

# Targeting the Eukaryotic Translation Initiation Factor 4E for Cancer Therapy

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## Abstract

**The eukaryotic translation initiation factor 4E (eIF4E) is frequently overexpressed in human cancers in relation to disease progression and drives cellular transformation, tumorigenesis, and metastatic progression in experimental models. Enhanced eIF4E function results from eIF4E overexpression and/or activation of the *ras* and phosphatidylinositol 3-kinase/AKT pathways and selectively increases the translation of key mRNAs involved in tumor growth, angiogenesis, and cell survival. Consequently, by simultaneously and selectively reducing the expression of numerous potent growth and survival factors critical for malignancy, targeting eIF4E for inhibition may provide an attractive therapy for many different tumor types. Recent work has now shown the plausibility of therapeutically targeting eIF4E and has resulted in the advance of the first eIF4E-specific therapy to clinical trials. These studies illustrate the increased susceptibility of tumor tissues to eIF4E inhibition and support the notion that the enhanced eIF4E function common to many tumor types may represent an Achilles' heel for cancer.** [Cancer Res 2008;68(3):631–4]

## eIF4E Function and mRNA Discrimination

Eukaryotic translation initiation factor 4E (eIF4E) binds the 5',7-methylguanosine cap structure of mRNAs, delivering these mRNAs to the eIF4F translation initiation complex, which is composed of eIF4E, the scaffolding protein eIF4G, and the ATP-dependent RNA helicase eIF4A. The eIF4F complex then scans through the 5' untranslated region (UTR), unwinding mRNA secondary structure to expose the translation initiation codon and enable translation (1). eIF4F complex assembly is rate limiting for translation initiation and is largely dependent on eIF4E availability. Under normal cellular conditions, eIF4F complex assembly is limited as eIF4E is sequestered from eIF4G by binding to 4E binding proteins (4EBP). Stimulation of the phosphatidylinositol 3-kinase/AKT/mTOR pathway leads to hierarchical 4EBP phosphorylation, dislodging 4EBP from eIF4E and enabling assembly of the eIF4F complex (1).

Enhanced eIF4F complex formation increases the translation of all cap-dependent mRNAs and thereby increases global protein synthesis rates. However, mRNAs vary widely in their inherent "translatability," largely as a function of differences in the length and structure of their 5' UTRs. Cellular mRNAs most sensitive to

alterations in eIF4E availability and eIF4F complex formation (i.e., weak mRNAs) have lengthy, G+C rich, highly structured 5' UTRs that encumber efficient RNA unwinding by eIF4F and subsequently prevent ribosome loading. Consequently, these mRNAs are most sensitive to eIF4E availability and are poorly translated under normal conditions when eIF4F complex formation is limiting. In contrast, most cellular mRNAs have relatively short, unstructured 5' UTRs (e.g.,  $\beta$ -actin) that enable efficient scanning, initiation codon recognition, and ribosome loading and translation even when eIF4F complex levels are limiting. Whereas strong mRNAs (such as  $\beta$ -actin, glyceraldehyde-3-phosphate dehydrogenase) are only minimally affected by alterations in eIF4F complex formation, weak mRNAs, which typically encode growth and survival factors [e.g., *c-myc*, vascular endothelial growth factor (VEGF), ODC, survivin], are preferentially and disproportionately affected by eIF4E availability (ref. 1; Fig. 1).

## eIF4E in Cancer

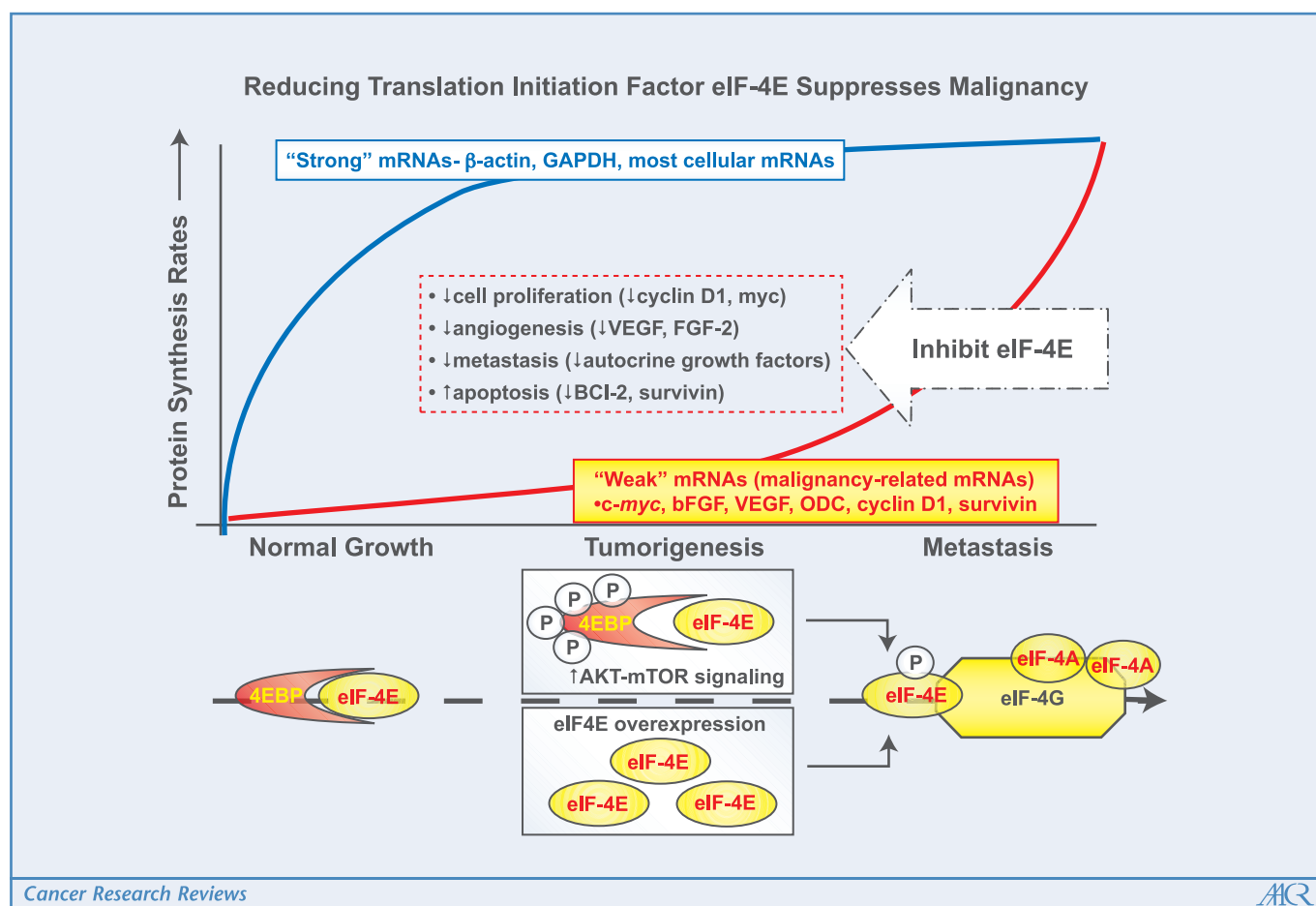
In experimental cancer models, elevated eIF4E function selectively and disproportionately increases translation of weak mRNAs, mRNAs encoding potent growth, and survival factors notoriously involved in malignancy (1). Increased eIF4E function increases the nucleocytoplasmic transport of mRNAs encoding potent growth regulatory proteins, such as cyclin D1 (2), and enhances ribosome loading of mRNAs with lengthy, G+C-rich 5' UTRs (e.g., *c-myc*, ODC, VEGF, survivin, etc.; ref. 1). Interestingly, most mRNAs that are characterized by short, unstructured 5' UTRs (e.g.,  $\beta$ -actin) are largely unaffected by changes in eIF4E activity (ref. 1; Fig. 1).

Not surprisingly then, by selectively increasing the protein expression of growth, angiogenesis, and survival factors, forced eIF4E overexpression in cultured fibroblasts or epithelial cells can induce cellular transformation and tumorigenesis (1, 3, 4). Moreover, in transgenic mice, ectopic eIF4E expression increases the incidence of multiple cancers, including lymphomas, lung adenocarcinomas, hepatomas, and angiosarcomas (5), and accelerates lymphomagenesis (5, 6). eIF4E overexpression can facilitate the establishment of autocrine stimulatory loops (4, 7), suppress apoptosis (8), and impart drug and radioresistance (1, 6)—phenotypic alterations integral to malignant progression.

Whereas enhanced eIF4E function promotes malignancy in experimental models, reducing or inhibiting eIF4E function suppresses malignancy, in concert with reduced expression of potent growth and angiogenesis factors (9–13). Peptides designed specifically to block the interaction of eIF4E with eIF4G rapidly induced apoptosis in MRC5 cells (12). Overexpression of 4EBP1 also suppressed tumorigenesis and growth in Src-transformed cells (11) and in breast cancers (13). Likewise, antisense RNA-mediated reduction of eIF4E in both epithelial and fibroblast tumor models suppressed tumor growth, invasion, and metastasis in concert with

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**Figure 1.** Reducing translation initiation factor eIF4E suppresses malignancy. Cellular mRNAs can be divided into two major categories—the strong mRNAs, which are well translated even when the availability of eIF4E is limiting, and the weak mRNAs, which are translated only when eIF4E availability is increased as in malignancy. eIF4E availability is increased with tumorigenesis and malignant progression by eIF4E overexpression and/or increased signaling through mTOR, which phosphorylates the 4EBPs, releasing eIF4E. Reducing eIF4E expression in tumors via systemic therapy would inhibit cell proliferation, suppress tumor-related angiogenesis, repress metastasis, and increase apoptosis by selectively repressing the expression of potent growth and survival factors (e.g., cyclin D1, c-myc, ODC, survivin, BCL-2, VEGF, FGF-2, etc.). The reduction of eIF4E would have a minimal effect on total protein synthesis and may therefore minimally affect normal tissue function. Figure adapted from *Oncogene* 2004;23:3189–99 (1).

reduced translation of key malignancy-related molecules such as ODC, VEGF, and fibroblast growth factor-2 (FGF-2; refs. 1, 11, 12).

In human cancers, the levels of free eIF4E are commonly elevated consequent to increased eIF4E expression or release of eIF4E from 4EBPs, a result of signaling through the AKT/mTOR and *ras* signaling pathways (Fig. 1), which are frequently activated in a diverse range of human cancers (1). Increased eIF4E expression has been documented repeatedly in association with malignant progression in multiple human cancers, including leukemias and lymphomas and cancers of the breast, colon, bladder, lung, prostate, and head and neck (1). Moreover, recent work has also now shown that 4EBP phosphorylation is also increased and associated with malignant progression in breast, ovarian, prostate, and colon cancers (14).

### eIF4E as an Attractive Anticancer Target

The data that have accumulated over the past 17 years have implicated eIF4E, and by extension the eIF4F complex, as promising targets for anticancer therapy. Indeed, the activation

of eIF4E, either by increased eIF4E expression or by increased phosphorylation of the 4EBPs, is a key event downstream of the *ras* and phosphatidylinositol 3-kinase/AKT/mTOR signaling pathways (1). As such, eIF4E activation may represent a node of convergence for the key signaling events that drive oncogenesis and malignant progression and thereby serve as an Achilles' heel for cancer (15). Moreover, eIF4E function is particularly critical for the expression of a wide array of proteins that contribute to all aspects of malignancy, including growth factors such as c-myc and cyclin D1, angiogenesis factors such as VEGF and FGF-2, degradative enzymes such as matrix metalloproteinase-9 and heparanase, and antiapoptotic proteins such as survivin and Bcl-2. Consequently, inhibition of eIF4E function (and the function of the eIF4F complex) would potentially be a viable strategy for the treatment of many different cancers and would be expected to simultaneously diminish the expression of multiple, important cancer targets (see Fig. 1). Yet, despite the attractiveness of targeting eIF4E (and the eIF4F complex), there have been no eIF4E-specific therapies developed and advanced to clinical trials until recently.

## Targeting eIF4E and the eIF4F Complex

The lack of an eIF4E-specific inhibitor may be attributed to numerous technical issues and concerns. First, to date, no well-defined, drug-like small-molecule inhibitors of the eIF4E: 7-methylguanosine cap interaction have been reported. Second, the potential exists that targeting a general protein synthesis factor could elicit substantial toxicity. Recent work in our laboratory and others has now begun to address these concerns and has identified the first eIF4E-specific therapy to be advanced to human clinical trials.

How might we develop a therapeutically viable, systemically deliverable eIF4E-specific therapy? The first option might be to target the interaction of eIF4E with the 7-methylguanosine cap structure, an interaction that has been described structurally. A recent report has indicated that the small-molecule ribavirin might interfere with this interaction and may therefore present a clinical opportunity as an eIF4E-targeted therapy. As anticipated, ribavirin treatment selectively diminished the expression of key, eIF4E-dependent proteins such as cyclin D1 and suppressed tumor growth. However, the direct inhibitory effect of ribavirin on the eIF4E-cap interaction has been controversial (16).

A second approach to developing a small-molecule inhibitor of eIF4E function has exploited the interaction of eIF4E with the scaffolding protein eIF4G. Wagner and colleagues were able to develop specific inhibitors of the eIF4E-eIF4G interaction. Treatment of cancer cells in culture with these agents elicited the expected reduction in expression of eIF4E-regulated proteins, such as c-myc and cyclin D1. Perhaps most compelling, these inhibitors blocked only the eIF4E-eIF4G interaction but did not block the eIF4E-4EBP1 interaction. These inhibitors show the exciting possibility that small molecules could be developed to specifically prevent eIF4F complex assembly (17).

Recent work from Pelletier and colleagues has now also revealed the plausibility of targeting the helicase function of the eIF4F complex. From a phenotypic screen for inhibitors of cap-dependent translation, they have discovered a natural product, pateamine A, which serves to promote the indiscriminant association of the RNA helicase eIF4A with RNA, thereby blocking the incorporation of eIF4A into the eIF4F complex (18). These data clearly show the possibility of exploiting this member of the eIF4F complex as a therapeutic target.

We have recently taken an alternative approach to targeting eIF4E and have now delivered the first eIF4E-specific therapeutic to advance to clinical trials (19). Exploiting advances in antisense oligonucleotide (ASO) chemistry, we have developed an eIF4E-specific, second-generation ASO. This 20-mer ASO has been engineered with five 2'-O-(2-methoxyethyl)-modified bases at either end of the ASO flanking an internal ten 2'-deoxyphosphorothioate bases (to maintain the capacity for RNase-H-mediated target mRNA degradation). These MOE modifications substantially enhance plasma and tissue stability as well as increase the affinity for target mRNA. In addition, these ASOs have been engineered specifically to avoid the immunostimulatory activity that stifled the development of earlier generation ASOs. Consequently, such MOE-gapmer ASOs can be systemically delivered and offer a much-improved capacity for therapeutic utility (19). Indeed, data from recent human clinical trials have shown that second-generation ASOs effectively reach tumor tissue and, in a dose-dependent manner, repress expression of the target RNA and protein (20).

The eIF4E MOE-gapmer ASO effectively reduces eIF4E RNA and protein in a wide array of transfected human and murine cells with an  $IC_{50}$  of <25 nmol/L. As expected, in cultured tumor cells, the reduction of eIF4E by this ASO decreased the expression of key, malignancy-related proteins—specifically cyclin D1, VEGF, c-myc, survivin, and BCL-2—but did not affect the expression of  $\beta$ -actin, a protein encoded by a “strong” mRNA. Moreover, unlike treatment with cycloheximide, which completely blocks translation elongation and thereby prevents all new protein synthesis, the eIF4E ASO reduced total protein synthesis by only ~20%. These data illustrate the selective effect that eIF4E inhibition has and that this effect is qualitatively and quantitatively distinct from the inhibition of global protein synthesis by cycloheximide.

The most prominent biological consequence of reducing eIF4E in cultured tumor cells was the dramatic induction of apoptosis and the concomitant reduction in cell viability. In human umbilical vein endothelial cells, the reduction of eIF4E suppressed the formation of vessel-like tube structures, suggesting that eIF4E plays a role in the response of endothelial cells to angiogenic stimuli. These results support the notion, born from 17 years of accumulated research, that targeting eIF4E would elicit an antitumor effect and would selectively diminish the expression of multiple proteins important to malignancy.

Numerous reports have shown that inhibition of eIF4E function would induce cell death and selectively suppress the expression of eIF4E-regulated proteins (1). However, these studies were always executed in transfected cell lines. Because eIF4E reduction in these studies was limited only to the engineered cell, there was no opportunity to address the critical question of whether the reduction of eIF4E function in normal tissues could be tolerated. Because these second-generation ASOs can be delivered systemically, simultaneously examining the effect of eIF4E reduction on normal and tumor tissues was possible. To accomplish this, the eIF4E ASO was specifically selected to recognize and target both murine and human eIF4E for reduction. Bolus i.v. administration of the eIF4E ASO to human tumor-bearing nude mice successfully reduced eIF4E expression in the human tumor xenograft tissues ~50% and substantially inhibited growth of human breast and prostate xenograft tumors. eIF4E ASO treatment induced apoptosis as measured by terminal deoxynucleotidyl transferase-mediated nick-end labeling staining and suppressed proliferation as monitored by Ki-67 positivity in the xenografted tumor tissue as well. In addition, eIF4E-ASO-treated xenografts showed a dramatic reduction in VEGF immunostaining and a reduction in the endothelial specific marker, von Willebrand's factor, suggesting that eIF4E ASO treatment reduced tumor vascularity. These data showed that the eIF4E ASO effectively reduced eIF4E expression in tumor tissue after systemic administration.

Perhaps most importantly, these xenograft studies also revealed that despite 80% reduction in eIF4E expression in liver, the eIF4E ASO was well tolerated with no evidence of weight loss or other signs of illness or distress in treated mice. In separate studies in normal mice with four different eIF4E-specific ASOs, i.v. administration also revealed substantial reduction of eIF4E in the murine liver tissue (70–80%) without any substantial effect on body weight, liver weight, spleen weight, or liver transaminase levels (19). Collectively, these data therefore provide the first direct evidence that tumor tissues would be more sensitive to the effects of eIF4E inhibition than normal tissues, a differential effect consistent with the conceptual understanding that eIF4E activity is elevated in, and required by, tumor tissue to sustain the expression of key growth

and survival factors that contribute to malignancy. These data thereby prompted the initiation of eIF4E ASO clinical trials in cancer patients starting in 2006.

## Concluding Remarks

Nahum Sonenberg's laboratory published the first report implicating eIF4E in oncogenesis in 1990 (3). Zimmer and colleagues quickly followed with reports demonstrating that the inhibition of eIF4E profoundly repressed tumor growth and malignancy in experimental models (1, 9, 10). Since these reports in the early 1990s, numerous articles have now substantially enhanced our understanding of the role for eIF4E in malignancy. These reports have shown that eIF4E function is commonly elevated in human cancers either by overexpression or by inactivating phosphorylation of the 4EBPs (1). These studies have also shown that eIF4E plays a role not only in cell growth and proliferation but also in the apoptotic response and in the acquisition of drug resistance (6, 8). These studies have established an ever-increasing list of mRNAs controlled by eIF4E that encode proteins with notable functions in all aspects of malignancy, including angiogenesis and invasiveness (1). Together, these reports have clearly implicated eIF4E as a promising target for anticancer therapy.

The most recent studies have now shown the plausibility of developing novel therapeutics that target eIF4E (19). These studies

have revealed that inhibiting eIF4E selectively suppressed expression of eIF4E-regulated proteins and substantially repressed tumor growth in preclinical tumor models. These studies also revealed that, in addition to a direct effect on tumor tissue, inhibiting eIF4E may have a substantial antiangiogenic effect. Importantly, these studies revealed that substantial reduction of eIF4E in normal tissues (i.e., liver) can be well tolerated by xenograft-bearing athymic nude mice and by normal immunocompetent mice (19). These studies support the concept that tumor tissues may indeed be preferentially susceptible to the inhibition of eIF4E. Clinical trials are presently under way to determine whether similar findings will be realized with the eIF4E ASO. Nevertheless, with the advance of this particular eIF4E-targeted therapy to the clinic and with continued research to uncover inhibitors of the eIF4F complex, it seems that targeting the protein translation machinery is an increasingly attractive, feasible, and potentially effective strategy for the treatment of human malignancies.

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