

eIF4E knockdown decreases breast cancer cell growth without activating Akt signaling

Alpana Soni,¹ Argun Akcakanat,¹ Gopal Singh,¹ David Luyimbazi,¹ Yuhuan Zheng,¹ Doyil Kim,¹ Ana Gonzalez-Angulo,² and Funda Meric-Bernstam¹

Departments of ¹Surgical Oncology and ²Breast Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Abstract

Activation of translation initiation is essential for the malignant phenotype and is emerging as a potential therapeutic target. Translation is regulated by the expression of translation initiation factor 4E (eIF4E) as well as the interaction of eIF4E with eIF4E-binding proteins (e.g., 4E-BP1). Rapamycin inhibits translation initiation by decreasing the phosphorylation of 4E-BP1, increasing eIF4E/4E-BP1 interaction. However, rapamycin also inhibits S6K phosphorylation, leading to feedback loop activation of Akt. We hypothesized that targeting eIF4E directly would inhibit breast cancer cell growth without activating Akt. We showed that eIF4E is ubiquitously expressed in breast cancer cell lines. eIF4E knockdown by small interfering RNA inhibited growth in different breast cancer cell subtypes including triple-negative (estrogen receptor/progesterone receptor/HER-2-negative) cancer cells. eIF4E knockdown inhibited the growth of cells with varying total and phosphorylated 4E-BP1 levels and inhibited rapamycin-insensitive as well as rapamycin-sensitive cell lines. eIF4E knockdown led to a decrease in expression of cyclin D1, Bcl-2, and Bcl-xL. eIF4E knockdown did not lead to Akt phosphorylation but did decrease 4E-BP1 expression. We conclude that eIF4E is a promising target for breast cancer therapy. eIF4E-targeted therapy may be efficacious in a variety of breast cancer subtypes including triple-negative tumors for which currently there

are no targeted therapies. Unlike rapamycin and its analogues, eIF4E knockdown is not associated with Akt activation. [Mol Cancer Ther 2008;7(7):1782–8]

Introduction

Translation initiation is deregulated in many human cancers including breast, head and neck, colorectal, bladder, prostate, lung, and cervical cancers and lymphomas (1). Translation initiation factor 4E (eIF4E) is rate limiting for cap-dependent translation (2). It has been proposed that the translational efficiency of mRNA with highly complex 5'-untranslated regions is especially dependent on eIF4E levels (1, 3). eIF4E overexpression leads to selective translation of mRNA such as cyclin D1, Bcl-2, Bcl-xL, and vascular endothelial growth factor *in vitro* (4–8). eIF4E expression shows significant correlation with cyclin D1 and vascular endothelial growth factor expression in human tumors (9). eIF4E enhances nucleocytoplasmic transport for selected mRNA such as cyclin D1. Thus, eIF4E expression affects the expression of important regulators of cell growth and survival. *In vitro* eIF4E overexpression mediates growth, proliferation, and survival signaling and has transforming activity in fibroblasts and mammary epithelial cells (10–12). Transgenic eIF4E-expressing mice show a marked increase in tumorigenesis, developing tumors of various histologies (13). Thus, eIF4E also directly acts as an oncogene *in vivo*.

Activated translation initiation is essential for the malignant breast cancer phenotype (14). Elevated eIF4E levels are critical for angiogenesis and progression of breast carcinomas (15). eIF4E is overexpressed in breast cancer and is a poor prognostic marker in retrospective and prospective studies (16–18). Graff et al. have recently shown that eIF4E expression can be down-regulated with second-generation antisense oligonucleotides, with reduction of *in vivo* tumor growth in a PC-3 prostate cancer model and MDA-MB-231 breast cancer model (19). These data suggest that eIF4E could indeed be pursued as a therapeutic target for breast cancer as well as a variety of other cancers.

The mammalian target of rapamycin (mTOR) kinase in a complex with raptor (mTORC1) leads to the phosphorylation of 4E-BP1 and S6K1, two other key proteins that regulate protein translation (20). Rapamycin and its analogues inhibit mTORC1 signaling. mTOR is being investigated as a potential target in many cancer types and is already a proven therapeutic target for renal cell carcinoma. The rapamycin analogue temsirolimus (Torisel, CCI-779, Wyeth) has been shown to improve overall survival among patients with metastatic renal cell carcinoma (21), leading to its Food and Drug Administration approval. However, eIF4E overexpression partially rescues

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Requests for reprints: Funda Meric-Bernstam, Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Unit 444, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-745-4453; Fax: 713-745-4926. E-mail: fmeric@mdanderson.org

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rapamycin-mediated G_1 arrest *in vitro* and confers resistance to rapamycin in transgenic lymphoma models *in vivo* (22, 23). Further, preclinical studies and sequential biopsies in patients treated in a phase I trial of mTOR inhibitors have determined that mTOR inhibition induces feedback loop activation of Akt, a mechanism that may potentially limit its antitumor activity (24). As this feedback loop activation is thought to be mediated through the inhibition S6K1 by mTOR inhibitors, there is rationale in targeting translation downstream of mTOR directly at eIF4E.

As activation of translation initiation is a common integral pathway for the malignant phenotype, we hypothesized that eIF4E down-regulation would lead to growth inhibition in breast cancer cells of differing tumor subtypes. Further, we hypothesized that targeting eIF4E directly rather than targeting mTORC1 signaling would not lead to feedback loop-mediated Akt activation.

Materials and Methods

Cell Lines and Cultures

MDA-MB-468, SK-BR-3, MCF7, MDA-MB-435S, and MDA-MB-231 were obtained from the American Type Culture Collection. MDA-MB-435 was obtained from National Cancer Institute. All cell lines were cultured in DMEM/F-12 (Cellgro Mediatech) supplemented with 10% (v/v) fetal bovine serum (SAFC Biosciences) at 37°C and humidified in 5% CO₂. For selected experiments, cells were treated with DMSO (Sigma) or rapamycin (LC Laboratories).

Small Interfering RNA

Small interfering RNA (siRNA) to eIF4E was custom-ordered duplexes from Dharmacon or Ambion. The four sequences tested were 4E1 (AAGCAAACCUGCGGCU-GAUCU; ref. 25), 4E2 (ACAGCAGAGACGAAGUGAC; ref. 25), 4E3 (GGAUGGUAUUGAGCCUAUG; ref. 26), and 4E4 (GGUGGGCACUCUGGUUUUU; ref. 26). Negative control siRNA were purchased from Ambion Transfection was done using DharmaFECT 1 reagent according to the manufacturer's protocol (Dharmacon).

Western Blots

Western blotting was done as described elsewhere (27). Membranes were immunoblotted with antibodies to eIF4E, phosphorylated eIF4E (S209), Bcl-2, Bcl-xL, 4E-BP1, phosphorylated 4E-BP1 (T70), S6K1, phosphorylated S6K1 (T389), Akt, phosphorylated Akt (p-Akt; T308), p-Akt (S473; all from Cell Signaling Technology), cyclin D1 (Santa Cruz Biotechnology), and actin (Sigma). Membranes were visualized by the Odyssey Infrared Imaging System (LICOR Biosciences) or ECL developer system (GE Healthcare Life Sciences).

Cell Growth and Cell Cycle Analysis

Cell growth was determined by either of two methods: by the relative percentages of metabolically active cells compared with untreated controls based on mitochondrial conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazine or by the protein content of treated and untreated cells through the sulforhodamine

B assay (28). The results were assessed in a 96-well format plate reader done in triplicates by measuring the absorbance at a wavelength of 570 nm. The IC₅₀ was determined based on the dose-response curves using sulforhodamine B assay. The commercial software package was obtained from CalcuSyn (Biosoft). Cell cycle was analyzed as described previously (29).

Colony Formation Assay

At 24 h incubation of siRNA, cells were trypsinized, counted, and plated at a density of 1×10^3 to 5×10^3 cells per 60 mm plate in triplicate for each treatment group. Approximately 2 to 4 weeks later, plates were fixed, stained with crystal violet, and scanned, and colonies were counted using NIH ImageJ v.1.33h software.

Sucrose Density Gradient

MDA-MB-468 cells were transfected by negative control or 4E3 siRNA and cultured for 72 h. The sucrose density gradient assay, cycloheximide (Sigma) was added to 100 µg/mL 5 min before harvesting. Polysome extracts were lysed in the following buffer: 10 mmol/L Tris-HCl (pH 7.5), 100 mmol/L KCl, 20 mmol/L MgCl₂, 0.5% Triton X-100, 0.5% sodium deoxycholate, 6% sucrose, 100 µg/mL cycloheximide, and 1,000 units/mL RNase inhibitor (Roche Diagnostic). Lysates were then centrifuged for 5 min at $12,000 \times g$ and applied to 15% to 50% (w/v) sucrose gradient prepared in 10 mmol/L Tris-HCl (pH 7.5), 100 mmol/L KCl, 20 mmol/L MgCl₂, and 100 µg/mL cycloheximide using an ISCO Model 160 Gradient Former (Teledyne Isco). Sucrose gradients were centrifuged at $38,000 \times g$ for 2 h 30 min in a Beckman SW40Ti rotor. After centrifugation, gradients were fractionated by using an ISCO Foxy Jr. fractionator (Teledyne Isco). The absorbance at 254 nm of gradient was analyzed with an ISCO UA-6 UV/VIS detector.

Statistical Analysis

Results were presented as mean \pm SD. Growth inhibition was normalized to the no treatment group, no treatment growth being set at 100%. For comparison between groups, data were analyzed by Student's *t* test. Differences between groups were considered statistically significant at $P < 0.05$.

Results

eIF4E Knockdown Inhibits Cell Growth in Breast Cancer Cell Lines

To determine the potential of eIF4E as a therapeutic target, we assessed the expression of eIF4E on Western blotting and observed that eIF4E is ubiquitously expressed in a panel breast cancer cell lines (Fig. 1A). The expression of total 4E-BP1 and phosphorylated 4E-BP1 was variable in the cell lines. To determine the rapamycin sensitivity of the cell lines, the cell lines were treated with varying concentrations of rapamycin ranging from 0.1 to 1,000 nmol/L for 5 days. Figure 1B shows that the panel of cell lines we selected for study varied in their sensitivity to the growth-inhibitory effects of rapamycin.

We tested a siRNA panel of sequences to eIF4E (data not shown) and identified two independent sequences

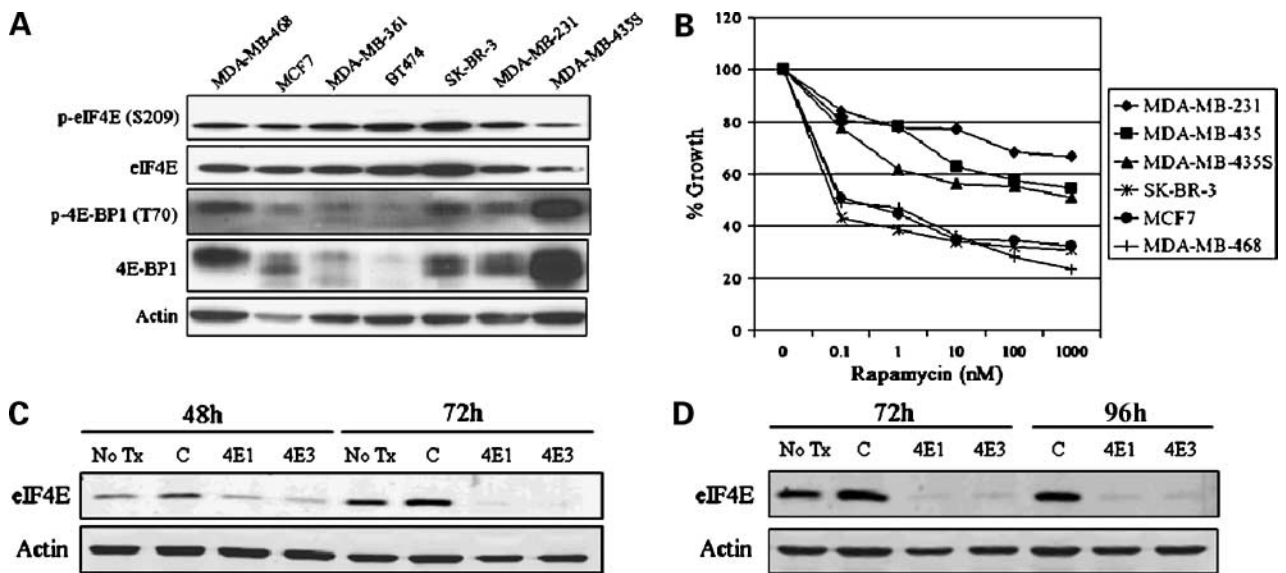


Figure 1. Expression of eIF4E and 4E-BP1 in panel of breast cancer cell lines. **A**, cell lysate was harvested from a panel of breast cancer cell lines and separated by SDS-PAGE. Western blotting was done for phosphorylated eIF4E (S209), total eIF4E, phosphorylated 4E-BP1 (T70), total 4E-BP1, and actin. **B**, rapamycin sensitivity of a panel of breast cancer cell lines. Cells plated in 96-well plates in triplicate and treated with increasing doses of rapamycin. Cell growth was assessed at 5 d by sulforhodamine B, and growth was compared with vehicle-treated controls (% control). Average of at least three independent experiments. **C** and **D**, MDA-MB-468 cells were transfected with control siRNA or one of two siRNA sequences for eIF4E (4E1 and 4E3). Cells were lysed at 48, 72, or 96 h. Western blotting was done for eIF4E and actin expression.

(4E1 and 4E3) that led to marked knockdown of eIF4E expression. Western blotting 48 h after siRNA transfection shows some decline of eIF4E levels, with further knockdown by 72 h (Fig. 1C) and maintained by 96 h (Fig. 1D).

We next assessed the effect of eIF4E knockdown by transfection of cells with control siRNA or eIF4E siRNA sequences 4E1 or 4E3 (Fig. 2A). In each experiment, eIF4E knockdown was confirmed with Western blotting for eIF4E and actin at 72 h (Fig. 2A). One day after transfection, cells transfected were trypsinized, counted, and plated in 96-well plates in triplicates. After 4 more days, cell growth was assayed (Fig. 2A). In all eight cell lines tested, eIF4E knockdown lead to a statistically significant decrease in growth compared with control siRNA-treated cells ($P < 0.05$ for all comparisons of 4E1 or 4E3 with control siRNA). Of note, the cell lines tested represent different subtypes of breast cancer: MCF7 cells being estrogen receptor (ER) positive, SKBR3 cells HER-2 positive, and MDA-MB-468, MDA-MB-231, and MDA-MB-435 cells being ER/progesterone receptor/HER-2 or triple negative. Thus, eIF4E knockdown inhibited growth in a variety of breast cancer subtypes, including triple-negative cells.

The effect of eIF4E knockdown on anchorage-dependent growth was also assessed by colony formation assay in MDA-MB-435S cells. One day after cells were transfected with control siRNA or one of two eIF4E siRNA sequences, cells were trypsinized, counted, and plated in 60 mm plates (1,000 per plate), and after 2 weeks, colonies were stained with crystal violet. eIF4E knockdown with either eIF4E siRNA sequence led to a dramatic decline in colony formation ability (Fig. 2B).

To elucidate its mechanism of action, we assessed the effect of eIF4E knockdown on the cell cycle with flow cytometry (Fig. 2C). MCF7 cells were transfected with control siRNA or one of two eIF4E siRNA sequences (4E1 and 4E3), and 4 days later, cell cycle analysis was done on attached cells. eIF4E knockdown was associated with a decline in % cells in S phase and a significant increase in % cells in G₁ phase (%G₁ cells 59% with control siRNA versus 86% with 4E1 and 83% with 4E3; $P < 0.0001$ for both).

eIF4E Knockdown Inhibits Translation and the Downstream Targets of eIF4E

We next evaluated the effect of eIF4E knockdown on translation. mRNA that are actively translated usually have multiple ribosomes associated with them, referred to as polysomes, whereas translationally inactive mRNA are often sequestered in messenger ribonucleoprotein particles or are associated with a single ribosome (monosome). Polysomal and messenger ribonucleoprotein particles can be separated by sucrose gradient centrifugation. Therefore, MDA-MB-468 cells were treated with control siRNA versus 4E siRNA, and after 3 days, cell lysates were fractionated on 15% to 50% sucrose gradients. eIF4E knockdown was associated with a decline of RNA in polysomal fractions and an enrichment in nonpolysomal fractions (Fig. 3A).

We next evaluated the effect of eIF4E knockdown on the expression of some of the mRNA proposed previously to be selectively regulated by eIF4E in four different cell lines (Fig. 3B). eIF4E knockdown was associated with down-regulation of cyclin D1 and Bcl-xL in all four cell lines and with Bcl-2 down-regulation in MCF7 and MDA-MB-231 cells.

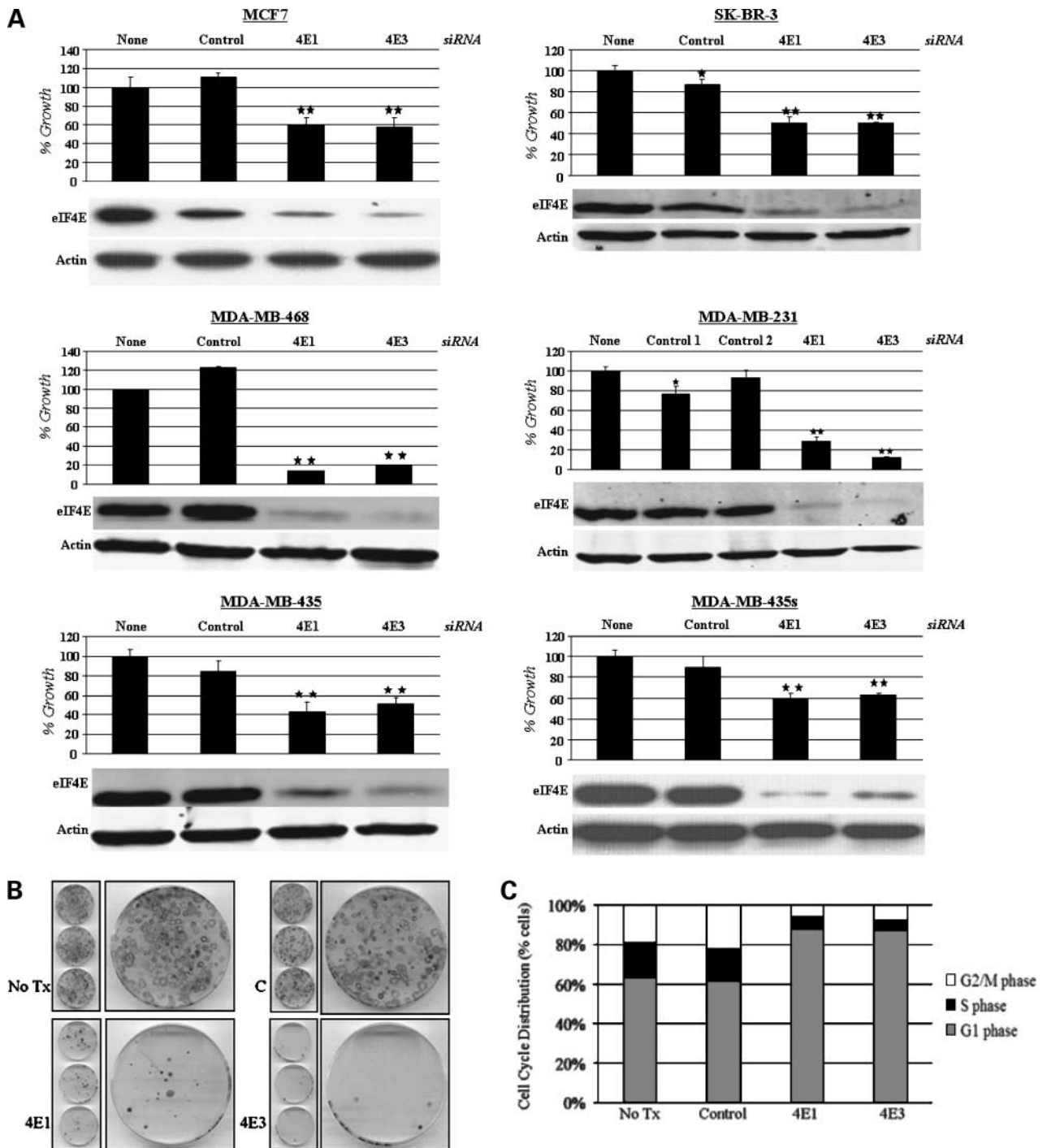


Figure 2. Effect of eIF4E knockdown on cancer cell growth. **A**, MCF7, SKBR-3, MDA-MB-468, MDA-MB-231, MDA-MB-435, and MDA-MB-435s cells were transfected with control siRNA or one of two eIF4E siRNA sequences (4E1 and 4E3). One day after transfection, the cells were trypsinized, counted, and plated in 96-well plates in triplicates. On day 4, cell growth was assayed and cell growth in untreated cells (*no tx*) was normalized to 100%. Representative of three or more experiments. *Columns, mean; bars, SD.* *, $P < 0.05$, untreated group versus control siRNA group by two-tailed Student's *t* test; **, $P < 0.05$, control siRNA group versus 4E1 or 4E3 siRNA by two-tailed Student's *t* test. Seventy-two hours after siRNA transfection, cells were lysed and Western blotting was done for eIF4E and actin expression. Representative Western blots showing eIF4E knockdown in each cell line. **B**, effect of eIF4E knockdown on anchorage-dependent growth. Cells were transfected with control siRNA or one of two eIF4E siRNA sequences (4E1 and 4E3). One day after transfection, the cells were trypsinized, counted, and plated in 60 mm plates (1,000 per plate). After 2 weeks, colonies were stained with crystal violet. **C**, effect of eIF4E knockdown on cell cycle. MCF7 cells were transfected with control siRNA or one of two eIF4E siRNA sequences (4E1 and 4E3). Four days after transfection, the attached cells were harvested, and FACS analysis was done. **, $P < 0.05$, % cells in G_1 in control siRNA group versus 4E1 or 4E3 siRNA by two-tailed Student's *t* test.

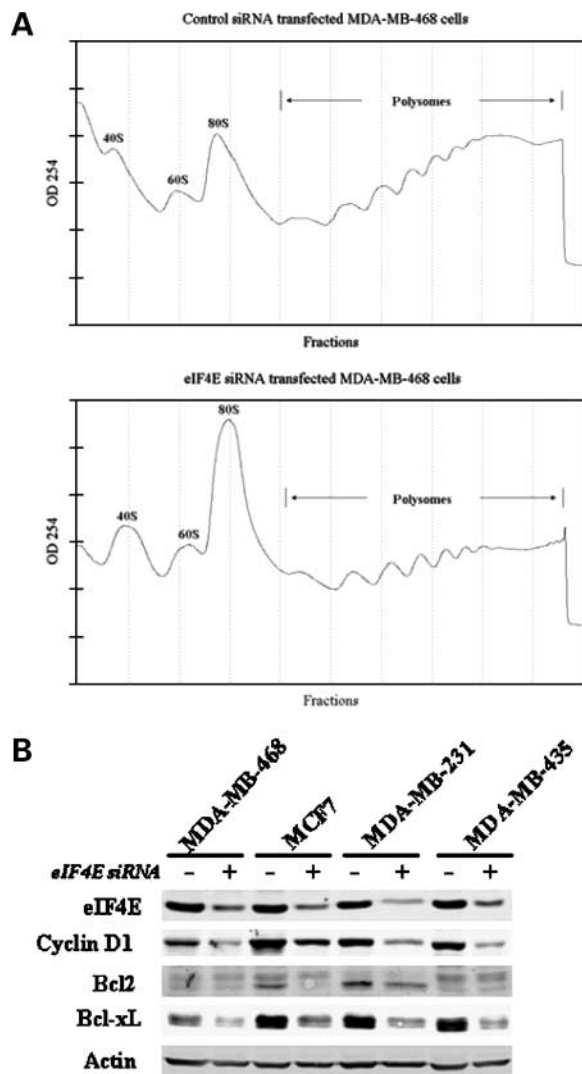


Figure 3. Effect of eIF4E knockdown on translation. **A**, MDA-MB-468 cells were transfected with control siRNA or eIF4E siRNA and were cultured for 3 d to allow the efficient eIF4E knockdown. Cell lysates were then separated on a 15% to 50% sucrose gradient. After centrifugation, gradients were fractionated by using an ISCO Foxy Jr. fractionator (Teledyne Isco). The A254 of gradient was analyzed with an ISCO UA-6 UV/VIS detector. **B**, effect eIF4E knockdown on the translational targets of eIF4E. MDA-MB-468, MCF7, MDA-MB-231, or MDA-MB-435 cells were transfected with control siRNA or eIF4E siRNA (4E3). Three days after transfection, cell lysate was harvested, and Western blotting was done for eIF4E, cyclin D1, Bcl-2, Bcl-xL, and actin.

eIF4E Knockdown Is Not Associated with Activation of Akt but Is Associated with a Decrease in 4E-BP1 Expression

In previous experiments, inhibition of translation via rapamycin was found to lead to an increase in p-Akt (24, 30). Therefore, we tested the effect of eIF4E knockdown on Akt phosphorylation. We found that eIF4E knockdown did not lead to an increase in Akt phosphorylation in MDA-MB-468, MCF7, MDA-MD-231, and MDA-MB-435 cells. MDA-MB-468 cells, a PTEN mutant cell line with consti-

tutive activation of Akt, had no change in its p-Akt levels, whereas the latter three cell lines had low p-Akt levels at baseline without activation with eIF4E knockdown (Fig. 4A). In contrast, rapamycin treatment enhanced p-Akt levels in MDA-MB-435 cells (Fig. 4B). Conditional and modest increases in p-Akt were observed in MCF7 cells, with less robust and less frequent effects noted in MDA-MB-231 and MDA-MB-468 (data not shown). We also evaluated the effect of eIF4E knockdown on the expression of 4E-BP1 and on the expression of S6K1, another mTORC1 target. Of note, Western blotting with anti-4E-BP1 antibodies detects multiple bands, representing isoforms generated by differential phosphorylation (29, 31–33), and Western blotting with anti-S6K1 antibodies detects multiple bands representing isoforms generated by alternative translation initiation (34).³ Interestingly, we found that eIF4E knockdown did decrease the expression of 4E-BP1 expression reproducibly in three of four cell lines, without altering the expression S6K1 (Fig. 4C).

Discussion

Activation of translation initiation is critical for cancer cell growth, survival, and tumor progression. Thus, translation is a rational target for novel cancer therapeutics (35). Here, we show that eIF4E knockdown inhibited growth in different breast cancer cell subtypes including triple-negative tumors for which currently there are no targeted therapies. Further, eIF4E knockdown inhibited rapamycin-insensitive as well as sensitive cell lines. eIF4E knockdown decreased polysomal recruitment and the expression of cyclin D1, Bcl-2, and Bcl-xL. eIF4E knockdown did not lead to Akt phosphorylation. These data suggest that eIF4E is a promising target for breast cancer therapy. eIF4E-targeted therapies may have wide efficacy, in tumors driven by different oncogenic stimuli, and may be effective in rapamycin-resistant tumors.

When targeting a critical process such as translation, a major question is whether the drug will have significant effects on normal cellular processes, leading to limiting toxicities and too narrow a therapeutic window. To address these issues, we attempted *in vivo* siRNA delivery in two independent experiments using neutral liposomal siRNA delivery technologies that have been previously described (36). Unfortunately, although we were able to deliver a fluorescent control siRNA into tumor xenografts and normal tissues, we did not observe knockdown of eIF4E at the RNA or protein level with two different 4E siRNA (data not shown). Thus, we were unable to address the question of *in vivo* efficacy or toxicity in our studies. However, recently Graff et al. did show that eIF4E knockdown can be achieved using antisense eIF4E oligonucleotides in a prostate cancer and a breast cancer xenograft model (19). They showed that eIF4E knockdown inhibits *in vivo* growth, and although 80% eIF4E

³ Kim et al., in preparation.

knockdown was observed in essential organs, no toxicity was observed in these mice models (19). These data provide *in vivo* evidence that cancers may be more susceptible to eIF4E inhibition than normal tissue and have provided the foundation for bringing eIF4E-specific antisense molecule LY2275796 into phase I clinical studies (37).

Another potential approach to inhibit translation is by interfering with its binding to the 7-methyl guanosine cap or by interfering with its binding to the multidomain adaptor protein eIF4G, thus interfering with assembly of the

translation initiation complex eIF4F. Kentsis et al. reported that the antiviral guanosine analogue ribovirin binds to eIF4E at the site used by the 7-methyl guanosine cap, competing with eIF4E binding and disrupting the transport and translation of mRNAs regulated by eIF4E (38). eIF4E-binding motif peptides can also interfere with eIF4E-eIF4G binding, translation initiation, cell cycle, and survival, providing proof of concept that eIF4E-binding small-molecule inhibitors may have utility in cancer therapy (39, 40). Recently, Moerke et al. did a high-throughput screen for inhibitors of the eIF4E/eIF4G interaction (41). The most potent compound exhibited *in vitro* activity against multiple cancer cell lines and appeared to have preferential effect on transformed cells (41).

Cancer therapy remains challenging due to survival pathways that emerge once a promising cancer target is inhibited. Our data suggest that eIF4E knockdown, unlike rapamycin-mediated translational inhibition, is not associated with feedback loop activation of Akt. However, eIF4E knockdown was associated with a decline in 4E-BP1 levels. Larsson et al. recently reported that eIF4E overexpression in human mammary epithelial cells led to an increased translational efficiency of 4E-BP1 transcripts (12). This suggests that 4E-BP1 is under eIF4E-mediated translational control; thus, eIF4E knockdown-induced decline in 4E-BP1 levels may at least in part be attributable to a decrease in its translation. As activation of translation initiation is critical to the malignant phenotype, the cell thus appears to respond to a decrease in eIF4E levels by trying to increase available eIF4E by decreasing its inhibitory protein, a potential compensatory mechanism to maintain active translation.

In conclusion, our results add to the rapidly increasing body of literature showing the critical role of activated translation initiation for cancer cell growth. Our data suggest that eIF4E is a promising therapeutic target in a variety of breast cancer subtypes, including triple-negative tumors for which no targeted therapies are available at this time. Strategies to decrease eIF4E expression such as antisense oligonucleotides or novel siRNA delivery systems as well as novel small molecular inhibitors of eIF4F complex formation may be pursued as potential antitumor strategies.

Disclosure of Potential Conflicts of Interest

F. Meric-Bernstam: Collaborator on Eli Lilly & Co. Clinical Trial (PI: Dr. Yun Oh, MDACC 2005-0770). The other authors disclosed no potential conflicts of interest.

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References

- De Benedetti A, Graff JR. eIF-4E expression and its role in malignancies and metastases. *Oncogene* 2004;23:3189–99.
- Zimmer SG, DeBenedetti A, Graff JR. Translational control of malignancy: the mRNA cap-binding protein, eIF-4E, as a central regulator of tumor formation, growth, invasion and metastasis. *Anticancer Res* 2000;20:1343–51.
- Koromilas AE, Lazaris-Karatzas A, Sonenberg N. mRNAs containing

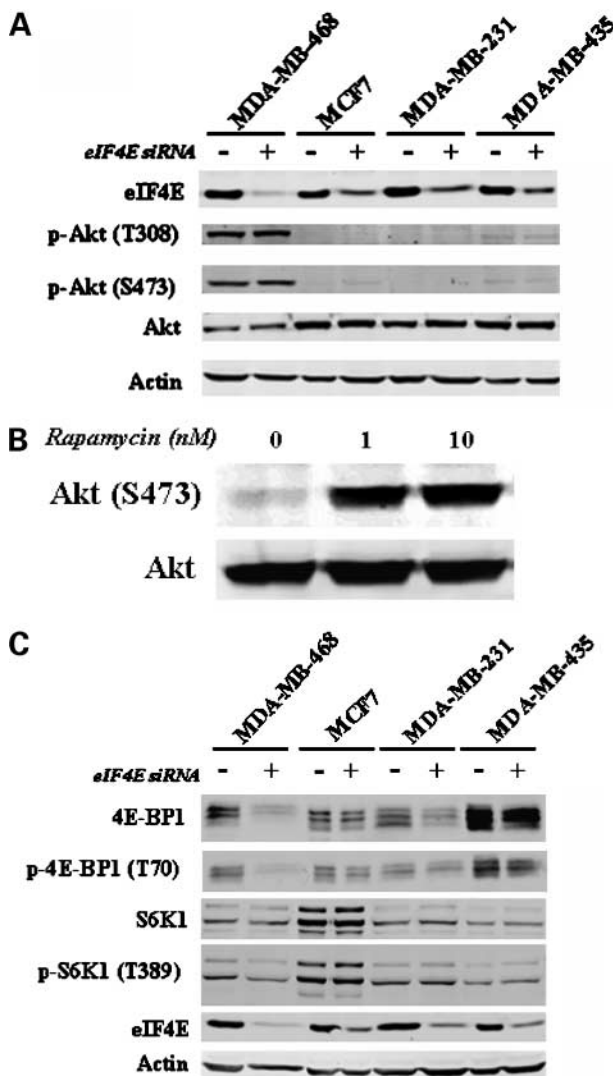


Figure 4. Effect eIF4E knockdown on Akt phosphorylation. **A**, MDA-MB-468, MCF7, MDA-MB-231, or MDA-MB-435 cells were transfected with control siRNA or eIF4E siRNA. Three days after transfection, cell lysate was harvested, and Western blotting was done for eIF4E, p-Akt (T308), p-Akt (S473), Akt, and actin. **B**, MDA-MB-435 cells were treated with vehicle or with 1 or 10 nmol/L rapamycin for 24 h. Western blotting was done for p-Akt (S473) and Akt. **C**, MDA-MB-468, MCF7, MDA-MB-231, or MDA-MB-435 cells were transfected with control siRNA or eIF4E siRNA. Three days after transfection, cell lysate was harvested, and Western blotting was done for 4E-BP1, phosphorylated 4E-BP1 (T70), S6K1, phosphorylated S6K1 (T389), eIF4E, and actin.

- extensive secondary structure in their 5' non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *EMBO J* 1992; 11:4153–8.
4. Rosenwald IB, Kaspar R, Rousseau D, et al. Eukaryotic translation initiation factor 4E regulates expression of cyclin D1 at transcriptional and post-transcriptional levels. *J Biol Chem* 1995;270:21176–80.
 5. Rhoads RE. Regulation of eukaryotic protein synthesis by initiation factors. *J Biol Chem* 1993;268:3017–20.
 6. Pelletier J, Sonenberg N. Insertion mutagenesis to increase secondary structure within the 5' noncoding region of a eukaryotic mRNA reduces translational efficiency. *Cell* 1985;40:515–26.
 7. Kozak M. Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. *Proc Natl Acad Sci U S A* 1986;83:2850–4.
 8. Li S, Takasu T, Perlman DM, et al. Translation factor eIF4E rescues cells from Myc-dependent apoptosis by inhibiting cytochrome *c* release. *J Biol Chem* 2003;278:3015–22.
 9. Yang SX, Hewitt SM, Steinberg SM, Liewehr DJ, Swain SM. Expression levels of eIF4E, VEGF, and cyclin D1, and correlation of eIF4E with VEGF and cyclin D1 in multi-tumor tissue microarray. *Oncol Rep* 2007;17:281–7.
 10. De Benedetti A, Rhoads RE. Overexpression of eukaryotic protein synthesis initiation factor 4E in HeLa cells results in aberrant growth and morphology. *Proc Natl Acad Sci U S A* 1990;87:8212–6.
 11. Fingar DC, Salama S, Tsou C, Harlow E, Blenis J. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev* 2002;16:1472–87.
 12. Larsson O, Li S, Issaenko OA, et al. Eukaryotic translation initiation factor 4E induced progression of primary human mammary epithelial cells along the cancer pathway is associated with targeted translational deregulation of oncogenic drivers and inhibitors. *Cancer Res* 2007;67:6814–24.
 13. Ruggero D, Montanaro L, Ma L, et al. The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. *Nat Med* 2004;10:484–6.
 14. 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.
 15. Nathan CA, Carter P, Liu L, et al. Elevated expression of eIF4E and FGF-2 isoforms during vascularization of breast carcinomas. *Oncogene* 1997;15:1087–94.
 16. Li BD, McDonald JC, Nassar R, De Benedetti A. Clinical outcome in stage I to III breast carcinoma and eIF4E overexpression. *Ann Surg* 1998; 227:756–61; discussion 61–3.
 17. Byrnes K, White S, Chu Q, et al. High eIF4E, VEGF, and microvessel density in stage I to III breast cancer. *Ann Surg* 2006;243:684–90; discussion 91–2.
 18. Li BD, Liu L, Dawson M, De Benedetti A. Overexpression of eukaryotic translation initiation factor 4E (eIF4E) in breast carcinoma. *Cancer* 1997; 79:2385–90.
 19. 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.
 20. Dancy JE. Therapeutic targets: mTOR and related pathways. *Cancer Biol Ther* 2006;5:1065–73.
 21. Hudes GR. mTOR as a target for therapy of renal cancer. *Clin Adv Hematol Oncol* 2007;5:772–4.
 22. Fingar DC, Richardson CJ, Tee AR, Cheatham L, Tsou C, Blenis J. mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor 4E. *Mol Cell Biol* 2004;24:200–16.
 23. Wendel HG, De Stanchina E, Fridman JS, et al. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 2004;428: 332–7.
 24. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006;66: 1500–8.
 25. Sachidanandam R. RNAi Codex; 2007. Available from: <http://codex.cshl.edu/scripts/newmain.pl?content=protocols.html>; cited 11/20/2007.
 26. Lynch M, Chen L, Ravitz MJ, et al. hnRNP K binds a core polypyrimidine element in the eukaryotic translation initiation factor 4E (eIF4E) promoter, and its regulation of eIF4E contributes to neoplastic transformation. *Mol Cell Biol* 2005;25:6436–53.
 27. Mondesire WH, Jian W, Zhang H, et al. Targeting mammalian target of rapamycin synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells. *Clin Cancer Res* 2004;10:7031–42.
 28. Voigt W. Sulforhodamine B assay and chemosensitivity. *Methods Mol Med* 2005;110:39–48.
 29. Noh WC, Mondesire WH, Peng J, et al. Determinants of rapamycin sensitivity in breast cancer cells. *Clin Cancer Res* 2004;10: 1013–23.
 30. Akcakanat A, Singh G, Hung MC, Meric-Bernstam F. Rapamycin regulates the phosphorylation of rictor. *Biochem Biophys Res Commun* 2007;362:330–3.
 31. Beretta L, Gingras AC, Svitkin YV, Hall MN, Sonenberg N. Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J* 1996;15:658–64.
 32. Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. *Genes Dev* 1998;12: 502–13.
 33. Gingras AC, Raught B, Gygi SP, et al. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev* 2001;15:2852–64.
 34. Grove JR, Banerjee P, Balasubramanyam A, et al. Cloning and expression of two human p70 S6 kinase polypeptides differing only at their amino termini. *Mol Cell Biol* 1991;11:5541–50.
 35. Meric F, Hunt KK. Translation initiation in cancer: a novel target for therapy. *Mol Cancer Ther* 2002;1:971–9.
 36. Landen CN, Jr., Chavez-Reyes A, Bucana C, et al. Therapeutic EphA2 gene targeting *in vivo* using neutral liposomal small interfering RNA delivery. *Cancer Res* 2005;65:6910–8.
 37. Isis Pharmaceuticals. Featured research: J Clin Invest; 2007. Available from: http://www.isispharm.com/featured_research_JCI_2007-09-05.html; cited 11/12/2007.
 38. Kentsis A, Topisirovic I, Culjkovic B, Shao L, Borden KL. Ribavirin suppresses eIF4E-mediated oncogenic transformation by physical mimicry of the 'methyl guanosine mRNA cap. *Proc Natl Acad Sci U S A* 2004;101: 18105–10.
 39. Salaun P, Boulben S, Mulner-Lorillon O, et al. Embryonic-stage-dependent changes in the level of eIF4E-binding proteins during early development of sea urchin embryos. *J Cell Sci* 2005;118:1385–94.
 40. Herbert TP, Fahraeus R, Prescott A, Lane DP, Proud CG. Rapid induction of apoptosis mediated by peptides that bind initiation factor eIF4E. *Curr Biol* 2000;10:793–6.
 41. 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.

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