Propyl gallate inhibits adipogenesis by stimulating extracellular signal-related kinases pathway in human adipose tissue-derived mesenchymal stem cells

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Abstract

Mesencymal stem cells (MSCs) are isolated from various mesenchymal tissues and differentiate into osteoblast, myocyte, and adipocyte. MSCs are easily obtained without ethical issue, and modulation of MSCs differentiation applies to various therapies. Moreover, the significance of modulating factor in MSCs differentiation is on the rise, we identified Propyl gallate, which can regulate differentiation of MSCs. PG is used as an additives in various foods that has antioxidant and anti-inflammatory effects. Although the function of PG is well identified in different cell types, it is unknown that PG has an effect on stem cell differentiation. We demonstrate that PG suppresses adipocyte differentiation in human adipose tissue-derived mesenchymal stem cells (hAMSCs) by reduced lipid droplets accumulation. Further, PG reduced expression of adipocyte-specific markers, including PPAR-γ, C/EBP-α, adipocyte fatty acid-binding protein and lipoprotein lipase. PG inhibited adipogenesis of hAMSCs through ERK pathway and the inhibition was recovered in response to blockage of ERK activation. Collectively these results demonstrate unusual function of PG on adipocyte differentiation in hAMSCs and PG inhibits adipocyte differentiation through ERK signaling pathway and decrease of PPAR-γ activity.

Contents

List of figures

Nomenclature

PG: Propyl gallate

hAMSCs: human adipose tissue-derived mesenchymal stem cells

BHA: butylated hydroxyanisole

BHT: butylated hydroxytoluene

TBHQ: tert-butylhydroquinone

EDTA: ethylenediaminetetraacetic acid

MTT: methyl thiazolyl tetrazolium

AIM: adipogenic induction medium

GM: Growth medium

PPAR-γ: peroxisome proliferator-activated receptor-γ

C/EBP-α: CCAAT enhancer binding protein- α

LPL: lipoprotein lipase

aP2: adipocyte fatty acid-binding protein

1. Introduction

Mssenchymal stem cells (MSCs) are multi-potent adult stem cells that derived from various tissues, containing liver, spleen, muscle, bone marrow and adipose tissue[23]. MSCs have self-renewal ability while sustaining their capacity to differentiate into multi-lineage tissues. MSCs differentiate into chondrocytes, osteocytes and adipocytes[3a, 24]. Adipose tissue is one of the richest sources of MSCs. Adipose tissue has more than 500 times more MSCs in each 1 gram of tissue when compared to bone marrow. Human adipose tissue-derived mesenchymal stem cells (AMSCs) are currently actively being researched in clinical trials for treatment in a variety of diseases. Based on these properties, regulating differentiation and suppressing adipocyte differentiation of MSCs might be very interesting treatment of obesity. Example, Berberine, is a quaternary ammonium salt from many herbs, inhibits adipocyte differentiation cause normal mouse weight in obesity mice[25]. Therefore, research of the factors that modulate MSC differentiation is mightily important for suppressing obesity.

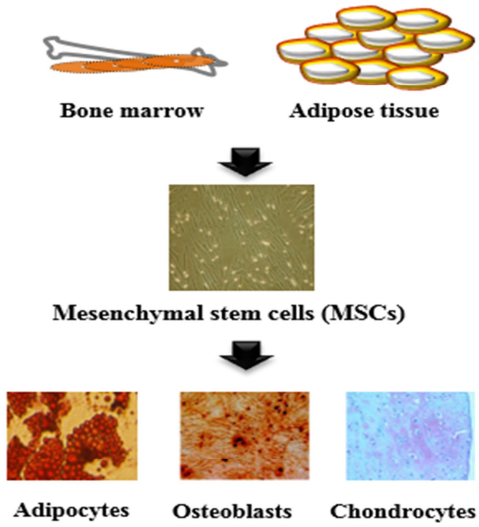

Figure 1 - 1. Mesenchymal stem cells

The adipocyte differentiation progress, which produces mature adipocytes from pre-adipocytes, considerably determines the number of adipocytes[22]. The progress of adipocyte differentiation consists of determination and terminal differentiation phase. The determination phase causes the transformation of the stem cell to a pre-adipocyte without morphological change. Subsequently, terminal differentiation phase obtains the property of mature adipocyte from pre-adipocyte, and causes adipogenic proteins secretion, lipid transport and synthesis. Furthermore, terminal differentiation phase goes along a succession of transcriptional progress. Stimulated both of C/EBP-β

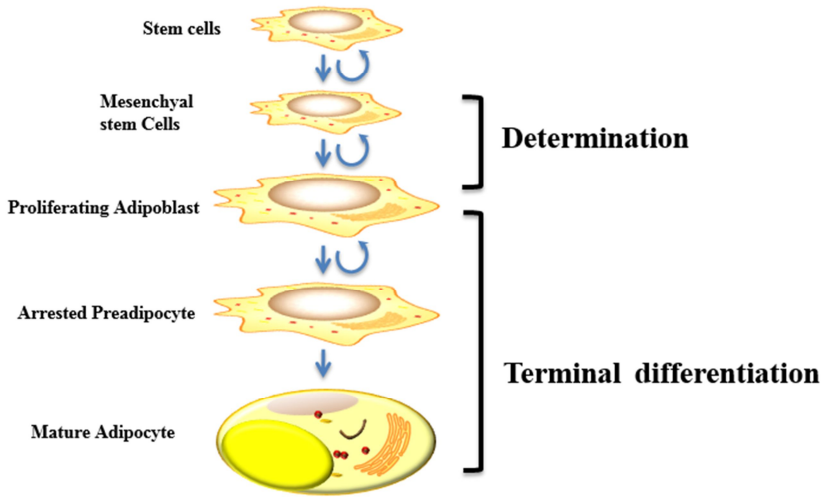

Figure 1 - 2. The process of adipocyte differentiation

and C/EBP-δ by adipocyte induced- hormone induces activation of PPAR-γ and C/EBP-α, and PPAR-γ play a role as transcription factors modulating genes expression in adipocyte differentiation[6,7,8]. Those PPAR-γ modulated adipogenic genes are lipoprotein lipase (LPL), perilipin, fatty acid transport protein-1 (FATP-1), adipocyte fatty acid–binding protein (aP2) or adiponectin, which work with lipid accumulation and the regulation of metabolism in adipocyte differentiation[9]. After determination and terminal adipocytes differentiation phase, MSCs become mature adipocyte and the result of mature adipocyte make up obesity in a broad sense. Therefore, comprehending adipocyte differentiation is not only important in biology, it is also necessary to treat metabolic diseases such as type II diabetes and obesity.

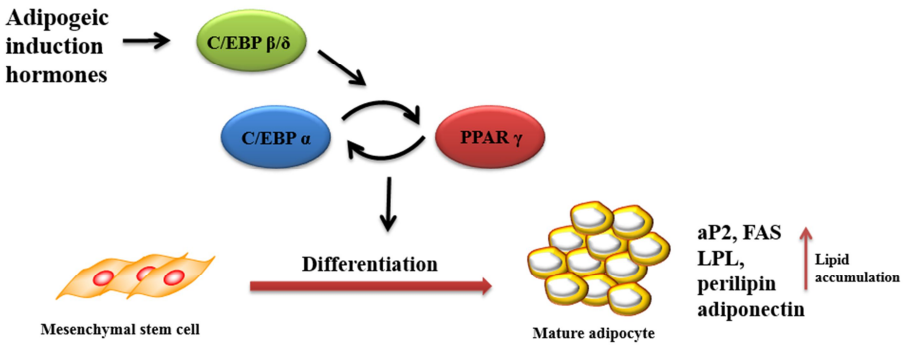

Figure 1 - 3. Transcriptional regulation in Adipocyte differentiation

One of the well-known signaling pathways in regulation of adipocyte differentiation is mitogen-activated protein kinases (MAPKs). MAPKs are serine/threonine-specific protein kinases, which are involved in diverse signaling pathways such as cell proliferation, cell death and differentiation. There are three typical MAPKs including the extracellular signal regulated kinase (ERK1/2), the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and the p38[26]. These MAPKs pathways are connected with diverse upstream activators and particular substrates[27]. Numerous reports demonstrate that MAPKs associate with regulation of adipocyte differentiation. ERK and JNK inhibit the adipocyte differentiation in hAMSC, whereas p38 positively regulates adipocyte differentiation[12]. Furthermore, In case of ERK, inhibition of ERK promotes adipocyte differentiation without adipocyte induced-hormone and it prevents osteogenic differentiation of hAMSCs[11].

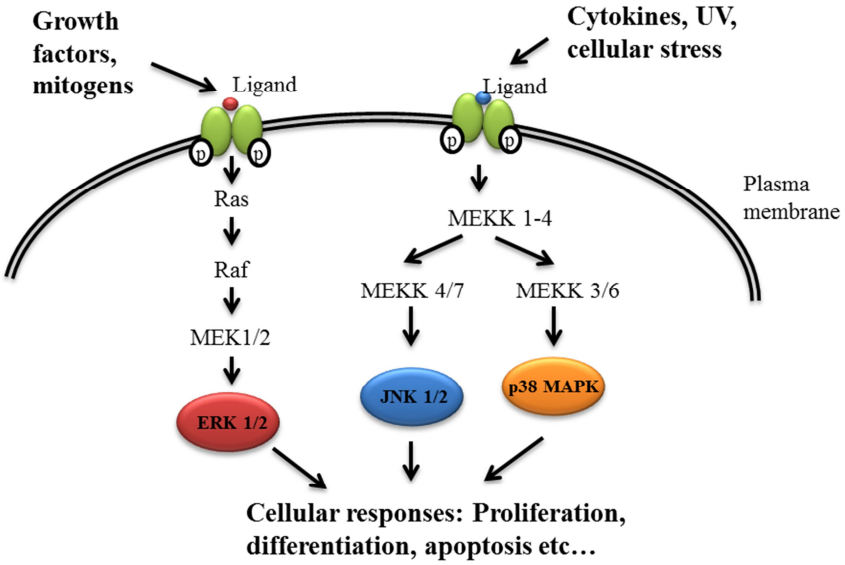

Figure 1 - 4. The MAPKs signaling pathway

To identify the novel factor that modulates adipocyte differentiation, we performed stem cell-based screen of the food additives library. As the result of screening, we found propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester, PG) has a novel function that inhibits adipocyte differentiation in AMSC. PG, which is an ester form of gallic acid with n-propanol, is synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), tert- ethylenediaminetetraacetic acid (EDTA), or butylhydroquinone (TBHQ)[15] in wide range of products in foods, cosmetics, hair products, adhesives, lubricants and food packing materials and pharmaceutical industry[13]. In food industry, PG is added to vegetable oil, mayonnaise, meat, soup, milk powder, spices, candies, snacks, vitamins and chewing gum. It is practically used as a food additive (especially oils and fats) permitted in the Korea and in many countries[16] The cellular toxicity of PG has been studied in vivo[17] and in vitro

to evaluate diverse toxicological characteristics, such as mutagenicity[18] and cytogenetic effects[19]. Nevertheless, although the properties of PG have been well investigated, the functions of PG remain unknown on stem cell differentiation.

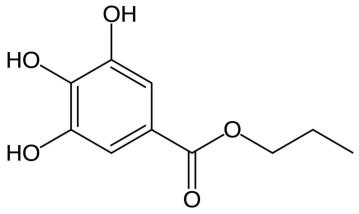

Figure 1 - 5. Structure of Propyl gallate

This study demonstrates influence of PG in adipocyte differentiation of hAMSCs. PG inhibits adipocyte differentiation program and lipid droplet formation in hAMSCs via activation of ERK and inhibition of PPAR-γ activity. These results suggest a new function of PG in stem cell differentiation and molecular mechanism of anti-adipogenic activity of PG.

2. Materials and Methods

2.1. Materials

PG, Oil red O, 3-isobutyl-1-methylxanthine, dexamethasone, indomethacin, insulin, rosiglitazone and methyl thiazolyltetrazolium (MTT) was from Sigma-Aldrich. Santa Cruz Biotechnology (Santa Cruz) provides PPAR-γ, C/EBP-α, aP2, and ERK2 antibodies. Additionally p-JNK, p-ERK1/2 and p-p38 antibodies were purchased from Cell Signaling Technology (Beverly). Antibody for Actin was from MP Biomedicals. Anti- rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibodies were from KPL (Gaithersburg). PD98059 was obtained from Calbiochem.

2.2. Cell culture

After getting informed consent, individual undergoing elective surgery provides subcutaneous adipose tissues. The patient was woman aged 28 years. hAMSCs were isolated from adipose tissue in accordance with previous described method[23a]. hAMSCs were cultured in growth medium (GM) consisting of α-MEM added with 100 U/mL penicillin, 100 mg/mL streptomycin (Gibco- Invitrogen) and 10% FBS. The hAMSC of Passage numbers 3-10 were used in experiments.

2.3. Induction of adipocyte differentiation

hAMSCs were cultured in GM before inducing differentiation. Culturing the hAMSCs with adipogenic induction medium (AIM, 10% FBS, 1 μM dexamethasone, 100 μM indomethacin, 500 μM 3-isobutyl-1-methylxanthine, and 1 μM insulin in α-MEM) for 14 days induce adipocyte differentiation. Every 3 days, AIM was changed and the cells were incubated in AIM but consisting 1 μM insulin and 10% FBS in α-MEM after 9 days. Oil red O, as an indicator of intracellular lipid accumulation, stains differentiated adipocytes

2.4. Oil red O staining

For 14 days, cells were cultured with AIM to induce adipocyte differentiation. Differentiated adipocytes have lipid accumulations from hAMSCs and accumulated lipid were stained by Oil red O solution. Phosphate buffered saline (PBS) wash differentiated cells and fixation solution(4% paraformaldehyde) were incubated with the cells at 4℃for 2 h. After washing the cells twice with PBS, cells were stained by 0.5% Oil red O solution (Isopropanol: Distilled water = 6: 4. Before dilute in the water, 0.5% Oil red O powder dissolve in 6 parts isopropanol) for 1 h at 4℃. And washing the cells twice with PBS again, pictures of the cells were taken with Olympus IX 71 microscope at the UNIST-Olympus Biomed Imaging Center (UOBC).

To measure quantitative analysis of Oil red O staining, the stained Oil red O of cells dissolve in isopropanol for half-hour. Absorbance of the Oil red O-consisted isopropanol was analyzed at 540 nm with SpectraMax® M5 Multi-Mode Microplate Reader (Molecular Devices).

2.5. Cell Viability Assay.

hAMSCs were prepared at 104cells/well in a 96-well plate . MTT stock solution contains Thiazolyl Blue Tetrazolium Bromid (5 mg/mL) in phosphate-buffered saline (PBS) and was filtered. A final concentration of MTT stock solution in culture medium should be 0.5 mg/mL and then incubation of the plates are required at 37°C for 3h 30min. MTT solution produce formazan crystals and it melt in 100 µl DMSO. The absorbance values of the samples was determined at 490 nm using SpectraMax® M5 Multi-Mode Microplate Reader (Molecular Devices).

2.6. Real-time quantitative polymerase chain reaction (RT-PCR)

Total RNA was prepared from hAMSCs using TRIzol reagent (Invitrogen). Moloney murine leukemia virus reverse transcriptase (Promega Corp.) efficiently reverse transcribe 1.5 µg total cellular RNA into cDNA with oligo(dT) primers. For real-time PCR , cDNA was amplified with the next primers by the Light Cycler 2.0 PCR system (Roche Diagnostics): C/EBP-α [5′-AACCTTGTGCCTTGGAAATG-3′, 5′-CCTGCTCCCCTCCTTCTCT-3′], PPAR-γ [’-ATGGAGTCCACGAGATCATT-3’, 5’-CGCAGGCTCTTTAGAAACTC-3’], LPL [5′-CTGGACGGTAACAGGAATGTATGAG-3′, 5′-CATCAGGAGAAAGACGACTCGG-3′] adiponectin [5′-ACCACTATGATGGCTCCACT-3′, 5′-GGTGAAGAGCATAGCCTTGT-3′], aP2 [5′-AACCTTAGATGGGGGTGTCCTG-3′, 5′-TCGTGGAAGTGACGCCTTTC-3′], ribosomal protein large P0 (RPLP0) [5′-GGAATGTGGGCTTTGTGTTC-3′, 5′-TGCCCCTGGAGATTTTAGTG-3′]. For amplifying cDNA, Real-time PCR conditions are made up a hot start 10-min at 95°C, 45 cycles of 15s at 95°C, 10s at 60°C, and followed by 30s at 72°C. Expression level of RPLP0 is used for normalization of quantitative real-time PCR.

2.7. Western blot analysis

The cells lysate were prepared with lysis buffer (1% Triton X-100, 10% glycerol, 150 mM NaCl, 50 mM HEPES, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM phenylmethysulfonyl fluoride, 10 mg/mL leupeptin, 10 mg/mL aprotinin and pH 7.3, 1 mM EGTA). Centrifugation (14,000rpm for 15 min at 4oC) of the cell lysate isolates total proteins and quantitative analysis of protein was measured by Bradford protein concentration assay (Bio-Rad). 8 - 12% SDS-polyacrylamide gel present the samples with electrophoresis and the samples were electrophoretically transferred to nitrocellulose membranes. After 5% skin milk block membranes, primary antibodies

were incubated with membranes and reach to the proteins of membranes (overnight at 4°C). After the suitable horseradish peroxidase-conjugated antibodies incubate with membranes, antibodies were developed using Enhanced chemiluminescence (ECL system; GE Healthcare).

2.8. Transfection and luciferase activity assay

For transfection, 3 x PPRE-tk-LUC plasmid, which has 3 copies of the PPAR-γ response element, pSV-PPAR-γ2 and pRL-SV40 construct, which expresses renilla luciferase (Promega Corporation), were prepared. The transfection was carried out following the procedure described by the Lipofectamine® 2000 instructions. To investigate the effect of PG on PPAR-γ activation, the Human embryonic kidney 293 (HEK293, 5×104cells/well) cells were prepared into 24-well plates. When the cells in 24-well plates reaches to approximately 70-80% of density, DNA mixture were transfected in the cells using Lipofectamine® 2000 for 24h. DNA mixtures consist of the pRL-SV-40 (25 ng), pSV-PPAR-γ2 (0.1 μg) and 3 x PPRE-tk-LUC plasmid (0.1 μg). After transfection, the cells were cultured with following treatments of rosiglitazone, PPAR-γ ligand, and PG for an additional 36 h. Acc0ording to the manufacturer' instruction, the luciferase activity assay was measured with the Dual-Luciferase Reporter Assay System (Promega Corporation). Renilla luciferase activity was in used for normalization of relative luciferase activity.

2.9. Statistical analysis

Data are expressed as mean ± standard deviation for independent experiments. Statistical significance test was estimated by Student's t-test and differences were significantly considered at $p<0.05$. All statistical tests were performed using SPSS for Windows version 18.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. PG reduces lipid droplet formation

hAMSCs were cultured with AIM in the presence of PG for investing the function of PG on adipocyte differentiation. At 10 days after the initiating differentiation, the lipid accumulation and a characteristic of the adipocyte formation process was monitored using Oil red O staining. PG-treated hAMSCs show major changes as compared with the vehicle control in adipocyte differentiation condition. Treatment with PG significantly reduced accumulation of lipid droplets in a dose-dependent manner (Fig. 1B). Quantification of the Oil Red O staining also indicates that PG suppresses adipocyte differentiation in hAMSCs (Fig. 1C). Additionally, we test the effect of PG on cellular viability with MTT assay in AIM. It presents that PG has no influence in cellular viability up to 30 μM (Fig. 1D). Therefore, these experiments demonstrate that PG suppresses adipocyte differentiation without the effect on cellular viability.

3.2. The expression of adipogenic genes are decreased by PG

During adipocyte differentiation, the expression of diverse transcriptional factors and adipogenic genes has significant role for regulating adipocyte differentiation[28]. To substantiate these phenotype results, we check the expression level of PPAR-γ and C/EBP-α, both major factors are required in adipocyte differentiation, with quantitative real-time PCR. We also examine expression of adipogenicgenes as LPL, aP2 and adiponectin, which are expressed during late or terminal stage of differentiation. These mRNA level was significantly decreased at the condition of AIM with 10uM PG. Inhibition of both PPAR-γ and C/EBP-α expressions was started by PG at 6days (Fig. 2A). Moreover, co-treatment of AIM and PG reduce the protein expression of adipogenic markers; PPAR-γ, C/EBP-α and aP2 (Fig. 2B). These results demonstrate that expression of adipogenic genes are suppressed by PG.

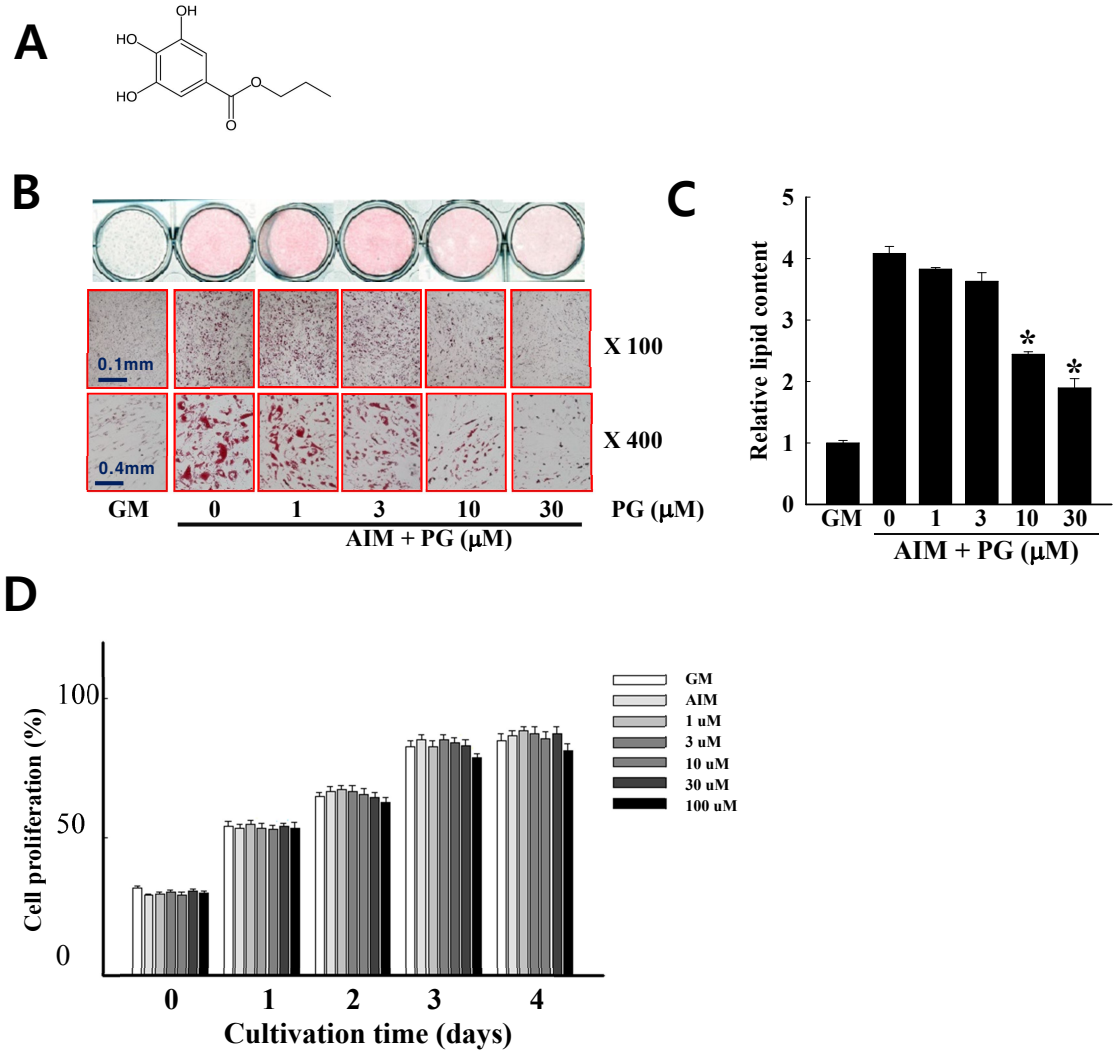

Figure 2 - 1. PG suppresses lipid accumulation in hAMSCs

(A) Structure of PG (B) Every 3 days, hAMSCs was cultured with indicated doses of PG in GM or AIM. On day 14, the differentiated cells were stained by Oil red O staining and the staining presents the lipid accumulation. (Magnifications×100 and ×400) (C) Oil red O staining was quantified by dissolving in isopropanol for a half hour and absorbance of isopropanol was measured at 540 nm. On the 14 days, GM-cultured cells were presented as 1 in measurement of the mean value. Values present the mean ± SD. (D) hAMSCs were incubated with or without 1 - 100 μM PG in AIM. MTT assay shows the cellular viability. The mean value of 4day-cultured cells were presented as 100. Values present the mean ± SD (*$p<0.05$). All of experiments are represented in triplicates. Error bars present the range of experiment thrice.

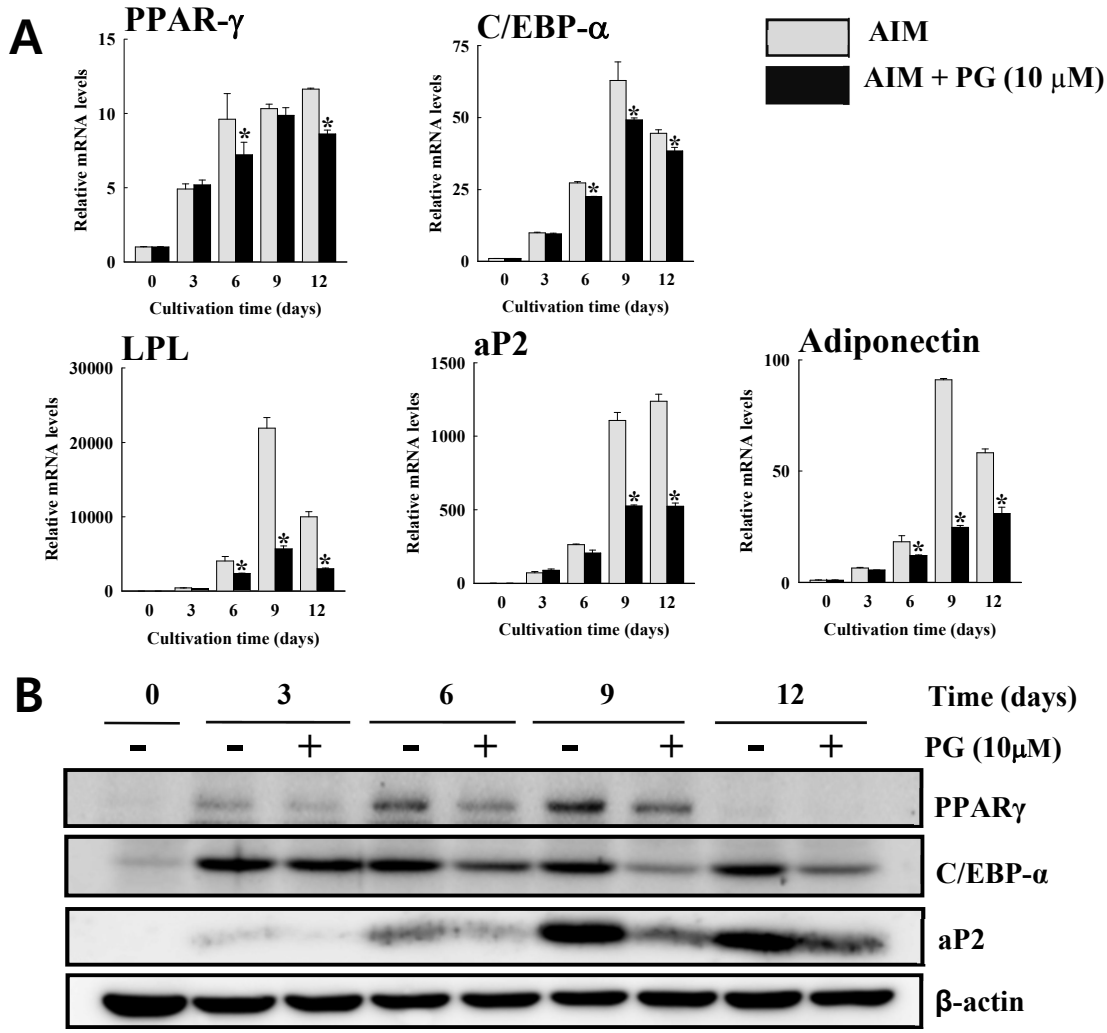

Figure 2- 2. mRNA and protein level of adipocyte-specific genes were decreased in PG condition

(A) hAMSCs were incubated with or without 10 μM PG in AIM. Gene expression of PPAR-γ, C/EBP-α, LPL, aP2, and adiponectin was examined by Real time-PCR. The mean values of 0day were presented as 1. (B) During adipocyte differentiation the protein level of PPAR-γ, C/EBP-α and aP2 was detected by western blot at the indicated time. All of experiments are represented in triplicates. β - actin are used as a normalization.

3.3. PG decreases PPAR-γ activity

For adipocyte differentiation, PPAR-γ is a significant transcriptional factor[29] and it has been known that some natural compounds modulate adipocyte differentiation by regulating PPAR-γ activity[30]. According to this reports, we test that whether PG reduces adipocyte differentiation by regulating PPAR-γ activity. In that hAMSCs has very low efficiency in the gene transfection, HEK293 cells were used to check the function of PG on PPAR-γ activity. To measure the PPAR-γ activity, three different plasmids, which are renilla luciferase expression vector, PPAR-γ2 expression vector and PPREx3-tk-luciferase reporter vector, were transfected in HEK293 cells. Rosiglitazone (100 nM, a PPAR-γ ligand) is used for inducing PPAR-γ activation and works as a positive control. When PPAR-γ was activated, co-treatment with PG effectively inhibits PPAR-γ activity of in a dose-dependent manner (Fig. 3). These results support that PG suppresses PPAR-γ activation.

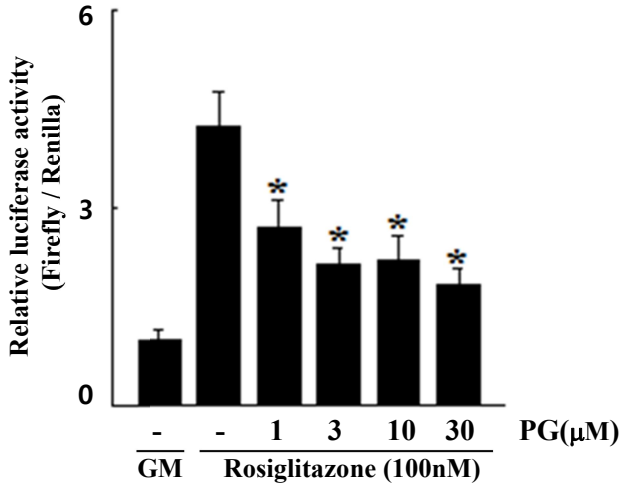

Figure 2 - 3. PG inhibits PPAR-γ activity

PPAR-γ2 expression vector, PPREx3-tk-luciferase reporter plasmid and pRL-SV40 vector were transfected (12 hours) in HEK293 cells. After transfection, the cells were incubated with 100nM rosiglitazone and 10uM PG for 36 h. The relative luciferase activity indicates normalized mean value of Firefly luciferase activity by activity of renilla luciferase. Renilla was used as transfection control. The mean values of vehicle-cultured cells were presented as 1. Values represent the mean ± SD.*$p<0.05$ (compared to control).

3.4. PG negatively regulate adipocyte differentiation via ERK activation

It has been known that MAPKs are involved in adipocyte differentiation from hMSCs. The activation of ERK and JNK signaling suppress the adipocyte differentiation whereas P38 promoted it[12]. The previous research demonstrated that a number of compounds regulate adipocyte differentiation through modulating MAPK activity[23a, 31]. Based on these studies, we demonstrate that PG reduces adipocyte differentiation by controlling MAPKs activity. In serum free condition, PG induced phosphorylation of ERK1/2 and p38, but not JNK phosphorylation (Fig. 4A). In addition, treatment of PG potentiated the ERK1/2 and p38 phosphorylation under AIM condition, compared with the vehicle control (Fig. 4B, C).

To establish whether PG inhibits adipocyte differentiation through ERK signaling pathway, we used the specific inhibitor of mitogen-activated protein kinase kinase (MEK), PD98059. Inhibition of MEK activity, which is the upstream kinase of ERK, blocks ERK signaling[32]. PG-induced phosphorylation of ERK1/2 was significantly decreased in response to PD98059 treatment (Fig. 5A). In adipocyte differentiation condition, co-treatment of PD98059 restored PG-mediated inhibition of lipid accumulation (Fig. 5B). Additionally, 20 μM PD98059 rescues the expression of adipocyte specific protein aP2 in a dose dependent manner (Fig. 5C). It demonstrates that PG supress adipocyte differentiation via ERK pathway. On the other hand, inhibition of p38 signal pathway by specific inhibitors SP203580 did not affect the anti-adipogenic activity of PG (Fig. 5B). Therefore, these results support that PG prevents adipocyte differentiation, possibly through sustained ERK activity without the activation of other MAPKs.

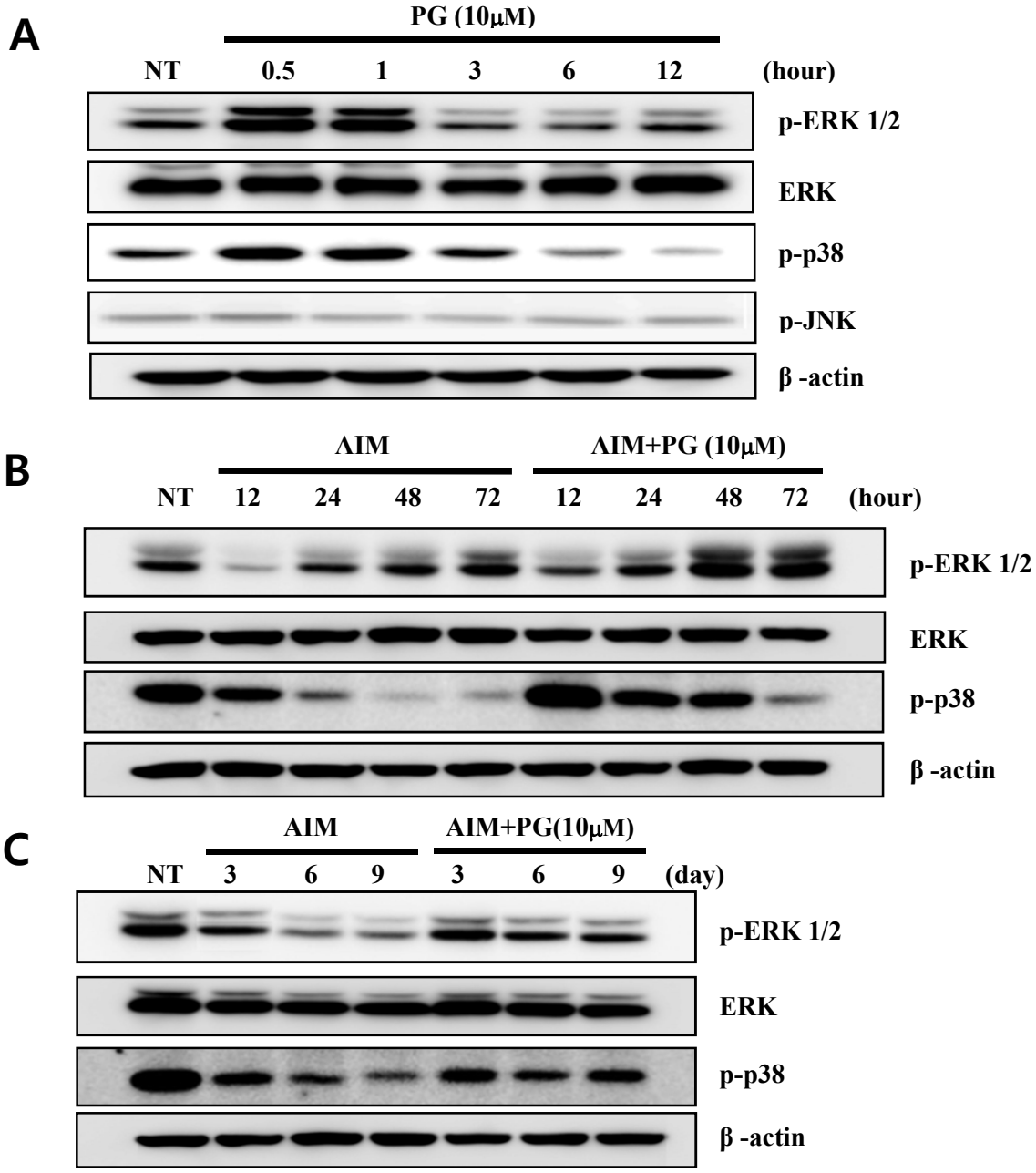

Figure 2 - 4. PG activates phosphorylation of ERK1/2 and p38 in hAMSCs

(A) hAMSCs were treated with 10uM PG in serum-starvation conditions and the cells were harvested at various indicated time. Phosphorylation of ERK1/2, JNK and p38 was examined with immunoblotting. (B), (C) hAMSCs were incubated with or without PG (10 μM) in AIM and cells are harvested at indicated time. Phosphorylation of ERK1/2 and p38 and expression of ERK2 and β -actin were examined by immunoblotting.

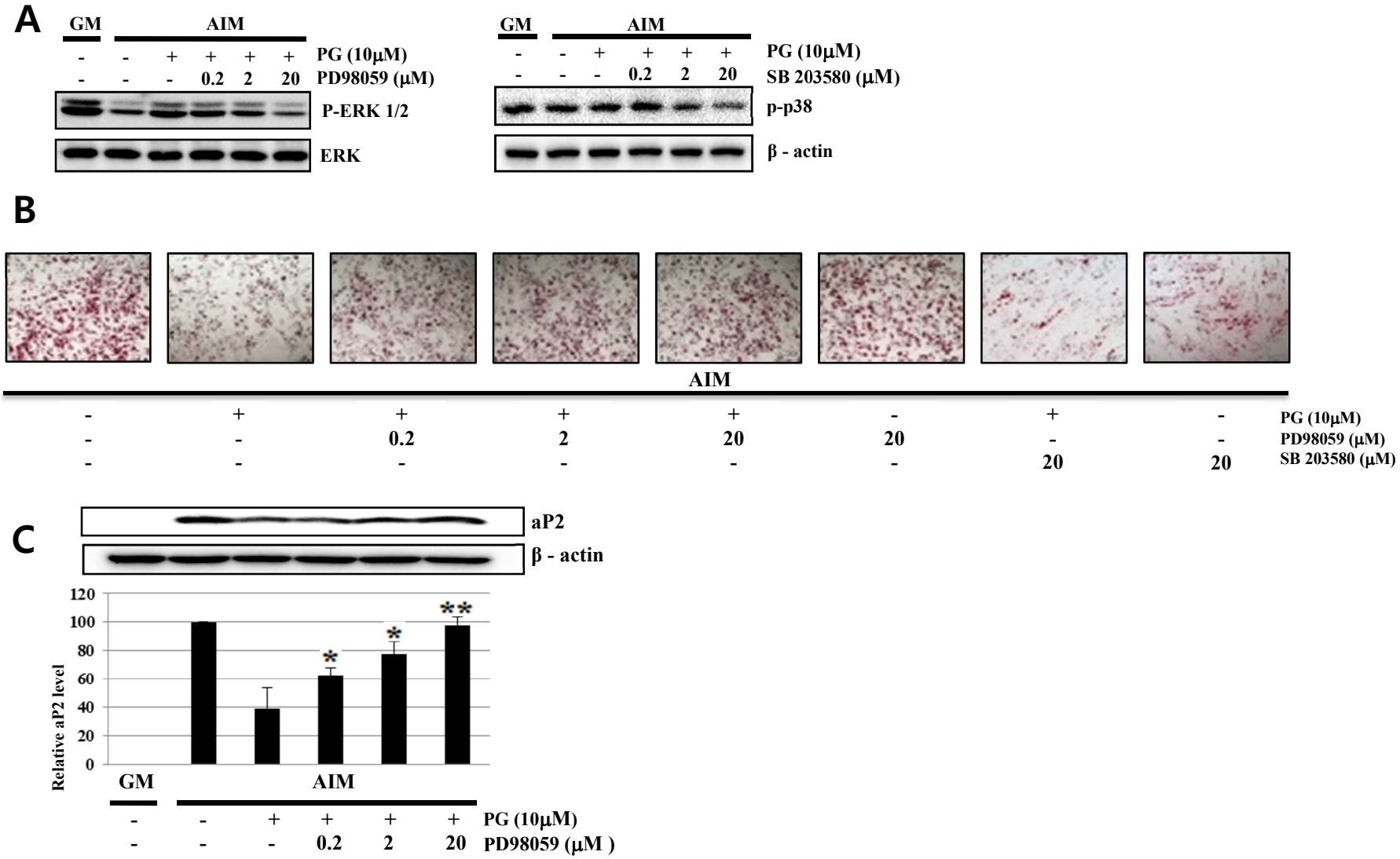

Figure 2 - 5. PG negatively regulate adipocyte differentiation through ERK activation

Figure 2 - 5. PG negatively regulate adipocyte differentiation through ERK activation

(A) Vehicle or PD98059 was pre-treated for 1 h, and hMSCs were additionally incubated with AIM containing 10 uM PG. At 30 min after treatment of PG with AIM, p-ERK1/2 was presented by immunoblotting. (B) hAMSCs were cultured in adipocyte differentiation condition with or without PG (10 uM) and effect of PD98059 or SB203580 was examined. After adipogenic induction, lipid accumulation was estimated by Oil Red O staining at 14 days. (C) Adipogenic marker, aP2, expression was examined in PG-treated cells with or without PD98059. Protein level of aP2 was examined at day 14 after adipogenic induction. The mean values of venicle -treated cells were presented as 100 and others were as its relative values. Data represent the mean $\pm$SD. *$p<0.05$, **$p<0.01$

4. Discussion

PG has been knwon as a synthetic antioxidant in cosmetics, processed food and food packaging materials to avoid spoilage and rancidity. The present, Drug Administration and US Food list assure safety and effectiveness of PG. Previous studies focused on PG have proprieties of antioxidant, a chemo preventive agent and anti-inflammatory factor[17b, 33]. However, a function of PG on stem cell differentiation has not been investigated. This study suggests anti-adipogenic activity of PG in hAMSCs. Our results show that PG significantly decreases adipocyte differentiation without cellular toxicity and PG inhibits lipid droplets formation. In response to PG treatment, adipogenic markers expression also was suppressed during adipocyte differentiation of hAMSCs and the activity of PPAR-γ was also decreased. Moreover, the inhibitory effects of PG require ERK activity, but not p38 activation. To our understanding, this is the first study of PG that inhibits adipocyte differentiation through ERK signaling pathway and regulating PPAR-γ activity in hAMSCs.

During adipocyte differentiation, induced PPAR-γ and C/EBP-α are the key transcriptional regulators[34]. Especially, PPAR-γ expression can promote adipocyte differentiation in absence of C/EBP-α, but C/EBP-α cannot induce adipocyte differentiation without PPAR-γ, it shows that PPAR-γ is a key transcriptional factor of adipocyte differentiation[35]. PPAR-γ is known as a member of the nuclear receptor superfamily of ligand-induced transcription factors[36]. No factor has been found out that induced adipocyte differentiation without PPAR-γ and most adipogenic factors can modulate the adipocyte differentiation via modulation of PPAR-γ activity and expression. For example, some flavonoids such as vitexin, orientin, and lovastatin inhibit adipocyte differentiation by inhibiting PPAR-γ expression in bone marrow-derived mesenchymal cell[37]. We also observed PG decreased PPAR-γ expression in adipocyte differentiation from hAMSC. Furthermore we examine that PG has the effect on PPAR-γ activation. The results show that PG inhibits the activity of PPAR-γ in HEK293 cells (Fig. 3). Taken together, those results indicate that PG suppresses the adipocyte differentiation of hAMSC through decreasing the expression and activation of PPAR-γ.

Recently, several reports demonstrated that anti-adipogenic compounds induce activation of MAPK and inhibit adipocyte differentiation through MAPK signal pathway[12]. For example, Ochratoxin A, is as food-contaminating mycotoxins, suppresses adipocyte differentiation via activation of ERK[23a]. Wedelolatone, which is naturally occurring coumestan and used for septic shock patient, has been known to suppress adipocyte differentiation via phosphorylation of ERK and JNK in bone marrow stromal cells[38]. ERK has significant control function on stem cell differentiation and ERK inhibitor, PD98059, is known for induction of adipocyte differentiation. PG promotes ERK and p38 under serum-free conditions (Fig. 4A). And in AIM condition, PG activates ERK1/2 phosphorylation, and treatment of MEK inhibitor, PD98059, rescues the inhibitory activity of PG in adipocyte

differentiation. But a p38-specific inhibitor could not recover adipocyte differentiation (Fig. 5B). Therefore, our results demonstrate that PG suppresses adipocyte differentiation in ERK-dependent manner. In addition, PG decreases PPAR-γ activity. PPAR-γ can be regulated through ERK activation. The ERK pathway causes S112 phosphorylation of PPAR-γ and phosphorylation of PPAR-γ inhibits the RXR/PPAR- heterodimers activity on PPREs and it can inhibit adipocyte differentiation[39]. Therefore, we can assume that activated ERK cascade by PG contributes to regulation of PPAR-γ genomic action through phosphorylation of PPAR-γ.

Obesity, which stands for as abnormal or immoderate lipid accumulation, cause global issue such as type II diabetes, hypertension, cardiovascular diseases and certain cancers[20]. A great deal of researchers has focused on study and treatment of obesity through inhibition of adipocyte differentiation. This report provides the possibility of obesity care through PG stimulated- inhibition of adipocyte differentiation. And then, to confirm the possibility, we need to discover the function of PG in animal model in further study.

5. Conclusion

We demonstrate a novel effect of PG in adipocyte differentiation of hAMSCs. PG reduces lipid droplets formation and adipogenic marker expression in hAMSCs. Especially, we reveal that activation of PPAR-γ, which works as a major transcriptional factor in adipocyte differentiation, was decreased in PG condition and PG inhibits adipocyte differentiation via ERK activity. Furthermore, these results suggest that PG can be used as anti-obesity food additives.

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엇이든 밝고 명랑하게 생활하는 신경진 후배에게 감사 드립니다. 또한 저희 실험실의 구성원으로 무엇이든 믿고 물어볼 수 있는 든든한 김영미 선생님, 지금은 포항에 계시지만 멋진 라이프 스타일을 보여주신 이혜린 선생님, 몸은 가녀리시지만 밝은 얼굴로 에너지 넘치게 일하시는 오지현 선생님 마지막으로 오신지는 얼마 되지 않으셨지만 열심히 따라 배우려고 하시는 남다름 선생님께 고맙다는 인사 드리며 같이 먹었던 점심시간은 정말 즐거웠다는 말 하고 싶습니다.

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대학원 수업을 하면서 친해진 친구들은 힘들 대학원 생활에서 서로 고민상담하고 서로 위로하며 만든 소중한 인연들 이였습니다. 제일 큰 고민부터 제일 사소한 고민까지 모두 털어놓으며 많은 이야기를 한 임지원 오빠, 밥 한번 먹자는 말로 서로에게 할 이야기가 있다는 것을 알게 되는 조주형 오빠, 멀리서 보여도 반가워서 손 먼저 흔들고 반갑게 인사하는 봉서, 언니 언니 하며 반갑게 인사하는 게 너무 귀여운 은진이, 의젓한 듯 보이지만 여린 마음에 고민이 많은 환희까지 모두 만나면 반겨주고 힘이 들 때 도와주고 챙겨줘서 고맙다는 마음을 전하고 싶습니다.

제가 공부하는 동안 저에게 가족들은 저를 항상 믿고 지지해주는 큰 버팀목 이였습니다.

늦게까지 공부하느라 힘들지 않냐며 항상 저를 걱정해주시는 우리 아빠, 점심 도시락을 준비해주시며 오늘 하루도 힘내라고 힘이 되어주신 우리 엄마, 언니 노릇을 할 수 있도록 언니를 항상 믿고 따라와주는 친구 같은 동생 지은이 그리고 울산 떠나 포항에서 공부하느라 힘든 막내 동생 건희까지 우리 가족 모두에게 항상 고맙고 사랑한다는 말을 전하고 싶습니다.

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