The synthetic ajoene analog SPA3015 induces apoptotic cell death through crosstalk between NF-κB and PPARγ in multidrug-resistant cancer cells

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Abstract

Multidrug resistance (MDR) caused by P-glycoprotein (P-gp) overexpression impedes successful cancer chemotherapy. In this study, we investigated the anticancer effects of SPA3015, a synthetic ajoene analog, in P-gp-overexpressing MDR cancer cells (KBV20C and MES-SA/DX5). Treatment with SPA3015 caused a dramatic decrease in the cell viabilities of both KBV20C and MES-SA/DX5 cells. This decrease was accompanied by apoptotic cell death without affecting the expression level or drug efflux function of P-gp. SPA3015 selectively suppressed NF-κB reporter gene activity, which led to decreased expression of NF-κB target genes such as CIAP1, CIAP2, XIAP, and Bcl-XL. Surprisingly, nuclear localization and DNA binding affinity of the p65 subunit were not affected by SPA3015, suggesting that SPA3015 inhibits the transcriptional activity of NF-κB at the nucleus. Indeed, SPA3015 treatment led to an increase in the physical interaction of p65 with PPARγ, which resulted in the inhibition of NF-κB activity. Our findings support the hypothesis that SPA3015 inhibits NF-κB transcriptional activity by facilitating the physical interaction of the p65 subunit and PPARγ, which leads to apoptotic cell death in MDR cancer cells.

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1. Introduction

Multidrug resistance (MDR), a significant impediment to chemotherapeutic cancer treatment, results from the elevated expression of proteins such as cell-membrane ATP-binding cassette transporters. These transporters are involved in the efflux of cytoxic drugs from cells, thus reducing intracellular drug concentrations (Bradley and Ling, 1994; Szakacs et al., 2006). A 170 kDa ATP-dependent P-glycoprotein (P-gp) acts as a major efflux pump; it is clear that the overexpression of P-gp is linked to MDR in many human cancer cells, including colon, kidney, adrenal, pancreas, and liver cancer (Ambudkar et al., 2003; Gottesman et al., 2002; Thomas and Coley, 2003). Abundant evidence links the failure of certain chemotherapeutic agents to the expression of P-gp (Thomas and Coley, 2003). Thus, the development of a chemotherapeutic strategy against MDR remains a major challenge in the treatment of cancer.

Since the first explanation of P-gp function in MDR, strong effort has been made to use P-gp inhibitors to reverse MDR phenotype in various cancers (Amin, 2013; Palmeira et al., 2012; Szakacs et al., 2006). First-generation P-gp inhibitors, such as verapamil, quinine, and cyclosporine, are less selective for and display low binding affinity to P-gp. This results in unwanted toxic side effects and failure to show improved outcome due to high serum concentrations of the inhibitor (Amin, 2013; Palmeira et al., 2012). The development of second-generation P-gp inhibitors such as dexverapamil and valsodar focused on improving their specificity and affinity for inhibiting P-gp, as well as decreasing their toxicity. However, they have been shown to inhibit the P450 CYP3A4 enzyme and other transport systems (Amin, 2013; Palmeira et al., 2012). Because of these limitations, the use of first and second-generation inhibitors is limited to clinical trials. The third-generation inhibitors such as zosuquidar and tariquidar, now under clinical development, are designed specifically for high...
transporter affinity, low pharmacokinetic interaction, and decrease of toxicity. Although P-gp is clearly established as a prognostic marker in several human tumors, the clinical benefit of modulating P-gp-mediated MDR is still in question. In addition to traditional pharmacological approaches, more creative searches for fourth-generation inhibitors are in progress, examining diverse mechanisms such as capturing, neutralizing, or evading of P-gp, and downregulation of P-gp gene expression (Szakacs et al., 2006; Thomas and Coley, 2003).

Ajoene (4,5,9-trithiadodeca-1,6,11-tiene 9-oxide: supplementary data; sfig. 1A) is one of the major active compounds derived from garlic (*Allium sativum*) (Apitz-Castro et al., 1983). It has been reported that ajoene possesses a broad spectrum of medical and biological activities including anti-thrombosis, antioxidant, antimicrobial, virucidal, and anticancer activity (Apitz-Castro et al., 1992; Dirsch et al., 1998; Kaschula et al., 2010; Naganawa et al., 1996; Weber et al., 1992). In particular, ajoene has anti-proliferative activities in several human cancer cells including leukemia, esophageal, and basal skin tumor (Kaschula et al., 2012; Li et al., 2002; Scharfenberg et al., 1990; Taylor et al., 2006). On the basis of this anticancer activity, efforts have been made to develop more active ajoene-based anticancer agents by substituting the terminal allyl groups with alkyl, aromatic, or heteroaromatic groups (Kaschula et al., 2010). However, the anticancer effects of ajoene and its analogs in MDR cancer cells remain largely undefined.

In this study, we examined the antiproliferative potential of newly synthesized ajoene analogs on P-gp-overexpressing MDR cancer cells. Since SPA3015 [(E)-1-phenyl-7-phenyl-2,3,7-trithiahepta-4-ene 7-oxide: supplementary data; sfig. 1B] shows the most potent antiproliferative effect, we studied possible molecular mechanisms. SPA3015 induced apoptotic cell death in MDR cancer cells without affecting the expression level and function of P-gp. These effects seemed to be mediated by suppression of anti-apoptotic genes through the inhibition of NF-κB function. Interestingly, we found that SPA3015 inhibits NF-κB transcriptional activity by enhancing the physical interaction of the p65 subunit and PPARγ at the nucleus. Our results suggest a novel mechanism for the ajoene analog SPA3015 in inhibiting the survival and proliferation of cancer cells and highlight the potential to develop a novel chemotherapeutic drug to reverse MDR phenotype in cancer.

2. Materials and methods

2.1. Cell culture

KB (Human oral squamous cell carcinoma), KBV20C (vincristine-resistant daughter cells), MES-SA (human uterine sarcoma), and MES-SA/DX5 (doxorubicin-resistant daughter cells) were obtained from American Type Culture Collection (ATCC, Manassas, VA). KB and KBV20C cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories, Logan, UT), 100 units/mL penicillin, and 100 μg/mL streptomycin (HyClone Laboratories). KBV20C cells were maintained with 20 mM vincristine in their growth media. MES-SA and MES-SA/DX5 cells were grown in DMEM supplemented with equal amounts of FBS and penicillin/streptomycin. Cells were maintained at 37 °C under 5% CO2 in a humidified chamber.

2.2. Reagents and cytotoxicity test

Synthetic ajoene analogs were obtained from Prof. R. Jeon (Sookmyung Women’s University). Doxorubicin and vincristine were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). All chemicals were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and stored at –80 °C until use. The cytotoxicity test was determined by the MTT assay (DUCHEA BIOCHEMIE, B.V., Netherlands) according to the manufacturer’s instruction.

2.3. Cell cycle analysis

Cells were treated with the indicated concentrations of drugs for 24 h. Cells were harvested by trypsinization, washed twice with PBS, fixed with 70% ethanol, and stored overnight at 4 °C. For staining, cells were washed with PBS, incubated with 100 μg/mL RNase A (BD Bioscience, Franklin Lakes, NJ) at 37 °C for 30 min, stained with 10 μg/mL propidium iodide (PI, BD Bioscience) solution, and analyzed by FACS Calibur (BD Bioscience).

2.4. Immunoblotting

For preparation of whole cell extracts, cells were lysed in RIPA buffer supplemented with 1 x protease inhibitor cocktail (Roche, Basel, Switzerland) and 1 x phosphatase inhibitor cocktail (GenDEPOT, Barker, TX) for 20 min in an ice bath. Cell lysates were centrifuged at 16,000 x g for 15 min at 4 °C, and the supernatants were collected in new tubes. After boiling in Laemmli sample buffer for 5 min, 30 μg of protein was used for SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skim milk/0.1% Tween 20/TBS (Tris-buffered saline) for 1 h at room temperature and incubated overnight with the appropriate primary antibody. β-actin (sc-47778), PARP (sc-7150), 1-x-b3a2 (sc-847), and p65/RelA (sc-372) were purchased from Santa Cruz, Dallas, TX. SP-1 antibody (ab13370) was purchased from Abcam, Cambridge, UK. α-tubulin (#2144), cleaved active caspase-3 (#9661), phosphor-p38 (#9211), p38 (#9212), phosphor-ERK (#4201), and ERK (#9102) were purchased from Cell Signaling Technology, Danvers, MA. The membranes were then washed with 0.1% Tween 20/TBS, incubated with secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA), and visualized with an enhanced chemiluminescence detection kit (Advanstar, Menlo Park, CA).

2.5. Reverse-transcriptase PCR

Total RNA was extracted using the easy-BLUE™ total RNA extraction kit (iNTRON Biotechnology, Seongnam, Korea), and the integrity of the RNA was checked by agarose gel electrophoresis and ethidium bromide staining. One microgram of RNA was used as a template for each M-MLV reverse transcriptase-mediated PCR reaction. The primer sets for P-gp were 5’-CCCCATCATGGCAA-TAGCAGG-3’ and 5’-GTTTAAACCTTGTCCCTGTA-3’; the primer sets for GAPDH were 5’-CTCATGACACAGTCCATGCATC-3’ and 5’-CTGTTCCACCACCTTTGATGTC-3’.

2.6. Quantitative real-time PCR

Total cDNA synthesized by M-MLV reverse transcriptase (Promega, Fitchburg, WI) was used as a template for PCR performed with the Eco Real-Time PCR System (Illumina, San Diego, CA) using SensiFAST SYBR® No-ROX Kit (Bioline, London, UK). Reaction parameters were as follows: cDNA synthesis at 37 °C for 60 min, transcriptase inactivation at 95 °C for 5 min, PCR cycling at 95 °C for 10 s, 58 °C for 30 s, and 72 °C for 20 s for 40 cycles. The expression levels of mRNA were normalized to the expression of 28S rRNA as an internal control. The following primers were used for real-time PCR: CIAP1 upstream (5’-ACTTCGACACCTGCTATACATC-3’) and downstream (5’-GCTAGATTTTCTCCTGAATTCTC-3’); CIAP2 upstream (5’-GCTGGTTCACACCAGTATACCA-3’) and downstream (5’-TTCACCCAGGTCTCTATTAAAGCC-3’); XIAP upstream (5’-GAAG-
ACCCCTGGAACAACTGTA-3') and downstream (5'-CGCCATTGTGTTTCTTCTGACTC-3'); Bcl-xl upstream (5'-CCCAGAAGGATACAGCTTG-3') and downstream (5'-GGATCCGACTACCAAAATCT-3'); and 28S rRNA upstream (5'-AACGAGATCCATGTCCC-3') and downstream (5'-CTTCACGTCGAGACTGAG-3').

2.7. Rhodamine 123 assay

KBV20C and MES-SA/DX5 cells were incubated with 10 μM Rhodamine 123 (Sigma-Aldrich) for 3 h under normal culture conditions. Cells were harvested, washed twice with ice-cold PBS, and resuspended in PBS just before use. Untreated cells suspended in PBS were used as blanks. Fluorescence intensity of intracellular Rhodamine 123 was determined by flow cytometry (FACSCalibur, BD Bioscience). Ten thousand cells per sample were analyzed.

2.8. Nucleus/cytosol fractionation

Cells were washed twice in PBS, harvested, and pelleted by centrifugation at 500 g for 3 min. Cell pellets were resuspended with hypotonic buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM MgCl2, 0.5 mM dithiothreitol, 1 x protease inhibitor cocktail (Roche)). Cells were pelleted by spinning at 1000 g for 5 min and lysed in ice-cold 0.5% NP-40 containing buffer A on ice for 10 min. The cytoplasmic fraction was obtained by spinning the cells for 10 min at 900 x g. To obtain the nuclear fraction, the remaining pellet was lysed on ice in lysis buffer B (20 mM HEPES, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, 25% glycerol, 1 x protease inhibitor cocktail) for 30 min and vortexed periodically. Nuclear fractions were cleared by centrifugation for 10 min at 16,000 x g.

2.9. Luciferase reporter gene assay

KBV20C cells were transfected with NF-κB-Luc, AP-1-Luc, or PPRE-Luc firefly luciferase reporter constructs plus SV40/Renilla luciferase plasmid using Lipofectamine2000 (Invitrogen, Carlsbad, CA). After incubation for 24 h, cells were treated with SPA3015 for an additional 24 h. The dual luciferase reporter assay kit (Promega) was used for the assay with 10 μl lysate, 100 μl luciferase assay reagent II (Promega), and 100 μl Stop&Glow™ (Promega) according to the manufacturer's protocol and quantified in a GloMax™ 20/20 (Promega). The data are represented as the mean ± SD of three independent experiments.

2.10. Co-immunoprecipitation (Co-IP)

Cells were collected and lysed in nonionic lysis buffer supplemented with protease inhibitors, incubated on ice for 15 min, and cleared by centrifugation at 16,000 x g at 4 °C for 15 min. The lysate (1000 μg of total protein) was subjected to immunoprecipitation with the agarose-immobilized antibody (1 μg of anti-PPARγ) overnight at 4 °C.

2.11. DNA pull down assay

Nuclear extracts were incubated with 5'-biotinylated double-strand NF-κB binding DNA probes (5'-AGT TGA GGG GAC TTT CCC AGC C-3' and 5'-GCC TGG GAA AGT CCC CTC AAC T-3', synthesized by Integrated DNA Technologies, Coralville, IA) for overnight at 4 °C. Protein-DNA complexes were pulled down by streptavidin agarose resin (Thermo Fisher Scientific, Waltham, MA). Precipitated proteins were detected by Western blotting using specific antibodies.

2.12. Statistics

The data are expressed as the mean ± standard deviation (S.D.) of three independent experiments. Differences between the mean values in the two groups were analyzed using one-way analysis of variance (ANOVA). P < 0.05 was considered statistically significant.

3. Results

3.1. The synthetic ajoene analog SPA3015 inhibits cellular proliferation and induces apoptosis in MDR cancer cells

First, we examined the antiproliferative effects of synthetic ajoene analogs on both KBV20C cells and its parental KB cells. All of the compounds tested significantly decreased the cell viability in both KB and KBV20C cells when treated at a concentration of 10 μM (data not shown). Among them, SPA3015 proved the most effective in decreasing the cell viability with very similar levels of decrease in both cell lines, suggesting the antiproliferative potential of SPA3015 on P-gp-overexpressing MDR cancer cells. We thus selected SPA3015 to investigate the molecular mechanism by which ajoene compounds exert antiproliferative effects in MDR cancer cells.

We next confirmed the antiproliferative effects of SPA3015 in two different MDR cancer cell lines, KBV20C and MED-SAX5. As shown in Fig. 1A, SPA3015 suppressed the proliferation of KBV20C cells with the IC50 at about 3.5 μM, which was similar to the effect on parental KB cells. In addition, similar effects were observed in another MDR cancer cell line MED-SAX DX5 (Fig. 1B). Next, we examined the effect of SPA3015 on cell cycle progression using flow cytometry. SPA3015 did not affect cell cycle progression in either KB or KBV20C cells (supplementary data; sFig. 2A). However, we found that the sub-G1 fraction, which often indicates DNA fragmentation, a marker of apoptotic cell death, was increased by SPA3015 in both cells (supplementary data; sFig. 2A and 2B). Further, to confirm the apoptotic potential of SPA3015, we analyzed the intracellular levels of active caspase-3 and PARP cleavage. As shown in Fig. 1C and D, KB and MES-SA cells are more sensitive to doxorubicin than KBV20C and MES-SA/DX5 cells. However, SPA3015 treatment led to the activation of caspase-3 and cleavage of PARP at similar levels in both MDR cancer cells and their parent cells, which correlates with our previous observations that SPA3015 suppresses the proliferation of MDR cancer cells.

3.2. SPA3015 does not influence the expression or drug efflux function of P-gp

Because most drugs targeting MDR affect transport function or the expression level of MDR-related efflux transporters (Szakacs et al., 2006), we examined the effect of SPA3015 on P-gp expression in MDR cancer cells. As shown in Fig. 2A and B, SPA3015 did not alter the expression level of P-gp in KBV20C or MES-SA/DX5 cells. Next, we determined whether SPA3015 affects the drug efflux function of P-gp. The efflux function of P-gp was analyzed by measuring the intracellular accumulation of Rhodamine 123, a well-known fluorescent P-gp substrate, using flow cytometry. Treatment with verapamil, an inhibitor of P-gp, led to a dramatic increase in the intracellular levels of Rhodamine 123 in both MDR cancer cells lines, whereas SPA3015 did not affect the intracellular accumulation of Rhodamine 123 (Fig. 2C and D), suggesting that SPA3015 has no effect on the efflux function of P-gp.

3.3. SPA3015 specifically inhibits NF-κB transcriptional activity at the nucleus

To investigate the molecular mechanism for the apoptotic
Fig. 1. The synthetic ajoene analog SPA3015 inhibits cellular proliferation and induces apoptosis in MDR cancer cells. (A) KB/KBV20C or (B) MES-SA/MES-SA/DX-5 cells were treated with serially diluted concentrations of SPA3015 from 30 μM for 48 h. Cell viability was determined using the MTT assay as described in Materials and Methods. Data represent the mean ± S.D. of three independent experiments. (C, D) Cells were treated with 1 or 10 μM SPA3015 or 1 μM doxorubicin for 24 h, and the cleavage of PARP and caspase-3 activation were assessed by immunoblot analysis.

Fig. 2. SPA3015 does not influence the expression or drug efflux function of P-gp. After treatment of 1 or 10 μM SPA3015 for 24 h, the expression level of MDR1 in KBV20C (A) or MES-SA/DX5 (B) cells was analyzed using RT-PCR. KBV20C (C) or MES-SA/DX5 (D) cells were pretreated with 10 μM verapamil or 10 μM SPA3015 for 1 h. Following treatment with 10 μM Rhodamine 123, the fluorescence intensity of intracellular Rhodamine 123 was measured using flow cytometry.
Fig. 3. SPA3015 specifically inhibits NF-κB transcriptional activity at the nucleus. (A) HEK293T cells were cotransfected with Renilla-SV-40-luc and firefly-NF-κB-luc plasmids for 24 h, then treated with 10 μM SPA3015 combined with 50 nM phorbol-dibutyrate (PDBu) for 4 h. Luciferase activities were measured as described in the Materials and Methods section. Data represent the mean ± S.D. of three independent experiments. *P < 0.05 vs. PDBU treatment. (B) After 24 h treatment with 10 ng/mL TNF-α combined with 1 or 10 μM SPA3015 in KBV20C cells, the mRNA levels of Bcl-xL, CIAP1, CIAP2, and XIAP were analyzed using quantitative real-time PCR. Data represent the mean ± S.D. of three independent experiments. *P < 0.05 vs. TNF-α treatment. (C) KBV20C cells were transfected with GFP-RelA and then pre-treated with 10 μM SPA3015 for 1 day. Green fluorescent images were captured after treatment with 10 ng/mL TNF-α. (D) Cytosol/nucleus fractionation was performed for KBV20C cells, followed by IBL with various antibodies, as indicated. (E) KBV20C cells were treated with 10 ng/mL TNF-α combined with 10 μM SPA3015 for the indicated times. Cell lysates were subjected to immunoblotting using the indicated antibodies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
potential of SPA3015, we examined whether SPA3015 affects the NF-κB signaling pathway, which functions in cell survival signaling (Karin et al., 2002). SPA3015 strongly inhibited NF-κB reporter gene activity (Fig. 3A), which was further confirmed by the dramatic decrease in the expression levels of NF-κB target genes (e.g., cIAP1, cIAP2, XIAP, and Bcl-xL) associated with cell survival following SPA3015 treatment (Fig. 3B). However, SPA3015 did not alter AP-1 reporter gene activity (supplementary data; sFig. 3), suggesting that inhibition of NF-κB activity by SPA3015 is a specific response. We next attempted to identify the detailed mechanisms for NF-κB inhibition by SPA3015. First, we examined the effects of SPA3015 on the nuclear translocation of the p65/RelA subunit. The nuclear translocation of p65/RelA reporter constructs containing the PPARγ-response element (PPARγ-luc) for 24 h, and treated with 1 or 10 μM SPA3015 or 10 μM troglitazone (TRO) for 24 h, followed by luciferase reporter activity measurement. Data represent the mean ± S.D. of three independent experiments. *P < 0.05 vs. control. (B) KBV20C cells were transfected with GFP-p65/RelA for 24 h and then treated with 1 or 10 μM SPA3015 for 6 h. Cell lysates were subjected to IP with control IgG or anti-PPARγ antibody, followed by IB with various antibodies, as indicated. (C) HEK293T cells were cotransfected with Renilla-SV-40-luciferase and firefly-luciferase reporter constructs containing the PPARγ-response element (PPARγ-luc) for 24 h, and treated with 1 or 10 μM SPA3015 or 10 μM troglitazone (TRO) for 24 h, followed by luciferase reporter activity measurement. Data represent the mean ± S.D. of three independent experiments. *P < 0.05 vs. PDBU treatment.

in resting conditions, this interaction was dramatically increased by SPA3015 (Fig. 4B), indicating that PPARγ activation by SPA3015 facilitates the physical interaction between PPARγ and NF-κB, which leads to an inhibition of NF-κB transactivation.

Further, to confirm that PPARγ activation by SPA3015 is required for the suppression of NF-κB transactivation, we examined the effects of standard PPARγ agonists, pioglitazone and troglitazone, on NF-κB transactivation. As shown in Fig. 4C, these two PPARγ agonists strongly suppressed NF-κB reporter gene activity. The suppression was significantly reversed by treatment with GW9662, a common PPARγ antagonist, suggesting that the transcriptional activity of NF-κB might be regulated by crosstalk with PPARγ. Interestingly, the effect of SPA3015 was quite similar to that of troglitazone and pioglitazone (Fig. 4C). Taken together, these results support our postulation that SPA3015 activation of PPARγ enhances the physical interaction with NF-κB, resulting in a suppression of NF-κB activity.

4. Discussion

Ajoene, a natural garlic compound, has an attractive anticancer effect in many human cancers, including mammary, bladder, colorectal, hepatic, nasopharyngeal, gastric, prostate, lung, pancreatic, lymphoma, leukemia, and skin, with an IC50 range of 5–41 μM (Kaschula et al., 2010). Several groups have reported that the anticancer effects of ajoene are largely attributed to inhibition of proliferation and induction of apoptosis (Li et al., 2002; Scharfenberg et al., 1990; Taylor et al., 2006). However, neither the molecular mechanisms of these anticancer effects nor the potency of ajoene in MDR cancer cells is well defined. In this study, our data indicate that the ajoene analog SPA3015 induces apoptosis in MDR cancer cells to a similar degree as in the parental cells, suggesting that SPA3015 could evade the drug efflux system and consequently inhibit the proliferation of MDR cancer cells. Interestingly, it has been reported that ajoene sensitizes the apoptotic effect of two chemotherapeutic drugs, cytarabine and fludarabine, in human KG1 (P-gp-expressing drug-resistant myeloid leukemia) cells through Bcl-2 inhibition and caspase-3 activation (Ahmed et al., 2001). Consistent with this study, we observed that SPA3015 induces apoptosis without any effect on either expression or efflux function of P-gp. Based on these observations, we propose...
that ajoene and its analog SPA3015 can be developed as a novel strategy against MDR in cancer chemotherapy.

Because of the pivotal role of NF-κB in cancer, it is a potential target of chemotherapeutic anticancer drugs (Baud and Karin, 2009; Hoesel and Schmid, 2013). Indeed, NF-κB-targeting drugs are increasingly being investigated for the treatment of human cancers (Baud and Karin, 2009). Regulation of the IKK/IκBα cascade has been thought to be a key step for controlling NF-κB activity. However, several studies demonstrate that the control of the NF-κB signaling pathway is more complex than simply IKK-mediated regulation of the IκBα/NF-κB interaction. Therefore, it is very important to develop an innovative NF-κB-targeting drug that is more specific and effective. In this study, we found that SPA3015 does not affect the steps of IκBα degradation in cytosol, NF-κB nuclear translocation, or NF-κB DNA binding activity, whereas de novo synthesis of IκBα is sustained by SPA3015 treatment. These data suggest that SPA3015 could be a novel type of NF-κB inhibitor, which acts at the nucleus, not in the cytosol, to inhibit NF-κB activity. Several underlying mechanisms for this notion are of interest: the recruitment of other transcriptional suppressors, or inhibition of co-activator. Although we cannot explain its exact mechanism, we suggest that the crossstalk between NF-κB and PPARγ might be associated with SPA3015 suppression of NF-κB.

PPARγ belongs to the family of nuclear hormone receptors that mainly governs lipid metabolism and insulin sensitization (Krishnan et al., 2007). The relationship between the overexpression of PPARγ and its roles in tumorigenesis in many human cancers have been identified (Dicitore et al., 2013; Kotta-Loizou et al., 2012; Tseng and Tseng, 2012). PPARγ activation by specific agonists leads to growth inhibition, apoptosis, and differentiation of tumor cells. Recently, it was reported that the crosstalk between NF-κB and PPARγ could lead to a suppression of the NF-κB pathway (Delerive et al., 1999; Jeon et al., 2014; Shih et al., 2014). Our study shows that SPA3015 functions as a PPARγ partial agonist and facilitates the physical interaction of NF-κB with PPARγ, leading to a suppression of the NF-κB pathway. However, we observed that thiazolidinedione PPARγ agonists (pioglitazone, rosiglitazone or troglitazone) did not suppress cell viability of KBV20C cells at physiologically functional concentrations of these agonists, but suppress the viability at high concentration (supplementary data; sFig 4). These results suggest that PPARγ agonists alone may not be enough to suppress cell growth of cancer cells, and PPARγ activation may be associated with, in part, the growth inhibition. Indeed, SPA3015 has no adiogenic potential in despite of its PPARγ agonistic effects (data not shown). All these results indicate that PPARγ activation mechanisms by SPA3015 might be different from thiazolidinediones, and either adiogenesis or NF-κB inhibition by PPARγ may be regulated by different and independent mechanisms.

In summary, we have demonstrated that the synthetic ajoene analog SPA3015 has pharmacological potential to induce apoptotic cell death of P-gp-overexpressing MDR cancer cells. The anticancer effects of SPA3015 are due to the suppression of antiapoptotic gene expression by inhibiting NF-κB activity through, in part, crosstalk between NF-κB and PPARγ. Collectively, we suggest that SPA3015 is a strong candidate for further development as an anticancer drug to reverse MDR phenotype in cancer chemotherapy.

Conflict of interest

We declare that we have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfct.2016.07.020.

Transparency document

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