

## Differential Cytotoxicity of Penta-O-galloyl- $\beta$ -D-glucose in Human Cancer and Normal Cell Lines of Various Origins

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Received June 8, 2016 / Revised August 1, 2016 / Accepted September 22, 2016

The present study examined the cytotoxic effects of 1, 2, 3, 4, 6-penta-O-galloyl- $\beta$ -D-glucose (PGG), known as the pentahydroxy gallic acid ester of glucose, in the various human cancer cell lines (A-549, MDA-MB-231, U87-MG, MCF-7 and PANC-1), normal MRC-5 fetal fibroblasts, and dental papilla tissue-derived mesenchymal stem cells (DPSCs). Significantly ( $p < 0.05$ ) lower half maximal inhibitory concentration ( $IC_{50}$ ) values were observed in the A-549 and MDA-MB-231 cells showing a high proliferation capacity, compared with other cancer and normal cell lines with a relatively low proliferation capacity. The population doubling time (PDT) was significantly ( $p < 0.05$ ) higher in the 10  $\mu$ M PGG-treated cell lines than those of untreated control cell lines. The present study demonstrated that the  $IC_{50}$  value increases proportionally to the extending PDT. A high cell number with senescence-associated  $\beta$ -galactosidase activity was also observed in the 10  $\mu$ M PGG-treated cells compared with those of untreated control cells. Moreover, the level of telomerase activity was significantly ( $p < 0.05$ ) decreased with 10  $\mu$ M PGG treatment, especially in A-549 and MDA-MB-231 cells showing a high proliferation capacity. Based on these observations, PGG could serve as a potent agent for cancer chemotherapy, as its treatment was more effective in cells with a high proliferation capacity.

**Key words** : Cancer cells, human, PGG, proliferation, telomerase activity

### Introduction

Globally, many people are suffering from various types of cancers. Cancer is generally characterized by an uncontrolled cell proliferation due to the failure in growth regulation and control of metastasis, consequently which invades into other tissues of the body. The untreated cancers lead to death of patients with severe pain. Although, various treatments such as surgery, radiation, chemotherapy, immunotherapy and others have been successfully applied for cancer therapy, more efficient and economic treatments are required to increase the survival rate of cancer patients. Several glycoside compounds, including amygdalin (cyanogenic glycoside) from mainly bitter almonds, plant saponins

and ginseng are often used as anti-cancer drugs in chemotherapy treatment [10, 30, 34]. Recently, 1, 2, 3, 4, 6-Penta-O-galloyl- $\beta$ -D-glucose (PGG) found in many plant species, a simple hydrolysable tannin, has been considered as a candidate anti-cancer drug. It has been previously demonstrated that PGG treatment induces cellular cytotoxic effects, including the induction of cellular apoptotic cell death, elevated expression of activating p53 tumor suppressor, arrest of cell cycle and blocking angiogenesis by inhibiting VEGF expression in several types of human cancer cells [5, 8, 12, 21]. Moreover, different studies have reported a wide variety of biological activities, such as anti-microbial, anti-platelet-coagulation functions as well as anti-tumor effects in human cancer cells by PGG treatment [4]. Additionally, PGG has been reported to be a strong inhibitor of fatty acid synthase (FAS), a type of lipogenic enzyme, through competition with the active domains of the enzyme subsequently leading to the anti-tumor activity in human cancer cells [35]. Generally, the FAS activity is over-expressed in the cell lines showing high rate of cell proliferation, such as cancer cells [33]. The fatty acid in the cells showing high proliferation rate could

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be continuously supplied for new cell membrane, probably presuming that arrest of cell cycle phase can potentially be induced in the cells treated with PGG. However, the cellular cytotoxicity related with cell proliferation rate and population doubling time by PGG treatment is not yet investigated. Furthermore, even though PGG possesses the remarkable cellular cytotoxicity against several human cancer cells, the anti-cancer effects in the various human normal and cancer cell lines is not fully understood until recently. More interestingly, the selective cellular cytotoxicity in the cancer cell lines is an important factor for the process of cancer therapeutic treatment while maintaining less cytotoxic effects in the normal cell lines.

Cancer cells generally form a cell lumps through unlimited cell division with loss of contact inhibition. The unlimited cell division of cancer cells is tightly regulated with the maintenance of telomeric repeats that consist of repetitive DNA sequences (GGTTAG<sub>n</sub>) at the end of each eukaryotic chromosome, and high telomerase activity is responsible for the maintenance of telomeric repeats [3]. Therefore, the telomerase activity has been considered as a main biomarker in cancer treatment *in vitro* [18, 28]. Previously, many studies have focused on telomere and telomerase-targeting drug in cancer cell lines [7, 25, 27]. However, the effects on telomerase activity are not fully investigated in the various human cancer and normal cell lines treated with PGG.

Herein, we investigated the cytotoxic effects by PGG treatment in various types of human cancer and normal cell lines, including A-549 lung adenocarcinoma, MDA-MB-231 breast adenocarcinoma, MCF-7 breast adenocarcinoma, U87-MG brain glioblastoma astrocytoma and PANC-1 pancreatic carcinoma cancer cells as cancer cell lines, and MRC-5 fetal lung fibroblasts and dental papilla tissue-derived mesenchymal stem cells (DPSCs) of third molar (wisdom tooth) as normal cell lines. The IC<sub>50</sub> value by 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay was preferentially decided in the each of cell lines treated with PGG. After PGG treatment at an established concentration, the cytotoxic effects of PGG were investigated by the changes of population doubling time and senescence-associated-β-galactosidase activity in the cell lines of various. Furthermore, the relative telomerase activity was also examined in the each of cell lines treated PGG.

## Materials and Methods

### Preparation and culture of cells

All chemicals and media used in this study were purchased from Sigma (Sigma, St. Louis, MO, USA) and Gibco (Invitrogen, Carlsbad, CA, USA), unless otherwise specified. The pH and osmolality in media was adjusted to 7.4 and 280 mOsm/kg, respectively. A-549 lung adenocarcinoma, MDA-MB-231 breast Adenocarcinoma, MCF-7 breast Adenocarcinoma, U87-MG brain glioblastoma astrocytoma, PANC-1 pancreatic carcinoma cancer cells and MRC-5 normal fetal lung fibroblasts were purchased from the American Type Culture Collection (Manassas, VA, USA). The DPSCs used for this experiments were derived from extracted third molar tooth and the basic characterizations of stem cell in the isolated DPSCs were demonstrated in the previous study [15]. All cells were maintained in advanced-Dulbecco's modified eagle medium (A-DMEM) supplemented with 3% fetal bovine serum (FBS) and 1.0% penicillin-streptomycin (10,000 IU and 10,000 μg/ml, respectively) at 37.5°C in a humidified atmosphere of 5% CO<sub>2</sub> in air and sub-cultured upon confluence (70-80%), while culture media were changed twice a week.

### Cytotoxicity test by MTT assay

The cellular cytotoxicity of PGG was analyzed by MTT assay and then the half maximal inhibitory concentration (IC<sub>50</sub>) value was determined in each of the cell lines. Briefly, both cancer and normal cells were seeded at 5×10<sup>4</sup> per well into a 6-well plate and incubated at 37.5°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. After overnight incubation, media were carefully removed, and replaced with complete A-DMEM media containing 0 (control), 2.5, 5.0, 10, 20 and 50 μM PGG for 48 hr. After washing with Dulbecco's phosphate-buffered saline (D-PBS) for 3 times, the MTT assay was performed by adding 1 ml of 5 mg/ml MTT stock solution into each well and incubated at 37°C for 4 hr. After removing MTT stock solution, wells were washed with D-PBS for 3 times. Finally, 200 μl dimethyl sulfoxide (DMSO) solution was added and incubated for 15 min. The DMSO solution was then collected and measured with an ELISA microtiter plate reader (Bio-Tek, Winooski, VT, USA) at 570 nm wavelength and the IC<sub>50</sub> value was calculated for each cell lines.

### Analysis of PDT and correlation coefficient

Both cancer and normal cell lines were seeded at 1×10<sup>4</sup> cells/well into a 6-well plate and cultured in complete A-DMEM media containing 0 (control) and 10 μM PGG for

7 days at 37.5°C in a humidified atmosphere of 5% CO<sub>2</sub> in air by changing culture medium for every 3 days. After 7 days, cells were harvested with 0.1% (w/v) trypsin and counted using a hemocytometer. The PDT value was calculated using the formula  $DT = t (\log 2) / (\log N_t - \log N_0)$ , where *t* represents the culture time, and *N<sub>0</sub>* and *N<sub>t</sub>* are the initial and final cell numbers respectively. Furthermore, when IC<sub>50</sub> values and PDT were determined, the correlation coefficient analysis was performed between two values using Excel program (Microsoft, Redmond, WA, USA).

#### Analysis of senescence-associated β-galactosidase activity

The activity of senescence-associated β-galactosidase was carried out with cell senescence assay kit (Cell Signaling Technology, Danvers, MA, USA) according to the manufacturer's protocols. Briefly, the each of cell lines were treated in the media containing with 0 (control) and 10 μM PGG in 6-well plate for 7 days. After being washed with PBS, the cells were fixed with 1 ml fixative solution for 10-15 min at room temperature. The cells were then incubated with 1 ml senescence-associated β-galactosidase staining solution at 37°C for overnight. The cells stained with blue color were examined under an inverted microscope (Nikon, Tokyo, Japan) equipped with CCD camera and image program.

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

The conventional ELISA-based TRAP assay was modified for the real time RQ-TRAP using the LightCycler 3.0 (Roche, Mannheim, Germany), and the telomerase activity was quantified as previously described [19]. Briefly, 1×10<sup>5</sup> cells/sample were harvested and lysed in 400 μl of iced 0.5% (v/v) 1·3-[3-cholamidopropyl] dimethylammonio] propanesulfonic acid (CHAPS) lysis buffer (pH 7.5) supplemented with 10 mM Tris-HCl, 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.1 mM benzamidine, 5 mM 2-mercaptoethanol and 10% glycerol for 30 min at 4°C. And the samples were centrifuged for 20 min at 12,000 × *g* at 4°C. Protein concentration was measured by a spectrophotometer (Mecasys, Daejeon, Korea) and 5 μg of total proteins were used for RQ-TRAP. The RQ-TRAP was performed using the PCR reagent LightCycler FastStart DNA Master SYBR Green 1 (Roche, Germany), containing lysed samples, 2.5 mM MgCl<sub>2</sub>, 0.02 μg of primer TS (5'-AAT CCG TCG GAG CAG AGT T-3'), 0.04 μg of primer ACX

(5'-GCG CGG CTT ACC CTT ACC CTT ACC CTA ACC-3'), according to the manufacturer's protocol and final volume was adjusted to 20 μl with RNase-free water. The RQ-TRAP protocol consisted of 30 min incubation at 30°C, followed by a denaturing cycle of 10 min at 94°C, and 40 cycles of PCR (94°C for 30 sec, and 60°C for 90 sec). All samples were quantified using the LightCycler Quantification Software's (Roche, Mannheim, Germany) second derivative method of crossing point (Cp) determination, and the relative telomerase activity of untreated control MRC-5 fibroblasts was considered as 100% for comparison with the treatment groups in five replicates.

#### Statistical analysis

Differences among the cell groups were analyzed by using one-way analysis of variance (ANOVA). Differences in the IC<sub>50</sub> value, population doubling time and relative telomerase activity were analyzed using a Student's *t*-test. Significance was set at *p*<0.05.

## Results

#### Determination of IC<sub>50</sub> value by MTT assay

The cytotoxicity test with IC<sub>50</sub> values assay was determined by MTT in the A-549, MDA-MB-231, U87-MG, MCF-7 and PANC-1 human cancer cell lines, and DPSCs derived from dental papilla tissues and MRC-5 fetal fibroblasts treated with PGG, as shown in Fig. 1. The IC<sub>50</sub> value (mean±SEM) in three replicate was 6.5±3.23, 7.8±4.12, 38.1±5.58, 53.5±6.71, 215.5±12.37, 36.1±10.09 and 23.1±8.35 μM in the A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, DPSCs and MRC-5 cell lines, respectively. The IC<sub>50</sub> values in the A-549 and MDA-MB-231 cancer cell lines were significantly (*p*<0.05) lower than other cell lines. Moreover, the IC<sub>50</sub> value of PANC-1 cancer cells was interestingly observed at a highest level among all other cell lines.

#### Analysis of population doubling time (PDT)

The PDT was analyzed in the untreated control and 10 μM PGG-treated cell lines, and the results were shown in Fig. 2A. The PDT (mean±SEM) in three replicate was 23.2±3.47, 25.47±3.45, 51.9±6.78, 66.05±6.87, 237.8±17.7, 85.8±7.89 and 49.1±15.23 hours in the untreated control A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, DPSCs and MRC-5 cell lines, respectively. And the high proliferation rate was exhibited in the A-549 and MDA-MB-231 cancer lines. Whereas,

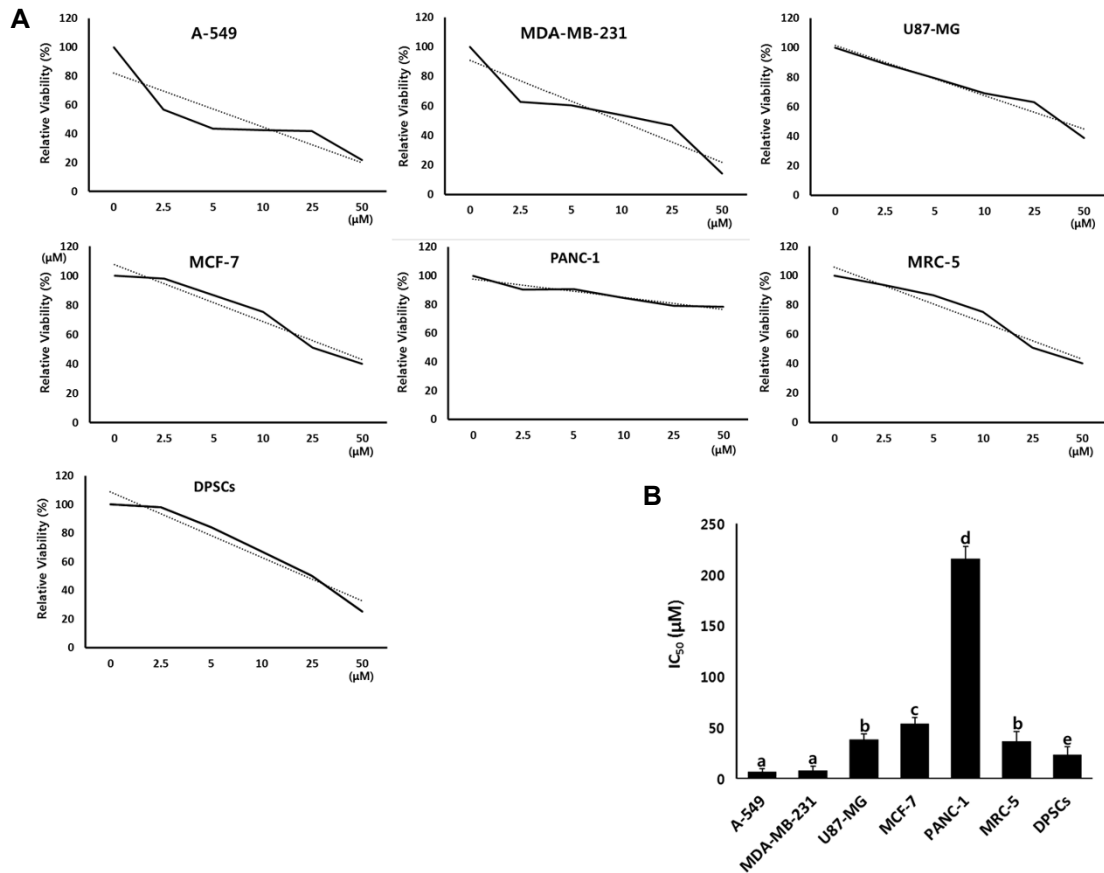


Fig. 1. Analysis of cytotoxicity and determination of IC<sub>50</sub> values by MTT assay in A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, MRC-5 and DPSCs cell lines treated with PGG. A: Inhibition curves by MTT assay in each cell line. B: Mean±SEM of IC<sub>50</sub> values of each cell line in triplicate. a, b, c, d and e indicate significant (*p*<.05) difference among each cell lines.

the PDT was 110.3±15.69, 106.2±26.89, 347.5±37.42, 359.5±8.33 and 237.8±27.22 hours in the U87-MG, MCF-7, PANC-1, DPSCs and MRC-5 cell lines treated with 10 μM PGG, respectively. The PDT was significantly (*p*<.05) increased by 10 μM PGG treatment in all of cell lines. Moreover, the cell numbers were exhibited at an extremely low increase in the 10 μM PGG-treated A-549 and MDA-MB-231 cancer cell lines with shorter PDT and higher proliferation rate compared with those of other cell lines, therefore, their PDT could not be determined.

In addition, the correlation coefficient was measured between IC<sub>50</sub> values and PDT in each of cell lines, as shown in Fig. 2B. A considerably high correlation coefficient degree (*r*<sub>2</sub>=0.9496) was exhibited between IC<sub>50</sub> values and PDT, and the IC<sub>50</sub> values were proportionally increased along with the increase of PDT in each cell lines.

**Activity of senescence-associated-β-galactosidase**

To examine the effect of cellular senescence by PGG treat-

ment, the enzymatic activity of senescence-associated-β-galactosidase was measured in all of cell lines treated with 10 μM PGG, as shown in Fig. 3. The high frequency of cells with senescence-associated-β-galactosidase activity was observed in all the PGG-treated cell lines, compared to those of untreated control cell lines. Furthermore, the cells treated with PGG were gradually changed to enlarged and star-shaped morphology, implying that cellular senescence is proceeding.

**Analysis of telomerase activity**

To examine the effect of PGG treatment on the telomerase activity, the RQ-TRAP protocol was employed in the untreated control and 10 μM PGG-treated cancer and normal cell lines. The level of telomerase activity was considered as 100% in the untreated control MRC-5 cells, and the level of telomerase activity in the other cell lines was relatively calculated on the basis of this level. The results are shown in Fig. 4. The level of relative telomere activity was 816±

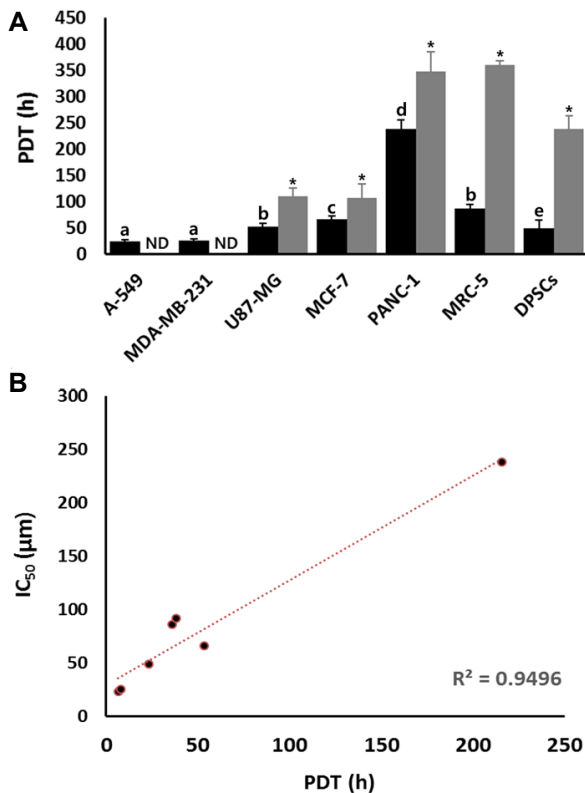


Fig. 2. A. Changes of population doubling time (PDT) in A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, MRC-5 and DPSCs cell lines treated with 10  $\mu$ M PGG for 7 days. a, b, c, d and e indicate significant ( $p < 0.05$ ) difference among cell lines. Asterisks (\*) indicates significant ( $p < 0.05$ ) difference between untreated control and 10  $\mu$ M PGG-treated cell lines, respectively. ND, not determined. B. Analysis of correlation coefficient between IC<sub>50</sub> value and PDT in each cell line. Increased IC<sub>50</sub> value against PGG was displayed in cell lines with high PDT than those of relatively low PDT with remarkably high correlation coefficient ( $r_2 = 0.9496$ ).

36.6%, 475 $\pm$ 23.3%, 545 $\pm$ 32.5%, 1016 $\pm$ 72.6%, 698 $\pm$ 33.3, 100 $\pm$ 15.5 % and 115 $\pm$ 26.2 in the untreated control A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, MRC-5 and DPSCs cell lines, respectively. As expected, the level of telomerase activity in cancer cell lines was significantly ( $p < 0.05$ ) higher, compared to those of normal MRC-5 and DPSCs cell lines at basal level, reaching the levels over ~8 fold that of MRC-5 fibroblasts. Whereas the level of relative telomerase activity was 85 $\pm$ 20.3, 92 $\pm$ 50.1, 292 $\pm$ 80.5, 745 $\pm$ 80.4, 650 $\pm$ 57.1, 88 $\pm$ 60.1 and 95 $\pm$ 45.1% in the 10  $\mu$ M PGG-treated A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, MRC-5 and DPSCs cell lines treated with 10  $\mu$ M PGG, respectively. Following PGG treatment, the level of telomerase activity was not significantly ( $p < 0.05$ ) changed in the normal MRC-5 and DPSCs cell lines at the

basal level. However, the level of telomerase activity was significantly ( $p < 0.05$ ) down-regulated in the PGG-treated cancer cell lines. Especially, A-549 and MDA-MB-231 cell lines exhibited highly down-regulated telomerase activity with lower IC<sub>50</sub> value, compared to those of other cancer cell lines.

## Discussion

In the present study, we evaluated the cytotoxic effects of PGG treatment on the cell proliferation rate, senescence-associated  $\beta$ -glucosidase activity and telomerase activity in the human cell lines of various origins, including cancer cell lines (A-549, MDA-MB-231, U87-MG, MCF-7 and PANC-1), normal MRC-5 fibroblasts and mesenchymal stem cells derived from dental papilla tissues (DPSCs). Our results demonstrated that PGG treatment has induced the cellular cytotoxicity, including inhibition of cell proliferation, high level of senescence-associated  $\beta$ -glucosidase activity and down-regulation of telomerase activity in the human cell lines of various origins. However, the cytotoxic effects of PGG were different among each cell lines. The higher cytotoxicity was exhibited in cells showing high proliferation capacity than those of low proliferation capacity. Thus, the differential cytotoxicity of PGG was directly associated with the proliferation rate of each cell lines.

PGG is a secondary metabolite with astringent taste, known as the pentahydroxy gallic acid ester of glucose found in the pomegranate, the root of peony, the gallnut of *Rhus chinensis* Mill and other plant species, and is used as a precursor of gallotannin compound. Several previous studies have demonstrated that PGG treatment is often used for cancer chemotherapy owing to its anti-tumor effects [5, 8, 16]. The cells treated with PGG were induced to apoptotic cell death through the mediation of caspase activity in several human cell lines [14, 29]. In the others studies, PGG treatment induced G0/G1 arrest and DNA replicative S-phase arrest of the cell cycles in human breast cancer cells, MDA-MB-231 and MCF-7 cells. Furthermore, in vivo growth of MDA-MB231 xenograft orally ingested was highly inhibited without any adverse effect on the host body weight in a dose-dependent manner and growth of DU145 xenograft was also inhibited by activating p53 tumor suppressor pathway and decreasing STAT3 oncogenic signaling [5, 14]. Otherwise, PGG combined to VEGF receptor, and blocked the angiogenesis by inhibiting endothelial cell growth and

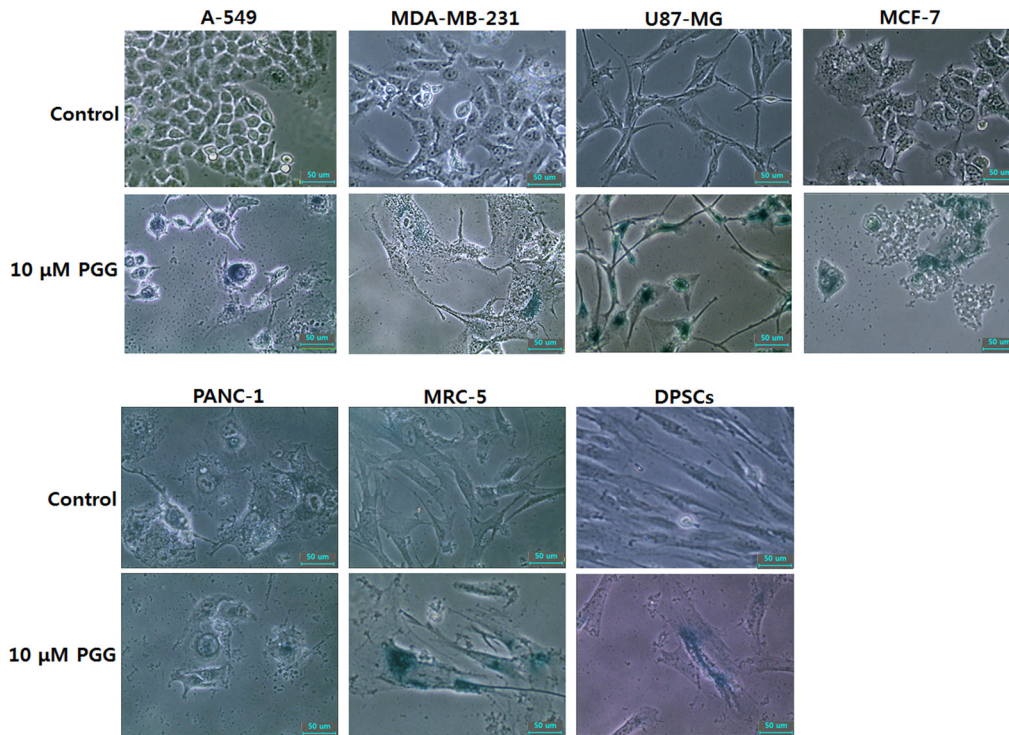


Fig. 3. Changes of cell morphology and senescence-associated-β-galactosidase activity in A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, MRC-5 and DPSCs cell lines treated with 10 μM PGG for 7 days (×200). The high incidences of senescence-associated-β-galactosidase activity (blue) were observed in each of the cell lines treated with 10 μM PGG. Scale bars; 50 μm.

tube formation of new blood vessels via down-regulation of VEGF activity [6, 16, 24]. Our results have also shown

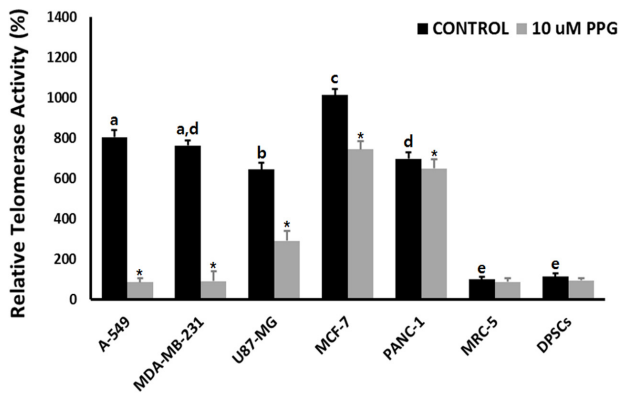


Fig. 4. Changes of telomerase activity analyzed by RQ-TRAP assay in A-549, MDA-MB-231, U87-MG, MCF-7, PANC-1, MRC-5 and DPSCs cell lines treated with 10 μM PGG for 7 days. Values indicated the mean telomerase activity (mean±SEM) of five replicates and the telomerase activity in untreated control MRC-5 fibroblasts was considered as 100% for comparison with other cell lines. a, b, c and d indicate significant ( $p < 0.05$ ) difference among untreated control cell lines. Asterisks (\*) significant ( $p < 0.05$ ) difference between control and 10 μM PGG-treated cell lines, respectively.

that the cells treated with PGG are induced to the cytotoxic effects, such as inhibition of cell proliferation with prolonged doubling time, induction of cellular senescence by elevated β-galactosidase activity and down-regulation of telomerase activity in various types of human cancer cell lines, and MRC-5 fetal fibroblasts and DPSCs. However, the differential cytotoxicity was exhibited accordingly to the cell lines used in the present study. Moreover, the high cytotoxicity was also exhibited in the normal cell lines (MRC-5 and DPSCs).

The cause of differential cytotoxic effect by PGG treatment in human cell lines of various origins is still unclear. It is well known that the plasma membrane in each cells consists of the phospholipid bilayer having hydrophilic phosphate part and two hydrophobic fatty acids with various proteins. High proliferating cells require a synthesis of large amount of fatty acids as well as proteins [31]. The synthesis of fatty acids consequently depends on the activity of fatty acid synthase (FAS), which is mainly responsible for the synthesis of palmitic acid from acetyl coenzyme A [31]. The activity of FAS enzyme is generally very low or undetectable in normal cells. However, the activity is found to be up-regulated in highly proliferating cells including cancer cells [30, 33].

Moreover, it has been reported that neuronal stem cells or neuronal progenitor cells with high proliferation capacity require a high activity of FAS enzyme-dependent lipogenesis for their proliferation [20], and over-expression of FAS activity is tightly associated with an early developmental process of prostate cancer cells and metastasis of cancer cells that spreads to other tissues of the body [32]. Therefore, the deficiency of fatty acid for plasma membrane of newly dividing cells may lead to the arrest of cell divisions. Based on this hypothesis, inhibition or down-regulation of FAS activity could be a potential target for cancer chemotherapy or alternative/adjuvant therapy. Several type of FAS inhibitors, such as C75, cerulenin, orlistat and others have already been tried for cancer treatment [10, 21, 30]. Previously, it has been demonstrated that PGG strongly binds to an active site of FAS, thereby down-regulating FAS activity [7]. However, the potential cytotoxic effects in normal cell lines as well as the anti-tumor effects in various type of cancer cells remains to be examined for clinical applications. Our results have demonstrated that PGG induces the cytotoxic effects, including the inhibition of proliferation rate, high senescence-associated- $\beta$ -galactosidase activity and down-regulation of telomerase activity. Especially, the cytotoxic effects was higher in A-549 and MDA-MB-231 cancer cell lines having high proliferation capacity and short PDT than those of other cell lines, and their  $IC_{50}$  values were also detected at a very low concentrations. As previously reported, the PDT in A-549 and MDA-MB-231 cancer cell lines was approximately below 30 hours and exhibited the high proliferation capacity, compared with other cell lines used in the present study [24]. We also observed a markedly low  $IC_{50}$  value in 3T3-L1 mouse embryonic fibroblasts treated with PGG, and these cells also possess rapid cell division capacity (data not shown). Furthermore, even though both MDA-MB-231 and MCF-7 cancer cell lines used in the present study were adenocarcinoma tumors derived from female breast epithelial cells, the cellular characterizations based on cancer property were considerably different between two cell lines. MDA-MB-231 cells are generally high malignant tumor cells and possess higher proliferation capacity and shorter PDT than that of MCF-7 cells [1, 12, 27]. However, our results have demonstrated that MDA-MB-231 cells exhibit the lower  $IC_{50}$  value and are more sensitive compared to MCF-7 cells. Furthermore, accordingly to correlation analysis between the  $IC_{50}$  value and proliferation rate, our data demonstrated that  $IC_{50}$  values against PGG are inversely proportional to the

rate of cell proliferation and directly proportional to the PDT. Whereas the lower  $IC_{50}$  values were observed in MDA-MB-231 and A-549 cell lines with high proliferation capacity and short PDT compared with those of other cell lines with relatively low proliferation capacity and long PDT. A previous study has shown that differential cytotoxic effects and  $IC_{50}$  value are observed in the PGG-treated U251 glioblastoma tumors and MDA-MB-231 breast adenocarcinoma tumors and U251 cells exhibit lower  $IC_{50}$  value than that of MDA-MB-231 cells [7]. It is well known that U251 cells also possess relatively shorter doubling times (~19 hr) than that MDA-MB-231 cells (~28 hr). Therefore, we assumed that the cytotoxic effect of PGG is probably induced by inhibiting synthesis of fatty acid and subsequent process of cell division.

Besides, the PDT was markedly expanded in the cells treated with PGG than those of non-treated control cells, implying that the cell proliferation rate is delayed. Other studies have shown that PGG induces arrest of cell cycle at the G1 and S-phase in the various types of human cancer cells treated with PGG by down-regulation of cyclin D1, and inhibition of DNA polymerase [5, 15]. The mis-regulation of control system for cell cycle induce the unscheduled and unlimited cell proliferation in most of the cancer cells and the arrest of cell cycle at G1 and S phase is a most common status that usually occurs with the inhibition of cell proliferation[13]. Furthermore, our results have also demonstrated that the high activity of senescence-associated- $\beta$ -galactosidase was increased upon PGG-treatment with cellular morphological alternations compared with those of non-treated control cells. The cell size was gradually expanded, and cells were changed into star-shaped and irregular morphology in the PGG-treated cells. It has been well demonstrated that the cells at senescent status display an enlarged and irregular cell shape with a large number of cells at G1/S phase of cell cycle [2]. Although, in the present study, the cell cycle analysis was not determined in the PGG-treated cells, we considered that the cells are suspended at G1/S phase of cell cycle. These dramatic alternations were predominantly observed in the PGG-treated A-549 and MDA-MB-231 with high proliferation capacity.

PGG treatment further also induced the down-regulation of telomerase activity resulting in cellular senescence in the human cancer cell lines with the exception for MRC-5 and DPSCs cell lines. Especially, the telomerase activity upon PGG treatment was dramatically decreased in the A-549 and



MDA-MB-231 cell lines, which had high proliferation capacity. To date, available information on the telomerase activity by PGG treatment is not yet fully reported in the various human cell lines. The telomerase activity and telomeric repeats are also related with cellular senescence [2]. The embryonic stem cells and most malignant tumors possess the up-regulated telomerase activity that continually maintains or extends telomeric repeats on the 3' end of the linear eukaryotic DNA strands by adding telomeric repeats [9]. On the other hand, most of the differentiated somatic cells with the down-regulation of telomerase activity are induced to shortening of their telomeric repeats, and the cells reached at crisis status with fully shortened telomeric repeats subsequently leads to cellular senescence [9]. Therefore, it has been reported that the level of telomerase activity and length of telomeric repeats are indices of the capacity of limitless cell division and high proliferation. Furthermore, our results have shown that PANC-1, U87-MG and MCF-7 cell lines with low proliferation capacity possess high level of telomerase activity, compared with those of A-549 and MDA-MB-231 cells. Therefore, we have assumed that high level of telomerase activity is only concerned with unlimited cell proliferation capacity, whereas unconcerned with rate of cell proliferation. Even though the shortening of telomeric repeats by down-regulated telomerase activity are certainly induced to cellular senescence and the senescent cells with high level of  $\beta$ -glucosidase activity were also observed in the present study by PGG treatment, we have presumed that the cellular senescence is probably induced by the cytotoxicity of PGG itself rather than shortening of telomeric repeats. Our previous study has shown that the decreased expression of the genes related with telomerase activity i.e. telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC) has resulted in down-regulation of telomerase activity [28]. Whereas, the significant changes in the telomerase activity was not observed in the MRC-5 and DPSCs cell lines. We have assumed that this might be due to telomeres activity reached at a critically low level, compared with those of cancer cell lines. Although the DPSCs exhibited outstanding characterization of stem cells, such as multi-lineage differentiation capacity, the cells exhibited critically low level of telomeres activity, as MRC-5 normal fibroblasts [27, 28].

Interestingly, our results have shown that PANC-1 cells with extremely low proliferation capacity exhibited outstanding resistance against PGG treatment and possess very

high  $IC_{50}$  value. As pointed out above, the cell lines with high proliferation capacity require a mass of fatty acid for newly synthesized cell membrane, and the PGG may induce more cytotoxicity effects in the high proliferation capacity, such as A-549 and MDA-MB-231. On the other hand, the present results have shown that PGG treatment also induces the cytotoxicity effects in the normal MRC-5 and DPSCs cell lines. Therefore, PGG treatment or oral administration may induce several side effects, i.e. dyspepsia, hair loss, anemia, weight loss and others by inhibiting the cell division or cell cycle in the normal cell lines. And clinical application of PGG should carefully be considered or examined as a potential toxic compound in various normal somatic cell lines. In the present study, although any intrinsic and cellular incidence was not investigated in the PGG-treated cancer cells, PGG against cancer cells have shown a kind of potential chemotherapy compound by inhibiting cell proliferation and down-regulating telomerase activity. Moreover, PGG is more susceptible in the cancer cells with high proliferation capacity.

## Acknowledgment

This study was supported by grants from Korean Foundation for the Advancement of Science and Creativity.

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### 초록 : 사람의 다양한 조직에서 기원하는 암세포 및 정상세포에 대한 penta-O-galloyl- $\beta$ -D-glucose의 세포독성 효과

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본 연구는 다당체의 한 종류인 penta-O-galloyl- $\beta$ -D-glucose (PGG)가 사람의 여러 조직에서 기원하는 여러 암세포주(A-549, MDA-MB-231, U87-MG, MCF-7 및 PANC-1), 정상 MRC-5 태아 섬유아세포 그리고 사랑니에서 유래한 간엽줄기세포(DPSCs)에 미치는 세포독성 효과를 조사하였다. IC<sub>50</sub>값은 다른 세포주에 비해 높은 증식률을 나타내는 A-549 및 MDA-MB-231 암세포주에서 유의적으로 낮게 관찰되었다. 10  $\mu$ M의 PGG가 포함된 배양액에서 세포를 7일 동안 배양한 결과, 세포배가시간은 모든 세포주에서 유의적으로 늘어났고, 세포배가시간과 IC<sub>50</sub>값의 관계를 조사한 결과, 세포배가시간이 늘어남에 따라 IC<sub>50</sub>값은 비례적으로 증가됨을 증명하였다. 또한, 10  $\mu$ M의 PGG로 처리된 세포주들은 노화와 관련된  $\beta$ -galactosidase의 활성도가 높게 관찰되었다. 특히, telomerase 활성도는 A-549 및 MDA-MB-231 암세포주에서 다른 세포주에 비하여 현저히 감소하는 것을 관찰하였다. 이러한 결과를 바탕으로 PGG는 높은 증식률을 보이는 암세포주에서 높은 세포독성효과를 나타내어 잠재적인 항암물질임을 증명하였다.