이학석사 학위논문

ALK와 mTOR 억제제인 alectinib와 everolimus 복합요법의 미분화 대세포 림프종 세포에 대한 증식억제 상승효과 분석

Synergistic effect of alectinib and everolimus on anaplastic large cell lymphoma cells via ALK−mTOR pathway inhibition

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서울대학교 대학원

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이 논문을 이학석사 학위논문으로 제출함

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김동찬의 이학석사 학위논문을 인준함

2018년 08월

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Synergistic effect of alectinib and everolimus on anaplastic large cell lymphoma cells via ALK-mTOR pathway inhibition

By

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A Thesis Submitted to the Department of Cancer Biology in partial fulfillment of the requirements for the Degree of Master of Science in Cancer Biology at Seoul National University College of Medicine

Aug 2018

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Abstract

Synergistic effect of alectinib and everolimus on anaplastic large cell lymphoma cells via ALK-mTOR pathway inhibition

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The purpose of this study was to investigate the synergistic effect of combination treatment of two drugs on ALK-positive cancer cells. The combination treat resulted in synergistic effect in Karpas299 cells but not in SU-DHL-1 cells. Viability of Karpas299 cell was decreased to 26.4% ($p<0.001$) by combination treatment of alectinib and everolimus compared with the single drug treated groups (alectinib: 59.5%, everolimus: 51.6%), and the value of Combination Index (CI) indicating synergism was 0.316. Moreover, combination treatment in Karpas299 augmented ALK-mTOR de-phosphorylation,

and cleavage of poly ADP ribose polymerase (PARP) molecules. Combination treatment significantly increased the numbers of Karpas299 cells arrested at subG1 (everolimus: p= 0.0096), and G0/G1 (alectinib: p= 0.0018; everolimus: 0.0013). We further investigated the biological differences between Karpas299 and SU-DHL-1, the expression pattern of ALK isoforms (ALK-201 and 203) was different between the two cell lines. Overall, Combination treatment with alectinib and everolimus synergistically reduced cell survival, augmented inhibition of mTOR signaling, and increased cell cycle arrest in Karpas299 but not in SU-DHL1 cells. This set of results can suggest that the possibility of a novel combination treatment for ALK-positive lymphoma patients. It also provides an approach to developing new therapies.

Keywords: Anaplastic large cell lymphoma (ALCL), Anaplastic lymphoma kinase (ALK), Alectinib, TOR Serine-Threonine Kinases, mTOR, Everolimus, Tyrosine Kinase Inhibitors (TKI), Combination treatment, Drug synergism

Student Number: 2016-25164

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Introduction

Anaplastic Lymphoma Kinase (ALK) is an oncogene found in various cancers including lymphoma, lung cancer, and leukemia.[1] ALK is not expressed in most normal cells, but is overexpressed in cancer cells due to various underlying genetic aberrations.[2] Anaplastic large cell lymphoma (ALCL) is dependent on constitutive ALK signaling resulting from the chromosomal translocation t(2;5)(p23;q35) leading to NPM/ALK fusion.[3,4] In non-small cell lung cancer (NSCLC), ALK can fuse with EML4, KIF5B, EIF2AK, PPM1B, or PRKAR1A.[5]

With the development of specific inhibitors, ALK has become even more important clinically. The first generation inhibitor crizotinib showed dramatic success in lung cancer and lymphoma with response rates reaching 65% in lung cancer[6] and 90% in lymphoma.[6] However, primary and secondary resistance to crizotinib has been discovered[7] and next generation ALK inhibitors are being developed.[8] Although more potent in terms of ALK inhibition, even second and third generation ALK

inhibitors including alectinib,[8] ceritinib,[9] and loratinib[10] cannot completely control ALK-driven cancers. Hence, optimization of drug strategies is necessary to inhibit ALK oncogenesis

The activation of ALK fusion proteins and downstream signaling molecules results in various abnormalities in cell function.[11] The ALK signaling pathway includes phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) of MAP kinase signaling (MAPK), phospholipase Cγ (PLCγ), Janus kinase-signal transducer and activator of transcription (JAK-STAT), and mammalian target of rapamycin (mTOR). Hence, these pathways are emerging as therapeutic targets for ALK-driven cancers.[12-14] Among the molecules in these pathways, mTOR is a crucial oncogenic protein. Ras-ERK signaling involves mTOR, and other signals involve PI3K-Akt signaling, which is directly associated with mTOR.[15] mTOR regulates ribosomal protein S6 kinase (p70S6K) and S6 ribosomal protein (S6RP) and inactivates the eukaryotic initiation factor 4E-binding protein 1(EIF4EBP1). As a result,

mTOR regulates the generation of ribosomes, protein synthesis, and cancer cell survival.[4,14,16]

Summarizing the above, we hypothesized that the simultaneous regulation of ALK and the ALK-mediated mTOR pathway might result in synergism and could indicate a novel therapeutic strategy in ALK-driven cancer.[14] In fact, similar strategies already have been reported as successful against cancers associated with other oncogenic mutations in EGFR,[17] and MET,[18] which provided the biological rationale for our hypothesis. In this study, we investigated whether the combination of the second generation ALK inhibitor alectinib and mTOR inhibitor everolimus was synergistic in ALK-positive ALCL cell lines.

Material and Method

Cell lines and culture

We used four cell lines in this study. The NPM-ALK-positive ALCL cell lines used were Karpas299 (Sigma-Aldrich, St. Louis, MO, USA) and SU-DHL-1 (ATCC, Manassas, VA, USA); the EML4-ALK-positive NSCLC cell line was NCI-H2228 (ATCC); and the aggressive T-cell lymphoma cell line Jurkat was used as an ALK-negative control (DSMZ, ACC-282, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). Karpas299 cells were cultured in RPMI-1640 medium supplemented with 20% inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (GIBCO, Grand Island, NY, USA). The remaining cell lines were cultured in RPMI-1640, 10% FBS, and 1% penicillin/streptomycin. All cell lines were incubated at 37° C in a 5% CO2 incubator. NCI-H2228 cells were washed with DPBS and detached from the culture vessel using 0.25% trypsin-EDTA (GIBCO).

Reagents and antibodies

The ALK inhibitors crizotinib (PF-02341066) and alectinib (CH542480), and the mTOR inhibitor everolimus (RAD001) were purchased from Selleck Chemicals (Houston, TX, USA). Antibodies used in immunoblot were: phospho-ALK (Tyr1282/Tyr1283), phospho-AKT (Ser473, Thr308), AKT, phospho-mTOR (Ser2481), phospho-mTOR (Ser2448), mTOR, phospho-p70S6K (Thr389), p70S6K, phospho-4E-BP1 (Thr37/46), and 4E-BP1 (Cell Signaling Technology (Beverly, MA, USA). The cleaved Poly ADP ribose polymerase (PARP) antibody was purchased from BD Pharmingen (San Jose, CA, USA). GAPDH (sc-25778), and horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabbit antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell viability assay

ALK-positive ALCLs and NSCLC cells were used at 24 hours after subculture. The NSCLC cells (NCI-H2228)

were washed once with DPBS and detached from the culture vessel. Harvested cells were counted and seeded into 96-well plates and then incubated at 37° C for 24 hours. All cell lines were treated individually with the indicated concentrations (0, 10, 100, 1000 nM) of crizotinib, alectinib, and everolimus for 24, 48, 72, 96, 120 hours. Karpas299 cells were treated with alectinib and everolimus mixed at fixed ratios and incubated for 72 hours. DMSO was used as the vehicle control for all experiments. After incubation, cell viability was assessed using a CCK-8 kit (Dojindo Molecular Technologies, Kumamoto, Japan). The CCK-8 solution (10 μl) was added to each well and incubated for 2 hours. Optical density (O.D.) was measured at 450 nm using a microplate reader (Becton Dickinson Labware, Le Pont de Claix, France).

Western blot analysis

The ALK-positive ALCL cell line, Karpas299, was used at 24 hours after subculture. Cells were harvested and

seeded into 6-well plates and then incubated at 37° C for 24 hours. In single treat experiment, Karpas299 cells were treated with crizotinib, alectinib, or everolimus in a dose escalation manner. For combination treatment, alectinib and everolimus were mixed at a fixed ratio (alectinib: everolimus = 5:1, based on result of the cell viability assay). DMSO was used as the control for all experiments. After 5 hours of incubation, cells were lysed in lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 0.5 mM Na3VO4, 50 mM NaF, 1 mM PMSF, and protease inhibitor cocktail [Thermo Scientific, Rockford, IL, USA]) for 1 hour on ice. Cell debris was removed by centrifugation at 16000xg for 15 min at 4° C. The protein concentration in cell lysates was determined using the Micro BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). An equal amount of protein for each sample was separated by 6% or 8% SDS-polyacrylamide gel electrophoresis (PAGE) then transferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA). After blocking with 5% skim milk for 1 hour in TBS-T (0.1% Tween 20 in Tris-buffered

saline [TBS]), membranes were incubated with primary antibodies and then with the respective HRP-conjugated secondary antibodies: Peroxidase-conjugated Affinipure Goat Anti-Rabbit IgG (111-035-003), Anti-Mouse IgG (115-035-003) (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Blots were developed with A WEST-ZOL (plus) Western Blot Detection System (iNtRON Biotechnology, Gyeonggi-do, Korea) and signals were detected with X-Ray Film Blue from AGFA (Septestraat, Mortsel, Belgium).

Cell cycle arrest assay

To determine cell cycle distribution, samples were stained with propidium iodide (PI; BD Pharmingen, San Jose, CA, USA) and analyzed by flow cytometry. Karpas299 cells were harvested at 24 hours after subculture. Cells were seeded into 6-well plates and then incubated at 37° C for 24 hours. Cells were treated with alectinib in the absence or presence of everolimus; DMSO was used as the control for all experiments. After

72 hours, cells were collected, washed with DPBS, and fixed with 70% ethanol. Fixed cells were incubated with DPBS containing 42 μg/ml PI, 140 ng/ml RNase (Invitrogen, Carlsbad, CA, USA), and 0.1% of bovine serum albumin (MP Biomedicals, Irvine, CA, USA). Stained cells were analyzed using the FACS Canto II (BD Biosciences, San Jose, CA, USA)

RNA sequencing data analysis

We obtained publicly available RNA sequence data, the Sequence Read Archive (SRA), from The National Center for Biotechnology Information and aligned the genome with the Spliced Transcripts Alignment to a Reference (STAR) tool.[19] Transcript quantification was performed using an analysis tool, RNA-Seq by Expectation-Maximization (RSEM), to compare protein expression levels. Expression of ALK and its isoforms was analyzed and compared.

Statistical analysis

All data were analyzed using GraphPad Prism v.5.00 (GraphPad Software Inc., San Diego, CA, USA). The Combination Index (CI) was assessed using CalcuSyn software version 2.11 (Biosoft, Cambridge, UK) using the median-effect methods described by Chou and Talalay.[20] The CI indicates whether the combinative effect of two drugs is synergistic (CI < 1), additive (CI = 1), or antagonistic (CI > 1) compared to treatment with the individual drugs. All of quantification data are presented as means ±SD. Student's t-test (unpaired, two tailed) and analysis of variance (ANOVA, single factor) were employed for binary and multiple comparisons, respectively. Statistical significance was defined as a $p < 0.05$.

Result

ALK inhibitors selectively reduced survival of ALK-positive cancer cell lines.

We evaluated the susceptibility of ALK-positive cell lines to ALK or mTOR inhibitors. To determine whether ALK inhibitors were more effective in ALK-positive cells compared to ALK-negative T-cell lymphoma cells, the Jurkat cell line was used as a negative control. Cells were treated with an ALK inhibitor (crizotinib, alectinib), or mTOR inhibitor (everolimus). These three inhibitors were used at increasing concentrations and for various durations to determine optimal treatment conditions. As shown as Figure 1A, ALK+/- cells were treated with alectinib at the indicated concentrations and survival of ALK-positive cells was significantly decreased to 28.0%, 31.6%, 44.8% 103.2% for Karpas299, SU-DHL-1, NCI-H2228 and Jurkat cells, respectively ($p<0.001$) compared to DMSO control. However, at 1000 nM alectinib, survival of ALK+/- cell lines was decreased to the same level. In Figure 1B, the effect of the other ALK inhibitor,

crizotinib, was tested against the cell lines. Although crizotinib was slightly less effective than alectinib in reducing viability of Karpas299 (34.4%), SU-DHL-1 (37.7%), and NCI-H2228 (53.6%), Jurkat (96.62%) cell lines ($p<0.001$), crizotinib also showed ALK-selective cytotoxicity in ALK-positive cell lines. In Figure 1C, all cell lines were treated with everolimus under the same conditions as the previous experiment. Survival of Karpas299, SU-DHL-1, NCI-H2228, and Jurkat cells started to decrease at 10 nM. At 100 nM, everolimus inhibited ALK-positive and -negative cell survival. These results indicated that ALK inhibitors had selective toxicity toward ALK-positive cells, and the mTOR inhibitor everolimus had no ALK-dependent toxicity.

Alectinib and everolimus inhibited oncogenic signaling in ALK-positive ALCL cells.

Each of these inhibitors was designed to inhibit a different target. Thus, we confirmed whether those target molecules were specifically inhibited by their

target inhibitor. The Karpas299 cell line was used as a representative cell line because it showed the strongest inhibitor susceptibility. For this experiment, the concentration of alectinib ranged from 0 to 100 nM, and everolimus ranged from 0 to 10 nM. In Figure 2A, at an early time point of treatment, phosphorylation of ALK was completely inhibited by alectinib in a dose-dependent manner. Phosphorylation of mTOR and its downstream molecules (p70S6K, 4EBP1) was slightly decreased by alectinib (left panel). However, phosphorylation and de-phosphorylation of Akt was irregularly increased and decreased. Everolimus never influenced the phosphorylation of ALK. However, mTOR, p70S6K, and 4EBP1 were completely blocked by everolimus even at a low concentration. Akt phosphorylation was induced consistently (right panel). In Figure 2B, at a late time point, phosphorylation of ALK was suppressed by alectinib (left panel) and ALK-related mTOR phosphorylation was weakly inhibited, but the inhibition of Akt phosphorylation was still in flux. This result indicated that ALK inhibition by alectinib did

not completely suppress ALK-dependent mTOR expression. Thus, we hypothesized that adding an mTOR inhibitor to alectinib might have a synergistic effect on inhibition of ALK signaling through mTOR signaling.

Synergistic activity of alectinib and everolimus treatment occurred at specific ratios.

We investigated whether alectinib had a synergistic effect when combined with everolimus for treatment of ALK-positive ALCL lines. The two inhibitors were mixed in various ratios and added to ALK-positive ALCL cells; cell survival was assessed by cytotoxicity assay and CI values were calculated using the equation of Chou and Talalay20. The three CI values for reference are CI < 1, = 1, and > 1 to indicate synergism, additive effect, and antagonism (Supplementary 1), respectively. Karpas299 and SU-DHL-1 cells were treated and viability was assessed; CI values, dose-effect curves, median-effect plots, and Fa-CI plots were calculated and drawn (Supplementary 2). The combination of two drugs

appeared to be mostly synergistic in the Karpas299 cell line, whereas synergism occurred in the SU-DHL-1 cell line only at a ratio of 5:1 (Table 1). The results were also analyzed by heatmap on R to confirm the discrepancy in drug susceptibility between Karpas299 and SU-DHL-1 (Figure 3). These results suggested that true synergism between the two drugs was observed only in Karpas299 cells, even at a low dose of alectinib (below 50 nM).

Alectinib combined with everolimus had a synergistic effect on inhibition of cell proliferation.

Based on the CI results of the various ratios, the best ratio was chosen for additional studies. We confirmed the ratios at low enough concentrations to exclude any toxicity associated with alectinib. Moreover, by referring to "clinical meaning" used for patients with ALK-driven cancer, the alectinib concentration must be minimized to be comparable to the Cmax values of alectinib in clinical trials.[21] ALK-positive ALCL cells

were treated with alectinib in the absence or presence of everolimus at a 5:1 (alectinib: everolimus) ratio using the same treatment and incubation conditions as in the above experiment. In Figure 4A, the alectinib 10 nM–everolimus 2 nM group showed the greatest synergistic effect. The combination of both inhibitors effectively reduced cell viability to 26.4% (compared to alectinib alone, decreased to 42.7%, compared to everolimus alone, 49.2%, $p<0.001$), respectively. Moreover, the CI value of the combination was 0.316, which was below the 0.85 value that indicates strong synergism (Figure 4B). However, SU–DHL–1 cells were not only less sensitive to each drug alone, but they also were not sensitive to the combination treatment (Figure 4C, 4D). The results of statistical analysis and CI values partially supported our hypothesis that alectinib and everolimus acted in a complementary manner on the inhibition of ALCL cell lines.

Effect of alectinib on cell signaling pathways was enhanced by combination with everolimus.

We evaluated the correlation between the synergistic effect and ALK-related signaling changes associated with oncogenesis by western blot analysis. Based on the results of the single treatment experiment, two inhibitors at the lowest concentrations (alectinib: 10nM; everolimus: 2nM) were used in Karpas299 cells. In Figure 5A, alectinib effectively suppressed phosphorylation of ALK, but not Akt (Serine473, Threonine308). Next, we investigated mTOR and PARP which are associated with the cell cycle, in order to identify the signaling molecules that were affected by inhibitors. The de-phosphorylation of mTOR and PARP cleavage were augmented by combination treat compared with single treat groups, the inhibition of phosphorylation of p70S6K, 4EBP1 showed the same tendency as the results above (Figure 5B). Although there was no synergism in combination treat, these phenomena were seen as additive effect on the inhibition of some molecules. This result could not fully explain the synergism observed in the cytotoxicity test

shown above. However, the accumulation of augmented effect in intracellular signaling pathways may have contributed to the toxicity on cancer cell physiology.

Combination treatment with alectinib and everolimus induced subG1 and G0/G1 cell cycle arrest in Karpas299 cells.

After we determined the correlation of drug synergism with the changes in cell cycle, we demonstrated the synergistic effect of the combination treatment on cell cycle progression. Karpas299 and SU-DHL-1 cells were treated with the drug combination. Inhibitor-treated cells were stained with PI, and analyzed by flow cytometry. In Karpas299 cells, the percentage of cells at G0/G1 cycle was increased to 72.9% by combination treat, compared to control or single treat groups (control: 45.6%; alectinib: 58.1%; everolimus: 50.9%, p= 0.0027, 0.0018, 0.0013) (Figure 6A, 6C), the percentage of cells arrested at subG1 cycle showed the same tendency as G0/G1 (combination treat: 3.63%; control: 2.45%;

alectinib: 2.93; everolimus: 1.94; p= 0.0142, 0.0283, 0.0096). As expected, subG1 and G0/G1 cell cycle arrest was augmented by the combination treatment only in Karpas299 cells. On the other hand, SU-DHL-1 cells were not affected by the combination treatment. (combination: 51.25%; control: 49.6%; alectinib: 39.3%; p= 0.2031, 0.0047) (Figure 6B, 6C). The DNA content at each phase of the cell cycle was statistically analyzed. When combined treatment, alectinib and everolimus inhibited simultaneously in the same cell cycle (subG1 and G0/G1) in Karpas299 cells. However, in SU-DHL-1 cell, alectinib increased the DNA content in S phase, but not G0/G1 (supplementary data 4). The above results indicate that the argument effect of combination treat only appears in the karpas299 cell line because both drugs inhibit the subG1, G0/G1 cell cycle at the same time.

Discussion

In our study, alectinib combined with everolimus effectively reduced cell survival and augmented the inhibitory effect of each drug on cell signaling and cell cycle progression. Despite the synergism on suppressive effect we have confirmed was clear, some results require further explanation.

In protein profiling experiments, the tendency of Akt dephosphorylation was not influenced by any concentration of alectinib (Figure 2A left, 2B left). mTOR feedback loop could explain this phenomenon. p70S6K, which was effectively inhibited by inhibitors suppresses the activation of PI3K. PI3K usually phosphorylates Akt in direct and indirect ways (through rapamycin-insensitive companion of mTOR, RICTOR).[22-25] And MET signaling salvage probably rescues Akt from inhibition. Unlike crizotinib, alectinib is a selective ALK inhibitor that does not affect MET. The activation of MET result in phosphorylation of Akt.[26]

In our combination treatment experiment, we calculated the CI values to determine whether there was a synergistic effect in ALK-positive ALCL lines. Interestingly, only in Karpas299 cells, the CI value was less than 1 and the Fa value exceeded 0.5 at low concentrations to be clinically applicable.[21] In the SU-DHL-1 cell line, CI values of the combination treatment at a ratio of 5:1 seemed to be synergistic (Table 1). However, we excluded those CI values for which the Fa values did not tend to also increase as the dose of inhibitor increased (Supplementary 2). In this way, we were able to conclude that the effect of the combination treatment on SU-DHL-1 was not synergistic, nor was it antagonistic. To determine why the combination treatment had such different effects in the two cell lines, further study was indicated.

To investigate the selective synergism between two cell lines, we examined the differences in gene expression between two cell lines by using a publicly available RNA sequence data. Specifically, we evaluated the expression rate of ALK, ALK-related genes and their

isoforms. The expression level of ALK-201 isoform (ENST00000389048.7), which has tyrosine kinase domain, accounts for 82.99%, 56.53% of the total ALK expression in the Karpas299, SU-DHL-1, respectively. Contrary to ALK-201, the expression of ALK-203 isoform (ENST00000453137.1), which has protein kinase-like domain similar to PI3Ks, accounts for 8.45%, 37.6% (Karpas299, SU-DHL-1) (Supplementary data 5). These differences can potentially be associated with drug responsiveness and synergism of combination therapy.

Taken together these results, we confirmed that the synergistic effect was seen in certain cell line. This phenomenon suggests that even cell lines with same mutations can not always be expected to have a synergism of combination treat, and the combination treat should be considered in various situations that are actually used in clinical studies. Furthermore, the deeper molecular and biologic considerations are also needed for successful drug combination therapy at the clinical treatment level.

Figure legend

Figure 1. Evaluation of cytotoxicity of ALK inhibitor and mTOR inhibitor in ALK positive / negative cancer cell lines. ALK-positive/negative cells were treated with inhibitors at the indicated doses and survival was measured using a cytotoxicity test. Results of (A) alectinib, (B) crizotinib, (C) everolimus treatment of ALK-positive/negative cell lines for 72 hours.

Figure 2. Identification of cellular signal changes by drugs in ALK-positive cell line. The inhibition of cell signaling by inhibitor treatment was confirmed by western blot analysis. Karpas299 cell was treated with alectinib or everolimus for (A) 5 h, (B) 24 h. showing the phosphorylation of ALK, Akt (serine 473, threonine 308), mTOR (serine 2481, serine 2448), p70S6K, p-4EBP1 and GAPDH.

Figure 3. The heatmap analysis of the combined treatment effects of both drugs. To determine the

optimum combination ratios of alectinib and everolimus that would result in synergism, Karpas299, SU-DHL-1 were treated with two-drug combinations for 72h.

Figure 4. Analysis of the synergistic effect of combination of ALK inhibitor and mTOR inhibitor. Effect of alectinib and everolimus at a 5:1 ratio on survival of ALK-positive ALCL cell lines. (A) Karpas299 and (C) SU-DHL-1 cells were treated with alectinib and/or everolimus at a 5:1 ratio to assess cell survival and (B), (D) CI values corresponding to values in (A).

Figure 5. Identification of cellular signal changes by synergistic combination of ALK inhibitor and mTOR inhibitor. Effect of combination treat on the phosphorylation of cell signaling molecules. Karpas299 cells were treated with 40 nM alectinib and 0.5 nM everolimus for 24 h and was analyzed by western blot. (A) ALK (NPM-ALK) and Akt. (B)

mTOR, p70S6K, 4EBP1, PARP. A= alectinib; E= everolimus.

Figure 6. Determination of cell cycle inhibition by combination therapy of ALK inhibitor and mTOR inhibitor. Effect of alectinib and everolimus on cell cycle. Temporal tracking of the cell cycle was demonstrated by propidium iodide (PI) DNA staining and flow cytometry. (A), (C) Karpas299 and (B), (C) SU-DHL-1 cells were treated with alectinib with or without everolimus for 72 h . PI stained cells were analyzed by flow cytometry (BD Calibur).

Table 1. Average values of Combination Index at various combined ratios.

Figures and Table

Figure 1. Evaluation of cytotoxicity of ALK inhibitor and mTOR inhibitor in ALK positive / negative cancer cell lines.

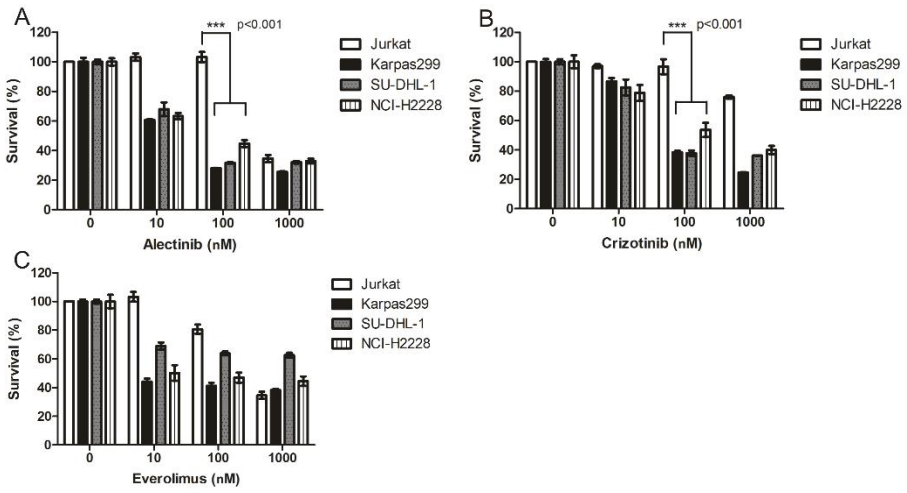


Figure 2. Identification of cellular signal changes by drugs in ALK-positive cell line.

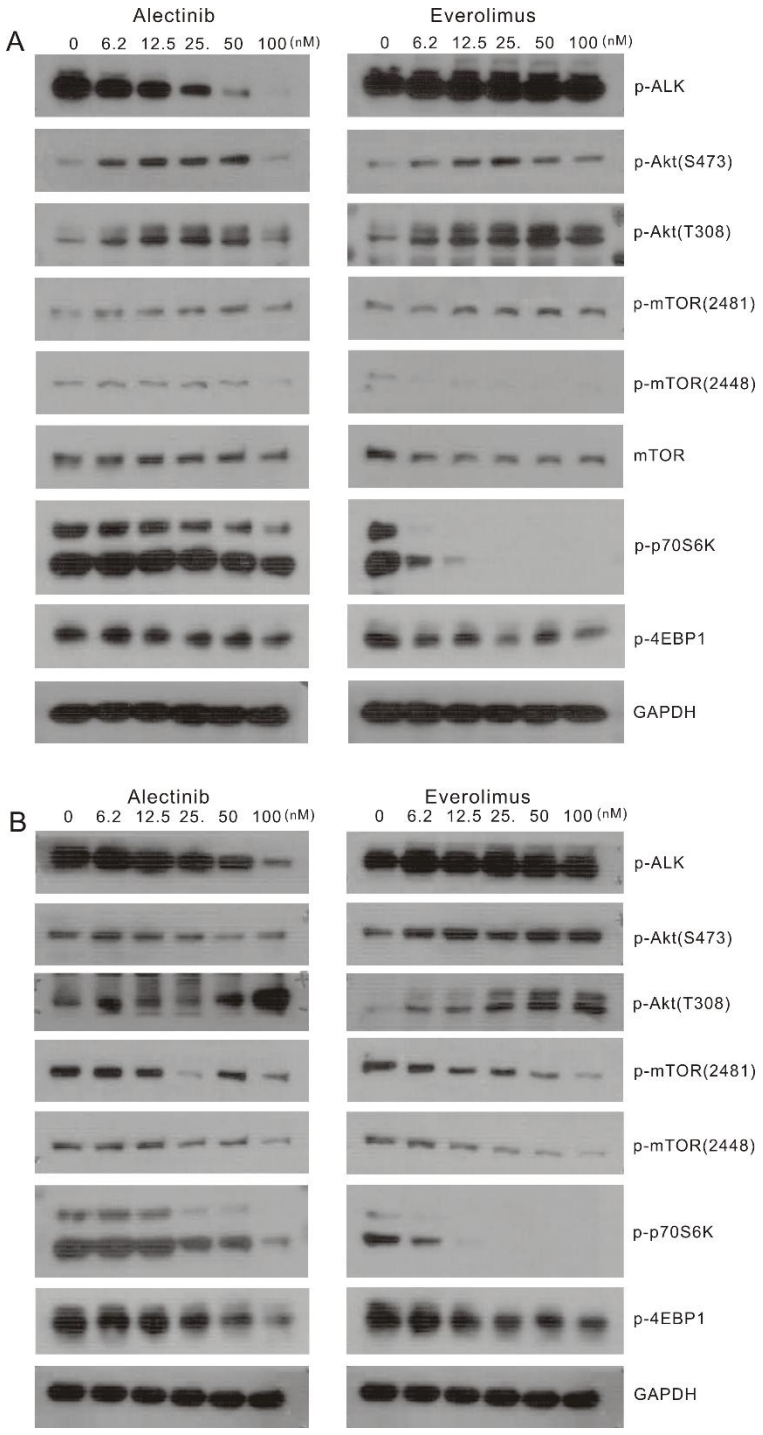


Figure 3. The heatmap analysis of the combined treatment effects of both drugs.

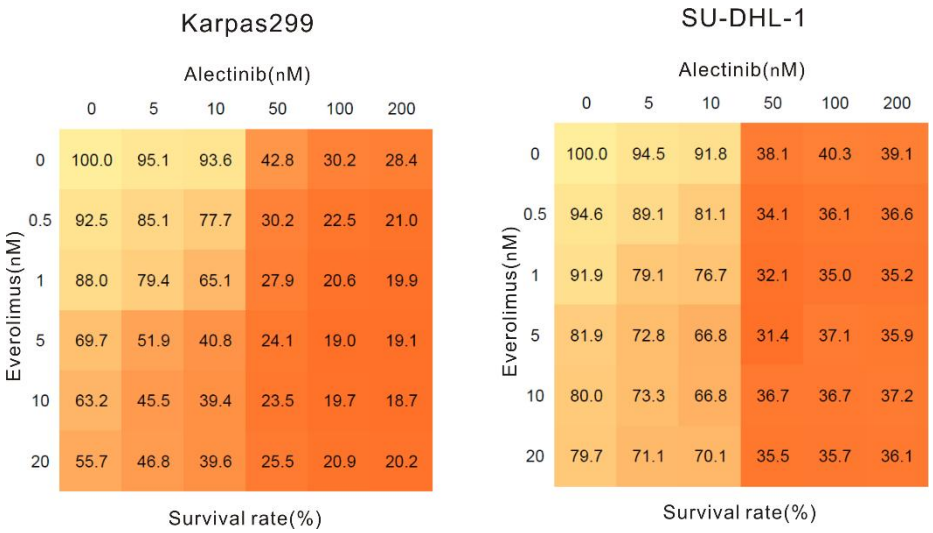
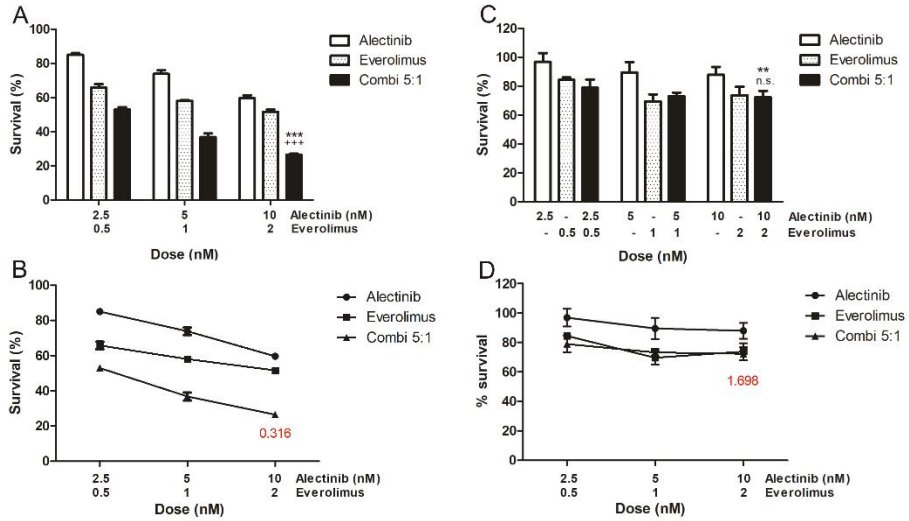


Figure 4. Analysis of the synergistic effect of combination of ALK inhibitor and mTOR inhibitor.



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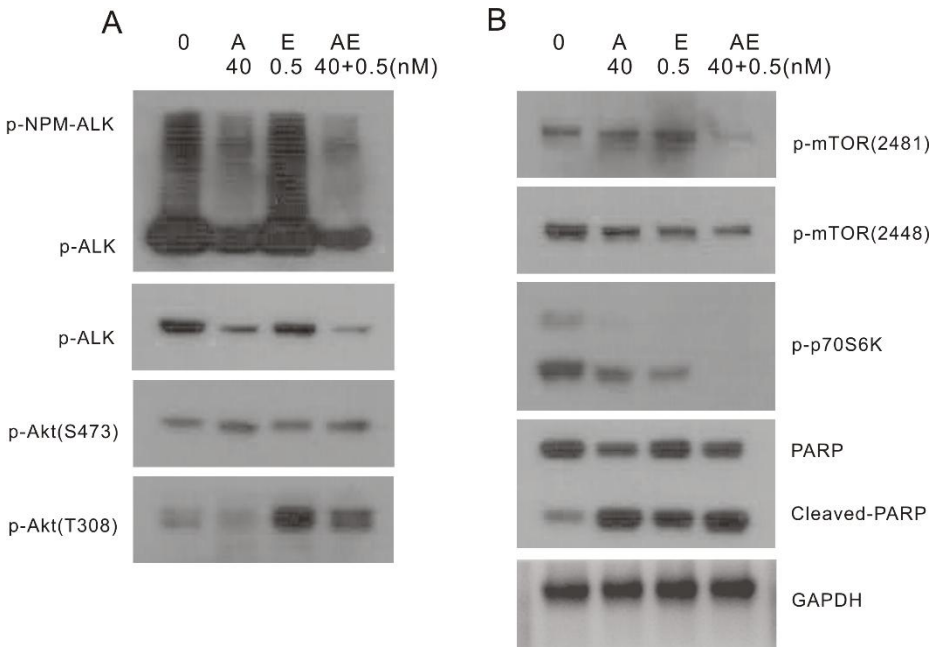

Figure 6. Determination of cell cycle inhibition by combination therapy of ALK inhibitor and mTOR inhibitor.

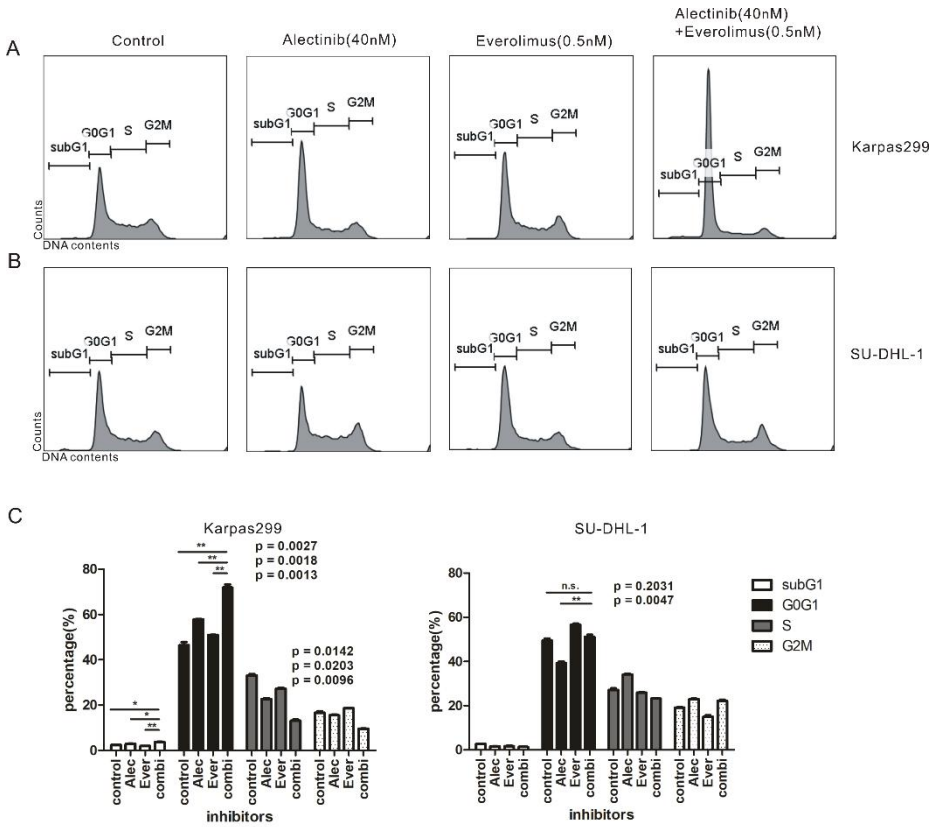

Table 1. Average values of Combination Index at various combined ratios.

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		Drug	Combination Index Values at				
			ED50	ED75	ED90	Average CI	Synergism Level
NPM-ALK+	Karpas299	combi 20:1	0.59525	0.69606	0.84456	0.711957	Synergism
		combi 10:1	0.48889	0.60906	0.81027	0.636073	Synergism
		combi 5:1	0.47101	0.49078	0.56864	0.510143	Synergism
		combi 1:1	0.31875	0.3421	0.43561	0.365487	Synergism
		combi 0.5:1	0.29479	1.45902	8.37348	3.375763	Antagonism
	SU-DHL-1	combi 20:1	0.95925	1.52618	2.44651	1.64398	Antagonism
		combi 10:1	0.6839	0.95754	1.36079	1.000743	Additive
		combi 5:1	0.04532	0.0745	0.12609	0.08197	Synergism
		combi 1:1	0.91835	3.83065	18.1468	7.631933	Antagonism
		combi 0.5:1	8.5253	269.3155	10584	3620.614	Antagonism

Combi A: B = alectinib : everolimus; ED: Effective dose

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Table 1. Average values of Combination Index at various combined ratios.....38

List of abbreviations

ALCL: Anaplastic large cell lymphoma

ALK: Anaplastic lymphoma kinase

mTOR: Mammalian target of rapamycin

IC50: Inhibitory concentration 50

CI: Combination index

Fa: Function of affected fraction

국 문 초 록

이 연구의 목적은 두 약물의 ALK 양성 암세포에 대한 조합처리 용법의 상승 효과를 확인하는 데있다. 조합처리 요법은 ALK 양성 림프종 세포주인 Karpas299 세포주에서 상승 효과를 나타냈지만 같은 ALK 양성 세포주인 SU-DHL-1 세포에서는 상승 효과를 나타내지 않았다. Karpas299 세포의 생존율은 단독 처리군 (alectinib: 59.5%, everolimus: 51.6%)과 비교하여 alectinib과 everolimus의 병용 요법으로 26.4% ($p<0.001$)로 나타났으며, 상승 효과지표인 combination index(CI)는 0.316이었다. 또한, Karpas299의 조합 치료는 ALK-mTOR의 탈 인산화와 poly ADP ribose polymerase (PARP) cleavage를 증가시켰다. 조합처리 요법은 subG1 기에서 (everolimus: $p = 0.0096$), G0 / G1 (alectinib: $p = 0.0018$, everolimus: 0.0013) 세포성장주기가 차단된 Karpas299의 수를 유의하게 증가시켰다. 우리는 Karpas299와 SU-DHL-1의 생물학적 차이를 더 조사하였으며, ALK 이형체(ALK-201, 203)의 발현 양상이 다르게 나타나는 것을 확인했다. 전반적으로 alectinib과 everolimus의 조합처리는 Karpas299의 세포의 약물에 대한 감수성과 mTOR 신호 전달 억제 그리고 세포성장주기를 효과적으로 억제했지만 SU-DHL1 세포에서는 억제하지 못했다. 이 결과는 ALK 양성 림프종 환자에 대한 새로운 병용 요법의 가능성을 시사할 뿐 아니라 새로운

치료법 개발에 대한 접근법을 제시한다.

주요어: 미분화 대 세포 림프종, 미분화 림프종, 인산화효소, Alectinib, 포유류 라파마이신 표적, Everolimus, 타이로신 인산화효소 억제제, 약제조합처리, 약물조합처리

학번: *2016-25164*