# Inhibition of the phosphatidylinositol 3-kinase/Akt pathway sensitizes MDA-MB468 human breast cancer cells to cerulenin-induced apoptosis

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## Abstract

**Fatty acid synthase is overexpressed in cancer especially in tumors with a poor prognosis. The specific fatty acid synthase inhibitor cerulenin can induce apoptosis in cancer cells. Likewise, phosphatidylinositol 3-kinase (PI3K)/Akt kinase activities are elevated in primary tumors and cancer cell lines. Here, we tested whether inhibition of PI3K/Akt pathway would sensitize cancer cells to cerulenin-induced apoptosis. We show that LY294002, an inhibitor of PI3K, sensitized MDA-MB468 breast cancer cells to cerulenin-induced apoptosis. In MDA-MB468 cells, cerulenin- and LY294002-mediated apoptosis was associated with caspase-3 activation and the release of cytochrome *c* from mitochondria to cytosol. In addition, we observed additional species of Bak in mitochondria, suggesting a possible Bak activation. Treatment of cells with cerulenin and LY294002 down-regulated the protein levels of X chromosome-linked inhibitor of apoptosis (XIAP), cellular inhibitor of apoptosis 1 (cIAP-1), and Akt, whereas the levels of mitogen-activated protein/extracellular signal-regulated kinase kinase and other antiapoptotic Bcl-2 family proteins (Bcl-2 and Bcl-xl) did not change. Interestingly, the nonspecific caspase inhibitor, z-VAD-FMK, inhibited the down-regulation of Akt, XIAP, and cIAP-1 in cerulenin- and LY294002-treated cells. In conclusion, these studies show that inhibition of PI3K can sensitize cerulenin-induced apoptosis in MBA-MB468 breast cancer cells via activation of caspases, down-regulation of antiapoptotic proteins, such as XIAP, cIAP-1 and Akt, and possibly, activation of Bak in mitochondria. [Mol Cancer Ther 2006;5(3):494–501]**

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## Introduction

Fatty acid synthase (FAS), a key metabolic enzyme that catalyzes the synthesis of long-chain fatty acids, is highly expressed in a variety of human cancers, including cancers from breast, colon, ovary, lung, and prostate (1, 2). FAS is overexpressed at both protein and mRNA level in prostate carcinoma (2). In addition, FAS is also regulated at nontranscriptional level because FAS mRNA and protein levels are discordant in a subset of prostate cancer (3).

The preferential expression of FAS in cancer makes it an attractive target for anticancer therapy. The specific FAS inhibitor, cerulenin, is able to inhibit tumor growth and induce apoptosis in a variety of cancer cell lines (4–6). Other FAS inhibitors, such as C75 and orlistat, show antitumor activity *in vivo* and *in vitro* (7–9). The effect of FAS inhibitor on cancer is further verified by the RNA interference experiment, where down-regulation of FAS by RNA interference in LNCaP prostate cancer cells renders them to undergo apoptosis (10).

One of the characteristics of cancer cells is their ability to evade programmed cell death through the activation of phosphatidylinositol 3-kinase (PI3K)/Akt pathway (11, 12). PI3K/Akt kinase activities have been shown to be elevated in primary tumors and cancer cell lines, due to gene amplification, protein overexpression, or mutation of tumor suppressor gene PTEN (12). PI3K/Akt pathway is activated upon growth factor stimulation (13, 14). The products of PI3K, especially phosphatidylinositol-3,4,5 triphosphate, can bind to the PH domain of Akt (15, 16). The binding of phosphatidylinositol-3,4,5 triphosphate to PH domain of Akt targets the protein to membrane (17), where it can be phosphorylated and activated by PDK1 and putative PDK2 (18–21). Activated Akt phosphorylates and inhibits proapoptotic proteins, including Bad, caspase-9, and forkhead transcription factors (22–25), thereby inhibiting apoptosis.

Recently, a molecular connection between PI3K/Akt pathway and FAS has been established (26–28). Activation of the PI3K/Akt pathway results in the overexpression of FAS through transcriptional regulation of FAS promoter, whereas inhibition of PI3K either by reintroduction of PTEN or by treatment with LY294002 dramatically reduces FAS protein levels in LNCaP prostate cancer cells (26). In addition, one of the HER2-regulated genes is *FAS* and HER2 mediates the induction of *FAS* through the PI3K/Akt pathway (27). On the other hand, inhibition of FAS down-regulates HER2/neu (erbB2) in HER2-overexpressing breast and ovarian cancer cells (28), indicating there is a bidirectional crosstalk between the FAS and HER2 pathways.

Although inhibition of PI3K/Akt pathway dramatically reduces the FAS protein levels and induces apoptosis in

LNCaP prostate cancer cells (26, 29), it is not sufficient for apoptosis induction in MDA-MB468 breast cancer cells. In this study, we investigated whether inhibition of PI3K/Akt pathway sensitizes FAS inhibitor–induced apoptosis in MDA-MB468 cells. Treatment with LY294002, an inhibitor of PI3K, increased the sensitivity to cerulenin-induced apoptosis in MDA-MB468 breast cancer cells. Cerulenin- and LY294002-mediated apoptosis was associated with caspase-3 activation and the release of cytochrome *c* from mitochondria to cytosol. Although the translocation of Bax from cytosol to mitochondria was not obvious, we did observe additional species of Bak in mitochondria, suggesting a possible Bak activation. Treatment of MDA-MB468 cells with cerulenin and LY294002 down-regulates the protein levels of Akt, X chromosome-linked inhibitor of apoptosis (XIAP), and cellular inhibitor of apoptosis 1 (cIAP-1) in a caspase-dependent manner, whereas the levels of mitogen-activated protein/extracellular signal-regulated kinase kinase and other antiapoptotic Bcl-2 family proteins (Bcl-2, Bcl-xl, and Mcl-1) did not change.

## Materials and Methods

### Chemicals

LY294002 and cerulenin were purchased from Calbiochem (San Diego, CA) whereas others were from Sigma (St. Louis, MO). Protein concentration was determined using BCA method (Pierce, Rockford, IL).

### Cell Lines

MDA-MB468 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were cultured under the conditions provided by the suppliers.

### Western Blot Analysis

Cells from 10 cm dishes were harvested and lysed in 200 μL buffer B [20 mmol/L HEPES (pH 7.5), 10 mmol/L NaCl, 20 mmol/L NaF, 1 mmol/L EDTA, 1 mmol/L EGTA, 5 mmol/L sodium PPi, 2 mmol/L sodium vanadate, 10 mmol/L β-glycerolphosphate, and 1% NP40] on ice for 30 minutes. The samples were centrifuged at 12,000 × *g* at 4°C for 10 minutes. Cell lysates were subjected to SDS-PAGE gel electrophoresis and Western analysis. Rabbit anti-Akt, anti-phospho-Akt (S473-P), anti-phospho–glycogen synthase kinase 3α/β, anti-phospho-FKHRL1, anti-phospho-p44/42 mitogen-activated protein kinase, anti-p44/42 mitogen-activated protein kinase, and anti-XIAP antibodies were purchased from Cell Signaling (Beverly, MA). Anti-FAS antibody was from BD Biosciences (San Jose, CA). Antiactin, anti-Bak (G-23), anti-Bak (N-20), anti-Bax, anti-Bcl2, and anti-Bcl-xl antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-cIAP-1 antibody was from R&D Systems (Minneapolis, MN). Immunoblot analysis was done using enhanced chemiluminescence detection reagents (Amersham, Piscataway, NJ) according to the instruction of the vendor.

### Flow Cytometry Analysis

Cells were harvested by pooling attached and detached cells and pelleted with centrifugation at 800 × *g* for 5 minutes at 4°C. The cells were washed with PBS and resuspended in 0.5 mL ice-cold staining solution (50 μg/mL propidium iodide, 40 units/mL RNase A, and 0.25% Triton X-100, in PBS). After incubated for 1 hour at 4°C in the dark, the cell cycle distribution was analyzed using a Becton Dickinson ExCalibur Flow Cytometer (Becton Dickinson, San Jose, CA). For flow cytometry analysis of control and apoptotic population using antiactive caspase-3 antibodies, cells were harvested, permeabilized, fixed, and stained for active caspase-3 (phycoerythrin-conjugated) as described in the Active Caspase-3 PE Staining Protocol provided by the manufacturer (BD Biosciences).

### Preparation of Cytosolic Fractions and Mitochondria Fractions from MDA-MB468 Cells

The isolation of cytosolic fractions from MDA-MB468 cells was carried out as described (30). Briefly, the cells were harvested and washed with ice-cold PBS and resuspended in 5 volumes of buffer A [20 mmol/L HEPES (pH 7.5), 10 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 1 mmol/L sodium EDTA, 1 mmol/L sodium EGTA, 1 mmol/L DTT, and 0.1 mmol/L phenylmethylsulfonyl fluoride] containing 250 mmol/L sucrose. The cells were homogenized with 10 strokes of a Teflon homogenizer. The homogenates were centrifuged twice at 750 × *g* for 10 minutes at 4°C. The supernatant was further centrifuged at 100,000 × *g* for 1 hour at 4°C, and the resulting supernatant was designated as cytosolic fraction, whereas the resulting pellet was designated as mitochondria fraction.

## Results

### LY294002 Increases Cerulenin-Induced Apoptosis in MDA-MB468 Cells

FAS expression is totally dependent on the activity of PI3K/Akt pathway in PTEN-negative LNCaP prostate cancer cells where LY294002 completely abolishes FAS protein expression (26). We tested the effect of LY294002 in PTEN-negative MDA-MB468 cells (31). Shown in Fig. 1A, LY294002 dramatically inhibited the phosphorylation of Akt. LY294002 also inhibited the phosphorylation of a downstream target of Akt, FKHRL1, in MDA-MB468 cells (Fig. 1A). Partial inhibition of glycogen synthase kinase 3α/β phosphorylation by LY294002 was observed at high concentration in MDA-MB468 cells (Fig. 1A), indicating that phospho-FKHRL1 was a better biochemical marker for inhibition of Akt activity in the experiment. However, FAS level was only partially reduced by LY294002 in MDA-MB468 cells (Fig. 1B). In addition, unlike the case in LNCaP cells, LY294002 did not induce apoptosis in MDA-MB468 cells after 24-hour treatment (Fig. 2A), although it inhibited the phosphorylation of Akt and the phosphorylation of FKHRL1 (Fig. 1A).

Because LY294002 partially reduced the FAS level in MBA-468 cells, we reasoned that there could be a synergy in inducing apoptosis when LY294002 was combined with FAS inhibitor cerulenin. Shown in Fig. 2A, treatment of MDA-MB468 with 2.5, 5, or 10 μg/mL cerulenin induced 3.4%, 15%, and 6.5% cell death, respectively, whereas LY294002 treatment alone resulted in no significant

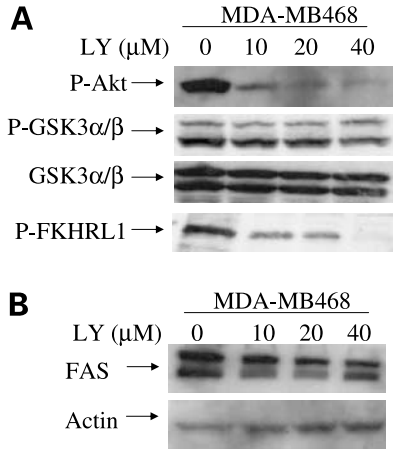


**Figure 1.** LY294002 inhibits phospho-Akt levels in MDA-MB468 cells. MDA-MB468 cells were treated with LY294002 at the indicated concentrations for 2 h (**A**) or 24 h (**B**) in DMEM with 0.1% fetal bovine serum (FBS). The cells were harvested and 50 μg of the cell lysate was used for Western blot analysis of phospho-Akt, phospho-FKHRL1, phospho–glycogen synthase kinase 3α/β, GSK3α/β, and FAS as described in Materials and Methods.

apoptosis. Cotreatment with 20 μmol/L LY294002 and 2.5, 5, or 10 μg/mL cerulenin induced 20%, 36%, and 33% cell death, respectively, as indicated by the sub-$G_1$ population according to fluorescence-activated cell sorting analysis (Fig. 2A). Similar phenomena were observed when 40 μmol/L LY294002 was used in the experiment (Fig. 2A). The sub-$G_1$ population decreased in MDA-MB468 cells when high cerulenin concentration (10 μg/mL) was used in the experiment (Fig. 2A). This was probably due to the reason that very late stage apoptotic cells were lost in the centrifugation step during the sample preparation for fluorescence-activated cell sorting. The synergistic effect on the induction of apoptosis by cerulenin and LY294002 was confirmed in immuno–flow cytometry using anti-active caspase-3 antibody (Fig. 2B).

### Cotreatment of LY294002 and Cerulenin in MDA-MB468 Cells Induces Caspase-3 Activation and Cytochrome *c* Release from Mitochondria to Cytosol

One of the biochemical hallmarks of apoptosis is the activation of caspases. Therefore, we carried out Western blot analysis on caspase-3 in MBA-MB468 cells treated with cerulenin alone, LY294002 alone, or the combination for 24 hours. Caspase-3 was activated at high concentration of cerulenin (10 μg/mL) and in conditions when low concentrations of cerulenin (2.5 and 5 μg/mL) were combined with LY294002 in MDA-MB468 cells (Fig. 3A). LY294002 itself did not induce caspase-3 activation (Fig. 3A). In a time course experiment, caspase-3 was activated 6 hours earlier in MDA-MB468 cells treated with the combination of cerulenin and LY294002 than that with cerulenin treatment alone (Fig. 3B).

To elucidate the molecular mechanism of the apoptosis induced by the combination of cerulenin and LY294002, we isolated cytosolic fractions and mitochondrial fractions of MBA-MD468 cells. Shown in Fig. 4A, cytochrome *c* was present in cytosolic fractions when MDA-MB468 cells were treated with either high concentration of cerulenin (10 μg/mL) or the combinations of low concentrations of cerulenin (2.5 and 5 μg/mL) with LY294002. The release of cytochrome *c* from mitochondria to cytosol correlated well with caspase-3 activation (Figs. 3A and 4A). We also carried out Western blot analysis in the mitochondria fractions to see whether there was a translocation of Bax or Bak from cytosol to mitochondria. Shown in Fig. 4B, the accumulation of Bax or Bak was not quite obvious. However, there were additional species of Bak that appeared in conditions where the release of cytochrome *c* was observed (Fig. 4A and B). We suspected that these may represent activated Bak in mitochondria. The nonspecific cross-reacting protein X with Bak antibody indicated the equal loading of the proteins.

The Bak antibody used in Fig. 4 is generated using a COOH-terminal Bak peptide [anti-Bak (G-23); Santa Cruz Biotechnology]. To verify that the additional species were indeed Bak, we did Western blot analysis using another Bak antibody that recognizes the $NH_2$-terminal of Bak [anti-Bak (N-20), Santa Cruz Biotechnology]. Both antibodies recognized Bak protein with same pattern: One new Bak band appeared below the main band in the cerulenin treatment and an additional band above appeared in the combination treatment of cerulenin and LY294002. The additional species of Bak may represent the activated Bak upon posttranslational modifications.

### Cotreatment of LY294002 and Cerulenin in MDA-MB468 Cells Resulted in Degradation of cIAP-1 and XIAP in a Caspase-Dependent Manner

Cotreatment of LY294002 with cerulenin did not result in significant changes in total protein levels of Bak, Bcl2, or Bcl-xl in MDA-MB468 cells, although the protein level of Bcl2 was decreased when 10 μg/mL cerulenin was included in the treatment. The protein levels of Bax were up-regulated slightly upon treatment with LY294002, but no further increase of Bax was observed in MDA-MB468 cells treated with cerulenin and LY294002 (Fig. 5A). The protein levels of cIAP-1 and XIAP were reduced when the cells were treated with low concentrations of cerulenin and LY294002, compared with those treated with cerulenin alone (Fig. 5A). Mcl1 protein was also degraded but the cotreatment did not enhance the degradation of Mcl1 further in MDA-MB468 cells (Fig. 5A).

To test whether the degradation of cIAP-1 and XIAP was due to caspase activation, we included the pan-caspase inhibitor Z-VAD-FMK in the treatment. As shown in Fig. 5B, the protein levels of XIAP, cIAP-1, and Akt were dramatically reduced in MDA-MB468 cells treated with cerulenin and LY294002 together. However, when Z-VAD-FMK was included in the treatment, the reduction in the protein levels of XIAP, cIAP-1, and Akt was attenuated (Fig. 5B). Addition of Z-VAD-FMK also prevented apoptosis. Thirty-five percent of cells were apoptotic when treated with 5 μg/mL cerulenin and 20 μmol/L LY294002.

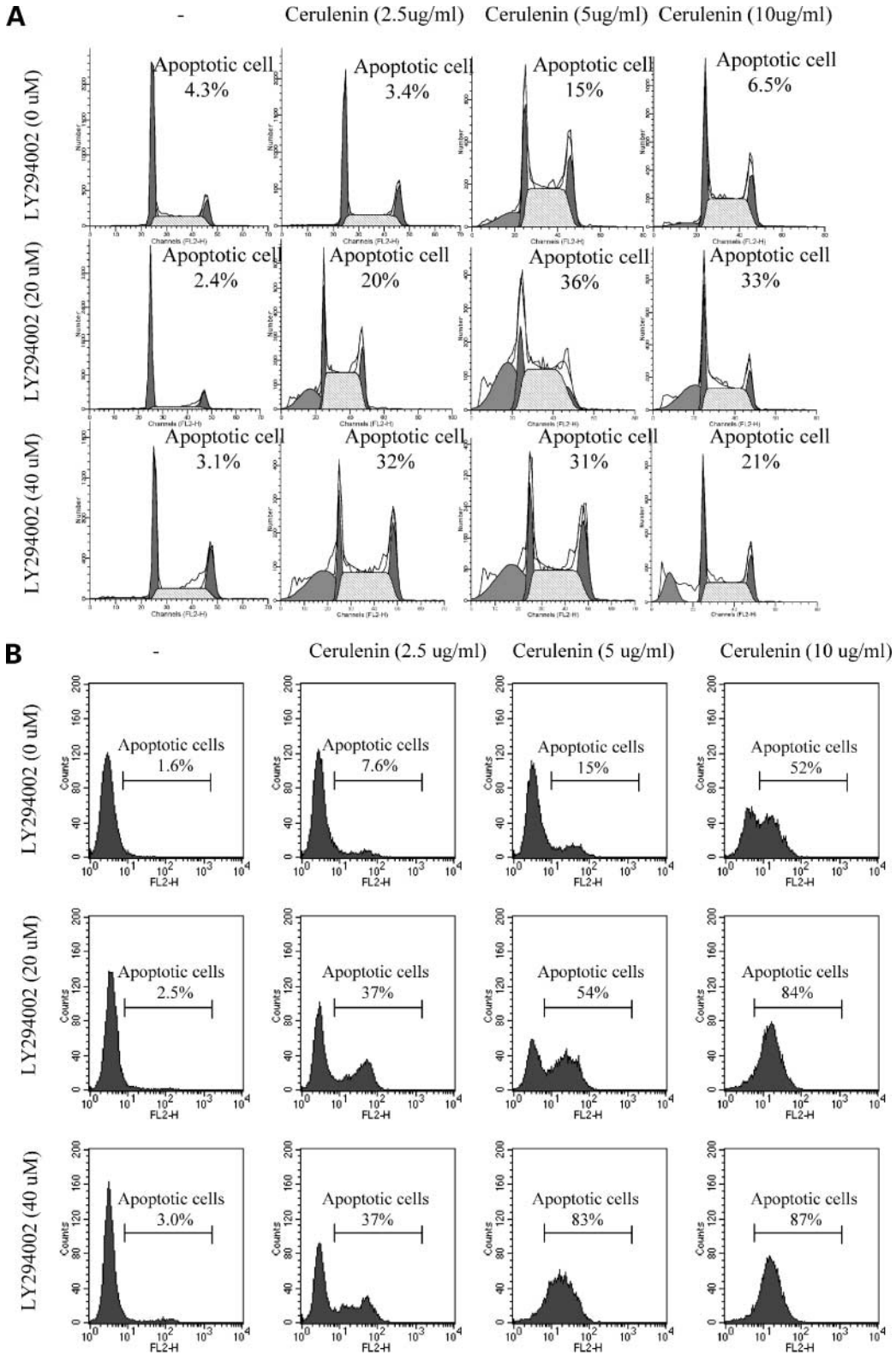


**Figure 2.** LY294002 sensitizes MDA-MB468 cells to cerulenin-induced apoptosis. **A** and **B,** MDA-MB468 cells were treated with cerulenin alone, LY294002 (20 μmol/L) alone, or the combination of cerulenin and LY294002 at the indicated concentrations for 24 h in DMEM with 0.1% FBS. Cells were harvested and fluorescence-activated cell sorting analysis was carried out using propidium iodide (**A**) or using antiactive caspase-3 antibodies (**B**) as described in Materials and Methods. Representative of two independent experiments.

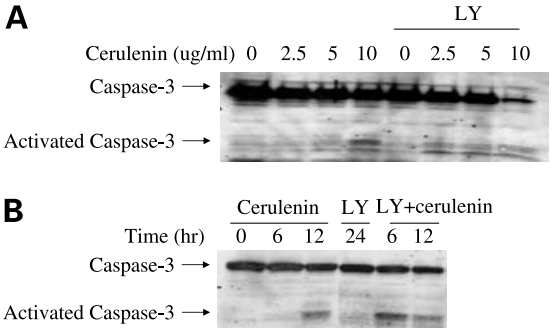


**Figure 3.** Cotreatment with LY294002 and cerulenin induces caspase activation in MDA-MB468 cells. **A**, MDA-MB468 cells were treated with cerulenin (2.5, 5, and 10 μg/mL) alone, LY294002 (20 μmol/L) alone, or the combination for 24 h in DMEM with 0.1% FBS. **B**, MDA-MB468 cells were treated with cerulenin (10 μg/mL) alone, LY294002 (20 μmol/L) alone, or the combination for the indicated times in DMEM with 0.1% FBS for the indicated time periods. The cells were harvested and 50 μg of the cell lysate were used for Western blot analysis of caspase-3 as described in Materials and Methods.

Addition of Z-VAD-FMK was able to reduce the apoptotic population to 1.7%, which is comparable with the treatment with DMSO alone (1.4%) or Z-VAD alone (0.55%) as indicated by the sub-$G_1$ population in the fluorescence-activated cell sorting analysis (Fig. 5C). Similar results were obtained in the immuno–flow cytometry analysis of using antiactive caspase-3 antibody (Fig. 5D).

### Cotreatment of LY294002 and Cerulenin in MDA-MB468 Cells Resulted in the Degradation of the Akt Protein and the Reduction of Phosphorylation of FKHRL1

Although phospho-Akt level was dramatically reduced in MDA-MB468 cells treated with LY294002 alone, it was further reduced in the cotreatment of LY294002 and cerulenin (Fig. 6). Examination of the total Akt protein levels indicated that Akt protein was also degraded upon the cotreatment (Fig. 6). Cotreatment with LY294002 and cerulenin also further reduced the phospho-FKHRL1 levels, correlating well with the phospho-Akt levels. FAS levels were also further reduced by LY294002. As a control, the total protein levels of p44/42 did not change when cotreatment of LY294002 with cerulenin (2.5 or 5 μg/mL) was compared with cerulenin alone, although treatment with LY294002 also reduced the phospho-p44/42 levels (Fig. 6). Therefore, the combination effect of cerulenin and LY294002 on Akt activity could also underlie the mechanism of synergy between the two agents in apoptosis induction.

## Discussion

Cancer is a disease that is characterized by multiple genetic defects, epigenetic changes, and plasticity in response to its environment. Multitarget therapy is thus necessary to achieve optimal efficacy. We have shown the potentiation of cerulenin-induced apoptosis by LY294002, providing a scientific rationale for a combination therapy of a FAS inhibitor and PI3K/Akt pathway inhibitor.

The breast cancer MDA-MB468 cells contain PTEN mutations, which result in high levels of Akt phosphorylation and activity (Fig. 1). FAS expression is totally dependent on the activity of PI3K/Akt pathway in LNCaP prostate cancer cells where LY294002 completely abolishes FAS protein expression (26). However, FAS level is only partially reduced by inhibition of PI3K/Akt pathway in MDA-MB468 breast cancer cells (Fig. 1). In addition, LY294002 is sufficient to induce apoptosis in LNCaP prostate cancer cells by itself (26, 29), whereas it is insufficient in MDA-MB468 breast cancer cells (Figs. 2 and 3), suggesting the important contribution of both Akt activity and FAS function to cell survival in these cells.

The Bcl2 family of proapoptotic and antiapoptotic proteins play critical role in apoptosis (32). Bax and Bak are multidomain proapoptotic proteins that are key components of the intrinsic cell death pathway (33, 34). Bax is a cytosolic protein that undergoes conformational change and translocates from cytosol to mitochondria in response to select apoptotic stimulations (35, 36). On the contrary, Bak is an integral mitochondrial membrane protein whose activity is inhibited by VDAC2 in viable

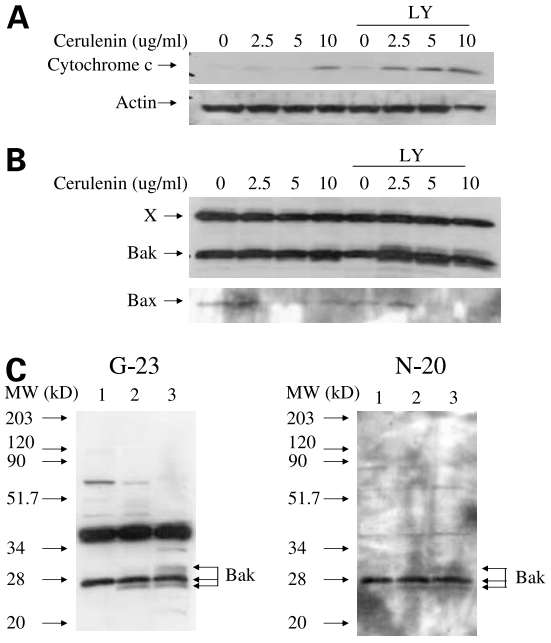


**Figure 4.** Cotreatment with LY294002 and cerulenin induces cytochrome *c* release from mitochondria to cytosol in MDA-MB468 cells. **A** and **B**, MDA-MB468 cells were treated with the indicated concentrations of cerulenin alone, LY294002 (20 μmol/L) alone, or the combination for 24 h in DMEM with 0.1% FBS. Cytosolic fractions (**A**) and mitochondrial fractions (**B**) were prepared and Western blot analysis was carried out using antibodies against cytochrome *c* Bax and Bak as described in Materials and Methods. The nonspecific cross-reacting protein X with Bak antibody indicated the equal loading of the proteins. **C**, the mitochondria fractions are isolated from MDA-MB468 cells treated with DMSO (*lane 1*), cerulenin (10 μg/mL; *lane 2*), or cerulenin (5 μg/mL) and LY294002 (20 μmol/L; *lane 3*) for 24 h. Fifty micrograms of the lysate were used for Western blot analysis using two different anti-Bak antibodies [anti-Bak (G-23) and anti-Bak (N-20)].

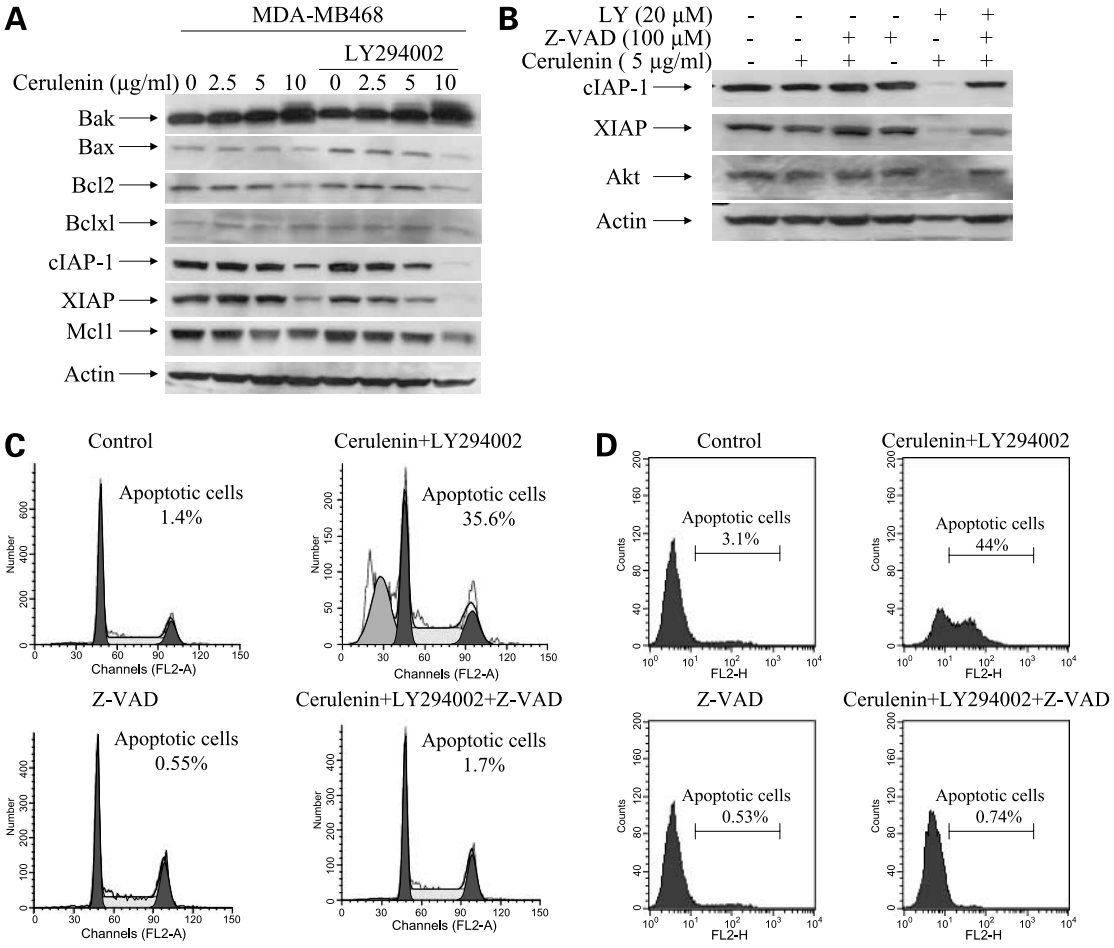


**Figure 5.** Cotreatment with LY294002 and cerulenin induces the degradation of XIAP and c-IAP-1 in MDA-MB468 cells in a caspase-dependent manner. **A**, MDA-MB468 cells were treated with the indicated concentrations of cerulenin alone, LY294002 (20 μmol/L) alone, or the combination for 24 h in DMEM with 0.1% FBS. The cells were harvested and cell extracts were prepared as described in Materials and Methods. Fifty micrograms of cell extracts were loaded onto 10% SDS ready gel and Western blot analysis was carried out using antibodies against Bak, Bax, Bcl2, Bcl-xl, c-IAP-1, XIAP, Mcl1, and actin as described in Materials and Methods. **B**, MDA-MB468 cells were treated with cerulenin (5 μg/mL) alone, Z-VAD (100 μmol/L) alone, the combination of cerulenin and Z-VAD, the combination of LY294002 and cerulenin, or the combination of LY294002, cerulenin, and Z-VAD for 24 h in DMEM with 0.1% FBS. The cells were harvested and cell extracts were prepared as described in Materials and Methods. Fifty micrograms of cell extracts were loaded onto 10% SDS ready gel and Western blot analysis was carried out using antibodies against c-IAP-1, XIAP, Akt, and actin. **C** and **D**, MDA-MB cells were treated with DMSO or 100 μmol/L Z-VAD for 1 h, followed by the treatment with DMSO or 5 μg/mL cerulenin and 20 μmol/L LY294002 for 24 h. Cells were harvested and stained with either propidium iodide (**C**) or antiactive caspase-3 antibody (**D**) as described in Materials and Methods. Representative of three independent experiments.

cells (37). Activated Bax and Bak undergo homo-oligomerization, which results in the permeabilization of the mitochondria outer membrane and the release of proteins, including cytochrome *c* from mitochondria to cytosol, which, in turn, triggers the activation of caspase cascade (38–40). It has been shown that mitochondria play a key role in cerulenin-induced apoptosis in many cancer cell lines (4), where induction of Bax expression correlates with the extent of apoptosis and seems to be regulated in a p53-independent manner. Our data suggest that mitochondria also play critical role in the sensitization of MDA-MB468 cells to cerulenin-induced apoptosis by LY294002. Although the induction of Bax expression and translocation of Bax from cytosol to mitochondria were not obvious in MDA-MB468 cells cotreated with cerulenin and LY294002, the appearance of the additional species of Bak was observed in apoptotic MDA-MB468 cells using two antibodies that recognize the different regions of Bak protein (Fig. 4C). This suggests that Bak may be activated in mitochondria, which, in turn, may be responsible for the cytochrome *c* release from mitochondria to cytosol. However, further investigation is necessary to characterize the role of Bak in regulating cytochrome *c* release and the molecular nature of the additional species of Bak.

In addition, cotreatment of MDA-MB468 cells with LY294002 and low concentration of cerulenin also results in the degradation of antiapoptotic proteins, including cIAP-1, XIAP, and Akt, which may also be responsible for

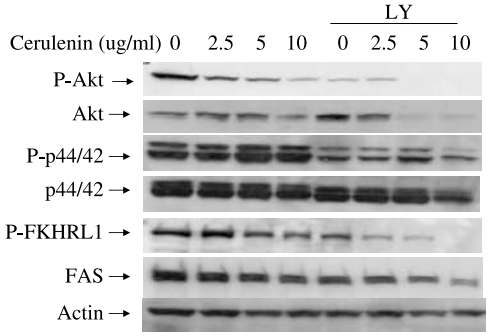


**Figure 6.** Cotreatment with LY294002 and cerulenin decreases the phospho-Akt and phospho-FKHRL1 by degradation of total Akt in MDA-MB468 cells. MDA-MB468 cells were treated with cerulenin (2.5, 5, and 10 μg/mL) alone, LY294002 (20 μmol/L) alone, or the combination for 24 h in DMEM with 0.1% FBS. The cells were harvested and 50 μg of the cell lysate were used for Western blot analysis of phospho-Akt, Akt, phospho-p44/42, p-44/42, phospho-FKHRL1, FAS, and actin as described in Materials and Methods.

the sensitization of MDA-MB468 cells to cerulenin-induced apoptosis by LY294002. It seems that the degradation of cIAP-1 and Akt is caspase-dependent, whereas the degradation of XIAP is partially dependent on caspase (Fig. 5). This indicates that there may be a feed-forward mechanism for inducing apoptosis by low concentration of cerulenin and LY294002 in MDA-MB468 cells: Cotreatment of MDA-MB468 cells with cerulenin and LY294002 causes release of cytochrome *c* and the subsequent activation of caspases; the activated caspases induces the degradation of the antiapoptotic proteins, such as XIAP and cIAP-1, which induces more cell death compared with cell death induced by cerulenin alone. Our data are consistent with previous reports that XIAP, cIAP-1, and Akt can be cleaved by caspases, and that XIAP cleavage facilitates cell death rather than protects cells from apoptosis (41–44).

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