Therapeutic Discovery

Molecular Cancer Therapeutics

Modulation of 4E-BP1 Function as a Critical Determinant of Enzastaurin-Induced Apoptosis

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Abstract

Enzastaurin (LY317615.HCl) is currently in a phase III registration trial for diffuse large B-Cell lymphoma and numerous phase II clinical trials. Enzastaurin suppresses angiogenesis and induces apoptosis in multiple human tumor cell lines by inhibiting protein kinase C (PKC) and phosphoinositide 3-kinase (PI3K)/AKT pathway signaling. PI3K/AKT pathway signaling liberates eukaryotic translation initiation factor 4E (eIF4E) through the hierarchical phosphorylation of eIF4E binding proteins (4E-BP). When hypophosphorylated, 4E-BPs associate with eIF4E, preventing eIF4E from binding eIF4G, blocking the formation of the eIF4F translation initiation complex. Herein, we show that enzastaurin treatment impacts signaling throughout the AKT/mTOR pathway leading to hypophosphorylation of 4E-BP1 in cancer cells of diverse lineages (glioblastoma, colon carcinoma, and B-cell lymphoma). Accordingly, enzastaurin treatment increases the amount of eIF4E bound to 4E-BP1 and decreases association of eIF4E with eIF4G, thereby reducing eIF4F translation initiation complex levels. We therefore chose to evaluate whether this effect on 4E-BP1 was involved in enzastaurin-induced apoptosis. Remarkably, enzastaurin-induced apoptosis was blocked in cancer cells depleted of 4E-BP1 by siRNAs, or in 4EBP1/2 knockout murine embryonic fibroblasts cells. Furthermore, eIF4E expression was increased and 4E-BP1 expression was decreased in cancer cells selected for reduced sensitivity to enzastaurin-induced apoptosis. These data highlight the importance of modulating 4E-BP1 function, and eIF4F complex levels, in the direct antitumor effect of enzastaurin and suggest that 4E-BP1 function may serve as a promising determinant of enzastaurin activity. *Mol Cancer Ther; 9(12); 3158–63. ©2010 AACR.*

Introduction

Enzastaurin (LY317615.HCl) is an orally available acyclic bisindolylmaleimide currently being evaluated in a phase III registration trial for diffuse large B-cell lymphoma (DLBCL). Enzastaurin is also in multiple phase II proof-of-concept trials as a single agent and in combination with standard oncolytics and targeted therapies. Enzastaurin has been shown to suppress signaling through the phosphoinositide 3-kinase (PI3K)/AKT pathway, blocking the phosphorylation of AKT, glycogen synthase kinase (GSK3β), and ribosomal protein S6 (rpS6; rev. 1). Consequently, enzastaurin directly induces tumor cell death and blocks angiogenesis, with the phosphorylation of GSK3β currently serving as a reliable *in vitro* and *in vivo* pharmacodynamic marker (1, 2).

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The eukaryotic translation initiation factor 4F (eIF4F) complex (composed of the mRNA cap-binding protein eIF4E, the scaffolding protein eIF4G, and the RNA helicase eIF4A) is responsible for initiating cap-dependent mRNA translation. eIF4E availability serves as the rate-limiting step in eIF4F complex formation and is largely dependent on binding to its negative regulators, the eIF4E binding proteins (4E-BP; refs. 3, 4). The hierarchical phosphorylation of the 4E-BPs by mTOR, which directly phosphorylates 4E-BP1 at residues T37/46, S65, and T70, liberates eIF4E (5). Once released from the 4E-BPs, eIF4E can engage eIF4G, promoting eIF4F complex assembly and enabling mRNA translation (6, 7). In addition to 4E-BP1 phosphorylation, mTOR also activates the S6 kinases to phosphorylate rpS6. Recent data, however, indicate that rpS6 phosphorylation is dispensable for lymphomagenesis, highlighting the role for the 4E-BP1: eIF4E interaction in malignant progression (8).

Experimental data indicate that eIF4E has a functional role in cellular transformation, tumor growth, and malignancy by selectively and disproportionately affecting the translation of mRNAs encoding key malignancy-related proteins including VEGF, c-myc, and cyclin D1 (6, 7). Clinical tissue studies indicate that eIF4E is overexpressed in tumor versus normal tissues and is related to disease progression and reduced patient survival in a

variety of cancers including prostate (9), lung adenocarcinoma (10), gastric (11), breast (12), head and neck (13), and others (7). eIF4E activity is attenuated by binding to the 4E-BPs. Experimental models show that the 4E-BPs are involved in tumorigenesis (14–16) and that 4E-BP1 overexpression suppresses tumor formation and growth (17, 18). Additionally, clinical data indicate increased phosphorylation of 4E-BP1 is associated with disease progression and poor patient survival in prostate (9), breast (19), and ovarian (20) cancers. Collectively, these data reveal that activation of eIF4E (either by direct overexpression or release from the 4E-BPs) promotes tumor formation and malignancy in experimental models and is related to reduced patient survival in numerous human cancers. Accordingly, 4E-BP1 has recently been shown to play a crucial role in transformation and that targeting eIF4E or the eIF4F complex provides an attractive, novel anticancer strategy (21–24).

We sought to understand more fully the critical determinants for the anticancer activity of enzastaurin. We now show that enzastaurin inhibits multiple phosphorylation events in the AKT/mTOR pathway leading to the hypophosphorylation of 4E-BP1. Consequently, enzastaurin increases the binding of eIF4E to 4E-BP1 and decreases the association of eIF4E with eIF4G. These data indicate enzastaurin treatment reduces the levels of the eIF4F translation initiation complex. Moreover, eIF4E expression is upregulated and 4E-BP1 expression is downregulated in cells selected for reduced sensitivity to enzastaurin-induced apoptosis. Importantly, 4E-BP1 reduction or 4E-BP1/2 knockout blocks enzastaurin-induced apoptosis. These data indicate that the modulation of 4E-BP is functionally important for enzastaurin-induced apoptosis and may serve as a critical determinant of enzastaurin activity.

Material and Methods

Cell culture

4E-BP1 and 4E-BP2 knockout (4E-BP1/2 KO) and wild-type (4E-BP1/2 WT) murine embryonic fibroblasts (MEF) were derived as previously described (14, 25). CRL2611 human glioma cells, HCT116 human colon cancer cells, and Farage B-cell lymphoma cells were obtained from the American Type Culture Collection. Cells lines were tested for mycoplasma and other pathogens and were authenticated using short tandem repeat genotyping in accordance with the referenced American Type Culture Collection genotype when available. CRL2611 and MEF cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, 11995) supplemented with 10% heat inactivated fetal bovine serum (HI FBS; Gibco, 10082). HCT116 cells were maintained in McCoy's 5A media supplemented with 10% HI FBS, 5% sodium pyruvate (Gibco, 11360), 5% sodium bicarbonate (Gibco, 25080), and 5% D-(+)-glucose (Sigma Aldrich, G8769). Farage B cells were maintained in RPMI-1640 media supplemented with 10% HI FBS, 5% sodium pyruvate (Gibco, 11360), 5% sodium bicarbonate (Gibco, 25080), and 5% D-(+)-glucose (Sigma Aldrich, G8769). Cells were routinely treated in media with 1% HI FBS as indicated (1). HCT116 cells were maintained in McCoy's 5A media supplemented with 1% HI FBS and 1 μmol/L enzastaurin for more than 100 passages to select for cells with reduced sensitivity to enzastaurin-induced apoptosis.

Western blotting

Western blots were done as previously described (1). The antibodies to 4E-BP1, p4E-BP1$^{Thr37/46}$, p4E-BP1^{Ser65}, p4E-BP1^{Thr70}, AKT^{Ser473}, AKT^{Thr308}, eIF4G, mTOR, pMTOR^{Ser2448} were used at 1:500 dilution (Cell Signaling), eIF4E and AKT were used at 1:500 dilution (BD Biosciences), β-actin at 1:20,000 dilution (Sigma Aldrich). All Western blots were incubated in 5% BSA in Tris-buffered saline Tween-20 (TBST) with 0.1% Tween-20. Secondary antibodies were used at 1:5,000 dilution (Santa Cruz) and incubated in 5% milk and 0.1% Tween-20 in PBS. 7-Methyl-GTP-sepharose (m7GTP) chromatography was used to assess the interaction of eIF4E with either eIF4G or 4E-BP1 from cell extracts as described (9).

siRNA transfection

A total of 1,000 cells (CRL2611 or HCT116) was plated in 96-well poly-D-lysine plates (Becton Dickinson) and transfected with 5 to 20 nmol/L 4E-BP1 siRNA (Ambion, s223471), 20nmol/L pooled Dharmacon ON-target plus Duplex 4E-BP1 siRNA (J-003005-11,12,13,14), or negative control siRNA no. 2 (AM4637, Ambion) in Oligofectamine (Invitrogen, 12252-011). Transfections were carried out in 100 μL DMEM 11995 for CRL2611 cells or McCoy's 5A supplemented with 1% FBS for HCT116 cells for 72 hours. After the 72 hour transfection, 100 μL of 2x dimethyl sulfoxide (DMSO) or enzastaurin was added for 48 to 72 hours.

Apoptosis assays

Induction of apoptosis was measured by oligonucleosomal fragmentation for 48 to 72 hours as indicated (Cell Death Detection Elisa; Roche Applied Science) or by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining for 48 hours, as described (1). Absorbance values were normalized to DMSO controls (error bars represent ±SEM).

Results and Discussion

Enzastaurin suppresses AKT/mTOR pathway phosphorylation

Enzastaurin inhibits signaling through the PKCβ and PI3K/AKT pathways specifically blocking the phosphorylation of GSK3β, rpS6, and AKT (1). We sought to determine to what extent enzastaurin treatment

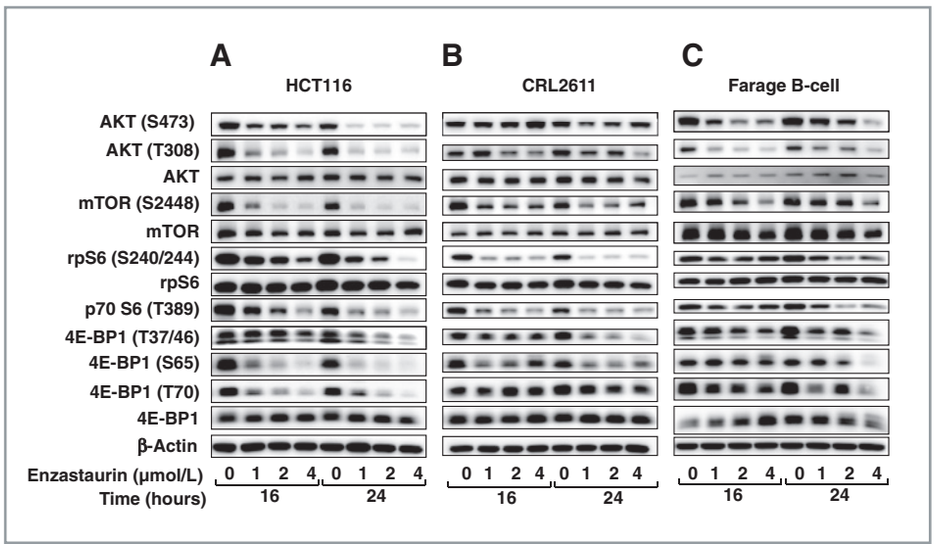

Figure 1. Enzastaurin suppresses cell signaling downstream of the AKT/mTOR pathway. Cancer cells were incubated with 0 to 4μmol/L enzastaurin in media supplemented with 1% HI FBS for 16 and 24 hours. Western blot analyses were done for total 4E-BP1, 4E-BP1$^{Thr37/46}$, 4E-BP1^{Ser65}, 4E-BP1^{Thr70}, AKT ^{Ser473}, AKT^{Thr308}, mTOR, mTOR^{Ser2448}, p70S6K^{Thr389}, rpS6 $^{Ser240/244}$, rpS6, AKT, and β-actin. A, HCT116 colon cancer cells; B, CRL2611 glioblastoma cells; C, Farage B-cell lymphoma cells.

would inhibit signaling throughout this pathway in cancer cell lines representing diverse cellular lineages (colon carcinoma, glioma, B-cell lymphoma). The chosen clinical dose for enzastaurin (500 or 525 mg per day) routinely yields 3 μmol/L mean plasma concentrations of total drug, reaching 8 μmol/L in some patients (26, 27). We therefore evaluated enzastaurin at 1 to 4 μmol/L.

At 16 and 24 hours, enzastaurin suppressed the phosphorylation of rpS6$^{Ser240/244}$, AKT^{Ser473}, and AKT^{Thr308} in HCT116 (Fig. 1A). This reduction in rpS6$^{Ser240/244}$, AKT^{Ser473}, and AKT^{Thr308} phosphorylation was also observed in the glioma cell line CRL2611 (Fig. 1B) and in B-cell Farage lymphoma cells (Fig. 1C) with the greatest reduction evident at 24 hours. We further observed dose-dependent inhibition of mTOR-^{Ser2448}, p70S6K ^{Thr389}, 4E-BP1$^{Thr37/46}$, 4E-BP1^{Ser65}, and 4E-BP1^{Thr70} in all 3 cell lines (Fig. 1A–C). These data highlight the inhibitory effect of enzastaurin on the phosphorylation of multiple proteins throughout the PI3K/AKT pathway confirming and extending previously published results (1).

Enzastaurin reduces the levels of the eIF4F translation initiation complex

Enzastaurin inhibits 4E-BP1 phosphorylation at multiple phosphorylation sites including T37/46, S65, and T70 (Fig. 1A–C). As the hypophosphorylation of 4E-BP1 increases the binding of 4E-BP1 to eIF4E (5), we sought to determine whether enzastaurin would increase this association using m7GTP cap analog beads to capture eIF4E and to pull down associated proteins. Enzastaurin treatment substantially increased the amount of 4E-BP1 bound to eIF4E in all 3 cell lines at all concentration levels [Fig. 2A (inset), B (left), and C (left)]. To confirm that this increased binding was not a reflection of changes in total eIF4E or 4E-BP1 levels we analyzed total levels of eIF4E and 4E-BP1 [Fig. 2B (right) and C (right)].

Hypophosphorylated 4E-BPs compete with eIF4G for a mutually exclusive binding site on eIF4E (3, 4). We therefore examined whether enzastaurin would affect eIF4G:eIF4E binding. Enzastaurin treatment decreased, in a dose-related manner, the amount of eIF4G associated with eIF4E in all 3 lines [Fig. 2A (inset), B (left), and C (left)]. Total eIF4G levels were unchanged [Fig. 2B (right) and C (right)]. These data show that enzastaurin treatment, by promoting the hypophosphorylation of 4E-BP1, sequesters eIF4E from eIF4G, thereby diminishing eIF4F complex levels. To quantify this reduction, we normalized the levels of cocaptured 4E-BP1 and eIF4G to eIF4E and then divided the normalized eIF4G values by the normalized 4E-BP1 values after m7GTP cocapture in HCT116 cells at 8, 16, and 24 hours. These data graphically illustrate the time-dependent reduction in eIF4F levels after enzastaurin treatment (Fig. 2A). We next sought to determine whether selection for decreased sensitivity to enzastaurin might involve alterations in eIF4E or 4E-BP1. Indeed, HCT116 cells selected for reduced sensitivity to enzastaurin-induced apoptosis showed increased eIF4E expression and decreased 4E-BP1 expression (Fig. 2D).

Reducing 4E-BP1 expression suppresses enzastaurin-induced apoptosis

Enzastaurin induces apoptosis in a wide variety of human cancer cell lines including glioblastoma multiforme (1, 28), cutaneous T-cell lymphoma (29), colon (1), gastric (30), multiple myeloma (31, 32), and Waldenstrom's macroglobulinemia (33). Our data

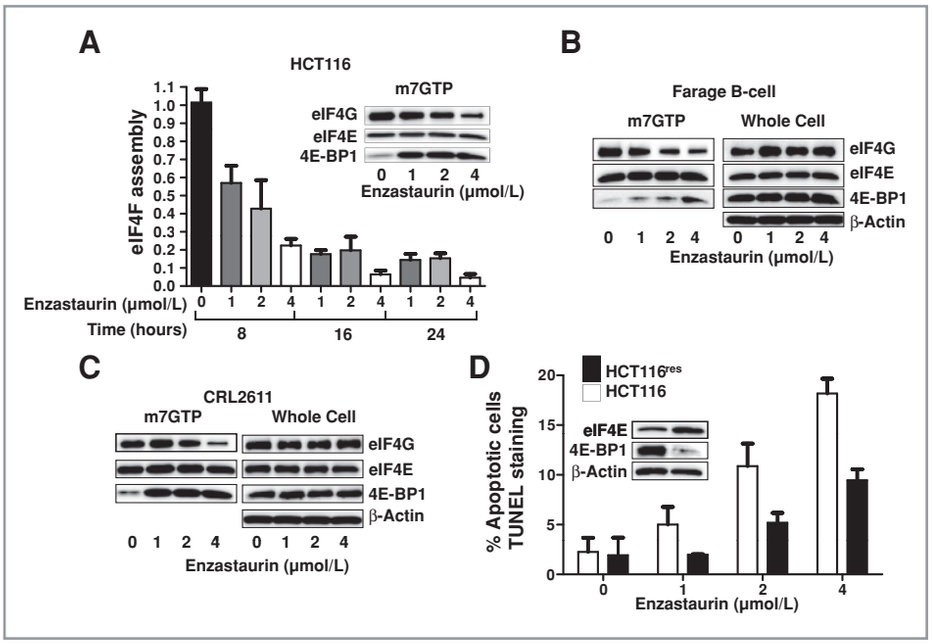

Figure 2. Enzastaurin decreases eIF4F complex levels. Cancer cells were incubated with 0 to 4 μmol/L enzastaurin in media supplemented with 1% HI FBS for 24 hours. Western blot analyses were done for total eIF4G, eIF4E, and 4E-BP1 from m7GTP pull-down lysates and whole cell lysates. Whole lysates were additionally probed for β-actin to control for loading and transfer. Graph represents eIF4G normalized to eIF4E divided by 4E-BP1 normalized to eIF4E [(eIF4G/eIF4E)/(4E-BP1/eIF4E)]. Data represent the mean of 3 separate experiments (±SEM). A, HCT116 colon cancer cells; B, Farage B-cell lymphoma cells; C, CRL2611 glioblastoma cells; D, percentage of apoptotic cells following enzastaurin treatment in HCT116 cells and in HCT116 colon cancer cells selected for reduced sensitivity (HCT116^{res}) to enzastaurin as measured by TUNEL staining. Inset shows the levels of eIF4E and 4E-BP1 by Western blot from whole cell lysates. β-Actin was assessed to control for loading and transfer.

indicate that enzastaurin treatment blocks 4E-BP1 phosphorylation and increases the association of eIF4E with 4E-BP1. We therefore examined whether manipulating 4E-BP1 levels would impact enzastaurin-induced apoptosis. Total 4E-BP1 expression was reduced 70% to 80%, 72 hours after 4E-BP1 siRNA transfection in HCT116 and CRL2611 cells (Fig. 3A). Enzastaurin was added after 4E-BP1 knockdown and assayed for the induction of apoptosis by oligonucleosomal fragmentation 48 to 72 hours thereafter. Apoptosis was evident in both HCT116 and CRL2611 cells treated for 48 hours with 1 to 2μmol/L enzastaurin, a concentration routinely achieved clinically [Fig. 3B (black bars) and C (black bars)]. Depletion of 4E-BP1 by siRNA transfection blocked enzastaurin-induced apoptosis in both cell lines [Fig. 3B (white bars) and C (white bars)]. These data indicate that enzastaurin-induced apoptosis can be significantly inhibited when 4E-BP levels are reduced.

To address the potential off-target effects of 4E-BP1 siRNA transfection and to confirm and extend these siRNA data, we also sought to determine whether complete knockout of 4E-BP1 and 4E-BP2 (4E-BP1/2 KO) would affect apoptosis and eIF4F complex levels after enzastaurin treatment. In wild-type (4E-BP1/2 WT) MEFs, enzastaurin-induced apoptosis (Fig. 4A, black bars) and increased the association of 4E-BPs with eIF4E, concomitant with 4E-BP hypophosphorylation (Fig. 4B). By contrast, in the 4E-BP1/2 knockout (4E-BP1 KO) MEFs, enzastaurin-induced apoptosis was blocked (Fig. 4A, white bars). To quantify the reduction in eIF4F complex levels, we normalized the levels of cocaptured 4E-BP1 and eIF4G to eIF4E and then divided the normalized eIF4G values by the normalized 4E-BP1 (Fig. 4C). The 4E-BP1/2 KO MEFs showed higher eIF4F assembly (i.e., eIF4G bound to eIF4E) than the 4E-BP1/2 WT MEFs. Further, the 4E-BP1/2 MEFs showed dose-dependent reduction of eIF4F levels after enzastaurin treatment (Fig. 4C). These data, along with the 4E-BP1 siRNA depletion data in cancer cells, further support a functional role for the 4E-BPs in enzastaurin-induced apoptosis.

Enzastaurin was originally advanced into the clinic as an antiangiogenic therapy and has subsequently been shown to induce apoptosis directly in cancer cells (1). The data in this article represent a continued effort to understand the critical effectors for the anticancer effect of enzastaurin. These data confirm and extend previously published data showing that enzastaurin inhibits multiple, key phosphorylation events throughout the PI3K/AKT pathway impacting phosphorylation not only of GSK3β, rpS6, and AKT (1) but also of mTOR, p70S6K, and 4E-BP1. These data show that, by augment-

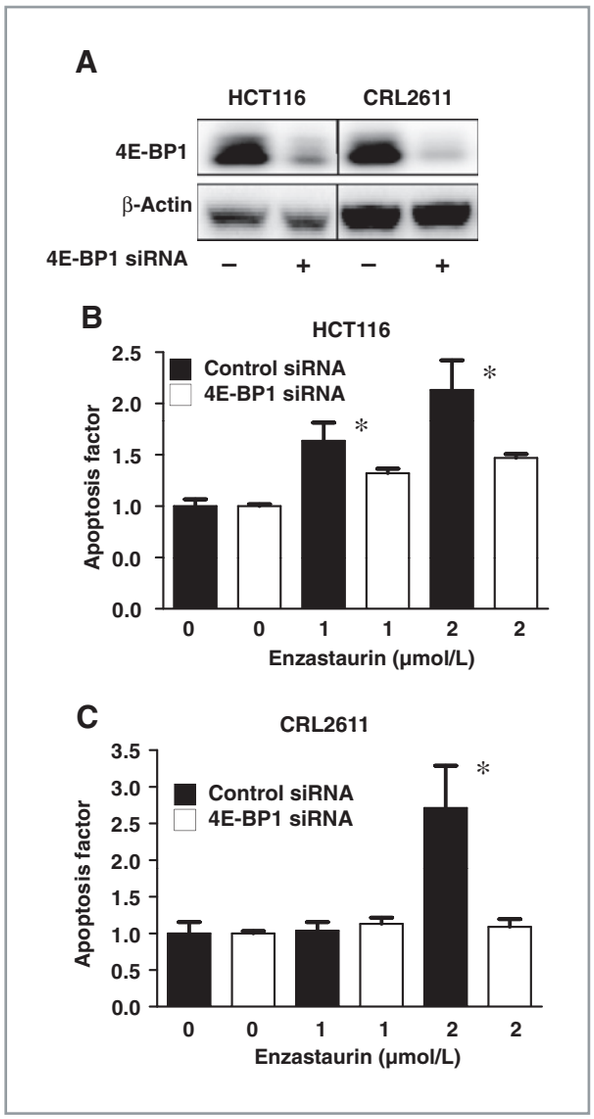

Figure 3. 4E-BP1 depletion reduces enzastaurin-induced apoptosis. A, Western blot analyses for total 4E-BP1 and β-actin 72 hours after 4E-BP1 siRNA transfection in HCT116 colon cancer cells and CRL2611 glioblastoma cells. Apoptosis after 72 hours 4E-BP1 siRNA treatment followed by incubation with 0 to 2 μmol/L enzastaurin in media supplemented with 1% HI FBS for 48 hours HCT116 (B) or 72 hours CRL2611 (C). Apoptosis was determined using the Cell Death Detection ELISA (Roche Applied Science). Graph represents mean (±SEM); *, $P < 0.05$ by unpaired t test.

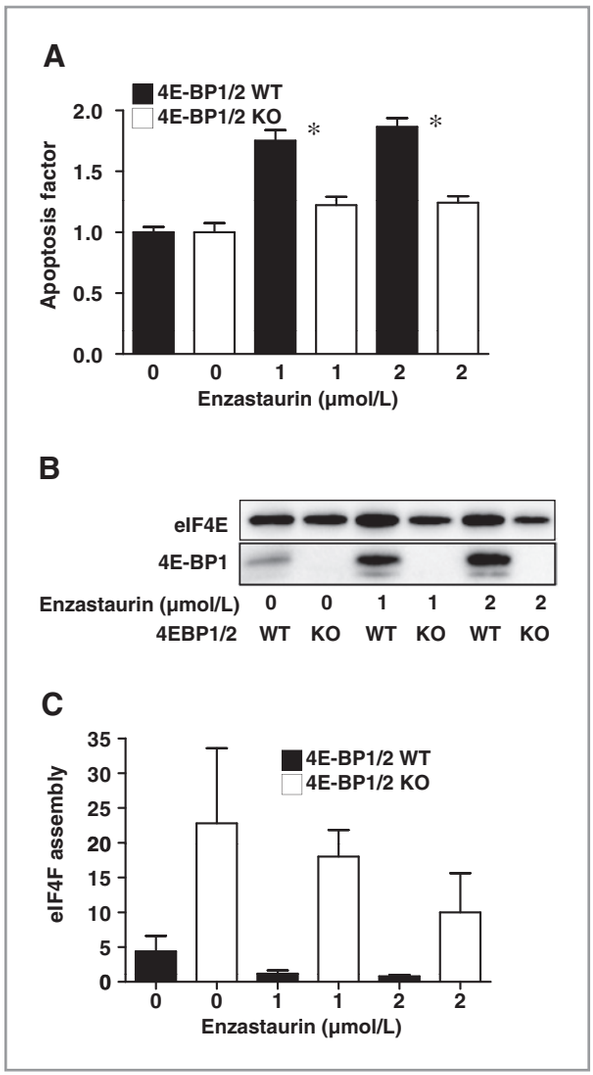

Figure 4. 4E-BP1/2 knockout increases eIF4F complex levels and reduces enzastaurin-induced apoptosis. Wild-type (WT) and 4E-BP1/2 knockout (KO) murine embryonic fibroblasts were incubated with 1 to 2 μmol/L enzastaurin in media supplemented with 1% HI FBS. A, apoptosis was determined 72 hours after enzastaurin treatment using the Cell Death Detection ELISA (Roche Applied Science). Graph represents mean (±SEM); *, $P < 0.05$ by unpaired t test. B, Western blot analyses from m7GTP pull-down lysates were done for total eIF4E and 4E-BP1 after 24 hours enzastaurin treatment. C, graph represents eIF4G normalized to eIF4E divided by 4E-BP1 normalized to eIF4E [(eIF4G/eIF4E)/(4E-BP1/eIF4E)]. Data represent mean of 3 separate experiments (±SEM).

ing eIF4E:4E-BP1 binding and decreasing eIF4E:eIF4G binding, enzastaurin substantially decreases levels of the eIF4F translation initiation complex. Importantly, knockout or depletion of 4E-BP obviates the apoptotic effect of enzastaurin on these cells highlighting that 4E-BP1 function is a critical determinant of enzastaurin activity in cancer cells of diverse lineage.

Disclosure of Potential Conflicts of Interest

All authors are employees of Eli Lilly and Company. N. Sonenberg is a consultant for Eli Lilly and Company.

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References

1. Graff JR, McNulty AM, Hanna KR, et al. The protein kinase C beta-selective inhibitor, Enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. Cancer Res 2005;65:7462–9.
2. Tekle C, Giovannetti E, Sigmond J, et al. Molecular pathways involved in the synergistic interaction of the PKC beta inhibitor enzastaurin with the antifolate pemetrexed in non-small cell lung cancer cells. Br J Cancer 2008;99:750–9.
3. Haghighat A, Mader S, Pause A, Sonenberg N. Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. EMBO J 1995;14:5701–9.
4. Mader S, Lee H, Pause A, Sonenberg N. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. Mol Cell Biol 1999;15;4990–7.
5. Gingras AC, Raught B, Gygi SP, et al. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. Genes Dev 2001;15:2852–64.
6. Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW, Sonenberg N. eIF4E–from translation to transformation. Oncogene 2004;23 (18):3172–9.
7. De Benedetti A, Graff JR. eIF-4E expression and its role in malignancies and metastases. Oncogene 2004;23:3189–99.
8. Hsieh AC, Costa M, Zollo O, et al. Genetic dissection of the oncogenic mTOR pathway reveals druggable addiction to translational control via 4EBP-eIF4E. Cancer Cells 2010;17:249–61.
9. Graff JR, Konicek BW, Lynch RL, et al. eIF4E activation is commonly elevated in advanced human prostate cancers and significantly related to reduced patient survival. Cancer Res 2009;69:3866–73.
10. Wang R, Geng J, Wang JH, et al. Overexpression of eukaryotic initiation factor 4E (eIF4E) and its clinical significance in lung adenocarcinoma. Lung Cancer 2009;66:237–44.
11. Chen CN, Hsieh FJ, Cheng YM, Lee PH, Chang KJ. Expression of eukaryotic initiation factor 4E in gastric adenocarcinoma and its association with clinical outcome. J Surg Oncol 2004;86:22–7.
12. Li BD, Liu L, Dawson M, De Benedetti A. Overexpression of eukaryotic initiation factor 4E (eIF4E) in breast carcinoma. Cancer 1997;79:2385–90.
13. Sorrells DL Jr., Ghali GE, De Benedetti A, Nathan CO, Li BD. Progressive amplification and overexpression of the eukaryotic initiation factor 4E gene in different zones of head and neck cancers. J Oral Maxillofac Surg 1999;57:294–9.
14. Petroulakis E, Parsyan A, Dowling RJ, et al. p53-dependent translational control of senescence and transformation via 4E-BPs. Cancer Cell 2009;16:439–46.
15. Kim YY, Von WL, Larsson O, et al. Eukaryotic initiation factor 4E binding protein family of proteins: sentinels at a translational control checkpoint in lung tumor defense. Cancer Res 2009;69:8455–62.
16. Avdulov S, Li S, Michalek V, et al. Activation of translation complex eIF4F is essential for the genesis and maintenance of the malignant phenotype in human mammary epithelial cells. Cancer Cell 2004;5:553–63.
17. Rousseau D, Gingras AC, Pause A, Sonenberg N. The eIF4E-binding proteins 1 and 2 are negative regulators of cell growth. Oncogene 1996;13:2415–20.
18. Jacobson BA, Alter MD, Kratzke MG, et al. Repression of cap-dependent translation attenuates the transformed phenotype in non-small cell lung cancer both in vitro and in vivo. Cancer Res 2006;66:4256–62.
19. Rojo F, Najera L, Lirola J, et al. 4E-binding protein 1, a cell signaling hallmark in breast cancer that correlates with pathologic grade and prognosis. Clin Cancer Res 2007;13:81–9.
20. Castellvi J, Garcia A, Rojo F, et al. Phosphorylated 4E binding protein 1: a hallmark of cell signaling that correlates with survival in ovarian cancer. Cancer 2006;107:1801–11.
21. Graff JR, Konicek BW, Vincent TM, et al. Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. J Clin Invest 2007;117:2638–48.
22. Assouline S, Culjkovic B, Cocolakis E, et al. Molecular targeting of the oncogene eIF4E in acute myeloid leukemia (AML): a proof-of-principle clinical trial with ribavirin. Blood 2009;114:257–60.
23. Moerke NJ, Aktas H, Chen H, et al. Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. Cell 2007;128:257–67.
24. She QB, Halilovic E, Ye Q, et al. 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. Cancer Cell 2010;18:39–51.
25. Le BO, Petroulakis E, Paglialunga S, et al. Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1 and 4E-BP2. J Clin Invest 2007;117:387–96.
26. Robertson MJ, Kahl BS, Vose JM, et al. Phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. J Clin Oncol 2007;25:1741–6.
27. Carducci MA, Musib L, Kies MS, et al. Phase I dose escalation and pharmacokinetic study of enzastaurin, an oral protein kinase C beta inhibitor, in patients with advanced cancer. J Clin Oncol 2006;24:4092–9.
28. Rieger J, Lemke D, Maurer G, et al. Enzastaurin-induced apoptosis in glioma cells is caspase-dependent and inhibited by BCL-XL. J Neurochem 2008;106:2436–48.
29. Querfeld C, Rizvi MA, Kuzel TM, et al. The selective protein kinase C beta inhibitor enzastaurin induces apoptosis in cutaneous T-cell lymphoma cell lines through the AKT pathway. J Invest Dermatol 2006;126:1641–7.
30. Lee KW, Kim SG, Kim HP, et al. Enzastaurin, a protein kinase C beta inhibitor, suppresses signaling through the ribosomal S6 kinase and bad pathways and induces apoptosis in human gastric cancer cells. Cancer Res 2008;68:1916–26.
31. Rizvi MA, Ghias K, Davies KM, et al. Enzastaurin (LY317615), a protein kinase Cbeta inhibitor, inhibits the AKT pathway and induces apoptosis in multiple myeloma cell lines. Mol Cancer Ther 2006;5:1783–9.
32. Neri A, Marmiroli S, Tassone P, et al. The oral protein-kinase C beta inhibitor enzastaurin (LY317615) suppresses signalling through the AKT pathway, inhibits proliferation and induces apoptosis in multiple myeloma cell lines. Leuk Lymphoma 2008;49: 1374–83.
33. Moreau AS, Jia X, Ngo HT, et al. Protein kinase C inhibitor enzastaurin induces in vitro and in vivo antitumor activity in Waldenstrom macroglobulinemia. Blood 2007;109:4964–72.