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***In vitro* anticancer activity of phosphatidylinositol 3-kinase alpha selective inhibitor BYL719 in head and neck cancer**

두경부 편평세포암 세포주에서 포스파티딜 이노시톨 3-키나제 알파 특이 억제제의 항암효과

2014년 07월

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***In vitro* anticancer activity of phosphatidylinositol 3-kinase alpha selective inhibitor BYL719 in head and neck cancer**

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A Thesis Submitted to the Department of Molecular Tumor Biology with Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in The Department of Molecular Tumor Biology, Seoul National University College of Medicine

August 2014

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ABSTRACT

Introduction: Activating mutations of the *PIK3CA* gene occur frequently in head and neck squamous cell carcinoma. The purpose of this study was to explore the antiproliferative effect of BYL719, a specific inhibitor for phosphatidylinositol 3-kinase (PI3K) p110 α , in human head and neck cancer cell lines, as a single agent or in combination with the irreversible *EGFR* tyrosine kinase inhibitor (TKI), dacomitinib.

Material and Methods: Six head and neck cancer cell lines consisting of 2 *PIK3CA* mutant cell lines, SNU-1076 and Detroit562 and 4 *PIK3CA* wild type cell lines, SNU-1066, SNU-1041, FaDu and SCC25, were analysed. The inhibitory effect of BYL719 on cellular proliferation was assessed using the tetrazolium bromide [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] assay. The cell cycle at various concentrations of BYL719 was analyzed by flow cytometry, and the protein expression of downstream molecules determined by Western blot analysis.

Results: The *PIK3CA* mutant cell lines (SNU-1076 and Detroit562) were more sensitive to BYL719 than the *PIK3CA* wild type cell lines (SNU-1066, SNU-1041, FaDu and SCC25). Following BYL719 treatment, all *PIK3CA* wild type cell lines, except the SNU-1066 cell line, exhibited higher IC₅₀ values (1.13, 20.65, 19.67 and 49.30 μ M, SNU-1066, SNU-1041, FaDu and SCC25, respectively) compared with the *PIK3CA* mutant cell lines (6.82 and 1.10 μ M, SNU-1076 and Detroit562, respectively). Administration of BYL719 in the *PIK3CA* mutant cell lines induced cell cycle G0/1 arrest and resulted in increased apoptosis in a dose dependant manner. Furthermore, the administration of BYL719 in the *PIK3CA* mutant cell lines reduced the level of p-mTOR, p-AKT and p-S6 expression indicating the down regulating of downstream signaling. BYL719 combined with dacomitinib had a synergistic inhibitory effect.

Conclusion: BYL719, PI3K alpha selective blocker, could be a promising treatment for head and neck cancer as a single agent or in combination with dacomitinib. The beneficial effects of BYL719 in *in vitro* studies for head and neck cancer warrant a further clinical investigation.

Keywords: head and neck squamous cell carcinoma, *PIK3CA*, BYL719, dacomitinibs

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer with an annual incidence of ~600,000 worldwide.¹ Despite advances in the diagnosis and treatment of HNSCC, HNSCC still consists of poor prognosis with a 5 year survival rate of 50%.¹ In an attempt to improve the poor prognosis associated with HNSCC, recent studies of whole exome sequencing in HNSCC have revealed a wide spectrum of unexpected genetic aberrations.^{2,3}

A promising pathway in terms of genetic aberrations in HNSCC is the phosphatidylinositol 3-kinase (PI3K) signaling pathway. It is well known that the PI3K signaling pathway regulates cell proliferation, cell survival and apoptosis.^{4,5} Dysregulation or genetic aberration of the genetic components involved in the PI3K signaling pathway, including *AKT*, *PTEN* and *PIK3CA*, has been associated with cancer development and cancer progression.^{4,5} The Class IA PI3K is a heterodimeric lipid kinase complex with two subunits namely the p110 α catalytic domain and the p85 regulatory domain. Upon ligand binding and receptor tyrosine kinase (RTK) autophosphorylation, PI3K is recruited to the cell membrane, binds to the intracellular arm of the RTK and catalyzes the conversion of phosphatidylinositol (4,5)-diphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3).⁴

Recently, high frequencies of somatic mutations in the *PIK3CA* gene have been reported in HNSCC with frequent genetic aberration and amplification.⁶⁻⁸ More than 75% of these mutations are clustered in the helical (exon 9: E542K, E545K) and kinase domains (exon 20; H1047R) of the *PIK3CA* gene.⁶⁻⁸ These hot spot mutations in the *PIK3CA* gene have been shown to elevate constitutive lipid kinase activity and lead to increased activation of the downstream AKT signaling pathway.^{9,10} As PI3K is constitutively activated in *PIK3CA* mutant tumors, PI3K appears to be an ideal target for drug development in the therapeutic treatment of *PIK3CA* mutant tumors. A newly developed PI3K inhibitor, BYL719¹¹ has higher selectivity for the PI3K p110 α subunit than any other PI3K subunits.

Dacomitinib (PF-00299804) is an orally administered, irreversible epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor (TKI). *In vitro* studies demonstrated that dacomitinib is potent and

specific inhibitor against EGFR, human epidermal growth factor receptor 2 (HER2), and HER4 tyrosine kinases.¹² In the preclinical setting, dacomitinib was active not only against EGFR wild-type cancers, but also in mutant models¹³, including those harboring EGFRvIII mutation, detected in up to 40% of HNSCC.¹⁴ Dacomitinib, as a single agent, is also effective in recurrent or metastatic HNSCC with response rate of 12.7%.¹⁵

The purpose of this study was to investigate the *in vitro* antiproliferative effects of BYL719 in human head and neck cancer cell lines as either a single agent or in combination with an irreversible *EGFR* TKI, dacomitinib, and to determine the molecular mechanisms underlying the cell proliferation inhibition and the chemo-sensitizing effects. To gain a better understanding of the mechanism of growth inhibition, protein expression of downstream molecules, particularly in the EGFR signal transduction pathways, and the alteration of cell cycle regulatory molecules were investigated.

MATERIALS AND METHODS

Cell lines and culture

Six human head and neck cancer cell lines, consisting of 2 *PIK3CA* mutant cell lines, SNU-1076 and Detroit562 and 4 *PIK3CA* wild type cell lines, SNU-1066, SNU-1041, FaDu, and SCC25 were purchased from the American Type Culture Collection (Manassas, VA, USA) and Korean Cell line Bank (Seoul, Korea). The SNU-1066, SNU-1041 and SNU-1076 cell lines were maintained in RPMI 1640 medium with 100 U/mL penicillin, 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Grand Island, NY, USA). The Detroit562, and FaDu and SCC25 cell lines were maintained in American Type Culture Collection (ATCC) Eagle's modified essential medium (EMEM) with 100 U/mL penicillin, 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (GIBCO, Grand Island, NY, USA). All cell lines were incubated under standard culture conditions (5% CO₂ at 37°C).

Mutation analysis for head and neck cancer cell lines

The mutational profiles of head and neck cancer cell lines were screened via The Cancer Cell Line Encyclopedia¹⁶ (CCLE) (<http://www.broadinstitute.org/ccle>). The CCLE is a compilation of gene expression, chromosomal copy number and massively parallel sequencing data from 947 human cancer cell lines. Mutation information was obtained by using both massively parallel sequencing of > 1,600 genes and by mass spectrometric genotype screening (OncoMap). The *PIK3CA* mutation status of the HNSCC cell lines were obtained from CCLE. The mutational profiles of 6 HNSCC cell lines were re-confirmed by whole exome sequencing incorporated with another study. Detailed methods of the whole exome sequencing method are described in prior report.¹⁷

Drugs and Reagents

Both BYL719 and dacomitinib was purchased from Selleck Chemicals LLC (Houston, TX, USA).

Both BYL719 and dacomitinib were initially dissolved in dimethylsulfoxide (Sigma Chemical Co., St. Louis, Missouri, USA) at a concentration of 10 mM/mL and stored in small aliquots at -20°C .

Cell growth-inhibition assay

A modified MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed using Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan). The inhibitory concentration at 50% (IC_{50}) was calculated. Cells ($3-5 \times 10^3$) were seeded on 96-well plates and incubated for 24 h and then treated with drugs (BYL719 or dacomitinib) for 3 days at 37°C . After drug treatment, CCK-8 solution was added to each well and the cells incubated for 4 hours at 37°C . Absorbance was measured at 450 nm in an Eon™ Microplate Spectrophotometer (BioTek, Winooski, VT, USA) in triplicate. Graphs were generated by nonlinear regression of the data points to a four-parameter logistic curve using SigmaPlot software (Statistical Package for the Social Sciences, SPSS, Inc., Chicago, IL, USA). All data are expressed as mean \pm standard deviation (SD) obtained from at least three independent experiments.

Cell cycle analysis

Cells were plated in each culture dish and were treated with 0, 5, 10mM/mL BYL719 for 72 h. Cell cycle analysis by flow cytometry was performed by detaching cells from culture plates with trypsin/ethylenediaminetetraacetic acid (EDTA), washing with phosphate buffered saline (PBS) and fixing in 75% ethanol. The pellet was re-suspended and incubated for 30 min in 5 μl PI (1 mg/mL) and RNase A 10 μl (10 mg/mL) in PBS. The suspension was then analyzed on a Becton Dickinson FACScan. The ratio of cells in the G0/G1, S and G2/M phase of cell cycle were determined by the relative DNA content per cell.

Annexin-V assay

Cells were treated with increasing doses of BYL719 (5 μM and 10 μM), incubated for 30 h collected and concurrently stained with Annexin-V and 7-AAD (Becton Dickinson Biosciences, San Jose, CA,

USA). Apoptotic cell death was determined by positive staining for Annexin-V and negative staining for 7-AAD using fluorescence-activated cell sorting analysis.

Western blot analysis and fluorescence in situ hybridization (FISH)

Cells were resuspended in lysis buffer (Cell Signaling Technology, Danvers, MA, USA), incubated on ice for 10 min and centrifuged for 15 min at 4°C. Samples containing equal quantities of total protein were resolved on SDS–polyacrylamide denaturing gel, transferred to PVDF membranes, and probed with antibodies, according to the manufacturer’s procedure. Antibodies against EGFR, p-EGFR, HER2, p-HER2, PTEN, mTOR, p-mTOR, AKT, p-AKT, S6, p-S6, ERK, p-ERK and β -actin were purchased from Cell Signaling Technology (Danvers, MA, USA). β -actin was used as the protein loading control. Detection was performed using an enhanced Lumi-Light Western Blotting Substrate kit (Roche, Indianapolis, IN, USA).

HER2 amplification of 6 cell lines were determined by FISH using PathVysion HER2 DNA Probe Kit (Vysis, Downers Grove, IL, USA). HER2 FISH was performed by previously described methods.¹⁸ Cell lines were considered to be amplified if they had a copy number ratio of HER2/ chromosome 17 centromere (CEP17) >2.0

Determination of synergism and antagonism

To evaluate the effects of BYL719 administered in conjunction with dacomitinib, cells were treated with serial dilutions of each drug individually and with both drugs simultaneously at a fixed ratio of doses that corresponded to the individual IC_{50} . After 72 h of exposure, cell viability was measured using the MTT assay. The combination index (CI) was calculated according to the Chou-Talalay method.¹⁹ Data were analyzed using the CalcuSyn software (Biosoft, Ferguson, MO, USA). The CI index has been used for data analysis of two-drug combinations. $CI < 1$, $CI = 1$ and $CI > 1$ indicate synergism, additive effect and antagonism, respectively.

RESULTS

Proliferation inhibition activity of BYL719 in head and neck cancer cell lines

Head and neck cancer cell lines (both *PIK3CA* mutant and *PIK3CA* wild type) were treated with increasing doses of BYL719 (no treatment, 0.1, 0.5, 1, 5, 10, 50, and 100 μM) for 72 h. The *PIK3CA* mutant cell lines (SNU-1076 and Detroit562) were more sensitive to BYL719 with a significant decrease in cell proliferation in a BYL719 dose dependent manner than the *PIK3CA* wild type cell lines (SNU-1066, SNU-1041, FaDu and SCC25,) (Figure 1). The IC_{50} values in the *PIK3CA* mutant cell lines were 6.82 μM in SNU-1076 and 1.10 μM in Detroit562. The *PIK3CA* wild type cell lines, except the SNU-1066 cell line, exhibited higher IC_{50} values for BYL719 treatment (IC_{50} values: 1.13 μM , 20.65 μM , 19.67 μM , and 49.30 μM in SNU-1066, SNU-1041, FaDu and SCC25, respectively).

Apoptotic effect and cell cycle analysis of BYL719 treatment in head and neck cancer cell lines

The *PIK3CA* mutant cell lines (SNU-1076, and Detroit562) and *PIK3CA* wild type cell line SNU-1066 that showed exceptionally low IC_{50} values to BYL719 treatment, were treated with BYL719 at various doses (no treatment, 5 and 10 μM) for 72 hours. As measured by flow cytometry, BYL719 increased the sub-G1 phase and induced G1 arrest in the Detroit562 cells. Increased sub-G1 phase and decreased S phase fraction arrest was also observed in the SNU-1076 and SNU-1066 cells (Figure 2A).

Apoptosis was detected by Annexin V staining in the Detroit562, SNU-1076 and SNU-1066 cells (Figure 2B). These cell lines showed increasing Annexin V staining cells in a BYL719 dose-dependent manner.

Effect of BYL719 on the PI3K downstream signaling

Changes in the protein expression of the downstream signaling pathway of PI3K were analysed via Western blot (Figure 3). The SNU-1076, Detroit562, and SNU-1066 cells were treated with various

doses of BYL719 (0, 0.1, 1 and 10 μM) for 48 h. Protein expression of p-mTOR and p-AKT were reduced in a dose-dependent manner.

As SNU-1066 which is *PIK3CA* wild type cell lines was sensitive to BYL719, we determined PTEN loss and HER2 amplification. PTEN loss was not observed in SNU-1066 (Figure3). However, HER2/CEP17 ratio of SNU-1066 cell line was 4.40, and HER2 amplification was observed in SNU-1066.

Synergistic effect of BYL719 combined with irreversible EGFR tyrosine kinase inhibitor, dacomitinib

The synergistic or additive effects of BYL719 combined with irreversible EGFR inhibitor, dacomitinib were evaluated after simultaneous exposure of dacomitinib to BYL719. As the IC_{50} value of dacomitinib were $4.73 \pm 0.21 \mu\text{M}$, dacomitinib concentrations of a lower level IC_{50} such as 0.01, 0.05 and 0.1 μM were chosen for analysis of drug combination. When all cell lines were treated with combined dacomitinib and BYL719, the IC_{50} values of BYL719 decreased except in the SCC25 cell line, indicating that dacomitinib can produce a synergistic effect with BYL719 treatment (Figure 4). The synergistic activity of BYL719 combined with dacomitinib was seen not only in the *PIK3CA* mutant cell lines, but also in *PIK3CA* wild type cell lines. SNU-1076, Detroit562, SNU-1066, SNUH-1041 and FaDu showed CI values <1 with the combination of BYL719 and dacomitinib, indicating a synergistic interaction (Table 1).

DISCUSSION

The present study shows the efficacy of BYL719, a novel PI3K 110 α specific inhibitor, in head and neck cancer cell lines. In the present study, BYL719 inhibited cell proliferation by inducing apoptosis, particularly in *PIK3CA* mutant head and neck cancer cell lines. In combination with dacomitinib, BYL719 treatment showed a synergistic cell proliferation inhibitory effect on *PIK3CA* mutant cell lines, whereas this synergistic effect was not observed in the *PIK3CA* wild type cell lines.

The recent elucidation of HNSCC genomics offers an opportunity to identify genotype based treatment decisions. Recently, Stransky *et al.*² and Agrawal *et al.*³ reported the mutational landscape of HNSCC using whole exome sequencing. These two studies provided new insights into the genetic understanding of HNSCC and highlighted the high frequency of mutations in tumor suppressors genes including: *TP53*; *CDKN2A*; *NOTCH1*; *NOTCH2*; *NOTCH3* and *FAT1*.^{2,3} Among the various altered genes observed in whole exome sequencing, the most targetable oncogene with sufficient mutation frequency was *PIK3CA*. The *PIK3CA* mutations comprised from 8.0% to 30.5% of HNSCC^{2,3,6-8,20}. In addition, *PIK3CA* mutations occur at a high frequency in HPV-positive oropharyngeal cancer⁷, of which the incidence is rising rapidly.²¹ Furthermore, more than 75% of *PIK3CA* mutations are hot spot mutations in the helical (exon 9: E542K, E545K) and kinase domains (exon 20; H1047R) of the gene, and these hot spot mutations constitutively activate the PI3K pathway. Activation of the PI3K pathway offers the possibility for personalized therapy with *PIK3CA* pathway inhibitors to improve the treatment outcomes of HNSCC.

Somatic mutations in *PIK3CA* have been identified in a variety of human cancers, including breast, colon, endometrial cancers and glioblastomas.²² Most of these mutations cluster to two hot spot regions in exon 9 which encodes the helical domain of p110 α , and in exon 20 which encodes the catalytic domain of p110 α . These mutations de-repress an inhibitory interaction between the N-terminal SH2 domain of p85 and the p110 α catalytic subunit.²³ These *PIK3CA* mutants leads to increased oncogenic potential *in vitro* and *in vivo*^{24,25} by causing constitutive activation of the PI3K

pathway in the absence of growth factors.

The findings of the current study show that BYL719 possesses a high cytotoxicity in *PIK3CA* mutant head and neck cancer cell lines. The growth inhibition of the 3 head and neck cell lines occurred in the micromolar range (1.10 – 6.82 μ M range) of BYL719, and no cell line examined in the present study was resistant to a single treatment with this agent. This is comparable with previous reports of BYL719 treatment in breast cancer cell lines²⁶ and myeloma cell lines.²⁷ In addition, the antitumor effects of BYL719 treatment in xenograft models have previously reported.²⁶ The data of the present study also suggest that BYL719 can have synergism with the irreversible *EGFR* TKI, dacomitinib, further inhibiting carcinoma cell proliferation. As HNSCC expresses high levels of *EGFR* on the cell surface²⁸ and dacomitinib, as a single agent, is also effective in preventing recurred or metastatic HNSCC¹⁵, BYL719 in combination with dacomitinib may be a promising therapeutic approach for *PIK3CA* mutant HNSCC.

In the present study, the *PIK3CA* mutant cell lines showed better sensitivity to BYL719 treatment compared to the *PIK3CA* wild type cell lines. Interestingly, one *PIK3CA* wild type cell line, SNU-1066 also had good sensitivity to BYL719 treatment as indicated by induced apoptosis even when compared with the 2 *PIK3CA* mutant cell lines. It is of note that BYL719 can be effective in *PIK3CA* wild type cell line. Synergistic interaction between BYL719 and dacomitinib were observed in both *PIK3CA* mutant and wild type cell line.

Such findings of the current study suggest that, besides the *PIK3CA* mutation, there may be additional working mechanisms involved in HNSCC such as upstream pathway activation or loss of PTEN or mutation of PTEN. In our study, it was suggested that HER2 amplification and the activation of upstream pathway of PI3K were the reason for BYL719 sensitivity in *PIK3CA* wild type cell line. A predictive factor for BYL719 in *PIK3CA* wild type cell line needs to be elucidated in order to establish the optimal cell type candidate for BYL719 treatment.

In conclusion, BYL719 has cell proliferation inhibitory effects in several head and neck cancer cell lines, in particular, *PIK3CA* mutation head and neck cancer cell lines. The results of the present study provide evidence that BYL719 is a potential target in the treatment of HNSCC with *PIK3CA* mutation.

Future clinical trials of BYL719 alone or in combination with an irreversible *EGFR* TKI, such as dacomitinib, in HNSCC, are warranted.

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FIGURES AND LEGENDS

Table 1. Combination index values of concurrent treatment with BYL719 and dacomitinib in head and neck cancer cell lines

	Combination index (CI) values	
	BYL719 (1uM)+ dacomitinib (0.01uM)	BYL719 (10uM)+ dacomitinib (0.01uM)
SNU-1076	0.223	0.308
Detroit562	0.140	0.266
SNU-1066	0.299	0.276
SNU-1041	0.107	0.353
SCC25	0.901	1.730
FaDu	0.118	0.315

CI < 1 synergistic; CI = 1 additive; CI > 1 antagonistic.

Figure 1. Antiproliferative activity of BYL719 in various head and neck cancer cell lines.

The 6 human head and neck cancer cell lines were treated with increasing concentrations of BYL719 (0, 0.1, 0.5, 1, 5, 10, 50, and 100 μM) for 72 h. The IC_{50} values and cell viability using an MTT assay were determined by measuring the absorbance at 540 nm in a microplate reader. Each value represents the means of 12 replication experiments: Bars, data are presented as mean \pm SD.

PIK3CA mutation	Mutant (H1047R)	Mutant (H1047R)	Wild type	Wild type	Wild type	Wild type
Cell line	Detroit562	SNU-1076	SNU-1066	FaDu	SNU1041	SCC25
IC_{50} (μM)	1.10 ± 0.07	6.82 ± 0.30	1.13 ± 0.22	19.66 ± 1.96	20.65 ± 1.80	49.30 ± 3.43

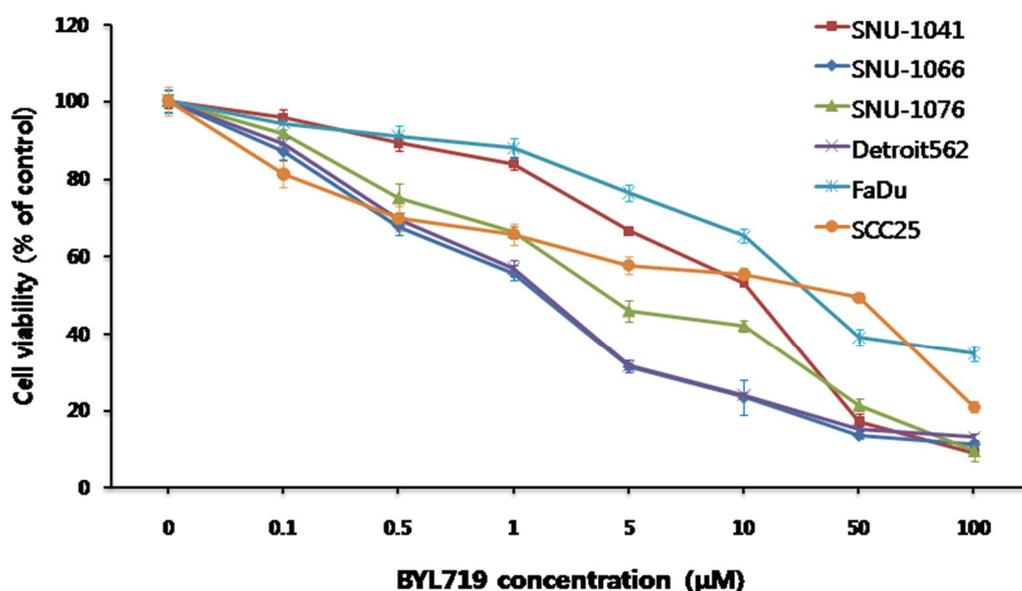


Figure 2. Effects of BYL719 on cell cycle.

(A) *PIK3CA* mutant cell lines (SNU-1076 and Detroit562) and the *PIK3CA* wild type cell line (SNU-1066) were treated with BYL719 (0, 5, and 10 μ M) for 72 h. Proportions of cells in the G1, S, and G2-M phase were quantified; total percentages of G1, S, and G2-M phases are presented as 100%. Treatment with BYL719 caused accumulation of Detroit562 cells in the sub-G1 phase. (B) Apoptosis of SNU-1076 and Detroit562 cells, the staining of Annexin V-phycoerythrin by fluorescence activated cell sorting analysis. Both the SNU-1076 and Detroit562 cells showed increasing annexin V staining cells in a BYL719 dose-dependent manner. The data are representative of six independent experiments. * $P < 0.001$

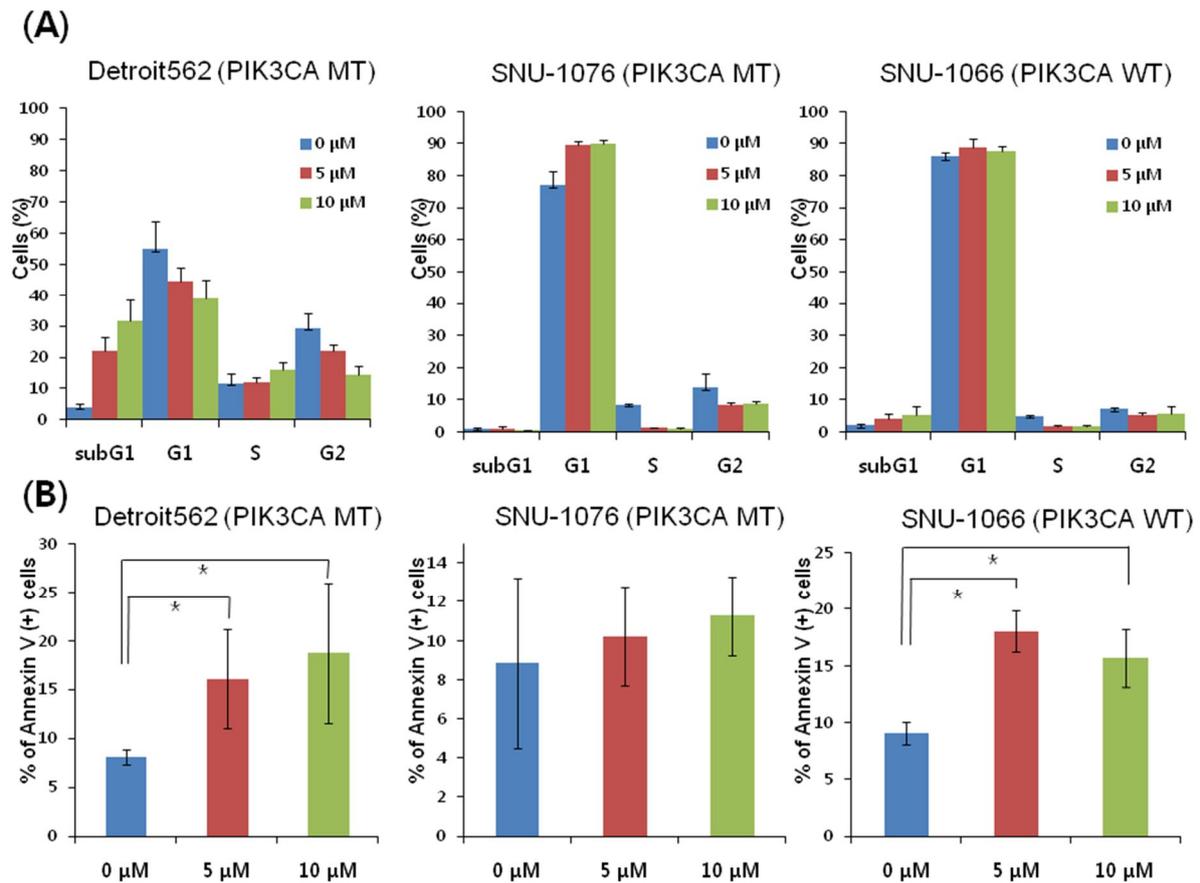


Figure 3. Western blot analysis for downstream signaling

Western blotting was conducted for *PI3K* downstream signaling after treatment with BYL719. Detroit562, SNU-1076, and SNU-1066 cells were treated with BYL719 (0, 0.1, 1 and 10 μ M) for 48 hours. Downstream signaling molecules (mTOR, AKT, S6) and the phosphorylated forms of each protein were observed with the same treatments. The protein expression of p-mTOR and p-AKT were downregulated

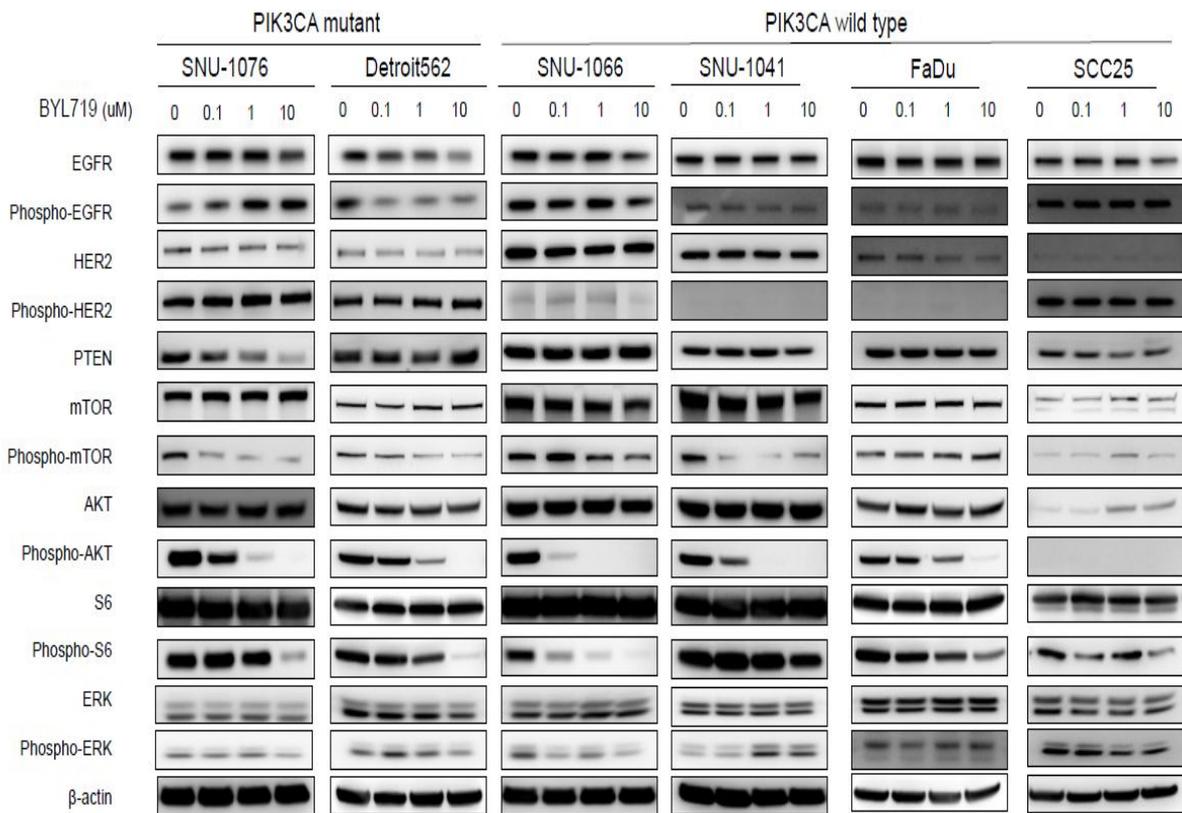
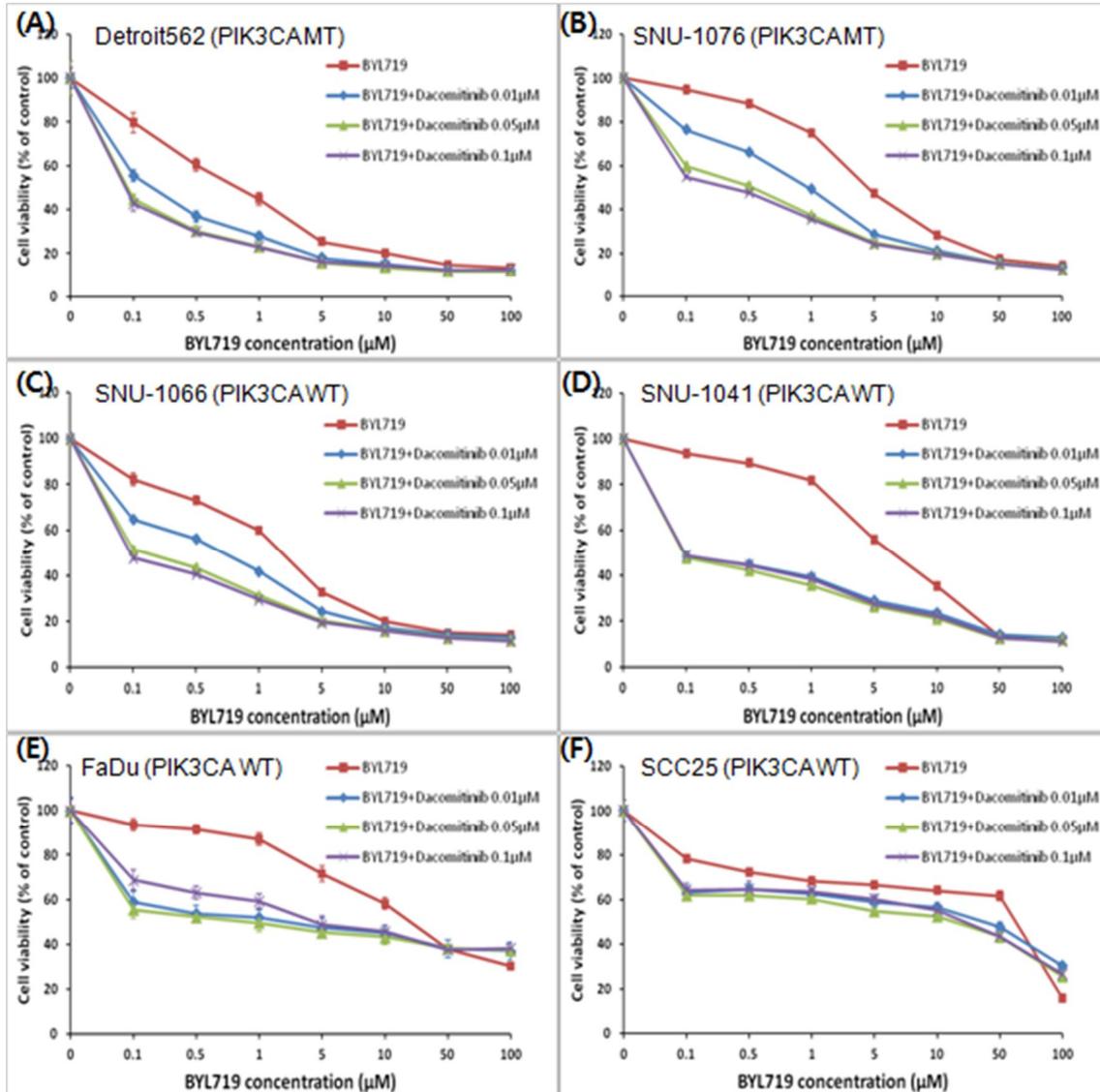


Figure 4. Synergistic interactions between BYL719 and dacomitinib

IC₅₀ values decreased with combined dacomitinib and BYL719 treatment. The IC₅₀ value of BYL719 was markedly decreased particularly in the 2 *PIK3CA* mutant cell lines (Detroit562, SNU-1072) in a dose dependent manner. The IC₅₀ value of BYL719 was not changed in the SCC25, *PIK3CA* wild type, cell line.



국문 초록

서론: *PIK3CA* 유전자의 활성화 돌연변이는 두경부 편평세포암에서 흔히 발견된다. 본 연구의 목적은 두경부암 세포주에서 포스파티딜 이노시톨 3 - 키나제 (PI3K) p110 α 특이 억제제인 BYL719의 항종양효과를 탐색하는 것으로 단일 약제 또는 비가역적 EGFR의 TKI인 dacomitinib과 함께 투여할 때 항종양효과를 살펴보고자 한다.

방법: *PIK3CA* 돌연변이 양성 세포주 2가지 (SNU-1076 및 Detroit562)와 *PIK3CA* 돌연변이 음성 세포주 4가지 (SNU-1066, SNU-1041, FaDu 및 SCC25)를 합하여 모두 6가지 두경부암 세포주를 이용하여 실험하였다. 세포주의 성장 억제 효과는 MTT분석 (테트라 졸륨 브로마이드 분석법)을 사용하여 평가하였다. BYL719의 다양한 농도에서 세포주기를 유세포분석기를 이용하여 평가하였다. 하위신호전달 분자는 Western 블롯 분석에 의해 살펴보았다.

결과: *PIK3CA* 돌연변이 양성 세포주 (SNU-1076 및 Detroit562)는 *PIK3CA* 돌연변이 음성 세포주 (SNU-1066, SNU-1041, FaDu 및 SCC25)보다 BYL719에 더 잘 반응하였다. SNU-1066 이외 *PIK3CA* 돌연변이 음성 세포주에서는 IC_{50} 값이 *PIK3CA* 돌연변이 양성 세포주의 IC_{50} 값에 비해 높았다 (돌연변이 음성 세포주인 SNU-1066, SNU-1041, SCC25, FaDu 에서는 각각 1.10, 20.65, 19.67 및 49.30 μ M, 돌연변이 양성 세포주인 SNU-1076, Detroit562에서는 6.82, 1.13 μ M). BYL719는 용량에 비례하여 G0/1를 억제하고 세포사멸을 유도하였다. 또한 BYL719는 용량 의존적으로 p-mTOR, p-AKT, p-S6의 발현이 감소시켰다. 또한 dacomitinib을 BYL719와 함께 투여하였을 때 항종양 효과에 상승효과가 있었다.

결론: PI3K 알파 선택적 억제제인 BYL719은 *PIK3CA* 돌연변이 양성 두경부암에서 단일 제제 혹은 dacomitinib와 함께 사용하여 유망한 치료법이 될 수 있다. 두경부암에서

BYL719의 인체 내 효과를 보기 위한 전향적 임상 연구가 필요하다.

주요어: 두경부암, PI3K 억제제, BYL719

학 번: 2007-30541