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의학석사 학위논문

Everolimus in combination with crizotinib synergistically inhibits the growth of ALK-positive anaplastic large cell lymphoma cells

2014 년 8 월

서울대학교 대학원

의학과 내과학 전공

Xu Wendan

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Everolimus in combination with crizotinib synergistically inhibits the growth of ALK-positive anaplastic large cell lymphoma cells

by

Xu Wendan

A Thesis Submitted to the Department of Medicine
in Partial Fulfillment of the Requirements for the
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Approved by Thesis Committee:

Professor _____ Chairman

Professor _____ Vice chairman

Professor _____

ABSTRACT

Everolimus in combination with crizotinib synergistically inhibits the growth of ALK-positive anaplastic large cell lymphoma cells

Xu Wendan

Department of Internal Medicine

The Graduate School

Seoul National University

Anaplastic large cell lymphoma (ALCL) is a rare, aggresive type of non-Hodgkin lymphoma, more than half of which harbors an aberrant NPM-ALK fusion protein. Both mTOR inhibitor everolimus and ALK inhibitor crizotinib have shown promising antitumor activity in ALK-positive cancer cell lines. However, their combined effect has not yet been investigated. This study aimed to evaluate the combined effect of

everolimus and crizotinib in ALK-positive ALCL cell lines, Karpas 299 and SU-DHL-1. Western blot and flow cytometry were performed to elucidate the mechanism of combined effect as well. The results show that the combination of everolimus and crizotinib synergistically inhibit the growth of ALK-positive ALCL cells. Crizotinib attenuated ERK and AKT phosphorylation caused by everolimus. The combination treatment induced G1 cell-cycle arrest and increased apoptosis in ALK-positive ALCL cells. The synergistic effect of everolimus and crizotinib was also found in the non-small cell lung cancer cell line, NCI-H2228, that harbors a *EML4-ALK* fusion gene. In summary, everolimus combined with crizotinib synergistically inhibited the growth of ALK-positive ALCL cells by inhibiting ALK and mTOR phosphorylation and thus indirectly preventing aberrant ERK and AKT phosphorylation. Our results suggest that this combination could be used to improve the therapeutic outcome in patients with ALK-positive ALCL.

Keywords: mTOR, Everolimus, Crizotinib, Anaplastic large cell lymphoma, Anaplastic lymphoma kinase

Student number: 2012-22672

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INTRODUCTION

Anaplastic large cell lymphoma (ALCL) is a rare, aggressive form of mature T-cell lymphoma, which comprises approximately 3% of adult non-Hodgkin lymphoma (NHL) and 10-15% of pediatric NHL (1). More than a half of ALCL harbors a nucleophosmin-anaplastic lymphoma kinase (*NPM-ALK*) fusion gene (2). The constitutively activated NPM-ALK fusion protein consequently activates a number of signaling pathways such as Ras/ERK, PI3K/AKT, and JAK3/STAT3 (3-5). Although *ALK* fusion gene-positive (ALK-positive) ALCL patients have a better prognosis than those without (5-year overall survival of 70% vs. 49%), those with relapsed or refractory disease generally had a poor prognosis (1, 2, 6). Thus, this genetic alteration could be a therapeutic target in this subset of patients.

Crizotinib (also known as PF-02341066) is an ALK tyrosine kinase inhibitor that has an anti-proliferative activity in ALK-positive ALCL cell lines (7). In patients with lung adenocarcinoma harboring an *EML4-ALK* fusion gene, crizotinib has shown substantial therapeutic efficacy (8, 9). However, in patients with ALK-positive ALCL, crizotinib has not yet been incorporated to standard clinical practice.

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase which belongs to PI3K related kinases family. It is comprised of mTORC1 and mTORC2, and the activity of mTORC1 is regulated by PI3K/AKT and Ras/ERK pathway through TSC complex, which directly regulates mTOR activator Rheb by converting Rheb-GTP (active form) into Rheb-GDP (inactive form) (10, 11). The mTOR signaling pathway is frequently deregulated in many human cancers including anaplastic large cell lymphoma (12-14). Aberrant activated mTOR pathway has been detected in ALK-positive ALCL cell lines and up to 83% of ALK-positive ALCL tumors (12). Activation of this pathway promotes the driving protein synthesis, cap-dependent translation of mRNA, angiogenesis, invasion, metastasis, and proliferation of cancer cells (15, 16). Everolimus (also known as RAD001), an oral rapamycin analog, is an mTOR inhibitor which has been approved for the treatment of patients with advanced renal cell carcinoma or hormone receptor-positive breast cancer (17, 18) .

Recently, in patients with diverse NHL subtypes, several clinical trials of everolimus have been performed and shown clinical activity (19, 20). However, the overall response rate of everolimus single treatment was only around 30% in these patients (21).

In this study, we aimed to evaluate the combined effect of everolimus and crizotinib in ALK-positive ALCL cell lines, Karpas 299 and SU-DHL-1. The biologic mechanism of this combination treatment was also investigated.

MATERIALS AND METHODS

1. Cell lines and culture

ALK-positive ALCL cell line Karpas 299 was obtained from Sigma-Aldrich (St. Louis, MO, USA) and SU-DHL-1 from American Type Culture Collection (Manassas, VA, USA). *EML4-ALK*-positive lung adenocarcinoma cell line NCI-H2228 was obtained from American Type Culture Collection (Manassas, VA, USA). Cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY, USA) and 1% penicillin/streptomycin (Lonza, Walkersville, MD, USA). All cells were incubated at 37℃ under an atmosphere of 5% CO_2.

2. Antibodies and reagents

Antibodies specific for phospho-mTOR (Ser2448), phospho-mTOR (Ser2481), phospho-AKT (Thr308), phospho-AKT (Ser473), phospho-p70S6K (Thr389), and phospho-4E-BP1 (Thr37/46) were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-GAPDH antibody was purchased from Santa Cruz Biotechnology (Dallas, TX,

USA). Anti-PARP antibody was purchased from BD Pharmingen (San Jose, CA, USA). Everolimus and crizotinib were purchased from Selleckchem (Houston, TX, USA), dissolved in dimethyl sulfoxide and stored at -20℃.

3. Cell viability assays

Cell lines were seeded into 96-well plates and then incubated at 37℃ for 72 hours. Cell viability was quantified using the Cell Counting Kit-8 (CCK-8) (Dojindo Molecular Technologies, Kumamoto, Japan). CCK-8 solution 10 μL was added to each well for 2 hours. The absorbance was measured at 450 nm in a microplate reader (Becton Dickinson Labware, Le Pont de Claix, France). All assays were performed 5 times. Data were normalized to the percentage of the control.

4. Western blot analysis

Karpas 299 cells were washed in ice-cold PBS, and then suspended in cell lysis buffer. The mixtures were placed on ice for 1 hour vortexed every 10 minutes. After centrifugation for 30 minutes at 13,000 rpm at

4℃ to remove cellular debris, the supernatant was collected. Protein quantification was performed by using Micro BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). The samples were boiled at 100℃ for 7 minutes, and 10-20 μg of each samples was loaded onto a 6%, 10%, 12%, or 15% polyacrylamide gels. After electrophoresis, gels were electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, Billerica MA, USA). After blocking with 5% skim milk and 5% BSA in Tris-buffered saline with Tween 20 (TBS-T) for 1 hour, the membranes were incubated with primary antibodies overnight at 4℃, followed by incubation with appropriate secondary antibodies for 1 hour the next day. A WEST-ZOL (plus) Western Blot Detection System (Gyeonggi-do, Korea) was used to develop the blot, detected by Medical X-Ray Film Blue from AGFA (Septestraat, Mortsel, Belgium).

5. Cell cycle analysis

The cell-cycle distribution was determined by propidium iodide (PI) staining. Karpas 299 cells were treated with everolimus and/or crizotinib. After 72 hours, cells were collected, washed with phosphate buffered

saline (PBS), fixed in ice-cold 70% ethanol for at least 2 days but less than 1 week. After stained with 42 μg/mL PI (Sigma-Aldrich), 140 ng/mL RNase A (Invitrogen, Carlsbad, CA, USA), and 0.1% bovine serum albumin (BSA)/PBS, the cells were analyzed using the FACSCalibur cytometer (BD Biosciences, San Jose, CA, USA). Cells fell in sub-G1 area were considered apoptotic and were excluded from the whole percentage of the cell cycle analysis.

6. Statistical analysis

Experimental data were expressed as the mean ± standard error. The combination index (CI) was assessed by CalcuSyn software version 2.11 (Biosoft, Cambridge, UK) using the median effect methods described by Ting-Chao Chou and Paul Talalay. A combination index of 1 indicates an additive effect of 2 drugs, less than 1 synergism, and more than 1 antagonism.

RESULTS

1. Both everolimus and crizotinib inhibit the growth of human ALCL cells

The cytotoxic effect of everolimus and crizotinib was evaluated in two ALK-positive ALCL cell lines, Karpas 299 and SU-DHL-1. These cells were treated with increasing dose of everolimus (0.001-1 μM) or crizotinib (0.1-1 μM) for 72 hours and cell viability was assessed using the CCK-8 assay. Both everolimus and crizotinib potently inhibited the cell growth in a dose-dependent manner in the two cell lines (Figure 1). Calculated half maximal inhibitory concentration (IC50) values for everolimus were 3.76 nM in Karpas 299 and 2.69 nM in SU-DHL-1. Calculated IC50 values for crizotinib were 54.1 nM in Karpas 299 and 53.4 nM in SU-DHL-1.

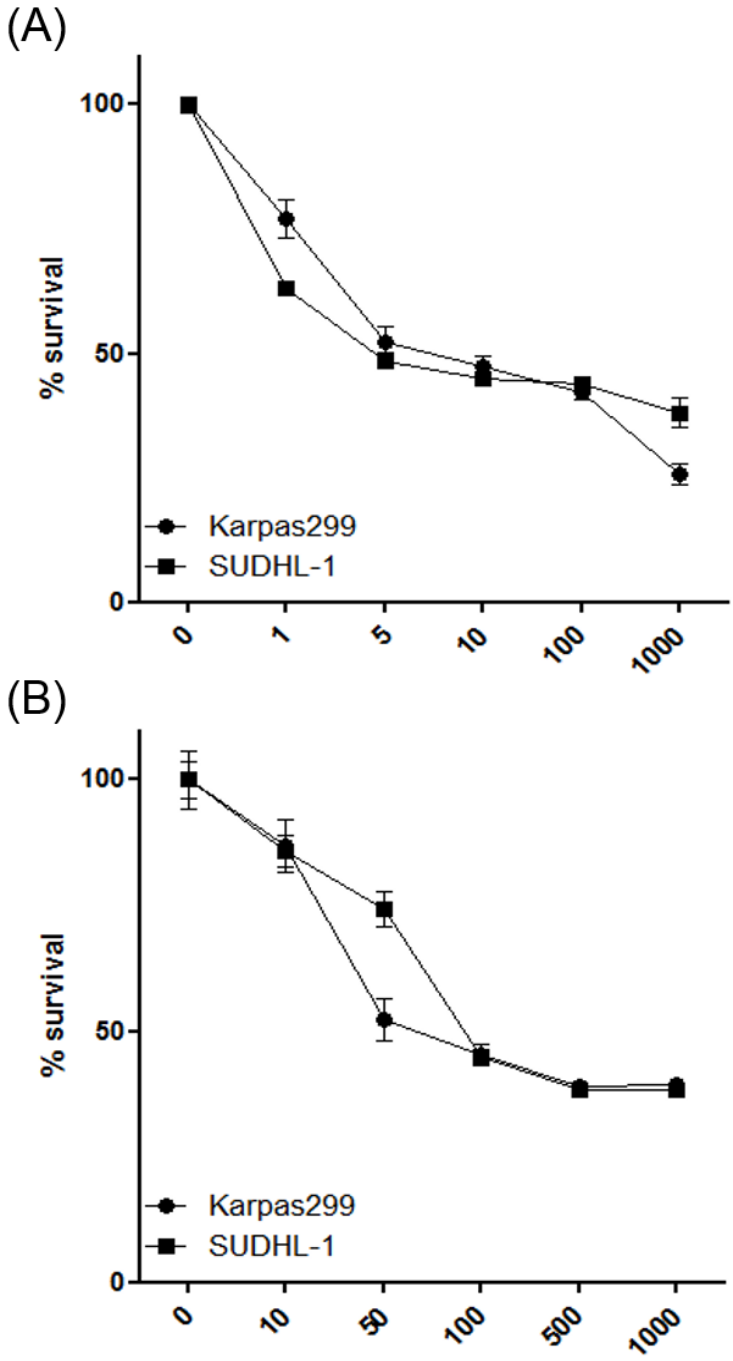

(A)
100
50
0
% survival
Karpas299
SUDHL-1
0
1
5
10
100
1000
(B)
100
50
0
% survival
Karpas299
SUDHL-1
0
10
50
100
500
1000
Concentrations of everolimus and crizotinib (nM)

Figure 1. Cytotoxic effect of everolimus and crizotinib on ALK-positive ALCL cells.

Human ALK-positive ALCL cell lines, Karpas 299 and SU-DHL-1, were treated with various concentrations of everolimus (A) or crizotinib (B) for 72 hours. Both everolimus and crizotinib potently inhibited the cell growth in a dose-dependent manner in the two cell lines.

2. Everolimus combined with crizotinib synergistically inhibits the growth of ALK-positive ALCL cells

The ALK-positive ALCL cell lines, Karpas 299 and SU-DHL-1, were incubated with increasing concentrations of everolimus and/or crizotinib at a fixed ratio of 1:40. As shown in Figure 2, the combination of everolimus and crizotinib was more effective than each agent alone in inhibiting the growth of ALCL cell lines. All CI values were less than 1, indicating synergistic effects that inhibit the growth of ALCL cells.

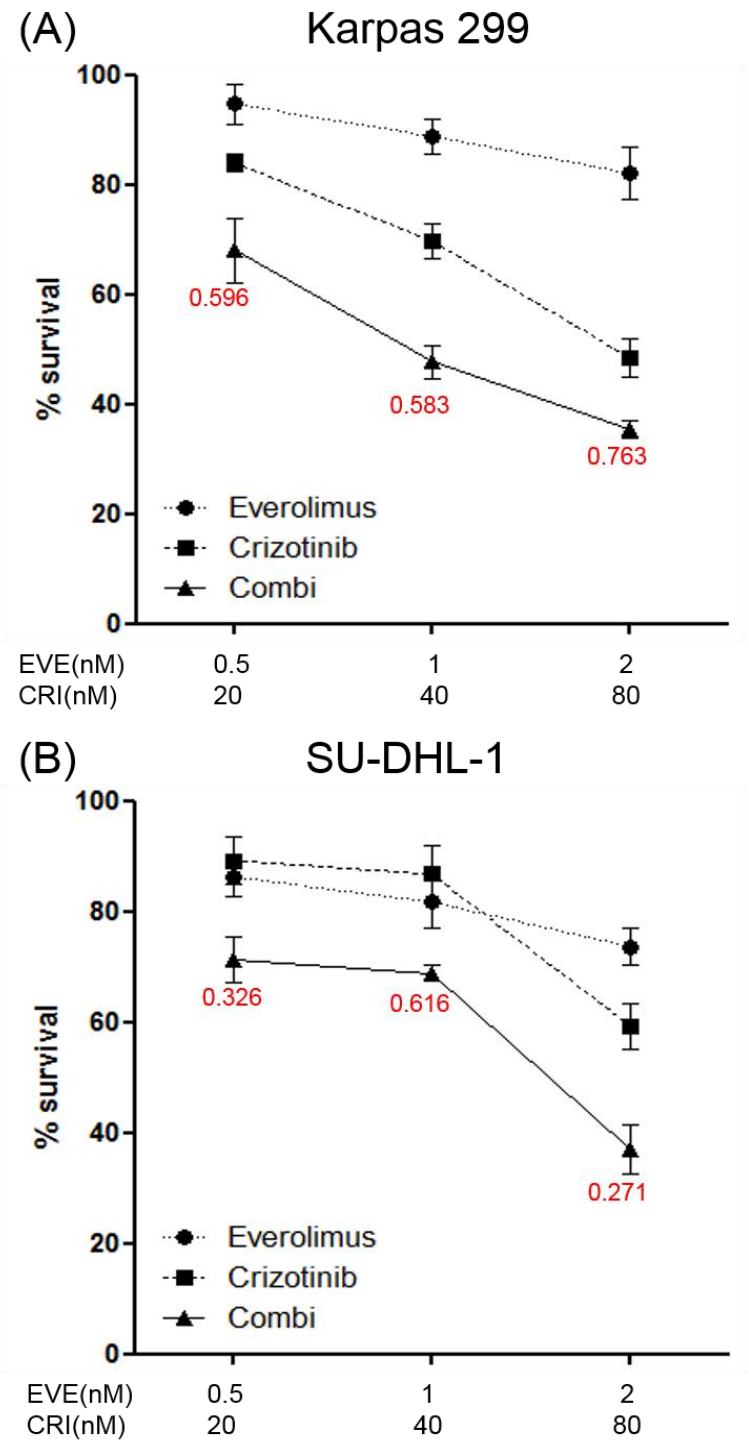

(A)
Karpas 299
100
80
60
40
20
0
% survival
0.596
0.583
0.763
Everolimus
Crizotinib
Combi
EVE(nM) 0.5 1 2
CRI(nM) 20 40 80
(B)
SU-DHL-1
100
80
60
40
20
0
% survival
0.326
0.616
0.271
Everolimus
Crizotinib
Combi
EVE(nM) 0.5 1 2
CRI(nM) 20 40 80

Figure 2. Synergistic cytotoxicity of everolimus and crizotinib in ALK-positive ALCL cells.

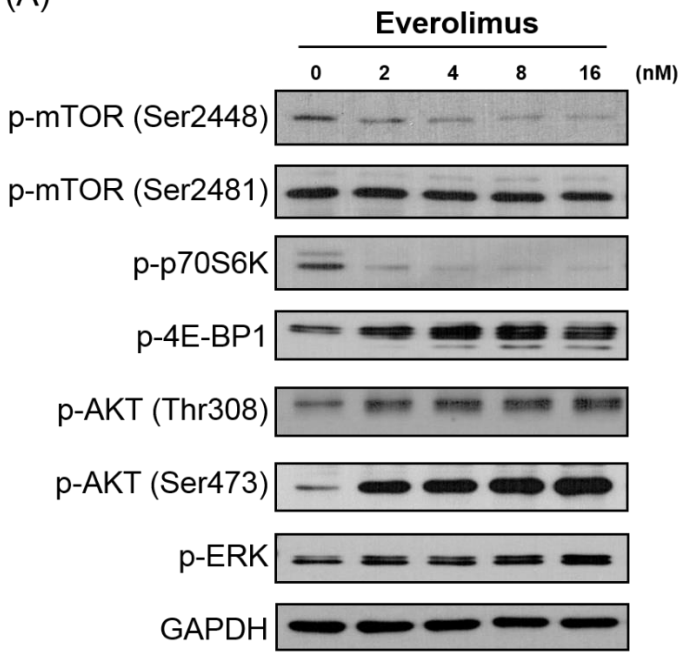
Karpas 299 cells (A) and SU-DHL-1 cells (B) were incubated with increasing concentrations of everolimus and/or crizotinib at a fixed ratio of 1:40. After 72 hours, the cell viability was estimated using the CCK-8 assay and the CI values calculated using CalcuSyn software were shown in the graphs. All CI values were less than 1, indicating synergistic cytotoxic effects of this combination treatment.

3. Everolimus and crizotinib have different effects on signaling pathways

Western blot assays were performed to investigate the effect of everolimus and crizotinib on down-stream signaling pathways in Karpas 299 cells. Previous studies showed that mTORC1 activity could be evaluated by examining the phosphorylation of Ser2448 on mTOR, while mTORC2 activity could be evaluated by the phosphorylation of Ser2481 (22) . Activity of mTORC1 was effectively decreased by an increased dose of everolimus, while the effect of crizotinib on mTORC1 was relatively less potent. On the other hand, mTORC2 activity was potently inhibited by crizotinib, while everolimus could not effectively suppress mTORC2 activity (Figure 3).

Phosphorylation of p70S6K was decreased by both everolimus and crizotinib treatment. In contrast, 4E-BP1 phosphorylation was increased by everolimus or crizotinib single treatments. Everolimus resulted in increased phosphorylation of AKT and ERK in Karpas 299 cells.

(A)


Everolimus
0
2
4
8
16
(nM)
p-mTOR (Ser2448)
p-mTOR (Ser2481)
p-p70S6K
p-4E-BP1
p-AKT (Thr308)
p-AKT (Ser473)
p-ERK
GAPDH

(B)

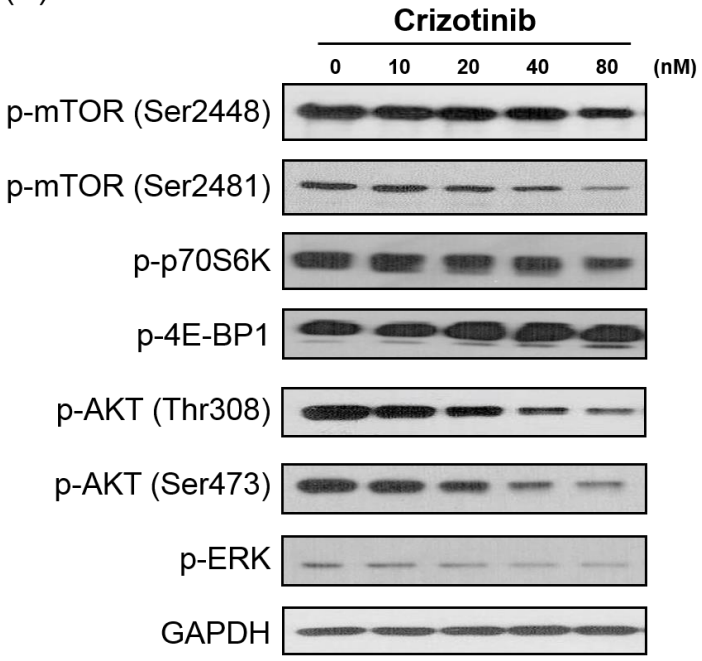

Crizotinib
0
10
20
40
80
(nM)
p-mTOR (Ser2448)
p-mTOR (Ser2481)
p-p70S6K
p-4E-BP1
p-AKT (Thr308)
p-AKT (Ser473)
p-ERK
GAPDH

Figure 3. Effects of everolimus and crizotinib on signaling pathways.

Karpas 299 cells were treated with 0 to 16 nM of everolimus (A) or with 0 to 80 nM of crizotinib (B) for 24 hours. Everolimus mainly inhibited mTORC1 activity, while crizotinib inhibited mTORC2 activity. Phosphorylation of p70S6K was decreased, while 4E-BP1 phosphorylation was increased in both cases. Everolimus increased ERK and AKT phosphorylation, while crizotinib decreased ERK and AKT phosphorylation.

4. Crizotinib down-regulates everolimus-induced ERK and AKT phosphorylation and strengthens mTOR inhibition

Combined effect of everolimus and crizotinib on signaling pathways was evaluated using western blot analysis. Karpas 299 cells were treated with 4 nM everolimus and/or 80 nM crizotinib for 24 hours. The combination treatment suppressed ERK and AKT phosphorylation caused by everolimus in Karpas 299 cells (Figure 4). Combination treatment with everolimus and crizotinib also decreased phosphorylation of mTOR (Ser2448), mTOR (Ser2481), and p70S6K compared to single treatments. Decreased phosphorylation of mTOR (Ser2481) indicated that everolimus combined with crizotinib inhibited mTORC2 as well as mTORC1 activity. Although the combination of everolimus and crizotinib reduced activity of mTORC1, p70S6K, and mTORC2, phosphorylation of 4E-BP1, another substrate of mTORC1, was not decreased by the combination treatment compared to the control group. Considering the effect of single treatments in Figure 3, we had hypothesized that the combination treatment could increase 4E-BP1 phosphorylation. However, the combination of everolimus and crizotinib appeared to slightly decrease 4E-BP1 phosphorylation compared to single treatments.

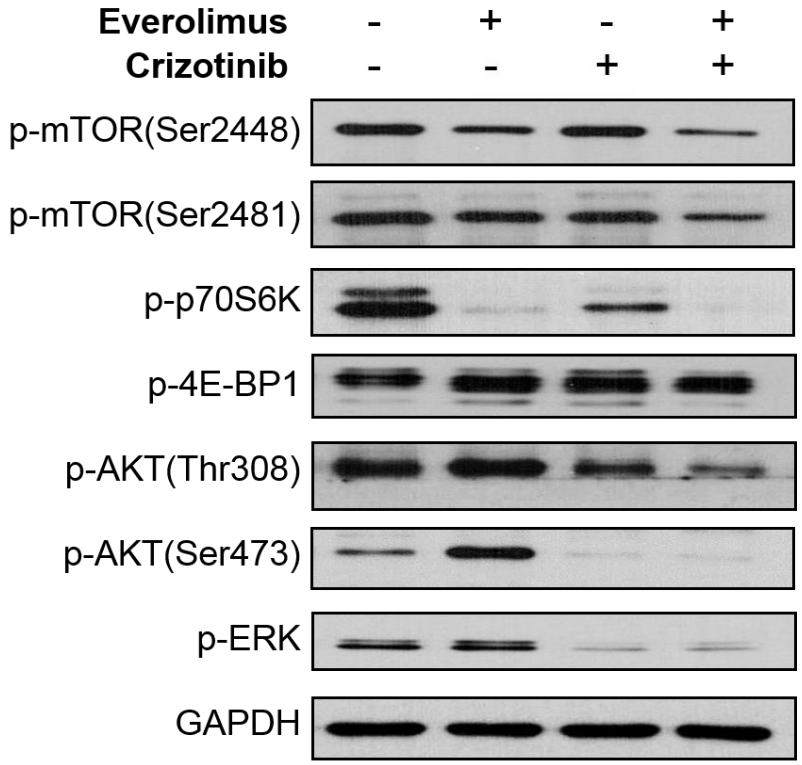


Figure 4. Effective inhibition of signaling pathways using everolimus plus crizotinib combination treatment in Karpas 299 cells.

Karpas 299 cells were treated with 4 nM everolimus and/or 80 nM crizotinib for 24 hours. The combination treatment more potently inhibited PI3K/AKT, Ras/ERK, and mTOR signaling pathways compared to single treatments.

5. Combination of everolimus and crizotinib enhances apoptosis and induces cell cycle arrest

To further evaluate the effect of crizotinib and everolimus combination on cell cycle and apoptosis, Karpas 299 cells were treated with 4nM everolimus and/or 80nM crizotinib for 72 hour, and then flow cytometry by PI staining was performed. As shown in Figure 5, the everolimus single treatment did not increase the fraction of cells in the sub-G1 region compared to the control (4.03% vs. 2.16%). In contrast, the fraction of cells in sub-G1 area was increased after crizotinib treatment compared to the control (11.88% vs. 2.16%). In addition, the combination of crizotinib and everolimus markedly increased the sub-G1 population (22.25%), which suggests increased apoptosis of the combination treatment. Crizotinib as a single agent significantly increased cell-cycle arrest in G0/G1 phase, while the effect of everolimus on cell cycle was not apparent. PARP cleavage was also increased after the combination treatment, suggesting increased apoptosis.

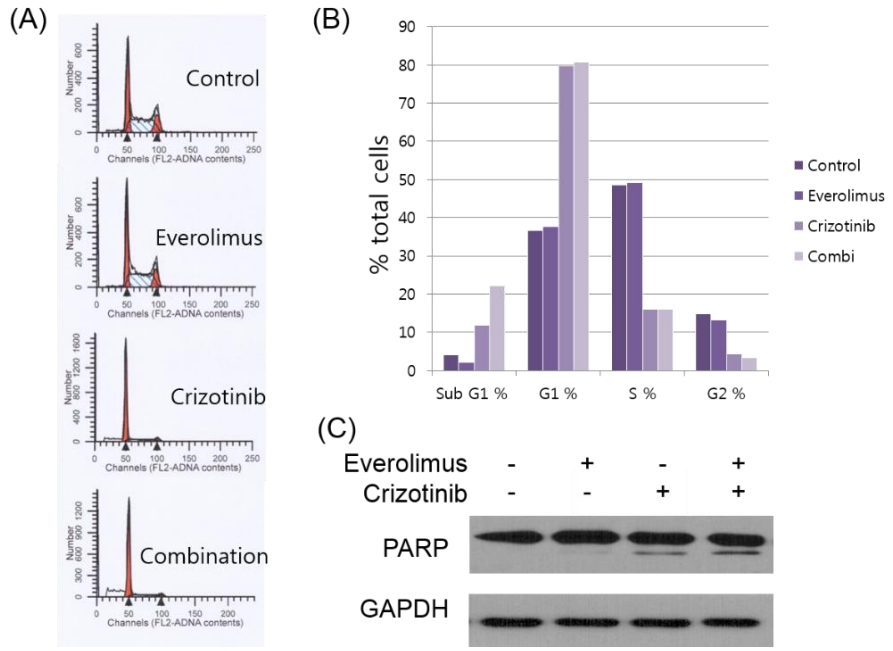


Figure 5. Cell cycle arrest and apoptosis induction by everolimus plus crizotinib combination treatment in Karpas 299 cells.

Karpas 299 cells were treated with 4 nM everolimus or/and 80 nM crizotinib for 72 hours and cell cycle distribution was assessed by flow cytometry (A). The cells were gated to sub-G1, G0/G1, S, and G2 phases according to their DNA contents (B). The combination of crizotinib and everolimus markedly increased the sub-G1 population compared to single treatments. In addition, crizotinib increased cell-cycle arrest in G0/G1 phase. PARP cleavage was increased after the combination treatment (C).

6. Everolimus combined with crizotinib synergistically inhibits the growth of ALK-positive lung adenocarcinoma cell line, NCI-H2228

On the basis of the synergistic effect of everolimus and crizotinib observed in ALK-positive ALCL cell lines, it was also speculated that this combination treatment would be synergistic in ALK-positive lung cancer cells. ALK-positive lung cancer cell line NCI-H2228 was treated with everolimus and crizotinib at a fixed ratio of 1:80 for 72 hours, and then cell viability was determined by CCK-8 assays. As expected, crizotinib combined with everolimus exhibited synergistic cytotoxicity (Figure 6).

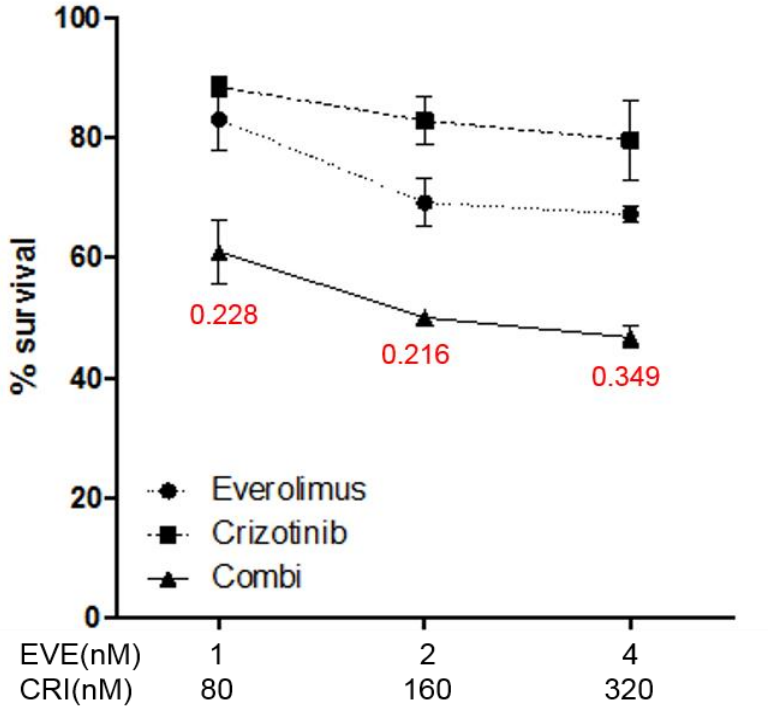


Figure 6. Synergistic cytotoxicity of everolimus and crizotinib in ALK-positive lung cancer cell line NCI-H2228.

NCI-H2228 cells were treated with everolimus and crizotinib at a fixed ratio of 1:80 for 72 hours. CI values calculated using CalcuSyn software were shown in the graphs. In all combinations, the CI values were less than 1, indicating synergistic cytotoxic effects of this combination treatment in ALK-positive lung adenocarcinoma cells.

DISCUSSION

The mTOR pathway is frequently deregulated in ALK-positive ALCL, indicating that mTOR could be a therapeutic target of ALK-positive ALCL (12, 23). However, the clinical efficacy of mTOR inhibitor monotherapy has not yet yield satisfactory results in patients with relapsed or refractory NHL (21), possibly due to the resistance mechanisms which activate several pro-surviving signaling pathways in response to mTOR inhibitors.

Previous reports showed that mTORC1 inhibition using rapalogs leads to up-regulation of ERK and AKT by several different mechanisms (24-27). The p70S6K, one of the major substrates of mTORC1, regulates the pathway via feedback mechanism by inhibiting PI3K through IRS1 down-regulation. Thus, mTORC1 inhibition using rapalogs, which down-regulates p70S6K, leads to PI3K activation (24). The p70S6K also inhibits mTORC2 through phosphorylation of Rictor on Thr1135 (25). Therefore, p70S6K down-regulation by rapalogs allows mTORC2 activity, leading to AKT phosphorylation on Ser473. In addition, mTOR inhibition increases PDGFR expression, leading to further AKT phosphorylation on Ser473 (26). ERK activation is also triggered by

mTORC1 inhibition through a p70S6K-IRS-Ras pathway in cancer patients as well as *in vitro* experiments and a preclinical model of prostate cancer (27). In our study, we observed that these resistance mechanisms to mTOR inhibitor were also found in ALK-positive ALCL cells (Figure 3). Recent studies revealed that simultaneous inhibition of PI3K/AKT and Ras/ERK pathways synergistically enhances the cytotoxic effect in a variety of malignancies, including lung and breast cancer, and rhabdomyosarcoma (27-29).

In ALK-positive ALCL cells, the aberrant *NPM-ALK* fusion gene activates Ras/ERK, STATs, and PI3K/AKT signaling pathways. Crizotinib effectively inhibits phosphorylation of ALK, AKT, ERK, PLC-γ, and STAT3, both *in vitro* and *in vivo* (30). A previous report showed that two ALK-positive ALCL patients could achieve rapid complete remission after crizotinib treatment although they had previously relapsed after CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy (31). Another clinical study of crizotinib monotherapy in patients with relapsed or refractory ALK-positive NHL exhibited the overall response rate of 91% (10 of 11) with 2-year overall and progression-free survival rates of 72.7% and 63.7%, respectively (32). However, a substantial portion of patients eventually

experienced relapse or progression despite crizotinib treatment. Therefore, we hypothesized that, rather than as monotherapy, crizotinib can be used more effectively as a part of a combination therapy with other chemotherapeutic agents. In this paper, we demonstrate that crizotinib used in conjunction with everolimus can create synergistic cytotoxicity in ALK-positive ALCL cells by suppressing aberrant ERK and AKT phosphorylation induced by everolimus.

As shown in Figure 7, the synergistic anti-proliferative effect of this novel combination suggested that ALK inhibition could help overcome everolimus resistance in ALK-positive ALCL cells by inhibiting down-stream ERK and AKT signaling pathways. In addition, the effect of everolimus on mTORC1 and p70S6K down-regulation was strengthened by the addition of crizotinib. In particular, mTORC2, which is not a target of everolimus was also down-regulated by crizotinib. However, 4E-BP1 phosphorylation was rather increased by everolimus or crizotinib single treatments. A previous study showed that rapamycin could induce 4E-BP1 phosphorylation within 6 hours despite initial inhibition within 3 hours (33). Crizotinib can also induce 4E-BP1 phosphorylation in ALK-positive lung cancer cells although the precise mechanism remains unknown (34). Nevertheless, the combination of everolimus and

crizotinib appears to slightly decrease 4E-BP1 phosphorylation compared to single treatments in our study (Figure 4). Thus, it is speculated that everolimus and crizotinib may have antagonistic effect on 4E-BP1 phosphorylation. In addition, rapamycin-induced 4E-BP1 phosphorylation could be abrogated by BKM120 (a PI3K inhibitor) or BEZ235 (a PI3K/mTOR dual kinase inhibitor) in lung cancer (35, 36) and bladder cancer (37) cells. Therefore, these novel drugs could be used to overcome the resistance of everolimus and crizotinib combination therapy.

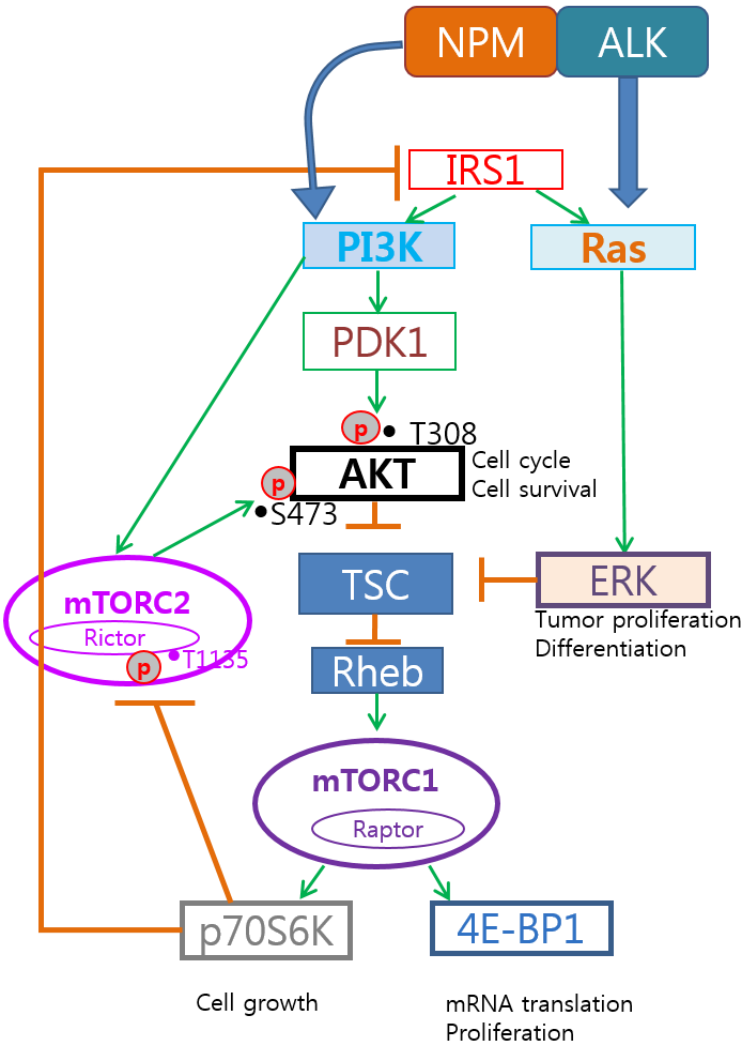
Everolimus and crizotinib have been known to induce G1 arrest in ALK-positive ALCL cells (30, 38). In accordance with these previous reports, crizotinib treatment showed G1 arrest in our study (Figure 5). However, G1 arrest was not apparent after everolimus treatment, possibly due to a much lower concentration of everolimus in this study compared to the previous study (4 nM vs. 10 nM) (38). The combination of everolimus and crizotinib resulted in G1 arrest, and the proportion of S and G2 population was decreased. Notably, the addition everolimus to crizotinib markedly increased Sub-G1 population, which reflects the proportion of apoptotic cells, compared to everolimus or crizotinib monotherapy. This phenomenon was in accordance with the increased

PARP cleavage after the combination treatment.

Several *ALK*-fusion genes have been identified in a variety of cancers, such as lung adenocarcinoma, inflammatory myofibroblastic tumor, and renal cell carcinoma (39). Therefore, it is speculated that our finding could be applied to other cancers harboring *ALK*-fusion genes. We tested this hypothesis in ALK-positive lung adenocarcinoma cell line NCI-H2228. As shown in Figure 6, the combination of everolimus and crizotinib showed synergistic cytotoxic effect in NCI-H2228 cells. This result suggest this combination might be an effective treatment in other cancers harboring *ALK*-fusion genes.

In summary, the combination of everolimus and crizotinib synergistically inhibits the growth of ALK-positive ALCL cells. Crizotinib inhibited aberrant ERK and AKT phosphorylation induced by everolimus, and more effectively suppressed the activity of mTOR than everolimus monotherapy. Our study may provide an evidence of further preclinical studies and clinical trials using everolimus and crizotinib combination in ALK-positive ALCL. We suggest that this novel combination can be used to improve therapeutic outcome in patients with ALK-positive ALCL.

(A)


NPM
ALK
IRS1
PI3K
Ras
PDK1
T308
AKT
Cell cycle
Cell survival
S473
TSC
ERK
Tumor proliferation
Differentiation
mTORC2
Rictor
T1135
Rheb
mTORC1
Raptor
p70S6K
4E-BP1
Cell growth
mRNA translation
Proliferation

(B)

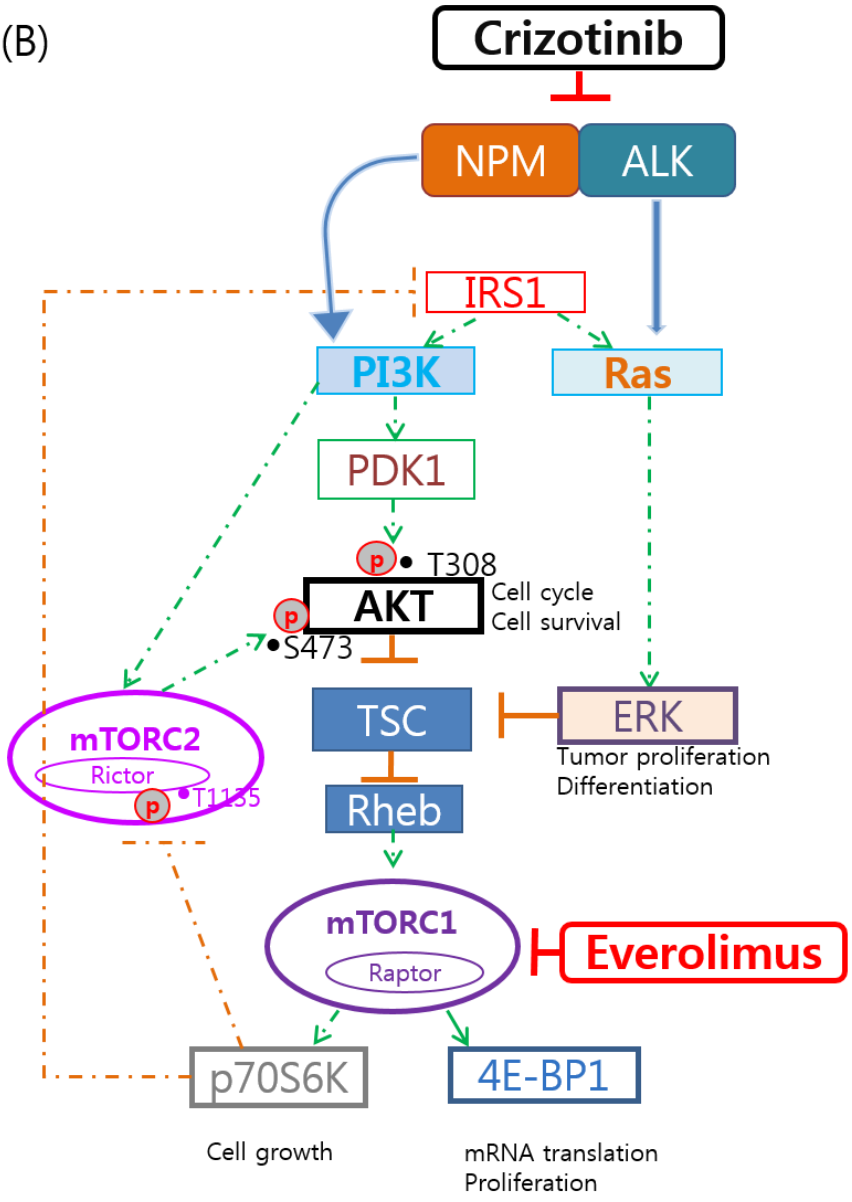

Crizotinib
NPM
ALK
IRS1
PI3K
Ras
PDK1
p
T308
AKT
Cell cycle
Cell survival
p
S473
mTORC2
Rictor
p
T1135
TSC
ERK
Tumor proliferation
Differentiation
Rheb
mTORC1
Raptor
Everolimus
p70S6K
4E-BP1
Cell growth
mRNA translation
Proliferation

Figure 7. Schematic diagram of signaling pathways in ALK-positive ALCL cells.

PI3K/AKT and Ras/ERK pathways are activated by NPM-ALK fusion protein in ALK-positive ALCL cells (A). Combination treatment of crizotinib and everolimus abrogates ERK and AKT up-regulation caused by everolimus (B).

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국문 초록

역형성 대세포 림프종은 비호지킨림프종의 한 종류로서 반수 이상의 환자에서 NPM-ALK 융합 유전자를 발현한다. ALK 억제제인 crizotinib 와 mTOR 억제제인 everolimus는 NPM-ALK 융합 유전자를 가진 역형성 대세포 림프종 세포주에 효과적인 항암 작용을 나타낼 것으로 기대된다. 그러나, 아직 이 두가지 약제의 병합요법의 효과에 대한 연구는 없다. 본 연구는 ALK 양성 역형성 대세포 림프종 세포주인 Karpas 299, SU-DHL-1를 사용하여 everolimus와 crizotinib 의 병합요법의 효과를 평가하였다. 또한, 다양한 실험을 통하여 본 병합요법의 작용 기전을 증명하였다. ALK 양성 역형성 대세포 림프종 세포에서 everolimus와 crizotinib 병합요법은 단일 요법에 비하여 세포 독성에대한 상승작용(synergism)을 나타내었다. 그리고, crizotinib은 everolimus에 의해 유도된 ERK와 AKT의 인산화를 효과적으로 억제하였다. 또한, 본 병합요법은 ALK 양성 역형성 대세포 림프종 세포에서 G1 세포주기정지 및

세포자멸사를 유도하였다. 본 병합요법에 의한 세포 독성 상승작용는 EML4-ALK 융합 유전자를 가진 비소세포폐암 세포주 NCI-H2228에서도 동일하게 관찰되었다. 요약하면, ALK 양성 역형성 대세포 림프종 세포에서 everolimus와 crizotinib 병합요법은 ALK와 mTOR의 인산화를 동시에 억제함으로써, everolimus에 의한 ERK와 AKT 활성화를 차단하여 세포 독성 효과를 향상시켰다. 본 연구결과를 토대로 향후 ALK 양성 역형성 대세포 림프종 환자의 치료 성적을 향상시키는 데 도움을 줄 수 있을 것이다.

주요어 : mTOR, Everolimus, Crizotinib, 역형성 대세포 림프종, Anaplastic lymphoma kinase

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