which was also previously reported[21]. Therefore, further investigation of lipid metabolism activity in TNBC is required.

ACOX1 expression showed the correlation with higher histologic grade, ER negativity, and PR negativity, which are known to be a poor prognostic factor. Previously, increased ACOX-1 expression was reported to be related to worsening histologic grade in brain glioma[41] and breast cancer[21], showing consistent finding with this study.

Lastly, FABP4 expression was correlated with poor disease-free survival in univariate analysis, however, statistical significance was not shown in multivariate analysis. Although this finding limits its clinical significance, as a previous study has shown that single-nucleotide polymorphism in the FABP4 gene rs1054135 in adipocyte has been suggested as a predictor of TNBC recurrence[42], it should investigated in future studies.

Taken together, in this study, we found that the metabolic interaction between adipocytes and breast cancer cells was present. Breast cancer cells increase the lipolysis in adipocytes and produces fatty acid, and fatty acid enters cancer cells. Also, adipocytes contribute to the survival and growth of cancer cells through increased mitochondrial β-oxidation by using fatty acid from adipocytes. The key molecule of the process is FABP4 which is combined with FFA and supports the migration to cancer cells. When FABP4 was suppressed, the metabolic interaction is reduced, suggesting its role as a potential therapeutic target.

V. CONCLUSION

Taken together, in this study, we found that the metabolic interaction between adipocytes and breast cancer cells was present. Breast cancer cells increase the lipolysis in adipocytes and produces fatty acid, and fatty acid enters cancer cells. Also, adipocytes contribute to the survival and growth of cancer cells through increased mitochondrial β-oxidation by using fatty acid from adipocytes. The key molecule of the process is FABP4 which is combined with FFA and supports the migration to cancer cells. When FABP4 was suppressed, the metabolic interaction is reduced, suggesting its role as a potential therapeutic target.

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ABSTRACT(IN KOREAN)

인체 유방암에서의 지방 세포에서 종양 세포로의 에너지 전달

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김 혜 민

지금까지 유방암에 대한 많은 연구가 진행되어 그에 따라 다양한 치료법이 개발되었음에도 불구하고, 현재 하나의 정립된 치료법이 없는 한계점이 있어 유방암에서 새로운 치료제의 필요성이 대두되고 있다. 최근 유방암에서도 종양 미세 환경을 대상으로 한 약물에 대한 관심이 높아지고 있다. 또한, 최근의 연구는 종양의 발생, 성장 및 전이에 있어서 종양 미세 환경을 구성하는 지방 세포의 역할을 제시하고 있다. 본 연구는 지방 세포와 유방암 세포 간의 대사 상호작용에 대해 조사하고, 유방암의 새로운 치료 표적으로서의 가능성에 대해 연구하였다. 유방암 세포주 및 인간 유방암 조직을 대상으로 조사하였다. 단독 배양한 유방암 세포, 즉 대조군에 비해 지방 세포와 함께 배양한 유방암 세포에서는 지방 세포로부터 유방암 세포로의 지질 이동이 관찰되었고, 그 양은 유방암의 분자 아형에 따라 달랐다. 또한 유방암 세포는 지방 세포의 지방 분해에 영향을 미쳤고, 지방 세포는 유방암 세포의 베타 산화 작용에 영향을 미쳤다.

이러한 일련의 과정에 관여하는 중요한 인자는 유리 지방산과 결합해 유리 지방산의 유방암 세포로의 이동을 도와주는 지방산 결합 단백질 4 (fatty acid binding protein 4, FABP4)이었다. FABP4의 발현이 억제될 때, 지방 세포로부터 유방암 세포로의 지질 이동, 지방 세포의 지방 분해, 유방암 세포의 베타 산화 작용이 감소하였다. 또한, 지질 대사 및 지방 분해 관련 단백질은 같은 유방암 조직에서도 그 영역에 따라 매우 상이한 발현을 보였다.

종합하면, 우리는 지방 세포와 유방암 세포 간의 대사 상호작용이 존재함을 증명하였다. 유방암 세포는 지방 세포의 지방 분해를 증가시켜 지방산을 생성하고, 이 지방산은 다시 암세포로 들어가게 된다. 또한, 지방 세포는 지방 세포로부터 들어온 이 지방산을 이용하여 유방암 세포 내 미토콘드리아에서의 베타 산화 작용을 증가시켜 유방암 세포의 생존과 성장에 기여한다. 이러한 과정에 관여하는 중요한 인자는 FABP4임을 알아냈고, FABP4의 발현이 억제되면 이러한 대사 상호작용이 감소하는 것을 관찰하여 FABP4의 잠재적인 치료 표적으로서의 역할을 제시하였다.

핵심되는 말: 에너지 전달, 유방암, 지방 세포, FABP4