Synergic Effect on Adipocytic Differentiation by a Combination of Thyroxine and Glucocorticoid in A549 Cells

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The present study examined the rate of cell growth and differentiation potential into adipocytes in A549 lung adenocarcinoma cells exposed to each adipogenic medium containing glucose metabolism hormones, such as thyroxine (T4) thyroid hormone and glucocorticoid (GC) adrenal steroid hormone, as well as pioglitazone (PGZ), a PPARγ agonist. Following each adipogenic treatment for 2 weeks, the rate of cell growth was significantly ($p<0.05$) inhibited, and the level of telomerase activity was significantly ($p<0.05$) decreased in the PGZ-based adipogenic medium containing both T4 and GC hormone compared with those containing each T4 or GC hormone. Moreover, the adiposome-like vesicles were highly reacted with Oil-Red O staining solution, and the levels of transcripts expressed in the differentiating adipocytes for adipogenesis, including adinopectin, leptin, and resistin, were significantly ($p<0.05$) increased in the PGZ-based adipogenic medium containing both T4 and GC hormone compared with those of the adipogenic medium containing each T4 or GC hormone, implying that adipocytic differentiation has fully occurred in the A549 cancer cells. Based on present observations, the PGZ-based adipogenic medium containing both T4 and GC efficiently induces inhibition of cell growth and cellular differentiation into adipocytes in A549 cancer cells rather than in the adipogenic medium containing only T4 or GC hormone. Adipogenic treatment could provide potential probability in cancer chemotherapy.

Key words : A549 adenocarcinoma, adipogenesis, anti-tumor, differentiation, human

Introduction

Tumors are abnormal tissue masses with unlimited cell growth patterns by mutation of proto-oncogenes and tumor suppressor genes, and benign tumors are gradually changed by more accumulation of gene mutations to cancers (malignant tumors) with metastatic characterization to another part of the body, and lead to ultimately death. Various treatments, including surgery, chemotherapy, and radiation therapy are recently applying for the removal and treatment of cancers, and new treatments, including immunotherapy and metabolic therapy, are continually developing and applying, depending on the types and stages of cancer [4, 14]. When cancers are observed by cancer detection techniques in our body, the visi-ble cancer mass is usually removed by surgery therapy, and chemotherapy and/or radiotherapy is progressively applying up to recently. However, the recent cancer treatment by chemotherapy is displayed with severe side effects by cellular cytotoxicity. Advanced chemotherapy with less side effects should be certainly developed for cancer treatment [4, 14].

The undifferentiated stem cells in our body are also displayed to unlimited cell growth characterization for the regeneration and repair of their cells and tissues, as shown in the cancer cells, however, the differentiated stem cells into specialized cell types of our body are gradually changed to limited cell growth pattern and finally reached at cellular senescence and apoptosis stage. Thus, it has also suggested that the cell growth of differentiated cancer cells might be reached at delayed cell growth and apoptosis stage, as shown in the differentiated stem cells, and the previous studies have demonstrated that the cancer cells, including A549 cancer cells, are induced to inhibition of cell growth and cellular senescence by adipocytic differentiation [24].

The glucose or glucose-similar monosaccharides are a major source of energy production in our body, and the inter-

mediate products derived from the glucose metabolism process are a precursor of other molecules, including nucleotides, amino acids, and lipids. Generally, most of the cancer cell lines were dramatically increased in glucose metabolism and uptake for their high and uncontrolled cell growth rate, survival, and cell maintenance, compared with those of normal somatic cells. In most somatic cells, the glucose is progressively hydrolyzed by the glycolysis pathway in the cytoplasm and the oxidative phosphorylation pathway in the mitochondria for ATP production. Whereas, ATP production in cancer cells is mainly produced by the glycolysis pathway than the oxidative phosphorylation pathway, even though oxygen is fully supplied to the cells and functional mitochondria normally existed. It was widely known as the Warburg effect [18, 29]. The glucose absorbed in the digestive tract of our body is entered into a cell by the functional insulin hormone. Except for the glucose used for basic energy production, excess-absorbed glucose can be converted to glycogen polysaccharides and further store lipid molecules derived from fatty acids and glycerol through biosynthesis pathways, known as adipogenesis [1, 16]. As well, the transport of glucose to the cytosol across the cell membrane was usually carried out by facilitative diffusion with glucose transporters (GLUTs) and/or sodium-dependent glucose transporters (SGLTs), depending on the concentration gradient of glucose in the normal cells. Whereas, tumor and cancer cells display a high glucose metabolism beyond that necessary for basic cell maintenance and metabolism, compared with those of normal cells [11, 38]. It has also been estimated that the main glucose transporters are GLUT1 and SGLT1 subtypes, and the expression of these transporters is highly increased in the cancer cells [2, 31].

Further, it has well known that key regulators for lipid metabolism and production in the adipogenesis process are CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptors (PPARs) transcription factors [1, 32]. Thus, undifferentiated stem cells and mouse 3T3-L1 pre-adipocytes were easily induced into adipocytes by treatment of PPARγ agonists, such as drugs of thiazolidinedione (TDZ) class in the previous studies [24, 39]. The previous study has also demonstrated that the cancer cells, including A549 cancer cells with high glucose metabolism and uptake capacity were easily induced to the adipocytic differentiation by treatment of PGZ, a kind of TDZ class [24]. Thus, it has finally been suggested that the adipogenic treatment by upregulation of PPARγ activation with TDZ drugs can probably be used for therapy of cancer treatment [19, 24]. In our body,

thyroid hormone (mainly thyroxine 4, T4) released from the thyroid gland is a main regulator for the increase of metabolic rate, protein synthesis, bone growth, neural development, and so on. And, another hormone associated with our body's glucose metabolism is a glucocorticoid (GC, cortisol) steroid hormone released from the adrenal cortex in the adrenal gland and its function is to increase new blood glucose through the gluconeogenesis process [26, 33]. However, the effect on growth inhibition is not fully demonstrated and is still unclear in the differentiation potential and pathway of cancer cells by adipogenic treatment containing only or combined T4 and GC hormone.

Herein, we have hypothesized that the increase of adipocytic differentiation potential might be prompted by treatment of the T4 and GC hormone related to glucose metabolism, and consequently induced to inhibition of cell growth. In the differentiating or differentiated adipocytes, several such as ADIPONECTIN, LEPTIN, and RESISTIN are secreted and contribute to the adipogenesis pathway, metabolism of adipocytes, and migration of immunocytes [15, 22]. And the present study has investigated the effect of glucose metabolism-related hormone treatment on the cell growth pattern and adipocytic differentiation potential in the A549 lung cancer adenocarcinomas.

Materials and Methods

Culture and treatment of cells

The Advanced-Dulbecco's modified eagle medium (A-DMEM) added with 3% fetal bovine serum and penicillin/streptomycin was the basic cell culture medium and purchased from Thermo Fisher Scientific (USA). All other supplement, including T4, GC, and PGZ was purchased from Sigma Chemical Company (USA). The A-549 adenocarcinomas derived from human lung was purchased from American Type Culture Collection (ATCC, USA). The A549 cells were cultured at 36.5℃ under 5% CO_2 in the basic cell culture medium. When the cells were grown at 80~90% confluent status, the cells were periodically sub-cultured, and the cell culture medium was changed every 3 days. The dexamethasone was used for a synthetic GC, and the stock solution was dissolved at 1 mM in methanol. The T4 was also dissolved at 10 mM in methanol. The stock solution for PGZ was dissolved at 100 mM in dimethyl sulfoxide (DMSO). For adipogenic treatment, the working solution for T4, GC, and PGZ was freshly prepared at 100 nM, 1 uM, and 50 uM by adding stock solution in the A-DMEM cell culture

medium, respectively. Each of the adipogenic treatments was cultured for 2 weeks, and each treated cell was harvested with trypsin treatment and either immediately examined or stored at 80℃ deep freezer for future analysis. Each adipogenic treatment consisted of 1) untreated control medium (A-DMEM basic medium), 2) A-DMEM medium containing only T4, 3) A-DMEM medium containing only GC, 4) A-DMEM medium containing both T4 and GC, 5) A-DMEM medium containing only PGZ, 6) PGZ-based A-DMEM medium containing T4, 7) PGZ-based A-DMEM medium containing GC, and 8) PGZ-based A-DMEM medium containing both T4 and GC.

Analysis of cell growth by population doubling time (PDT)

The value of population doubling time (PDT) was the amount of time that needs for a quantity to double in cell number. The A549 cells were cultured in each adipogenic medium for 2 weeks, and the rate of cell growth was investigated by PDT analysis. The A549 cells for each treatment were counted and seeded 1×10^3 cells into a 25 cm^2 culture flask for 14 days (2 weeks), and each A-DMEM media was regularly changed every 2 days. After 2 weeks, the treated cells were harvested by trypsinization, and the number of cells was calculated with a hemacytometer. An equation to use for the calculation of PDT was as follows: PDT = t (log 2)/(log N_t-log N_o), and t is cell culture time (hrs), N_o and N_t are the cell numbers initially at time 0, and the number of cells at time t, respectively.

Analysis of telomerase activity by relative-quantitative telomerase repeat amplification protocol (RQ-TRAP)

The enabling replicative immortality by high telomerase activity is one of a hallmark of cancer cells. The relative telomerase activity in each treated A549 cell was quantified by RQ-TRAP assay using SYBR Green real-time PCR machine (Rotor-Gene Q, Qiagen, USA) modified from PCR-based assay, as previously described by Jeon et al [21]. Briefly, the samples derived from each adipogenic treatment were harvested at 1×10^5 cells per sample and were lysed with 400 μl of 1X CHAPS cell lysis buffer (TRAPeze®, Millipore, USA) at 4℃ or 30 min. The lysed samples were centrifuged for 30 min at 12,000 ×g and collected 70% of the supernatant. The protein concentration of each sample was then measured with a spectrophotometer (Microdigital, Korea). The reactions for RQ-TRAP were contained with 1 μg protein of each lysed sample, Rotor-Gene^{TM} 2× SYBR green kit (Qiagen, USA), telomerase TS primer (0.02 μg), and anchored return ACX primer (0.04 μg), and adjusted with ddH$_2$O to the 20 μl volume. The sequences of TS and ACX primer were described in Table 1. The reactions for RQ-TRAP amplification were firstly run at 30℃ for 30 min, and the amplification protocols were accomplished in 40 cycles with each cycle consisting of 94℃ for 30 sec, 60℃ for 90 sec, and 72℃ for 0 sec. The level of telomerase activity in each treated cell was relatively estimated to those of untreated control cells with the Cp value of the second derivative curve analysis using Rotor-Gene Q Series Software (Qiagen, USA) in five replicative.

Analysis of Oil Red O staining

The adiposome-like organelles or vesicles in the adipogenic treated cells were stained with Oil Red O solution. Briefly, each adipogenic treatment medium was removed and gently washed with Dulbecco's Phosphate Buffered Saline (D-PBS) twice and successively added with 3.7% paraformaldehyde fixation solution for 1 hr. The fixed cells were washed with distilled water (DW), and incubated with 60% isopropanol for 5 minutes. After being discarded 60% iso-

Table 1. Primer sequences and PCR product size used for RQ-TRAP and RT-PCR.

Gene	Primer sequences (5'–3')	Amplification size (bp)
RQ-TRAP TS	AATCCGTCGGAGCAGAGTT	
RQ-TRAP ACX	GCGCGGCTTACCCTTACCCTTACCCTAACC	
GAPDH	GAAGGTGAAGGTCGGAGTC	228
	GAAGATGGTGATGGGATTTC	
ADIPONECTIN	TATGATGGCTCCACTGGTA	125
	GAGCATAGCCTTGTCCTTCT	
LEPTIN	TCCCCTCTTGACCCATCTC	110
	GGGAACCTTGTTCTGGTCAT	
RESISTIN	TCTAGCAAGACCCTGTGC	153
	CAGGTTTATTTCCAGCTCC	

propanol, the cells were stained with Oil Red O working solution for 2 hr at room temperature along with the rotation of the culture flask. After being washed DW, the adiposome-like organelles with red color were observed under an inverted microscope equipped with a CCD image system (Nikon, Japan). For quantification of lipid accumulation, the stained cells with adiposome-like organelles were extracted with 100% isopropanol, and the extracted solution was transferred to a 96-well plate, and absorbance ratio at 492 nm was measured using a spectrophotometer (Microdigital, Korea).

Analysis of transcripts by RT-PCR

The adipokine transcripts expressed from differentiated/differentiating A549 cells were analyzed by RT-PCR assay. Each adipogenic treated cell were harvested and the total RNA was extracted with an RNA extraction kit (Ribospin™, GeneAll, Korea), according to the provided protocol, and quantified with a spectrophotometer (Mecasys, Korea). 1 μg total RNA was synthesized with an Omniscript reverse transcription kit (Qiagen, USA), according to the provided protocol. The amplification of cDNA was employed with a real-time PCR machine (Rotor-Gene Q, Qiagen, USA), and 20 μl of PCR reactions containing 2 μl of cDNA sample, and each primer pair was amplified in 35 PCR cycles. The relative level of expression was calculated by a threshold value (Ct value) using Rotor-Gene Q software (Qiagen, USA), according to the expression level of GAPDH, as used for a reference gene. If necessary, the PCR products amplified in the real-time PCR machine were collected and confirmed on the agarose gel. The details of primers are described in Table 1.

Statistical Analysis

The statistical differences among the adipogenic treatments were analyzed with a one-way analysis of variance (ANOVA, SPSS 15.0 version, USA). The acquired data were displayed as mean ± standard error of the mean (SEM). The significant differences among treatments were tested at $p < 0.05$.

Results

Cell growth by adipogenic treatment

Following each adipogenic treatment for 2 weeks, the morphological alteration in A549 cells was observed under an inverted microscope, as representatively shown in Fig. 1. The adiposome-like vesicles were nearly displayed in the majority of the cells treated with all adipogenic medium, especially, and the high number of large vesicles were displayed in the PGZ-based adipogenic A-DMEM medium containing both T4 and GC, compared with those of other adipogenic A-DMEM medium.

To investigate the effect on the rate of cell growth by adipogenic treatment, the mean PDT was investigated in the A549 cells treated with each adipogenic medium, and the results are displayed in Fig. 2A. The mean PDT in the untreated control A-DMEM medium was 38.1±2.88 hr. Whereas the mean PDT was 54.8±1.27, 63.7±3.34, and 78.6± 2.54 hr in the adipogenic A-DMEM medium containing only T4, only GC, and both T4 and GC, respectively. In comparison to adipogenic A-DMEM medium containing T4 or GC, the rate of cell growth was also significantly ($p<0.05$) inhibited in the adipogenic A-DMEM medium containing GC, compared with those of T4. The significantly ($p<0.05$) increased PDT by the inhibition of cell growth was also exhibited in the adipogenic A-DMEM medium added with both T4 and GC, compared with untreated control A-DMEM medium, A-DMEM medium containing only T4 or GC. Further, the mean PDT was 64.6±2.54 hr in the adipogenic A-DMEM medium containing only PGZ, and 70.9±4.01, 89.9±5.01 and 107.6±3.88 hr in the PGZ-based adipogenic A-DMEM me-

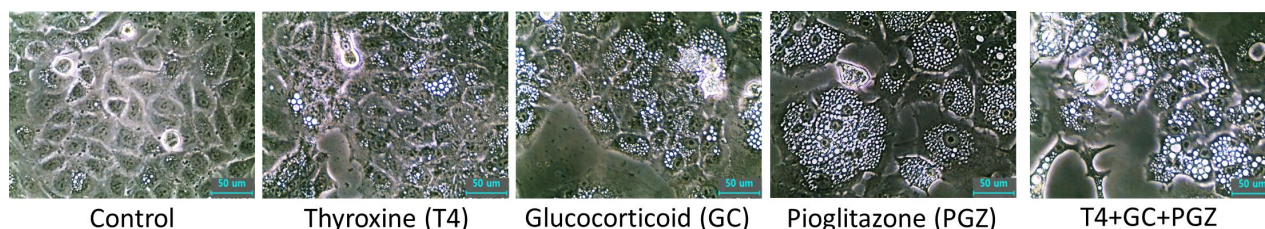| Control | Thyroxine (T4) | Glucocorticoid (GC) | Pioglitazone (PGZ) | T4+GC+PGZ |

Fig. 1. Changes of cell morphology by adipogenic treatment in the A549 cells for 2 weeks. A representative example in the untreated control A-DMEM medium, A-DMEM medium containing only T4, A-DMEM medium containing only GC, A-DMEM medium containing only PGZ, and PGZ-based adipogenic A-DMEM medium containing both T4 and GC is shown. The cells with adiposome-like vesicles were easily observed in each adipogenic medium, especially in the PGZ-based adipogenic A-DMEM medium containing both T4 and GC (×200). Scale bars: 50 μm.
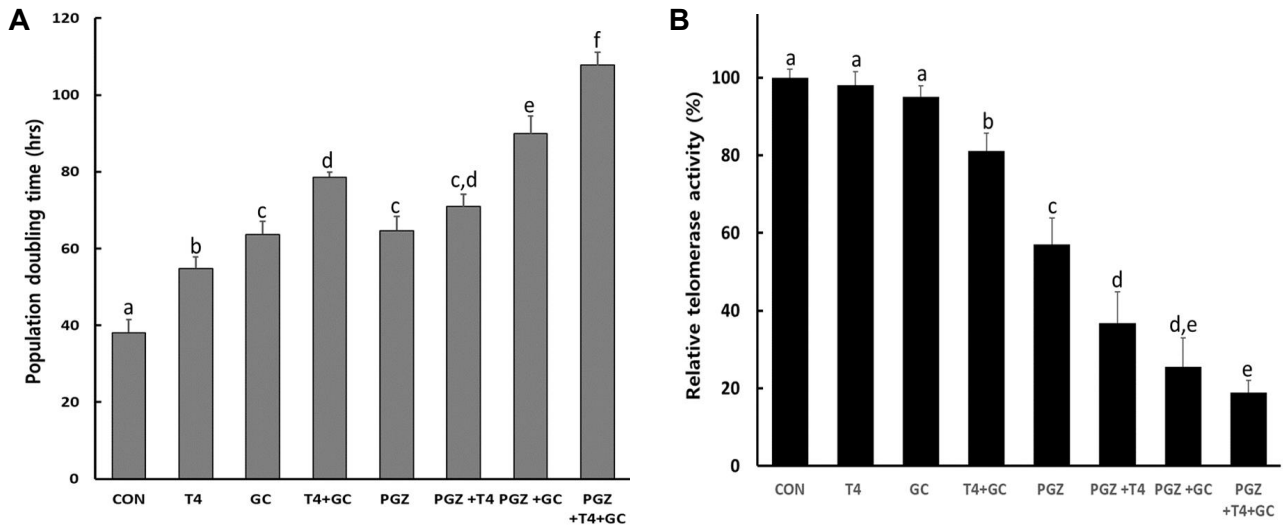
Fig. 2. A) Analysis of PDT by each adipogenic treatment in the A549 cells for 2 weeks. The cell growth was highly inhibited in the PGZ-based adipogenic A-DMEM medium containing both T4 and GC. B) Analysis of telomerase activity in the A549 cells treated with each adipogenic treatment for 2 weeks. The telomerase activity was highly decreased in the PGZ-based adipogenic A-DMEM medium containing both T4 and GC, compared with the untreated control A-DMEM medium. a, b, c, d and e indicate significant ($p<0.05$) differences among each adipogenic treatment, respectively.

dium containing only T4, only GC and both T4 and GC, respectively. The PDT was highly ($p<0.05$) increased by inhibition of cell growth with combination treatment of both T4 and GC to the PGZ-based adipogenic A-DMEM medium, rather than PGZ-based adipogenic A-DMEM medium containing only T4 or only GC.

The telomerase activity was investigated in the A549 cells exposed to each adipogenic medium, as shown in Fig. 2B. The level of telomerase activity in the untreated control A549 cells was considered as 100% for comparison with other adipogenic treatment groups. The level of mean telomerase activity was 98±2.2, 95±3.5, and 81±4.6% in the adipogenic A-DMEM medium containing only T4, only GC, and both T4 and GC, respectively. The telomerase activity was significantly ($p<0.05$) decreased in the adipogenic A-DMEM medium containing both T4 and GC, compared with the adipogenic A-DMEM medium containing only T4 or GC. Further, the mean telomerase activity was 57±6.8, 36±8.1, 25±7.5, and 18.9±3.1% in the adipogenic A-DMEM medium containing only PGZ, PGZ-based adipogenic A-DMEM medium containing only T4, PGZ-based adipogenic A-DMEM medium containing only GC, and PGZ-based adipogenic medium containing both T4 and GC, respectively. The level of telomerase activity was also significantly ($p<0.05$) decreased in the combination treatment of both T4 and GC to the PGZ-based A-DMEM adipogenic medium, rather than other adipogenic medium.

Analysis of cellular differentiation into adipocytes

The A549 cancer cells were treated with each adipogenic medium for 2 weeks, and the Oil Red O assay was employed to analyze the staining of intercellular neutral triglycerides in the adiposome-like vesicles of the cells, as displayed in Fig. 3. The A549 cells with red spots under a microscope were implied into adipogenic differentiation by an accumulation of neutral triglycerides and lipids (Fig. 3A). The frequency of the cells with the red spot was highly increased in the cells treated with PGZ-based adipogenic A-DMEM medium containing both T4 and GC. Further, the Oil Red O staining was extracted with isopropanol, and the extracted Oil Red O solution was measured by spectrophotometer (Fig. 3B). As expected, the intensity of Oil Red O solution was highly increased in the cells treated with PGZ-based adipogenic A-DMEM medium containing both T4 and GC, compared with other adipogenic media.

Analysis of adipokine transcripts

The expression level of adiop0nesis-related genes was investigated with by RT-PCR assay in the differentiating/differentiated A549 cells, and the results were shown in Fig. 4. The expression level of ADIPONECTIN, LEPTIN, and RESISTIN transcript was used in the study, and relatively compare with the expression level of GAPDH, as a control reference gene. The relative expression level of ADIPONECTIN, LEPTIN, and RESISTIN transcript was significantly ($p<0.05$)
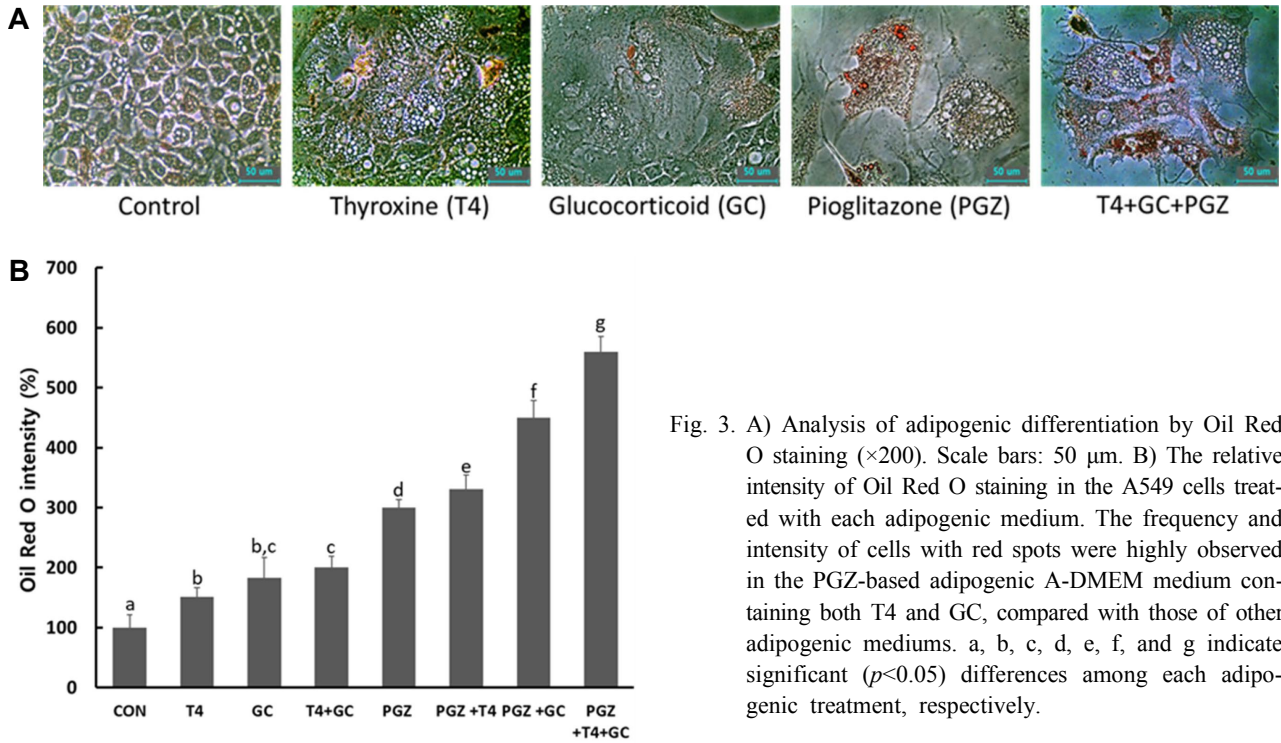
Fig. 3. A) Analysis of adipogenic differentiation by Oil Red O staining (×200). Scale bars: 50 μm. B) The relative intensity of Oil Red O staining in the A549 cells treated with each adipogenic medium. The frequency and intensity of cells with red spots were highly observed in the PGZ-based adipogenic A-DMEM medium containing both T4 and GC, compared with those of other adipogenic mediums. a, b, c, d, e, f, and g indicate significant (*p*<0.05) differences among each adipogenic treatment, respectively.
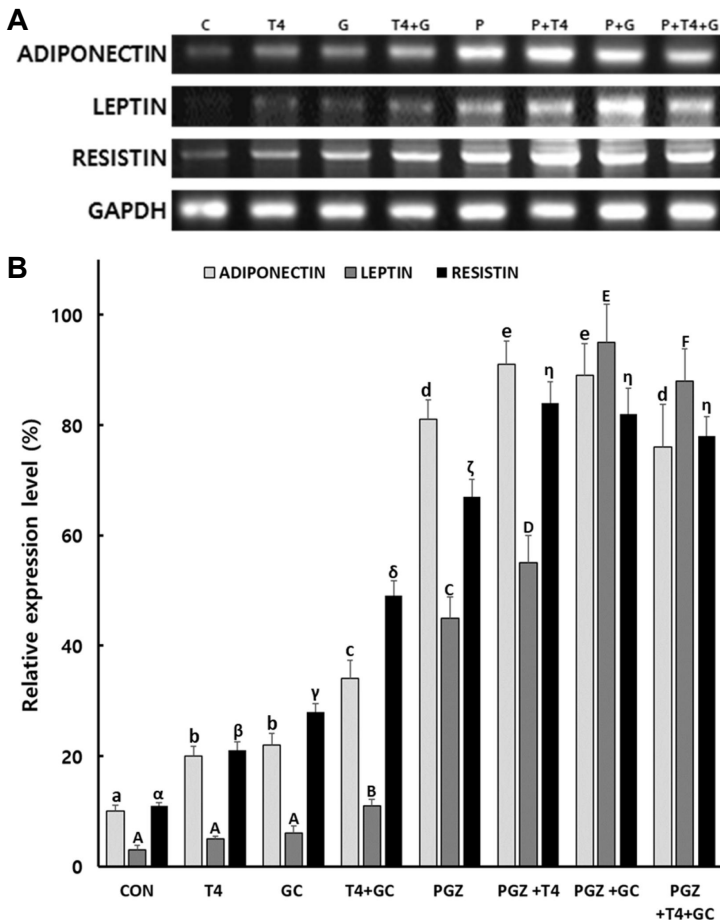

Fig. 4. Analysis of expression level of adipogenesis-related transcripts, including ADIPONECTIN (■), LEPTIN (■), and RESISTIN (■) in each adipogenic medium. a, b, c, d, and e indicate significant (*p*<0.05) differences in the expression of ADIPOINECTIN among each adipogenic treatment, respectively. A, B, C, D, E, and F indicate significant (*p*<0.05) differences in the expression of LEPTIN among each adipogenic treatment, respectively. α, β, γ, δ, ε, and ζ indicate significant (*p*<0.05) differences in the expression of RESISTIN among each adipogenic treatment, respectively.

increased in the PGZ-based A-DMEM adipogenic medium containing only T4, only GC, or both T4 and GC, compared with those of untreated control adipogenic A-DMEM medium and medium adipogenic containing only T4, GC or both T4 and GC.

Discussion

The present study investigated the rate of cell growth by PDT assay, adipocytic differentiation by Oil Red O staining, and expression level of transcript related to adipogenesis, including ADIPONECTIN, LEPTIN, and RESISTIN in the A549 lung adenocarcinomas. The A549 cells were exposed to each adipogenic medium containing T4 and GC hormone related to energy metabolism, and PGZ as an activator of PPARγ or a combination of those materials. The present results demonstrated that A549 cells were highly induced to growth inhibition and adipocyte differentiation in the PGZ-based adipogenic medium containing both T4 and GC, compared with those of only T4 and GC.

In our body, it was reported that several endocrine hormones, including T4 (mainly T4 than T3) from the thyroid gland, GC (cortisol) from the adrenal cortex and insulin from the pancreas, and growth hormone from the anterior pituitary gland have important effects on the increase of cell metabolism, and these hormones are rhythmically secreted from the endocrine cell of the tissue. The blood level of T4 is secreted at approximately 20 to 55 nM in adults [30]. Whereas, the side effect of overdosing on T4 to our body exhibit disruption in consciousness, cold and clammy skin. It has been reported that the bioactive thyroid hormone is T3 than T4 in the body, however, T4 is easily changed into T3 by the deiodinase which is a peroxidase enzyme in the cells [34]. And the cellular differentiation capacity into osteoblast was similar in the mouse treated with each T3 and T4 [8]. In early other studies, T4 and T3 thyroid hormones were also induced into adipocytes by the increased expression of adipogenesis-related transcription factors and genes in the brown adipose tissue [34]. However, the cellular differentiation into adipocytes by the treatment of thyroid hormones in the cancer cell lines is still unclear and not found in early studies, and further additional studies will be needed for the investigation of adipocytic differentiation capacity using T3 than T4.

The secretion level of GC is at approximately 10 to 20 ug/dl (275 to 550 nM) in the early morning time and 3 to 10 ug/dL (82 nM to 275 nM) in the afternoon time [12]. The GC hormone mainly controls fats, proteins, and carbohydrates metabolism, regulation of stress response and blood glucose level, suppression of inflammation, and so on [10]. The continuous overdose of GC also exhibits weight gain in the face and chest, flushed face, high blood pressure, and weakened bones [18]. For adipogenesis, the GC hormone was generally supplemented in the adipogenic medium and was easily induced in the human mesenchymal stem cells or 3T3-L1 mouse preadipocytes [25, 27]. Further, the GC treatment was also induced to increase of glucose uptake, high expression of glucose transporter 4 (GLUT4), glucocorticoid receptors β (GRβ), PPARγ as well as growth inhibition in the lung A549 and MCF-7 cells [25]. In a similar study, the GC treatment was also induced in adipocytic differentiation in the MCF-7 cancer cells [6]. The secretion level of insulin is at approximately 60 to 100 mg/dl (3.3 to 5.6 mM) between meal time and 140 mg/dl after meal (7.8 mM) [6]. The functions of insulin are the increased absorption of blood glucose into cells, and the regulation of the metabolism of carbohydrates, fats, and proteins. And the side effects of high-dose insulin are slight gain and constipation. The insulin was also supplemented with an adipogenic medium for the differentiation of adipocytes in the mesenchymal or embryonic stem cells [20, 21].

The PGZ as well as rosiglitazone and troglitazone is generally known as a kind of thiazolidinedione (TZD) class. These chemicals are an activator of PPARγ, a main key regulator of adipogenesis and receptors of steroid hormones, such as GRα and GRβ. And the oral administration of PGZ can be induced to increase glucose uptake and control of high blood glucose in patients with type 2 diabetes [12]. As mentioned above, early studies have emphasized that activation of C/EBPs and PPARs transcription factor is induced in the adipocyte-related genes, and subsequent cellular differentiation into adipocytes [1, 32]. For adipogenesis, PGZ treatment also induced adipocyte differentiation in the 3T3-L1 [39]. Further, it has been demonstrated that the PGZ treatment also increased the expression level of GLUT4 and PPARγ and cellular differentiation into adipocytes in the A549 cells [24]. In the previous present studies, the cells with adiposome vesicles or droplets strongly support that these vesicles are displayed in the differentiating or differentiated adipocytes [21, 23], and the present study has shown that the A549 cells treated with each adipogenic medium were exhibited in the adiposome vesicle stained with Oil Red O assay. Moreover, it has been reported that the expression level of adipokines, including ADIPONECTIN, LEPTIN, and RESISTIN was increased in the differentiated brown adipocytes and tumor cells

[3, 40]. And treatment of PGZ was induced to the increased level of plasma ADIPONECTIN in *in vivo* patients [36]. Our present results have also shown that expression of ADIPO-NECTIN, LEPTIN, and RESISTIN adipokine is increased in the adipogenic medium, especially in the PGZ-based adipogenic A-DMEM medium. However, the expression level of the adipokines was slightly decreased in the PGZ-based A-DMEM adipogenic medium containing both T4 and GC showing high adipocytic differentiation capacity, compared with those of PGZ-based A-DMEM adipogenic medium containing only T4 or GC. In the early study, the cellular senescence was gradually increased in the A549 cells treated with PGZ, as the cells progressed into highly differentiated adipocytes, [24]. Thus, the slightly decreased adipokines are thought to be a can be a consequence of cellular damage by more increased senescence. Taken together, PGZ treatment might certainly be induced to adipocytic differentiation in the A549 cells. Whereas, it has been suggested that prostate and pancreatic cancer may be increased by prolonged intake of PGZ [28].

Most of all, the PGZ treatment was exhibited to arrest or inhibition of cell growth in various cancer cells, including colorectal and anaplastic thyroid cancer cells [35, 41]. In our previous study, inhibition of cell growth was displayed in the A549 cells treated with PGZ as per increasing PGZ treatment time, and the growth arrest in the G1 phase of the cell cycle was also exhibited in the A549 cells by PGZ treatment [24]. Early studies have suggested that determining cell fate for cellular differentiation, proliferation, senescence or apoptosis is the G1 phase [5]. As shown in these results, the rate of cell growth was highly delayed by adipogenic treatment, and we assumed that the cell cycle is the G1 phase in the differentiated A549 cells. Previous studies also suggested that the PPAR-γ agonists, such as TDZ induce inhibition of cell growth by blockade of MAPK and TGFβ/SMADs signaling pathway [10], and the intrinsic mechanism(s) on inhibition of cell growth caused by TDZ treatment will further be examined in the various cancer cells. Besides, the unlimited cell growth showing in cancer cell lines and stem cell lines are tightly associated with a high level of telomerase activity that maintains/extends telomere repeats in the 3' end of DNA telomeres, whereas loss or shortening of telemetric repeats is induced to cellular senescence and apoptosis [23, 37]. In the present study, telomerase activity was gradually decreased in the differentiating/differentiated A549 cells, depending on each adipogenic treatment, and the rate of cell growth was gradually inhibited. The early study has shown that the short-ening of telomeric repeats is induced by the decreased telomerase activity in the A-549, MDA-MB-231, and U87-MG cancer cell lines, and the cells were consequently reached at cellular senescence stage [24]. Therefore, we also assumed that the decreased telomerase activity and inhibited cell growth in the differentiated A549 cells lead to increased cellular senescence and apoptosis stage.

In conclusion, the present study has demonstrated that a combined supplement of T4 and GC to adipogenic medium is found to be synergistically increased the cellular differentiation into adipocytes by adiposomes stained with Oil Red O solution and high expression of adipokine transcripts in A549 cancer cell lines as well as inhibition of cell growth by PDT assay. Above all, it was known that the side effects of various hormones related to energy and glucose metabolism, such as insulin, T4, and GC under normal body conditions occur very seldom. Therefore, the adipogenic treatment using increased energy metabolism-related hormones is easily induced to the inhibition of cell growth and might be efficient in cancer therapy, rather than traditional anti-cancer chemotherapy with high cytotoxicity and side effects. However, the adult stem cells in many different parts of our body are also found for the regeneration of cells and tissues, and especially mesenchymal stem cells showing differentiation capacity into specific cell types for regeneration of connective tissues were not discriminatively induced into adipocytes in each adipogenic medium (data not shown). However, dental tissue-driven mesenchymal stem cells were induced into adipocytes for prolonged in vivo cell culture [21]. The side effects by treatment of adipogenic treatment are to be carefully examined in many different types of normal and stem cancer cells for prolonged *in vivo* treatment.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : A549 폐암세포주의 지방세포 분화에 미치는 티록신 및 당질 코르티코이드 호르몬의 상승 효과

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이 연구는 우리 몸에서 포도당 및 에너지 대사에 관련된 호르몬으로 알려진 티록신 및 당질코르티코이드를 지방분화배양액에 단용 혹은 혼용 첨가하여 A549 폐암세포주가 지방세포로의 분화에 미치는 영향을 조사하였다. 각 지방분화배양액에서 A549세포를 2주 동안 배양한 후, A549세포의 세포 성장률과 말단효소 복원효소를 비교하였을 때, 기본 지방분화배양액이나 PGZ기반 지방분화배양액에서 티록신 및 당질코르티코이드가 단용으로 첨가된 경우보다, 두 호르몬이 혼용으로 첨가되었을 때, 세포의 성장의 유의적으로 억제되는 것을 알 수 있었다. 또한, 세포내 축적된 지방 분자를 염색할 수 있는 Oil Red O 염색과 분화된 지방세포에서 분비되는 여러 아디포카인의 발현을 조사하여 각 지방분화배양액에서 A549 세포의 지방분화능력을 비교하였다. 지방세포로의 분화 능력 역시 티록신 및 당질코르티코이드가 단용으로 첨가된 경우보다, 두 호르몬이 혼용으로 첨가되었을 때, Oil Red O 염색액으로 염색된 세포내 지방 과립의 수와 크기가 유의적으로 증가하는 것을 알 수 있었고, 아디포카인의 발현 유의적으로 증가하는 것을 알 수 있었다. 이러한 연구 결과를 바탕으로 A549 세포에서 지방세포의 분화를 유도할 때, 포도당 대사 관련 두 호르몬의 혼용 처리가 더욱 더 세포 분화를 촉진한다는 것을 알 수 있었고, 여러 다른 암세포주를 두 호르몬을 혼용하여 첨가한 지방분화배양액에서 처리하여 지방 분화 유도에 의한 세포 성장 억제 효과가 가능할 것으로 판단된다. 그러나, 체내의 다른 세포, 특히 미분화 줄기세포에 미치는 영향에 대한 추가적인 연구가 필요할 것으로 판단된다.