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Blockage of Stat3 enhances the sensitivity of NSCLC cells to PI3K/mTOR inhibition

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ABSTRACT

The PI3K/Akt/mTOR axis in lung cancer is frequently activated and implicated in tumorigenesis. Specific targeting of this pathway is therefore an attractive therapeutic approach for lung cancer. However, non-small cell lung cancer cells are resistant to BEZ235, a dual inhibitor of PI3K and mTOR. Interestingly, blockage of Stat3 with a selective inhibitor, S3I-201, or siRNA dramatically sensitized the BEZ235-induced cell death, as evident from increased PARP cleavage. Furthermore, inhibition of Stat3 led to enhancement of cell death induced by LY294002, a PI3K inhibitor. Treatment of cells with a combination of BEZ235 and S3I-201 significantly induced the proapoptotic transcription factor, CHOP, and its targets, Bim and DR4. Knockdown of CHOP or Bim suppressed cell death stimulated by the combination treatment, implicating the involvement of these BEZ235/S3I-201-induced factors in pronounced cell death. Moreover, the BEZ235/S3I-201 combination enhanced TRAIL-induced cell death. Our results collectively suggest that blockage of Stat3 presents an effective strategy to overcome resistance to PI3K/Akt/mTOR inhibition.

1. Introduction

The mammalian target of rapamycin (mTOR) is a downstream mediator in the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway, and plays a critical role in regulating cell growth and proliferation [1,2]. Since PI3K/AKT/mTOR signaling is deregulated in many cancer types, including brain, prostate, breast, lung, and liver tumors [3–6], mTOR is considered an attractive target for cancer therapy. The first generation of mTOR inhibitors, rapalogs, have been shown to be effective in a range of preclinical models [7]. However, the clinical success of rapalogs is limited to a few rare cancers, such as mantle cell lymphoma, renal cell carcinoma and TSC-related tumors [8]. The mechanisms proposed to account for therapeutic resistance to rapalogs to date include feedback loops, parallel signaling pathways, and limited drug targeting of mTOR [8]. More recently, second generation mTOR-targeting agents, such as dual mTORC1/2 or mTOR/PI3K inhibitors, have been developed, with several clinical trials currently in progress. NVP-BEZ235 (BEZ235) is an orally administered novel dual PI3K and mTOR kinase inhibitor. This compound acts as a potent, reversible inhibitor of both class I PI3K and mTOR kinase catalytic activities by competing for binding at their ATP binding sites [9]. BEZ235 is currently under evaluation in phase I/II clinical trials. Preclinical studies have shown that BEZ235 induces significant anti-proliferative effects in both transgenic mice and non-small cell lung cancer (NSCLC) cell lines [10]. However, BEZ235 is also reported to induce PI3K- and/or MYC-dependent tumor resistance to PI3K–mTOR-targeted therapy [11].

Aberrant activation of the signal transducer and activator of transcription (Stat)3 occurs in the majority of cancer cell lines and several primary tumors [12]. Constitutive Stat3 activity induces specific target genes that stimulate cell proliferation, prevent apoptosis, promote angiogenesis and facilitate tumor immune evasion [13]. Thus, Stat3 presents an attractive molecular target for the development of novel cancer therapeutics. In the present study,

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we showed that concurrent inhibition of PI3K/Akt/mTOR and Stat3 pathways promotes cell death to a greater extent in non-small cell lung cancer cells. Moreover, this sensitization effect is induced through upregulation of CHOP and its downstream target, Bim. Accordingly, we propose that combined inhibition of the PI3K/Akt/mTOR axis and Stat3 signaling represents a novel therapeutic strategy for non-small cell lung cancer.

2. Materials and methods

2.1. Cell culture and reagents

A549 and H460 lung cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI1640 (Welgene, Daegu, Republic of Korea) supplemented with 10% fetal bovine serum. BEZ235 and rapamycin were purchased from LC Laboratories (Woburn, MA, USA), S3I-201 from BioVision (Milpitas, CA, USA) and TRAIL from Enzo Life Sciences (Farmingdale, NY, USA).

2.2. Evaluation of cell death

Cells were stained with Annexin V-FITC and propidium iodide (PI) (BD Biosciences, San Jose, CA, USA) for the assessment of apoptosis, as described previously [14].

2.3. RNA isolation and RT-PCR

RNA isolation and RT-PCR were conducted as described earlier [15]. The following primers were used: Bim (5′-GGCCCCTA CCTCCCTACA-3′ and 5′-GGGGTTTGTGTTGATTTGTCA-3′; 258 bp product) [16], CHOP (5′-CAGACTGATCCAACTGCAG-3′ and 5′-GACT GGAATCTGGAGAGTG-3′; 280 bp product) [17], DR4 (5′-CTGAGC AACGCAGACTCGCTGTCCAC-3′ and 5′-TCCAAGGACACGGCAGAG CCTGTGCCAT-3′; 506 bp product) [17], DR5 (5′-CGCTGCACCAG GTGTGATTC-3′ and 5′-GTCTCCTCCACAGCTGGGAC-3′; 248 bp product) [17], and β-actin (5′-GGATTCCTATGTGGGCGACAG-3′ and 5′-C GCTCGGTGAGGATCTTCATG-3′; 438 bp product) [17]. PCR products were subjected to electrophoresis on 1.5% agarose gels and visualized with ethidium bromide.

2.4. siRNAs and transfections

Bim (sc-29802), CHOP (sc-35437), DR4 (sc-35218), Stat3 (sc-29493) and control (sc-37007) siRNAs were purchased from Santa Cruz Biotechnology (San Jose, CA, USA). Transfection experiments were performed with Lipofectamine™ 2000, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

2.5. Western blot analysis

Cell lysates were separated via SDS–PAGE and transferred to PVDF membrane, followed by immunoblotting with specified primary and horseradish peroxidase-conjugated secondary antibodies. Immunoreactive bands were visualized with SuperSignal West Pico chemiluminescent substrates (Thermo Scientific Pierce, Rockford, IL, USA). Antibodies specific for p-Akt at Ser473 (#9271), p-S6 at Ser240/244 (#4838), p-Stat3 at Ser727 (#9134), p-Stat3 at Tyr705 (#9145), and cleaved PARP (#9541) were obtained from Cell Signaling Technology (Beverly, MA, USA), while the β-actin antibody (#A5316) was purchased from Sigma.

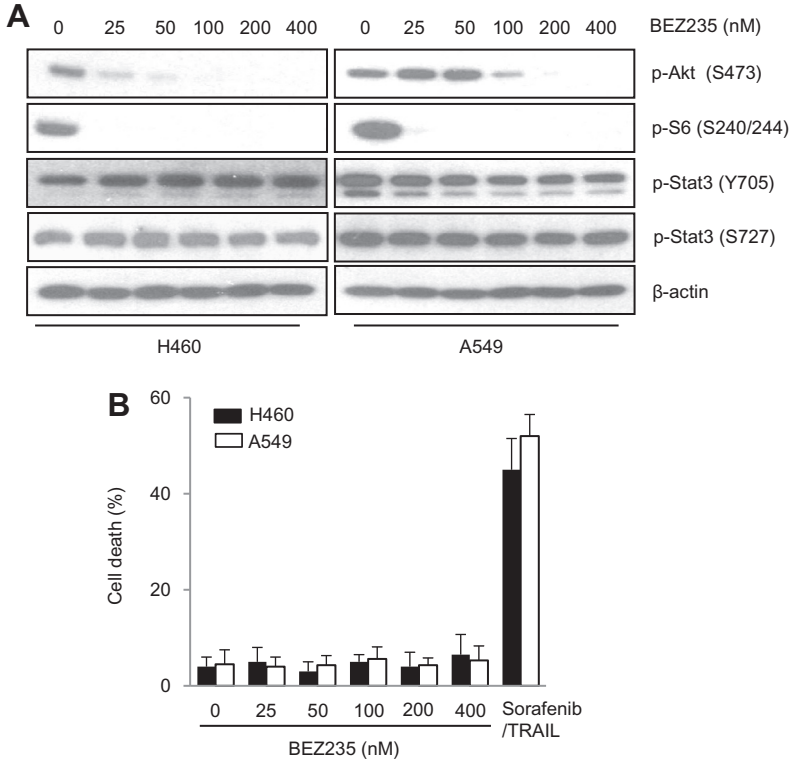

Fig. 1. Effect of BEZ235 on lung cancer cell viability. H460 and A549 cells were treated with the indicated concentrations of BEZ235 for 24 h, and the indicated protein levels estimated using Western blot analysis (A). The blot is representative of three independent experiments. Cell death was evaluated via flow cytometry after Annexin V and PI staining (B). Data are presented as means of triplicate samples and error bars reflect SD. As a positive control, H460 and A549 cells were treated with 5 μM sorafenib and 50 ng/mL TRAIL for 24 h.

3. Results

3.1. BEZ235 inhibits PI3K and mTOR activities, but does not induce cell death

We initially examined the effects of BEZ235 on PI3K and mTOR activities in non-small cell lung cancer cells. BEZ235 suppressed both PI3K and mTOR activities in a dose-dependent manner, as evident from the decreased phosphorylation of Akt at Ser473 and S6 at Ser240/244 (Fig. 1A). However, assessment of apoptosis based on Annexin V/PI positivity revealed no cell death in either H460 or A549 cells treated with BEZ235 (Fig. 1B). The data suggest that the inhibitory activity of BEZ235 alone is insufficient for induction of apoptosis in lung cancer cells. In view of the accumulating evidence that Stat3 contributes to cancer progression and resistance to chemotherapy [12,13], we further investigated the Stat3 phosphorylation level in BEZ235-treated cells. As shown in Fig. 1A, BEZ235 did not affect Stat3 phosphorylation at Tyr705 or Ser727 in H460 and A549 non small-cell lung cancer cells.

3.2. Combined treatment with a Stat3 inhibitor and BEZ235 induces cell death

Next, we examined whether inhibition of Stat3 potentiates cell sensitivity to BEZ235. S3I-201 is a cell-permeable Stat3 inhibitor that binds to the SH2 domain, preventing Stat3 phosphorylation, dimerization, and DNA binding. Notably, co-treatment with BEZ235 and S3I-201 led to dramatic induction of apoptosis, while either agent alone had no effect (Fig. 2A). The induction of cell death was evidenced by microscopic images and PARP cleavage (Fig. 2B and C).

To further establish the effects of Stat3 on BEZ235, we transfected cells with siRNA targeting Stat3, followed by BEZ235 treatment. Stat3 siRNA induced considerable knockdown of Stat3

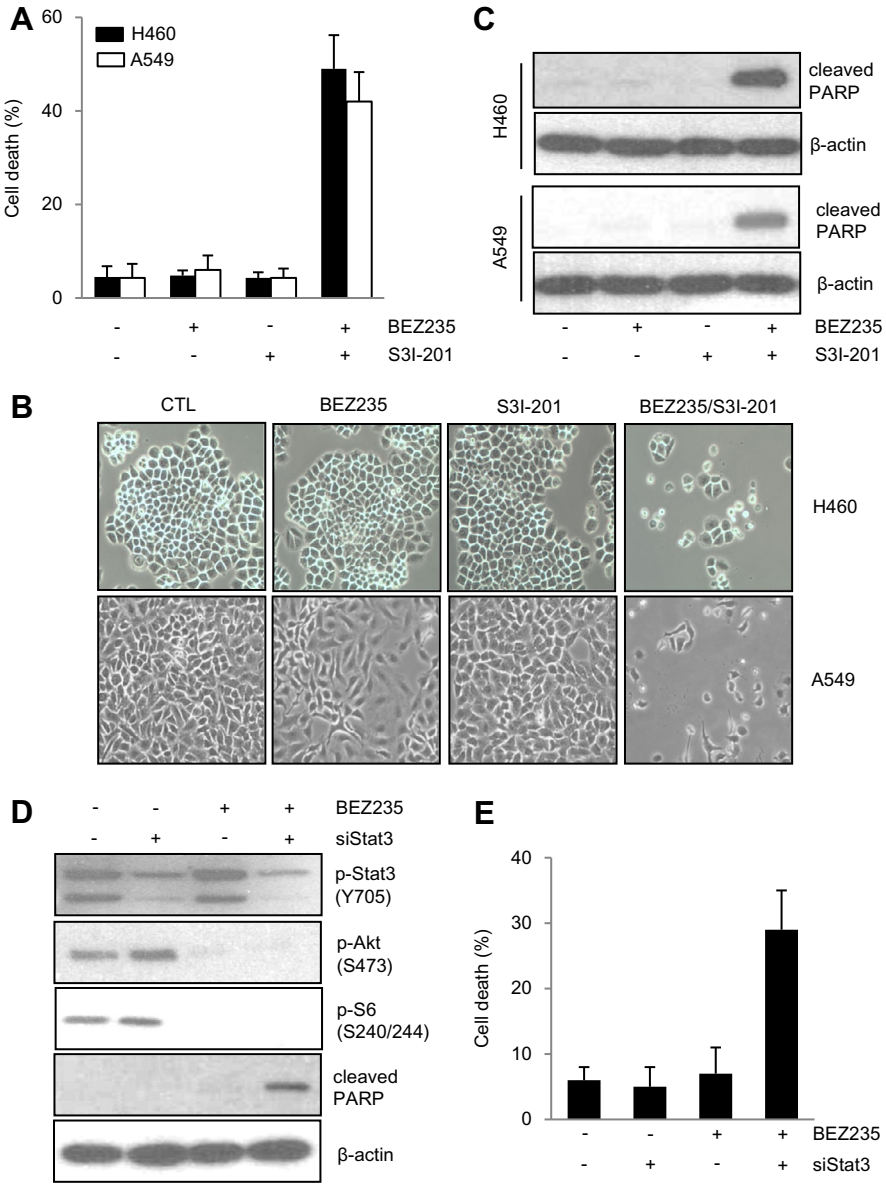

Fig. 2. Combination treatment with a Stat3 inhibitor and BEZ235 induces cell death. (A–C) H460 and A549 cells were treated with 100 nM S3I-201 and/or 300 nM BEZ235 for 24 h. (D and E) A549 cells were transfected with control or Stat3 siRNA for 16 h, followed by treatment with 300 nM BEZ235 for 24 h. Cell death was evaluated via flow cytometry after Annexin V and PI staining (A and E). Data are presented as means of triplicate samples and error bars reflect SD. Cell morphology images were obtained under a microscope (B). The indicated protein levels were estimated using Western blot analysis (C and D). The blot is representative of three independent experiments. CTL; control.

expression in A549 cells and consistently led to enhancement of BEZ235-induced cell death and PARP cleavage (Fig. 2D and E). Clearly, targeting of Stat3 activation effectively enhances cell sensitivity to BEZ235 in non-small cell lung cancer.

3.3. Dual inhibition of PI3K and mTOR potentiates cell death induced by the Stat3 inhibition

To elucidate the specific roles of PI3K and mTOR in cell death induced by Stat3 inhibition, we treated cells with LY294002 (PI3K inhibitor) and rapamycin (mTOR inhibitor) in H460 cells with S3I-201. LY294002 suppressed Akt and S6 phosphorylation (Fig. 3A), signifying inhibition of both PI3K and mTOR kinase at the same concentration. The combination of LY294002 and S3I-201 significantly enhanced cell death (Fig. 3B and C). Interestingly, however, co-treatment with rapamycin and S3I-201 led to only slight induction of cell death (below 10%; Fig. 3D), although rapamycin inhibited mTOR activity, as evident from S6 phosphorylation (Fig. 3A). Our data suggest that Stat3 inhibition-potentiated cell death is mediated via simultaneous blockage of mTOR and PI3K activities.

3.4. CHOP and its downstream target, Bim, are implicated in cell death induced by BEZ235/S3I-201

To determine the mechanisms underlying induction of cell death by the BEZ235 and S3I-201 combination, we examined expression of CHOP and its downstream target genes, Bim and DR4/5, using RT-PCR. As shown in Fig. 4A, expression levels of CHOP mRNA were markedly increased upon treatment with both S3I-201 and BEZ235, compared to S3I-201 or BEZ235 alone. To evaluate the effects of CHOP upregulation in response to BEZ235/S3I-201, its expression was silenced with specific siRNA, followed by BEZ235/S3I-201 co-treatment in A549 cells. Knockdown of CHOP led to significant reduction in the expression of downstream target genes, Bim, and DR4 (Fig. 4B), and suppression of cell death and PARP cleavage induced by BEZ235/S3I-201 (Fig. 4C). Furthermore, knockdown of Bim suppressed cell death induced by BEZ235/S3I-201 (Fig. 4D). These results indicate that CHOP-dependent Bim upregulation plays a role in cell death induced by BEZ235 and S3I-201.

We further investigated whether upregulation of DR4 by BEZ235/S3I-201 sensitized cells to TRAIL-mediated cell death. As shown in Fig. 4E, BEZ235/S3I-201 enhanced cell death to TRAIL, whereas TRAIL alone did not induce cell death. To verify the effects of DR4 on cell death by BEZ235/S3I-201, its expression was silenced with specific siRNA, followed by BEZ235/S3I-201/TRAIL treatment in A549 cells. As shown in Fig. 4F, DR4 siRNA led to considerable reduction in DR4 mRNA expression and inhibition of cell death induced by BEZ235/S3I-201/TRAIL. These results suggest that combined BEZ235/S3I-201 treatment sensitizes cells to TRAIL-induced apoptosis to a greater extent in non-small cell lung cancer. Moreover, this process is mediated via CHOP-dependent DR4 upregulation.

4. Discussion

Deregulation of PI3K/AKT/mTOR is considered to play a critical role in cancer progression and oncogenesis [3–6]. mTOR is one of

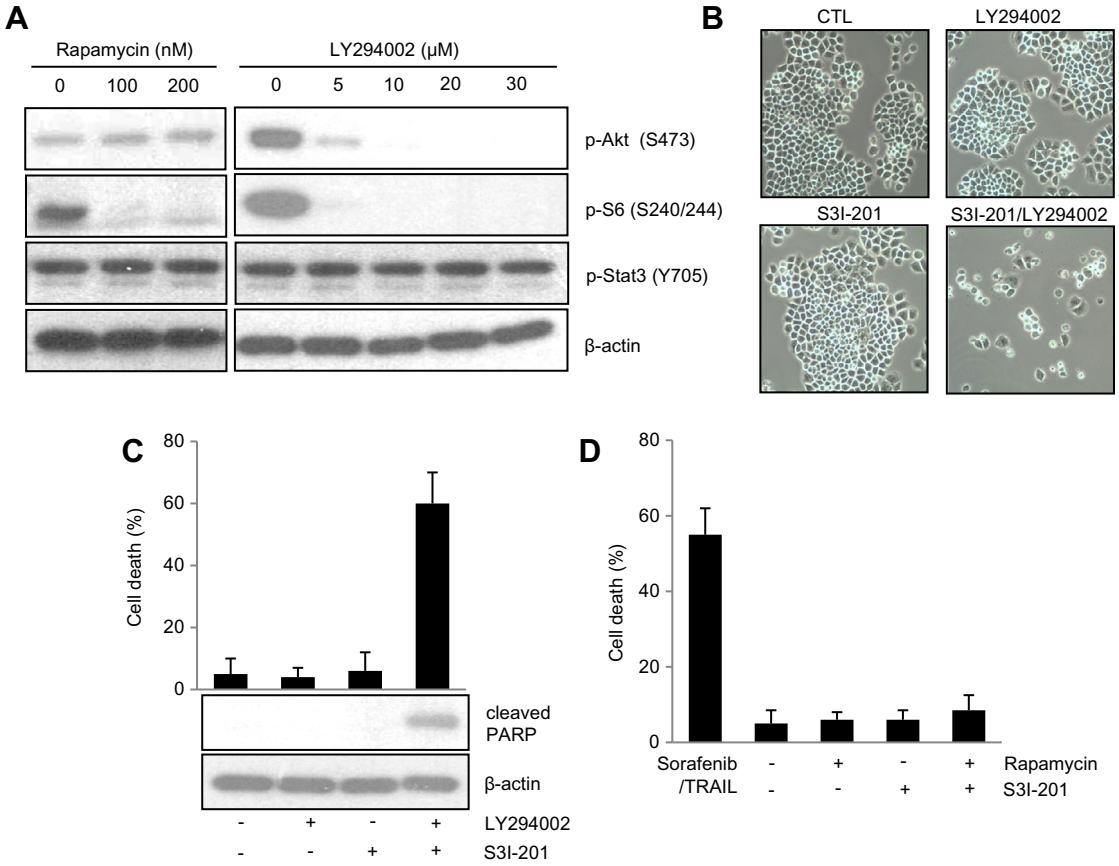

Fig. 3. The Stat3 inhibitor, S3I-201, potentiates cell death induced by LY294002, but not rapamycin. (A) H460 cells were treated with the indicated concentration of rapamycin or LY294002 for 24 h. (B and C) H460 cells were treated with 100 nM S3I-201 and/or 5 μM LY294002 for 24 h. (D) H460 cells were treated with 100 nM S3I-201 and/or 200 nM rapamycin for 24 h. The indicated protein levels were estimated using Western blot (A and C). Cell morphology images were obtained under a microscope (B). Cell death was evaluated via flow cytometry after Annexin V and PI staining (C and D). Data are presented as means of triplicate samples, and error bars reflect SD. As a positive control, H460 cells were treated with 5 μM sorafenib and 50 ng/mL TRAIL for 24 h.

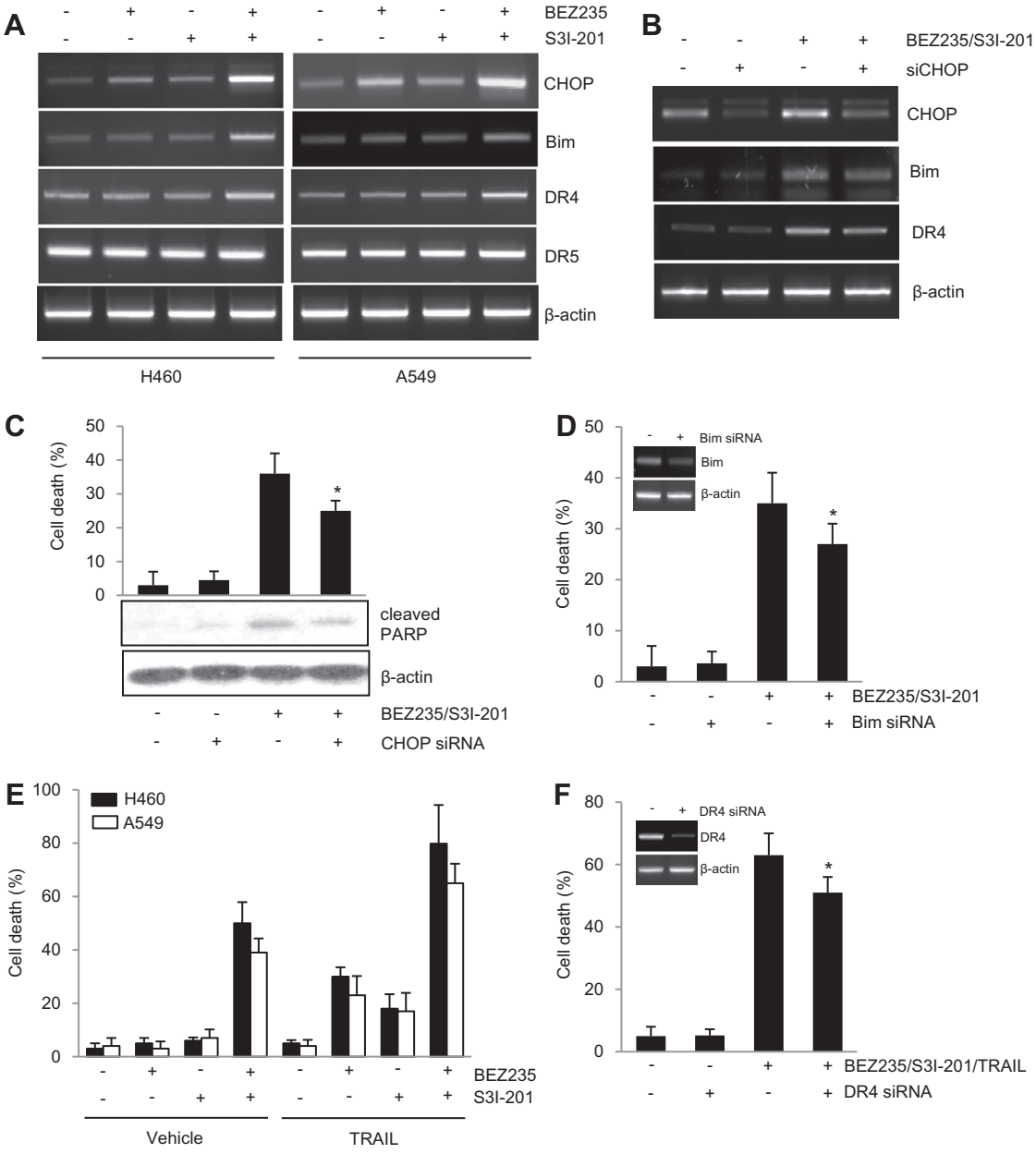

Fig. 4. Combination treatment with BEZ235 and S3I-201 leads to cell death via CHOP-induced Bim activity. (A) H460 and A549 cells were treated with 100 nM S3I-201 and/or 300 nM BEZ235 for 24 h. (B and C) A549 cells were transfected with control or CHOP siRNA for 16 h, followed by treatment with 300 nM BEZ235 and 100 nM S3I-201 for 24 h. (D) A549 cells were transfected with control or Bim siRNA for 16 h, followed by treatment with 300 nM BEZ235 and 100 nM S3I-201. (E) H460 and A549 cells were treated with 50 ng/mL TRAIL or/and 300 nM BEZ235 or/and 100 nM S3I-201 for 24 h. (F) A549 cells were transfected with control or DR4 siRNA for 16 h, followed by treatment with 300 nM BEZ235/100 nM S3I-201/50 ng/mL TRAIL for 24 h. Cell death was evaluated via flow cytometry after Annexin V and PI staining (C–F). Data are presented as means of triplicate samples, and error bars reflect SD. The indicated protein levels were estimated using Western blot (C). The blot is representative of two independent experiments. The indicated mRNA levels were estimated using RT-PCR analysis (A, B, D and F). $^{*}p < 0.05$ versus the BEZ235/S3I-201-treated group. $^{*}p < 0.05$ versus the BEZ235/S3I-201/TRAIL-treated group.

the most promising targets for cancer therapy identified to date. However, in past clinical trials, the mTOR inhibitor, rapamycin, and its analogs (CCI-779, RAD001, AP23573) failed to achieve a significant therapeutic effect [8]. mTOR inhibitors enhance PI3K/AKT activity, and therefore promote resistance to cell death [18,19]. In a previous study by our group, mTOR inhibition induced by Redd1 promoted phosphorylation of Akt at Ser473 in lung cancer cells, indicating that mTOR inhibition triggers Akt activation [20]. Therefore, dual targeting of mTOR and PI3K/Akt was subsequently proposed as an effective approach for cancer therapy. BEZ235 is an orally administered dual PI3K/mTOR inhibitor [9]. Previous studies have shown antitumor activity of BEZ235 on several cancer types, such as lung cancer, glioma cancer, neuroendocrine tumor, breast cancer, melanoma and pancreatic cancer [21–25]. However, while BEZ235 completely inhibited phosphorylation of Akt and S6, cell death was induced at a minimal level (<10%) in NSCLC, as determined using Annexin V/PI staining (Fig. 1), implying that other survival signaling pathways are involved in the response to BEZ235. In the present study, co-treatment with a Stat3 inhibitor and BEZ235 effectively induced apoptosis in NSCLC cells. Mechanistically, Stat3 inhibition, in combination with BEZ235, resulted in upregulation of CHOP and its downstream target gene, Bim, at the transcriptional level (Fig. 4). Downregulation of CHOP or Bim with specific siRNA led to significant reduction in cell death, suggesting that these

factors are required for BEZ235-mediated sensitization to the effects of Stat3 inhibition. Our results suggest that blockage of Stat3 presents an effective strategy to overcome BEZ235 resistance in NSCLC.

Additionally, suppression of Stat3 potentiated LY294002-induced cell death. LY294002 is a well-known PI3K inhibitor that blocks both PI3K and mTOR activity (Fig. 3). These data suggest that dual inhibition of PI3K/Akt and mTOR activities is necessary and sufficient to further enhance cell sensitivity to Stat3 inhibition. Several studies have described the inhibitory effects of Stat3 on tumor suppressor genes, suggesting that additional mechanisms exist by which Stat3 promotes cancer cell survival and suppresses apoptosis [26–28]. Inhibition of Stat3 induced breast cancer cell death via activation of proapoptotic genes, including CHOP, ERG2, NR4A2, PHLDA, and GADD45a [29]. Moreover, we observed a slight increase in CHOP mRNA in response to S3I-201, a Stat3 inhibitor (Fig. 4). Treatment with a combination of S3I-201 and BEZ235 led to significantly increased levels of CHOP and its downstream genes, Bim and DR4, compared to either single agent alone, in the lung cancer cell lines, H460 and A549 (Fig. 4). Previous studies reported that proapoptotic BH3-only proteins, Bim or puma, are required for CHOP-induced cell death [30–32]. However, we did not observe changes in puma levels in cells subjected to BEZ235/S3I-201 treatment (data not shown). Silencing of CHOP suppressed the expression of Bim and cell death by BEZ235/S3I-201. Additionally, knockdown of Bim suppressed cell death induced by BEZ235/S3I-201. Based on these results, we suggest that CHOP-dependent Bim upregulation plays a role in NSCLC cell death induced by BEZ235/S3I-201. The findings that BEZ235/S3I-201 enhances TRAIL-induced cell death in NSCLC cells and knockdown of DR4 inhibits apoptosis induced by BEZ235/S3I-201/TRAIL further suggest that TRAIL sensitization by BEZ235/S3I-201 is mediated via CHOP-dependent DR4 upregulation. In summary, a combination of Stat3 inhibition and the PI3K/mTOR inhibitor, BEZ235, acts synergistically to promote NSCLC cell death. This synergistic effect presents a novel strategy to enhance the efficacy of PI3K- and/or mTOR-targeted cancer therapy.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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References

[1] T. Schmelzle, M.N. Hall, TOR, a central controller of cell growth, Cell 103 (2000) 253–262.
[2] S. Sudarsanam, D.E. Johnson, Functional consequences of mTOR inhibition, Curr. Opin. Drug Discov. Dev. 13 (2010) 31–40.
[3] C.B. Knobbe, V. Lapin, A. Suzuki, T.W. Mak, The roles of PTEN in development, physiology and tumorigenesis in mouse models: a tissue-by-tissue survey, Oncogene 27 (2008) 5398–5415.
[4] A. Efeyan, D.M. Sabatini, MTOR and cancer: many loops in one pathway, Curr. Opin. Cell Biol. 22 (2010) 169–176.
[5] L. Salmena, A. Carracedo, P.P. Pandolfi, Tenets of PTEN tumor suppression, Cell 133 (2008) 403–414.
[6] R. Hill, J.H. Calvopina, C. Kim, Y. Wang, D.W. Dawson, T.R. Donahue, S. Dry, H. Wu, PTEN loss accelerates KrasG12D-induced pancreatic cancer development, Cancer Res. 70 (2010) 7114–7124.
[7] H. Pópulo, J.M. Lopes, P. Soares, The mTOR signalling pathway in human cancer, Int. J. Mol. Sci. 13 (2012) 1886–1918.
[8] S.A. Wander, B.T. Hennessy, J.M. Slingerland, Next-generation mTOR inhibitors in clinical oncology: how pathway complexity informs therapeutic strategy, J. Clin. Invest. 121 (2011) 1231–1241.
[9] S.M. Maira, F. Stauffer, J. Brueggen, P. Furet, C. Schnell, C. Fritsch, S. Brachmann, P. Chène, A. De Pover, K. Schoemaker, D. Fabbro, D. Gabriel, M. Simonen, L. Murphy, P. Finan, W. Sellers, C. García-Echeverría, Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity, Mol. Cancer Ther. 7 (2008) 1851–1863.
[10] G. Konstantinidou, E.A. Bey, A. Rabellino, K. Schuster, M.S. Maira, A.F. Gazdar, A. Amici, D.A. Boothman, P.P. Scaglioni, Dual phosphoinositide 3-kinase/mammalian target of rapamycin blockade is an effective radiosensitizing strategy for the treatment of non-small cell lung cancer harboring K-RAS mutations, Cancer Res. 69 (2009) 7644–7652.
[11] J. Tan, Q. Yu, Molecular mechanisms of tumor resistance to PI3K–mTOR-targeted therapy, Chin. J. Cancer 32 (2013) 376–379.
[12] B.B. Aggarwal, G. Sethi, K.S. Ahn, S.K. Sandur, M.K. Pandey, A.B. Kunnumakkara, B. Sung, H. Ichikawa, Targeting signal-transducer-and-activator-of-transcription-3 for prevention and therapy of cancer: modern target but ancient solution, Ann. NY. Acad. Sci. 1091 (2006) 151–169.
[13] B.B. Aggarwal, A.B. Kunnumakkara, K.B. Harikumar, S.R. Gupta, S.T. Taharakan, C. Koca, S. Dey, B. Sung, Signal transducer and activator of transcription-3, inflammation, and cancer: how intimate is the relationship?, Ann NY. Acad. Sci. 1171 (2009) 59–76.
[14] H.N. Choi, H.O. Jin, J.H. Kim, S.E. Hong, H.A. Kim, E.K. Kim, J.K. Lee, I.C. Park, W.C. Noh, Inhibition of S6K1 enhances glucose deprivation-induced cell death via downregulation of anti-apoptotic proteins in MCF-7 breast cancer cells, Biochem. Biophys. Res. Commun. 432 (2013) 123–128.
[15] H.O. Jin, Y.H. Lee, H.A. Kim, E.K. Kim, W.C. Noh, Y.S. Kim, C.S. Hwang, J.I. Kim, Y.H. Chang, S.I. Hong, Y.J. Hong, I.C. Park, J.K. Lee, Inhibition of vacuolar H+ ATPase enhances sensitivity to tamoxifen via up-regulation of CHOP in breast cancer cells, Biochem. Biophys. Res. Commun. 437 (2013) 463–468.
[16] R. Gogada, N. Yadav, J. Liu, S. Tang, D. Zhang, A. Schneider, A. Seshadri, L. Sun, C.M. Aldaz, D.G. Tang, D. Chandra, Bim, a proapoptotic protein, up-regulated via transcription factor E2F1-dependent mechanism, functions as a prosurvival molecule in cancer, J. Biol. Chem. 288 (2013) 368–381.
[17] Y.S. Kim, H.O. Jin, S.K. Seo, S.H. Woo, T.B. Choe, S. An, S.I. Hong, S.J. Lee, K.H. Lee, I.C. Park, Sorafenib induces apoptotic cell death in human non-small cell lung cancer cells by down-regulating mammalian target of rapamycin (mTOR)-dependent survivin expression, Biochem. Pharmacol. 82 (2011) 216–226.
[18] O.J. Shah, Z. Wang, T. Hunter, Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies, Curr. Biol. 14 (2004) 1650–1656.
[19] A. Carracedo, L. Ma, J. Teruya-Feldstein, F. Rojo, L. Salmena, A. Alimonti, A. Egia, A.T. Sasaki, G. Thomas, S.C. Kozma, A. Papa, C. Nardella, L.C. Cantley, J. Baselga, P.P. Pandolfi, Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer, J. Clin. Invest. 118 (2008) 3065–3074.
[20] H.O. Jin, S.E. Hong, J.H. Kim, H.N. Choi, K. Kim, S. An, T.B. Choe, C.S. Hwang, J.H. Lee, J.I. Kim, H.A. Kim, E.K. Kim, W.C. Noh, Y.J. Hong, S.I. Hong, J.K. Lee, I.C. Park, Sustained overexpression of Redd1 leads to Akt activation involved in cell survival, Cancer Lett. 336 (2013) 319–324.
[21] P. Cao, S.M. Maira, C. Garcia-Echeverria, D.W. Hedley, Activity of a novel, dual PI3-kinase/mTor inhibitor NVP-BEZ235 against primary human pancreatic cancers grown as orthotopic xenografts, Br. J. Cancer 100 (2009) 1267–1276.
[22] T.J. Liu, D. Koul, T. LaFortune, N. Tiao, R.J. Shen, S.M. Maira, C. Garcia-Echevrria, W.K. Yung, NVP-BEZ235, a novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor, elicits multifaceted antitumor activities in human gliomas, Mol. Cancer Ther. 8 (2009) 2204–2210.
[23] M.C. Manara, G. Nicoletti, D. Zambelli, S. Ventura, C. Guerzoni, L. Landuzzi, P.L. Lollini, S.M. Maira, C. García-Echeverría, M. Mercuri, P. Picci, K. Scotlandi, NVP-BEZ235 as a new therapeutic option for sarcomas, Clin. Cancer Res. 16 (2010) 530–540.
[24] D.C. Cho, M.B. Cohen, D.J. Panka, M. Collins, M. Ghebremichael, M.B. Atkins, S. Signoretti, J.W. Mier, The efficacy of the novel dual PI3-kinase/mTOR inhibitor NVP-BEZ235 compared with rapamycin in renal cell carcinoma, Clin. Cancer Res. 16 (2010) 3628–3638.
[25] C. Santiskulvong, G.E. Konecny, M. Fekete, K.Y. Chen, A. Karam, D. Mulholland, C. Eng, H. Wu, M. Song, O. Dorigo, Dual targeting of phosphoinositide 3-kinase and mammalian target of rapamycin using NVP-BEZ235 as a novel therapeutic approach in human ovarian carcinoma, Clin. Cancer Res. 17 (2011) 2373–2384.
[26] G. Niu, K.L. Wright, Y. Ma, G.M. Wright, M. Huang, R. Irby, J. Briggs, J. Karras, W.D. Cress, D. Pardoll, R. Jove, J. Chen, H. Yu, Role of Stat3 in regulating p53 expression and function, Mol. Cell. Biol. 25 (2005) 7432–7440.
[27] G. Niu, K.H. Shain, M. Huang, R. Ravi, A. Bedi, W.S. Dalton, R. Jove, H. Yu, Overexpression of a dominant-negative signal transducer and activator of transcription 3 variant in tumor cells leads to production of soluble factors that induce apoptosis and cell cycle arrest, Cancer Res. 61 (2001) 3276–3280.
[28] O. Zhang, H.Y. Wang, M. Marzec, P.N. Raghunath, T. Nagasawa, M.A. Wasik, STAT3- and DNA methyltransferase 1-mediated epigenetic silencing of SHP-1

tyrosine phosphatase tumor suppressor gene in malignant T lymphocytes, Proc. Natl. Acad. Sci. USA 102 (2005) 6948–6953.
[29] O.A. Timofeeva, V. Gaponenko, S.J. Lockett, S.G. Tarasov, S. Jiang, C.J. Michejda, A.O. Perantoni, N.I. Tarasova, Rationally designed inhibitors identify STAT3 N-domain as a promising anticancer drug target, ACS Chem. Biol. 2 (2007) 799–809.
[30] A.P. Ghosh, B.J. Klocke, M.E. Ballestas, K.A. Roth, CHOP potentially co-operates with FOXO3a in neuronal cells to regulate PUMA and BIM expression in response to ER stress, PLoS One 7 (2012) e39586.
[31] H. Puthalakath, L.A. O'Reilly, P. Gunn, L. Lee, P.N. Kelly, N.D. Huntington, P.D. Hughes, E.M. Michalak, J. McKimm-Breschkin, N. Motoyama, T. Gotoh, S. Akira, P. Bouillet, A. Strasser, ER stress triggers apoptosis by activating BH3-only protein Bim, Cell 129 (2007) 1337–1349.
[32] Z. Galehdar, P. Swan, B. Fuerth, S.M. Callaghan, D.S. Park, S.P. Cregan, Neuronal apoptosis induced by endoplasmic reticulum stress is regulated by ATF4-CHOP-mediated induction of the Bcl-2 homology 3-only member PUMA, J. Neurosci. 30 (2010) 16938–16948.