의학박사 학위논문

Efficacy of GDC-0980 (Apitolisib) for the Treatment of Cholangiocarcinoma: A Preclinical Study

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2020 년 8 월

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ABSTRACT

Cholangiocarcinoma (CCA) is a highly aggressive, lethal cancer, which is typically diagnosed at an advanced stage with poor prognosis. Since conventional chemotherapy (gemcitabine and cisplatin) has marginal survival benefit in patients with advanced CCA, an effective targeted therapeutic agent is urgently required. Activation of the PI3K/Akt/mTOR signaling pathway is frequently observed in CCA and plays a central role in tumor initiation, cell growth, and metastasis, and thus, PI3K and mTOR are promising therapeutic targets in CCA. Recently a new dual PI3K/mTOR inhibitor GDC-0980 (apitolisib) was introduced. This study was undertaken to examine the activity of apitolisib against CCA cells in vitro and in vivo. Apitolisib treatment strongly reduced Akt and mTOR active phosphorylation levels and attenuated cell growth in two different CCA cell lines (SNU478 and SNU1196). In addition, the cytotoxic activity of apitolisib enhanced the effects of gemcitabine or cisplatin in vitro and increased PARP cleavage. Moreover, we observed these co-treatments significantly reduced colony formation by SNU478 and SNU1196 cells and potently inhibited tumor growth in a Balb/c nude mouse xenograft model. The results of the present study show that apitolisib effectively reduces CCA cell growth by suppressing the PI3K/Akt/mTOR pathway. In addition, co-treatments with apitolisib and gemcitabine or cisplatin synergistically enhanced apitolisib activity, which suggests a means of improving the chemotherapeutic sensitivity of CCA.

Keywords: GDC-0980, Apitolisib, Cholangiocarcinoma, Treatment
Student number: 2014-30919

CONTENTS

LIST OF FIGURES

apitolisib (Api), cisplatin (Cis)+gemcitabine (Gem), and cisplatin (Cis)+gemcitabine (Gem)+ apitolisib (Api). $^{**}P < 0.01$. **(C)** Average mouse weights on different experimental days. $^{**}P = 0.01$. **(D)** % TUNEL positive cells in tumor tissues. $^{**}P < 0.05$. **(E)** % BrdU positive cells in xenograft tumor tissues. $^{**}P < 0.001$.

LIST OF ABBREVIATIONS

CCA, cholangiocarcinoma

PI3K/Akt/mTOR, phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin

RCC, renal cell carcinoma

RPMI, Roswell Park Memorial Institute

BrdU, 5-Bromo-2'-deoxyuridine

TUNEL, terminal deoxynucleotidyl transferaseYmediated dUTP nick end labeling

PARP, poly (ADP-ribose) polymerase

PTEN, phosphatase and tensin homolog

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

AKT1, AKT serine/threonine kinase 1

ERBB2, erb-b2 receptor tyrosine kinase 2

INTRODUCTION

Cholangiocarcinoma (CCA) is a highly aggressive, lethal adenocarcinoma arising from epithelial cells of intrahepatic and extrahepatic bile ducts. CCA is classified as intrahepatic, perihilar, and extrahepatic, and these three types exhibit different molecular profiles, pathogeneses, and clinical behaviors. Typically, CCA is diagnosed at an advanced stage (inoperable or metastatic), and thus, prognosis is poor.[1] The 5-year survival rate of CCA is 5-10%, and median survival of advanced CCA is less than 12 months.[2] Cisplatin plus gemcitabine has been used as a standard chemotherapy for advanced CCA since the ABC-02 trial (2010), but the survival gain this combination therapy provides is relatively small as compared with gemcitabine alone (11.7 vs 8.1 months).[3] Furthermore, no second line chemotherapy or molecular targeted therapy has been established although several molecular alterations have been identified.[4-6] Therefore, a potent, targeted therapeutic agent is required that acts alone or in conjunction with conventional chemotherapeutic agents.

The phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway is viewed as a promising therapeutic target for various cancers including CCA.[7,8] In particular, alterations in this pathway have been identified in all three CCA types.[5] The PI3K/Akt/mTOR pathway is a central regulator of cell metabolism, growth, and homeostasis,[9] and its dysregulation has been identified in a variety of human cancers including breast cancer, renal cell cancer (RCC), and in neuroendocrine tumors. Moreover, mTOR inhibitors (e.g., everolimus) have been approved for the treatment of these cancers.[10] However,

clinical evidence on the efficacies of PI3K/Akt/mTOR pathway inhibitors in CCA are lacking, though several positive preclinical reports have been issued.[11-14]

GDC-0980 (apitolisib) is a novel, dual inhibitor of PI3K and mTOR that was introduced in the early 2010's, and was expected to have potent effects because it inhibits two components of the PI3K/Akt/mTOR pathway.[15] Preclinical data demonstrate apitolisib is active against breast, prostate, lung cancer,[16] and pancreatic cancer.[17] Clinical trials have been conducted on RCC,[18] endometrial cancer,[19] and several types of advanced cancer[20], but despite acceptable tolerabilities, efficacies were limited. To the best of our knowledge, the effects of apitolisib have not been previously evaluated in CCA. Thus, the present study was performed to examine the effects of apitolisib on CCA cells *in vitro* and *in vivo*. In addition, we also evaluated the effects co-administering apitolisib and conventional chemotherapeutic agents (cisplatin and/or gemcitabine).

MATERIALS AND METHODS

Cells and cell culture

SNU478 and SNU1196 cells (human CCA cell lines) were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea), and maintained in RPMI-1640 containing L-glutamine (300 mg/L), 25 mM HEPES, 25 mM $NaHCO_3$, 10% heat-inactivated fetal bovine serum (FBS), and antibiotics (Invitrogen, Carlsbad, CA, USA) in a 5% CO_2 atmosphere at 37° C.

Cell proliferation

Cells seeded in 6- or 12-well plates were treated for 48 h with various concentrations of apitolisib (Api) and cisplatin (Cis) and/or gemcitabine (Gem). After three times washes in PBS, pH 7.4, cells were detached with 0.05% trypsin/EDTA. Cells were resuspended with two times volumes of growth medium. Cell numbers were calculated by trypan blue exclusion using an automated cell counter (Countess II FL, Invitrogen). Cell growths were expressed as percentages of non-treated control cells. Cells morphologies and confluencies were monitored using the IncuCyte™ Live-cell Imaging System (Essen BioScience, Ann Arbor, MI, USA).

Protein analysis

Cells were washed with phosphate-buffered saline (PBS) twice, and the cell pellets obtained were lysed in radioimmunoprecipitation assay (RIPA) buffer containing protease and proteasome inhibitors (Invitrogen), rotated at 4° C for 10 min, and sonicated for 10 sec. Protein lysates were separated by 8% or 10% (v/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membranes. Membranes were incubated with primary antibodies against to Ser-473-phosphorylated Akt (Cell Signaling, Danvers, MA, USA #9721), Akt (Santa Cruz Biotechnology, CA, USA #sc-5298), Ser-2448-phosphorylated mTOR (Cell Signaling #2791), mTOR (Cell Signaling #2927), Thr-389-phosphorylated S6K1 (Cell Signaling #9205), S6K1 (Cell Signaling #9202), GAPDH (Santa Cruz Biotechnology #sc-47724), cleaved PARP (Cell Signaling #9541), PARP (Cell Signaling #9532), cleaved PARP (Cell Signaling #9541), and PARP (Cell Signaling #9532). After incubation with horseradish

peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), protein bands were visualized by chemiluminescence.

Colony formation

SNU478 cells (5×10^3 cells) were seeded in 6-well plates and treated with Api, Cis, and/or Gem for 10 days. Colonies were washed in PBS, fixed/stained for 1 hr in 0.5% w/v crystal violet/methanol. Plates were then rinsed with tap water and dried. Macroscopically visible colonies were counted manually.

Mouse xenograft studies

The animal study protocol was approved by the Institutional Animal Care and Use Committee at Seoul University Hospital (No. 15-0128-S1A0). Five-week-old male Balb/c nude mice were purchased from Central Laboratory Animals (Seoul, Korea). SNU478 cells (10^6 cells/mouse) in 1 mL of Matrigel (BD Biosciences, Bedford, MA) were inoculated subcutaneously into both flanks of each mouse. Tumor bearing mice were divided into the following five groups (five mice per group): (1) vehicle alone (the vehicle control group), (2) Api (10 mg/kg via oral gavage; the Api group),[16] (3) Cis (5 mg/kg, i.p.; the Cis group), (4) Gem (200 mg/kg, i.p.) and Cis (the Gem+Cis group), and (5) Gem, Cis, and Api (the Gem+Cis+Api group). All drugs were administered on experimental days (ED) 1, 5, 9, and 13. Tumor sizes and body weights were also measured on these days and volumes were calculated using the following formula: volume = (length × width^2)/2. Mice were sacrificed on ED 15, and xenograft tumor tissues were resected.

Histological examination, BrdU and TUNEL assays

On ED 15, mice were administered 5-Bromo-2'-deoxyuridine (BrdU) (100 mg/kg, i.p.) and sacrificed 2 h later. Resected xenograft tumor tissues were sectioned at 4 ㎛ using a microtome, stained with hematoxylin and eosin (H&E), and placed on slide glasses. Sections were immersed in 3% H_2O_2 solution to block endogenous peroxidase activity, incubated with anti-BrdU antibody (Sigma, St. Louis, MO, USA) for 12 h at 4℃, with biotinylated anti-mouse antibody (Promega, Madison, WI, USA) for 1 h, and then with avidin-biotin peroxidase complex (Promega, Madison, WI, USA) for 30 min. The peroxidase activity was visualized by a color reaction using 0.04% diaminobenzidine and 0.02% hydrogen peroxide. The terminal deoxynucleotidyl transferaseYmediated dUTP nick end labeling (TUNEL) assay was used to assess degrees of apoptosis. TUNEL staining was performed by standard methods using 4 ㎛ section. Cell proliferation and apoptosis were quantified by counting numbers of BrdU stained and TUNEL-positive cells per 100 tumor cells, respectively, in 10 random microscopic fields (400×).

Statistical analysis

The significances of intergroup differences were determined using the Student's *t* test. The analysis was performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Results are presented as means±SDs and statistical significance was accepted for p values < 0.05.

RESULTS

PI3K/mTOR regulated cholangiocarcinoma cell viability and growth.

Initially, we determined the effect of Api on the PI3K/Akt/mTOR axis in SNU478 and SNU1196 cells. When these cell lines were treated with Api for 24 hr, the phosphorylations of Akt (Ser 473), S6K1 (Thr 389), and mTOR (Ser 2448) (key downstream markers of the PI3K/Akt/mTOR pathway) were dose-dependently diminished (Fig. 1A). To determine whether Api plays a crucial role in CCA cell proliferation, we monitored cell morphologies and confluencies, and measured cell numbers after Api treatment (Figs. 1B and 1C). Api treatment for 48 hr significantly inhibited cell growth. Next, we compared the anti-proliferative effects of Api with or without Cis or Gem in CCA cell lines. Api, Cis, and GEM all dose-dependently reduced cancer cell growth, and all three drugs Api substantially and similarly reduced CCA viability (Fig. 1C).

Co-treatment with apitolisib and cisplatin and/or gemcitabine additively inhibited CCA cell growth

In SNU478 cells, treatments with Api, Cis, or Gem significantly inhibited cell growth (Fig. 2A). In addition, Api co-treatment enhanced the anti-proliferative effects of Cis or Gem. Further, the growth of CCA cells treated with Cis or Gem was significantly reduced by Api co-treatment. Colony formation assay data also showed Api enhanced the inhibitory effects of Cis, Gem, or Gem plus Cis on cell proliferation (Figs. 2B and 2C). Under the same treatment conditions, western blotting showed that co-treatment with Api synergistically increased PARP

cleavage (an apoptosis marker) in the cells treated with Cis, Gem, or Gem plus Cis (Fig. 2D). These observations suggest that Api inhibits the PI3K/Akt/mTOR signaling pathway and CCA cell proliferation, and that co-treatment with Api plus Cis and/or Gem synergistically suppresses cell growth and apoptosis.

Treatment with apitolisib plus cisplatin and/or gemcitabine increased growth inhibition in the mouse tumor xenograft model

To compare the anti-tumor effects of Api, Cis, Gem+Cis, and Gem+Cis+Api *in vivo*, we administrated them to an SNU478 mouse xenograft model. Tumor volumes in these four groups and in vehicle controls were compared on the final day of treatment (ED 15) (Figs. 3A and B). Tumor volumes in the Cis and Cis+Gem groups were not significantly smaller than in the vehicle control group, whereas tumor volumes in the Api group were significantly smaller than in the Cis and Cis+Gem groups. Furthermore, tumor growth was most inhibited in the Gem+Cis+Api group. Throughout the experimental period, mouse weights in all four treatment groups remained similar to those in the control group. However, mean weight was significantly lower in the Gem+Cis+Api group than in control group on ED 15 (fig. 3C). TUNEL assays showed the percentage of apoptotic cells in tumor tissues was greatest in the Gem+Cis+Api group, and higher in the Api group than in the Cis+Gem group (Fig. 3D). Furthermore, BrdU assays showed proliferation index was lowest in the Gem+Cis+Api group (Fig. 3E).

DISCUSSION

Accumulating evidence indicates dysregulation of the PI3K/Akt/mTOR pathway is central to the initiation and progression of many types of cancer and that this pathway represents a promising therapeutic target. However, preclinical studies have shown single inhibitors of the PI3K/Akt/mTOR pathway (i.e., Akt, PI3K, or mTOR inhibitors) induce signaling feedback loops that limit their anti-tumor effects,[21] and thus, it has been suggested that the use of combinations of drugs targeting this pathway might increase antitumor effects. In this preclinical study, we investigated the role of PI3K/Akt/mTOR pathway in CCA cells and found that dual targeting of PI3K/mTOR using apitolisib and cisplatin or cisplatin plus gemcitabine dose- and time-dependently reduced CCA cell growth, viability, and colony formation. In addition, the cytotoxic effects of cisplatin and/or gemcitabine were enhanced by apitolisib. Although the antitumor activity of apitolisib was similar to those of cisplatin or gemcitabine alone, combinatorial treatments showed apitolisib enhanced these effects, which suggests the use of apitolisib in combination with conventional agents in clinical practice might enhance therapeutic effects.

In previous research studies, apitolisib was evaluated as a single treatment, and no comparisons were made of its effects when administered in combination with conventional drugs,[16,17] and thus, it was difficult to predict the efficacy of apitolisib in clinical practice. However, based on the results of the present study, we postulate that the activity of apitolisib when administered alone is similar to those of conventional drugs, and thus, because single agent therapies are

inadequate in CCA, combinatorial gemcitabine and cisplatin therapy has become a treatment standard.[3]

A previous phase I/II clinical trial on everolimus combined with gemcitabine/cisplatin failed to exhibite a synergistic effect in metastatic triple-negative breast cancer,[22] and a phase II clinical trial that compared apitolisib and everolimus in RCC concluded apitolisib was less effective than everolimus in metastatic RCC.[18] In particular, median progression-free survival was significantly shorter for apitolisib than everolimus (3.7 vs 6.1 months, $P<0.01$). The authors commented sustained dual inhibition of the PI3K/Akt/mTOR pathway in RCC is severely limited by a narrow therapeutic index and toxicity concerns. Another phase II single-arm clinical trial was conducted on apitolisib in patients with endometrial cancer.[19] However, the efficacy of apitolisib was limited by poor tolerability. In both studies, it was pointed out that the side effects of apitolisib were the most important problem. Therefore, clinical trials have been started to examine the effects of reducing apitolisib dosage[23] and increasing dosage intervals.[24] The toxicities of dual PI3K/Akt/mTOR pathway inhibitors have been suggested to be caused by the fact that increased mTOR and PI3K signaling is pervasive in highly proliferating normal cells.[21] Strategies are clearly required to reduce the toxicity of apitolisib.

In the present study, cisplatin and gemcitabine plus cisplatin both failed to show antitumor activity in our SNU478 xenograft model, despite the presence of effective drug concentrations sufficient to affect cell proliferation *in vitro*. We believe this may have been caused by the use a cell line continuous passaged *in vitro* to produce the xenograft model,[25] though drug delivery issues may also

explain this result. Nevertheless, even in the *in vivo* study, apitolisib/cisplatin/gemcitabine triple therapy exhibited more potent anti-tumor effects than monotherapies.

The development of next generation sequencing enhances comprehensive molecular profiling in clinical practice, and apitolisib treatment may be considered suitable only in patients expected to respond well based on molecular profiling results. Alterations in the expressions of the PTEN, PIK3CA, AKT1, or ERBB2 biomarkers indicate favorable response to apitolisib,[19] and cancers with these genetic alterations are likely to be more sensitive to lower doses of apitolisib. Although the effect of apitolisib on RCC has not been demonstrated, further research is needed on the effect of apitolisib on neuroendocrine cancers currently treated with mTOR inhibitors.

In summary, the present study shows apitolisib (a dual inhibitor of the PI3K/Akt/mTOR pathway) has potent therapeutic effects against CCA. The preclinical data obtained on the efficacy of apitolisib in CCA cell lines shows apitolisib administered in combination with gemcitabine and cisplatin has therapeutic potential. We suggest a clinical trial be conducted to determine the efficacy of gemcitabine/cisplatin/apitolisib combination therapy in CCA, but caution that toxicity concerns be fully addressed.

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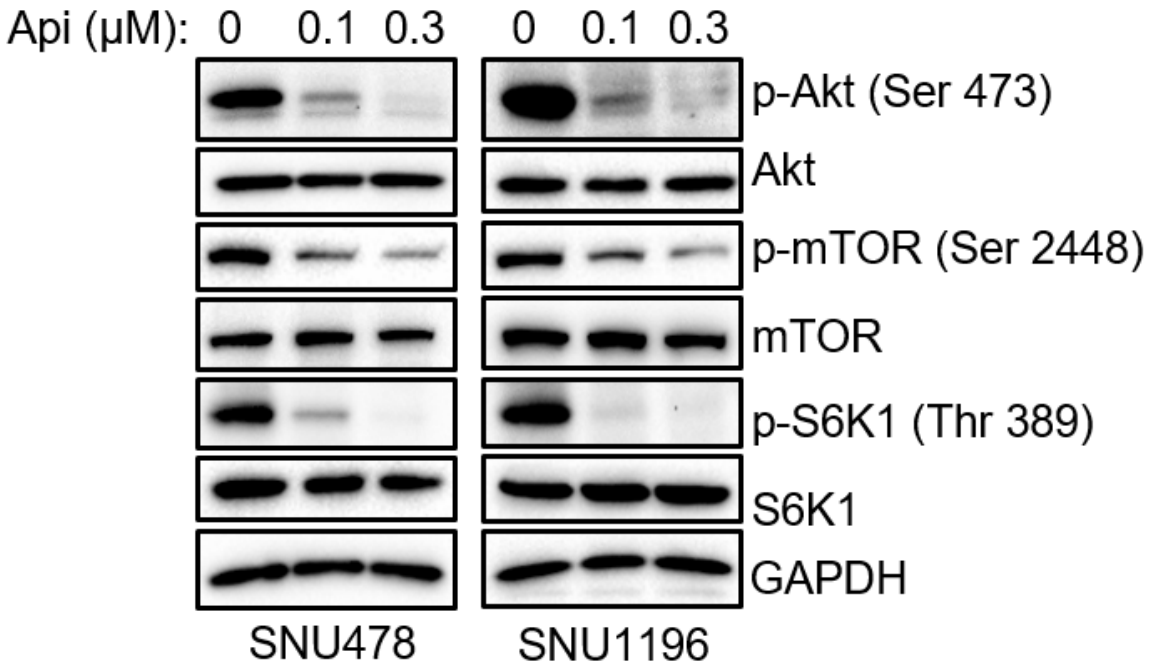
Figure 1. PI3K/mTOR is a crucial regulator of cholangiocarcinoma viability and growth. **(A)** SNU478 or SNU1196 cells were treated with the indicated concentration of apitolisib (Api) for 24 hr. Total cell lysates were analyzed by western blotting. p, phosphorylated. **(B)** SNU478 or SNU1196 cells were seeded in 12-well plates and treated with the indicated concentrations of Api. An IncuCyte™ Live-cell Imaging System (Essen BioScience, Ann Arbor, MI, USA) was used to monitor cellular morphology and cell confluency (scale bar: 400 μm). **(C)** SNU478 or SNU1196 cells were treated with the indicated concentrations of apitolisib (Api), gemcitabine (Gem), or cisplatin (Cis) for 48 hr. Cell proliferation was measured by direct cell counting. Mean ± SD (n = 3).

(A)



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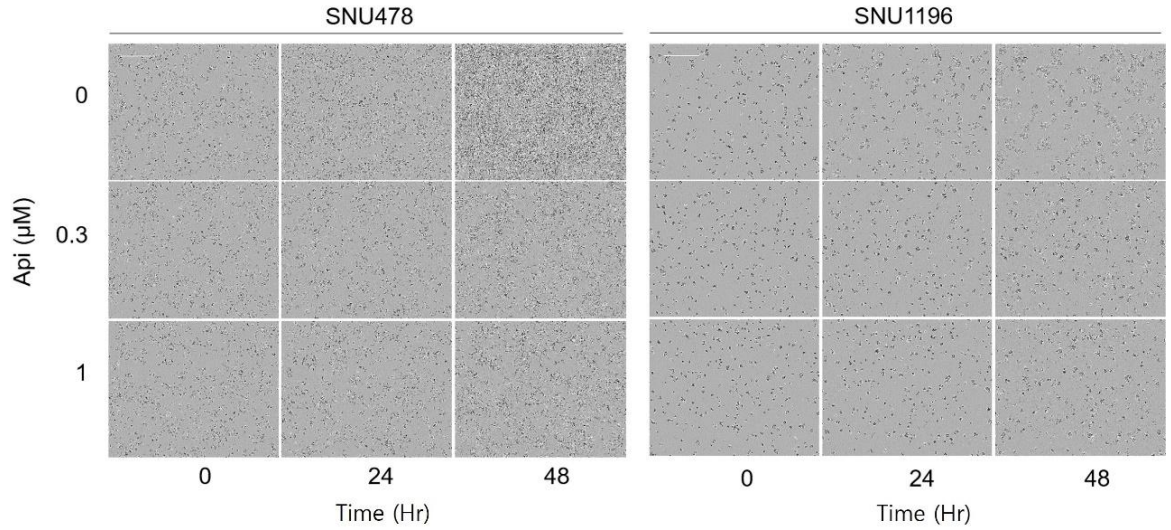
SNU478
SNU1196
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1
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24
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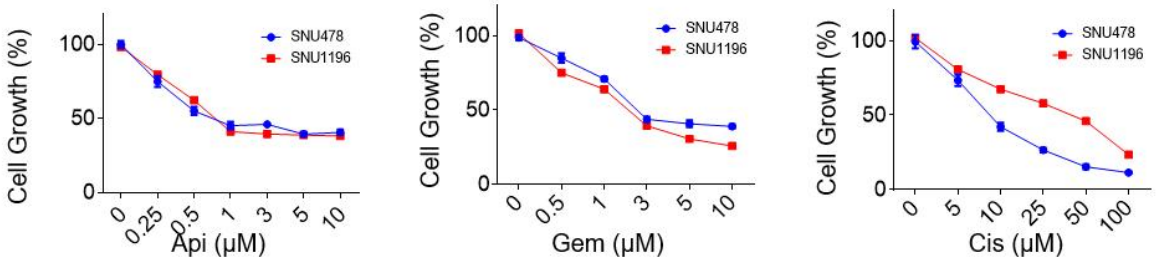
Time (Hr)
0
24
48
Time (Hr)

(C)


Cell Growth (%)
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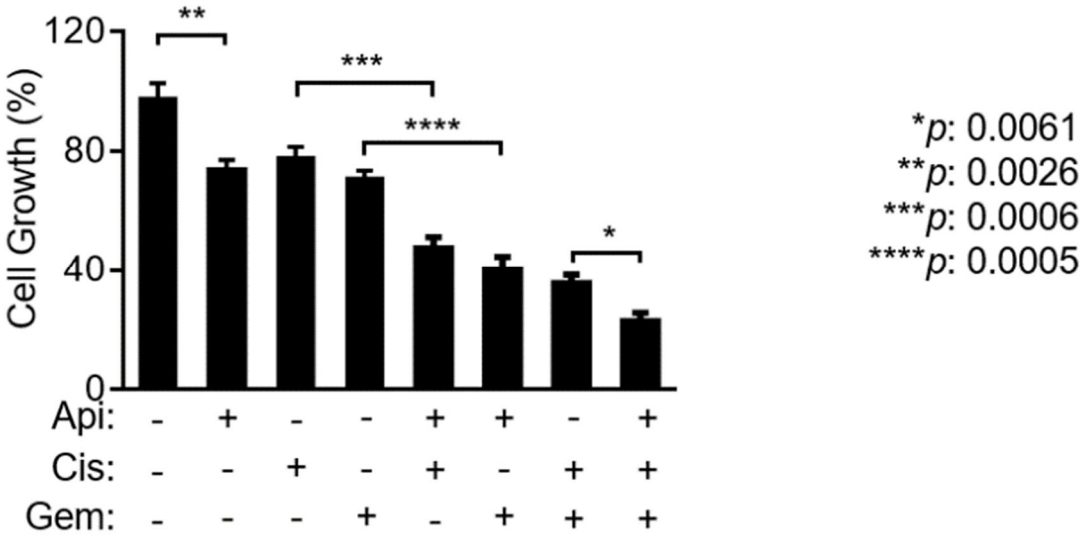
50
0
SNU478
SNU1196
0
0.25
0.5
1
3
5
10
Api (μM)
Cell Growth (%)
100
50
0
SNU478
SNU1196
0
0.5
1
3
5
10
Gem (μM)
Cell Growth (%)
100
50
0
SNU478
SNU1196
0
5
10
25
50
100
Cis (μM)

Figure 2. Apitolisib co-administered with cisplatin and/or gemcitabine additively inhibited cholangiocarcinoma cell growth. **(A-D)** SNU478 cells were co-treated with apitolisib (Api, 0.3 μM), cisplatin (Cis, 10 μM), gemcitabine (Gem, 1 μM) plus Cis, or with Api, Cis, plus Gem for 48 hr. **(A)** Cell proliferation was analyzed by direct cell counting. Mean ± SD (n = 3). *P = 0.0061, **P = 0.0026, ***P = 0.0006, ****P = 0.0005. **(B)** Colony formation was quantified by crystal violet staining after 10 days of treatment. **(C)** Mean ± SD (n = 3). *P = 0.0223, **P = 0.0016, ***P = 0.0012, ****P = 0.0005. **(D)** Total cell lysates were analyzed by western blotting. Clv, cleaved; p, phosphorylated.

(A)



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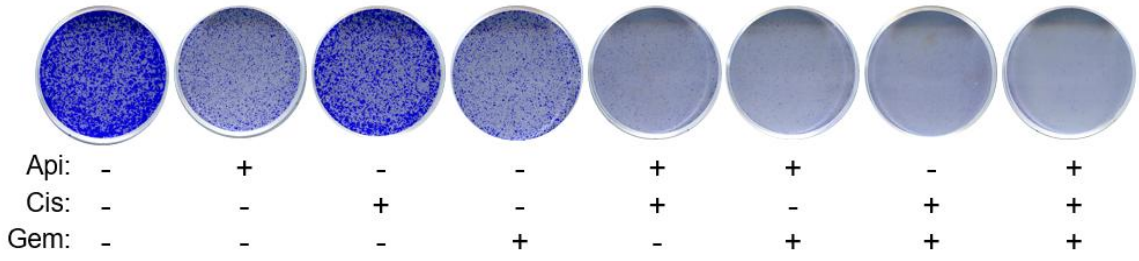

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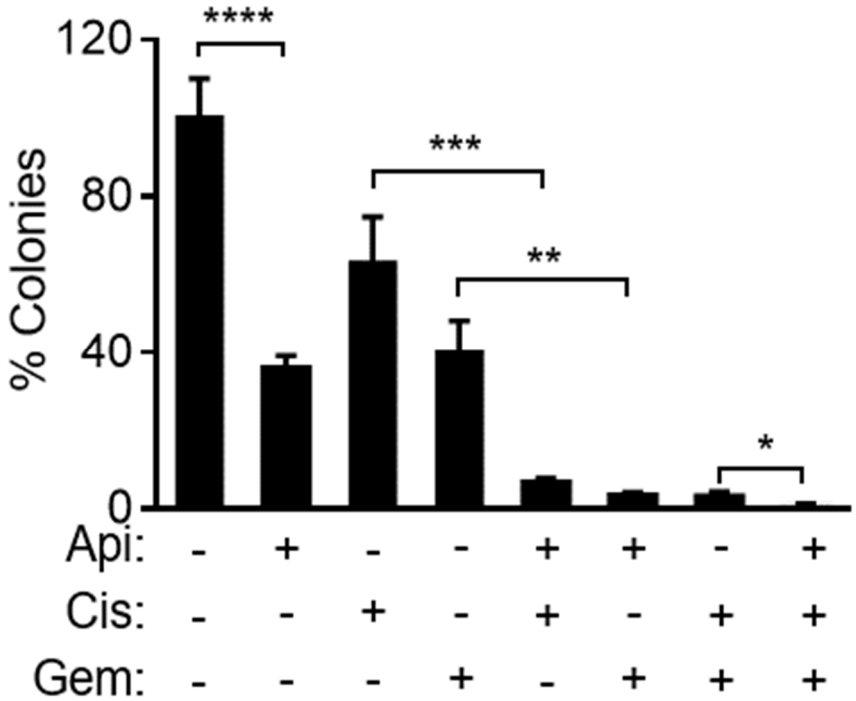
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120
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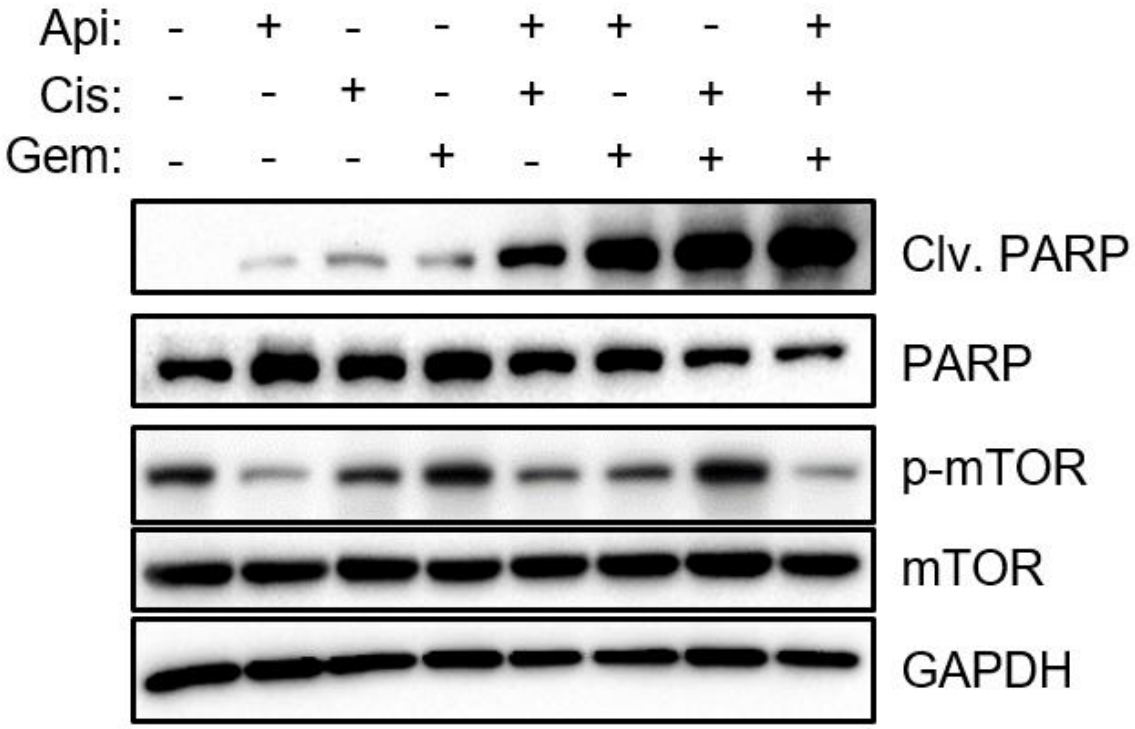
(D)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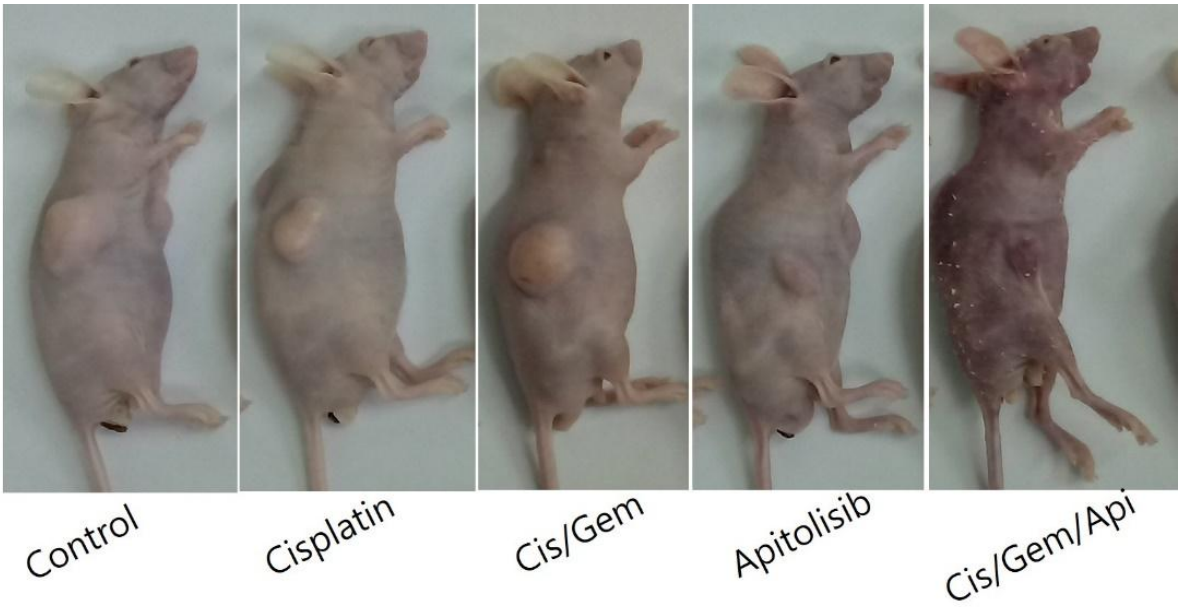
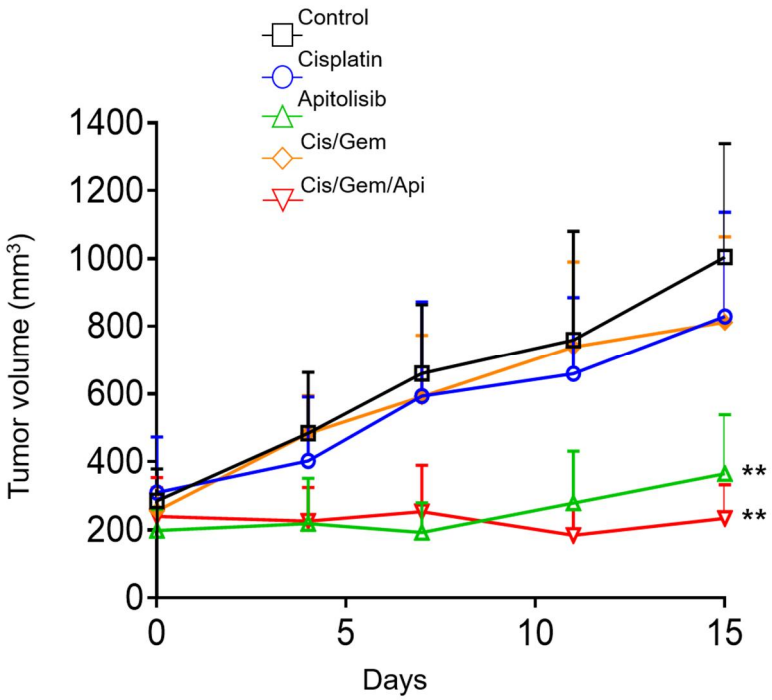
Cis: - - + - + - + +
Gem: - - - + - + + +
Clv. PARP
PARP
p-mTOR
mTOR
GAPDH

Figure 3. Apitolisib in combination with cisplatin or cisplatin plus gemcitabine enhanced growth inhibition in our tumor xenograft mouse model. **(A)** Differences between gross findings at the end of the 15−day experimental period. **(B)** Average tumor volumes on different experimental days in mice treated with cisplatin (Cis), apitolisib (Api), cisplatin (Cis)+gemcitabine (Gem), and cisplatin (Cis)+gemcitabine (Gem)+ apitolisib (Api). $^{**}P < 0.01$. **(C)** Average mouse weights on different experimental days. $^{**}P = 0.01$. **(D)** % TUNEL positive cells in tumor tissues. $^{**}P < 0.05$. **(E)** % BrdU positive cells in xenograft tumor tissues. $^{**}P < 0.001$.

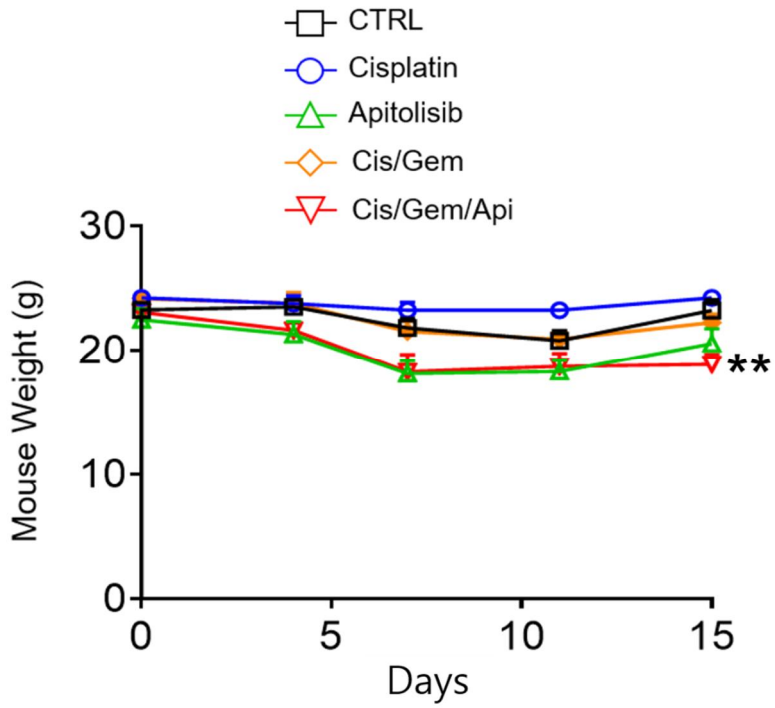
(A)



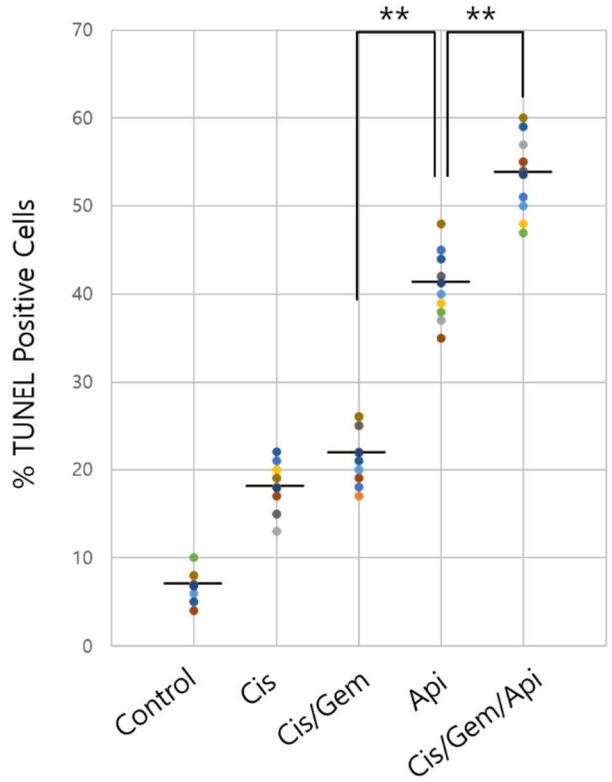
(B)


Control
Cisplatin
Apitolisib
Cis/Gem
Cis/Gem/Api
Tumor volume (mm3)
1400
1200
1000
800
600
400
200
0
0
5
10
15
Days
**
**

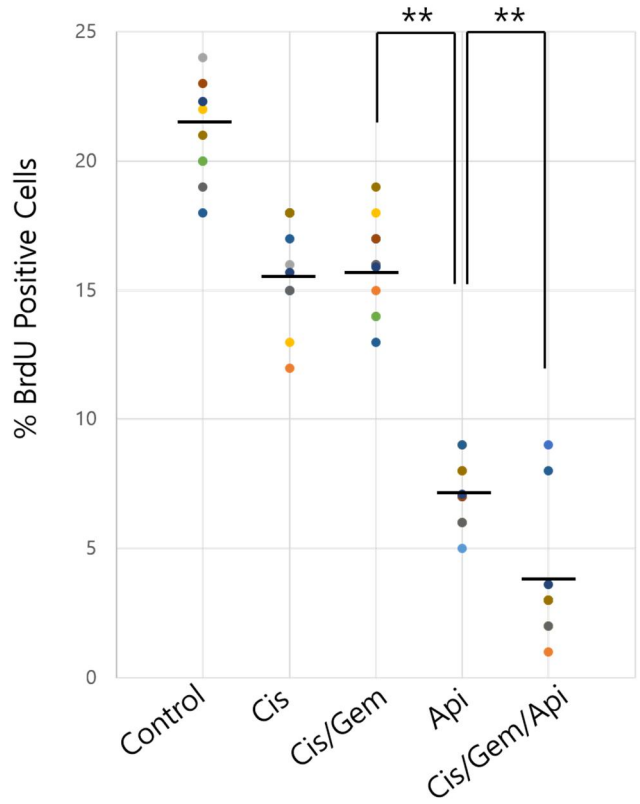
(C)


CTRL
Cisplatin
Apitolisib
Cis/Gem
Cis/Gem/Api
Mouse Weight (g)
30
20
10
0
0
5
10
15
Days
**

(D)


**
**
70
60
50
40
30
20
10
0
% TUNEL Positive Cells
Control
Cis
Cis/Gem
Api
Cis/Gem/Api

(E)


**
**
25
20
15
10
5
0
% BrdU Positive Cells
Control
Cis
Cis/Gem
Api
Cis/Gem/Api

국문 초록

담도암은 매우 위험하고 치명적인 암으로, 주로 진행된 상태에서 진단이 되어 예후가 좋지 않다. 진행성 담도암에 대한 gemcitabine 및 cisplatin 을 투약하는 전통적인 항암치료는 제한적인 생존 향상을 보여주고 있으므로, 효과적인 표적치료제의 개발이 시급하다. PI3K/Akt/mTOR 신호전달체계의 활성화는 담도암에서 자주 발견되며, 암세포의 발생, 성장, 전이에 중요한 역할을 한다. 따라서 PI3K 와 mTOR 는 담도암에서 효과적인 치료목표가 될 수 있다. 최근 PI3K 와 mTOR 의 이중저해제인 GDC-0980 (apitolisib)이 도입되었다. 본 연구는 apitolisib 의 담도암 세포에 대한 항암효과를 세포 및 동물실험을 통해 평가하고자 하였다. Apitolisib 치료는 Akt 와 mTOR 의 인산화정도를 낮추고 SNU478, SNU1179 두 가지 세포주의 세포성장을 충분히 억제하였다. 게다가 Gemcitabine, cisplatin 에 apitolisib 을 병합하는 경우 cleaved PARP 가 증가하는 것도 관찰되었다. 또한 apitolisib 의 병합은 담도암 세포주 균락의 생성을 억제할 뿐 아니라 이종이식 동물 모델에서의 암 성장도 효율적으로 억제하는 것이 확인되었다. 따라서 본 연구의 결과는 apitolisib 은 PI3K/Akt/mTOR 신호전달체계를 억제함으로써 효율적으로 담도암의 성장을 억제한다는 점을 시사한다. 그리고 gemcitabine 및 cisplatin 과의 병합요법은 apitolisib 에 동반상승효과를 부여하므로 향후 담도암의 치료효과를 높일 수 있을 것으로 기대된다.

주요어: GDC-0980, Apitolisib, 담도암, 치료

학 번: 2014-30919

감사의 글

남들보다 늦게 의학을 전공하고 의학박사 학위를 받는 데까지 참 오랜 시간이 걸렸습니다. 그 동안 수많은 시험을 통과하고, 이제 정말 마지막 시험을 마친 기분입니다. 의사 면허, 전문의 자격을 취득하는 과정은 정해진 길을 따라 지나왔다면, 의학박사학위를 취득하는 과정은 보이지 않는 곳을 향해 터널을 만들어가는 느낌이었던 것 같습니다. 의학을 전공하기 이전에 생명과학을 전공했지만, 의학에 매진하던 사이 생명과학에는 너무도 많은 발전과 변화가 있어 제가 뒤쳐져 있었기에 본 박사연구과정은 더욱 쉽지 않았던 것 같습니다. 이를 계기로 임상의 뿐 아니라 의과대학 교수, 과학자로서 제 소임을 다하리라 다짐해봅니다.

지도교수님이신 류지곤 교수님, 심사위원장 김용태 교수님, 그리고 본 연구결과를 내기 위해 직·간접적으로 많은 도움을 주신 이상협, 이준규 교수님께 먼저 깊은 감사를 드립니다. 한편 바쁘신 중에도 기꺼이 논문 심사를 해주신 이광혁, 김혜령 교수님 감사합니다. 그리고 20 살 포항공대 기숙사 같은 방에서 저와 새로운 인생을 함께 시작했고, 지금은 훌륭한 연구자가 되어 저에게 정말 많은 도움을 준 UNIST 채영찬 교수와 이유건 박사님 감사합니다.

그리고 저를 지금 이 자리에 있게 해준 부모님, 모자란 남편 옆에서 1 인 다역을 훌륭히 해주고 있는 인생의 동반자이자 원더우먼인 아내 김여형 교수, 나의 어린 시절을 그대로 보는 것 같은 첫째 아들 서진이, 개구쟁이 귀여운 둘째 아들 우진이에게 깊은 사랑을 전합니다. 마지막으로 동국대학교 일산병원 식구들, 저와 아내를 물심양면으로 뒷바라지 해주시는 장인어른, 장모님께 깊은 감사와 존경을 표합니다.

2020 년 7 월 장 동 기