CHAPTER 12

Inhibition of Translation Initiation as a Novel Paradigm for Cancer Therapy

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Contents

1. INTRODUCTION

The regulation of gene expression at the level of translation initiation is critical for proper control of cell growth, proliferation, differentiation, and apoptosis. Deregulation of translation initiation is frequently observed in tumors and plays an important role in the genesis, progression, and

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maintenance of some cancers. This is because unrestricted translation favors expression of genes that promote cell proliferation, malignant transformation, and cancer progression. Conversely, restricting the translation initiation by molecular and chemical genetic approaches reverts the malignant phenotype because it preferentially reduces translation of mRNAs that code for proteins important for the genesis and progression of cancer. The growing understanding of the structural biology and the mechanistic insight into the translation initiation cascade led to the identification of pharmacological targets for the development of mechanism-specific anticancer agents: a new paradigm for anticancer therapy. This report will not cover the impact of changes in signaling pathways such as PI3K-Akt and Ras-Raf MAPK on translation initiation.

Translation initiation is the process of assembling the translation competent ribosome on the AUG start codon of the *bona fide* open reading frame (ORF). This requires, at a minimum, the assembly of mRNA, 43S preinitiation complex, and 60S ribosomal subunits in a complex such that anticodon of Met-tRNA (Met-tRNAi) is paired with the AUG start codon of the ORF (Figure 1). The translation initiation machinery is made up of a host of translation initiation factors. The ternary complex, which comprises of the Met-tRNAi and the GTP-coupled eukaryotic initiation factor 2 (eIF2)

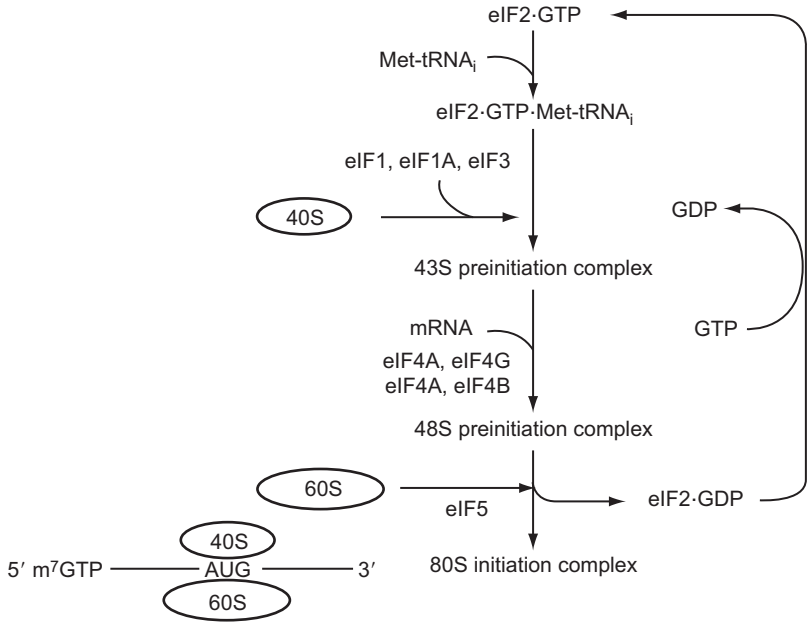


Figure 1 Eukaryotic cap-dependent translation initiation cascade.

binds to the small ribosomal subunit (40S) forming part of the 43S preinitiation complex. This complex also includes other translation initiation factors such as eIF1, eIF1A, and eIF3. The interaction of the 43S preinitiation complex with mRNA is facilitated by the eIF4F complex formed by the eIF4G, the scaffolding protein, eIF4A, the DEAD-box RNA helicase, and the eIF4E, the mRNA cap (the 7-methyl-guanosine 5′-triphosphate) binding protein. The binding of eIF4E to the cap at the 5′-end of mRNA is critical for scanning to locate the AUG initiation codon. The RNA helicase eIF4A in the eIF4F complex unwinds the secondary structure of the mRNA and allows the 43S preinitiation complex to scan the 5′ untranslated region (5′UTR) of the mRNA for the AUG start codon. The scaffolding protein eIF4G also interacts with other translation initiation factors such as eIF3, eIF2, eIF1, and eIF5 and polyadenylate binding protein. The eIF1 in the preinitiation complex plays an important role in the processivity of the 5′UTR scanning and, together with eIF1A and eIF5B, contributes to selection of the *bona fide* start codon. In addition, the hydrolysis of GTP in the ternary complex by eIF5 and eIF5B plays an important role in the binding of the large ribosomal subunit to the 48S preinitiation complex and formation of the 80S initiation complex that is competent for polypeptide synthesis [1–3].

1.1. Role of translation initiation in cancer

Tight regulation of translation ensures that the appropriate quantity and mix of proteins is synthesized. For example, quiescent cells synthesize mostly metabolic and housekeeping proteins. In proliferating cells, not only is the overall rate of protein synthesis increased, but also the mix of newly synthesized proteins is changed to include proteins required for DNA synthesis, chromosome segregation, and cell division. Relieving the physiological restraints on translation initiation induces preferential synthesis of oncogenic proteins and results in malignant transformation.

1.2. Weak and strong mRNAs

The secondary structures in the 5′UTR of mRNAs reduce the processivity of and may cause termination of scanning, while the upstream ORFs (uORFs) cause unproductive initiations. Both these factors lead to reduced translational efficiency. The mRNAs that contain stable secondary structure in the 5′UTR require ATP and the helicase activity of eIF4A to enhance the scanning by the 43S preinitiation complex [1]. Frequent initiation and robust RNA helicase activities are needed to overcome inefficiencies caused by complex secondary structures and uROFs. Interestingly, strong mRNAs coding for housekeeping proteins usually possess a relatively short and simple 5′UTR, while those coding for most pro-proliferation and survival proteins contain a rather long

5′UTR usually burdened by stable secondary structures and/or uORFs. The stable secondary structures and uORFs reduce while strong Kozak consensus sequences [4] increase the efficiency of translation. The stable secondary structures in the 5′UTR, presence of uORFs, and weak Kozak sequences render the mRNA translation highly dependent on the activity of translation initiation factors. This differential dependence of mRNA translatability on the activity of the translation initiation factors forms one of the bases of gene specific regulation at the level of translation initiation [5–7]. Perhaps not surprisingly, malignant transformation is associated with a selective increase in the translation of weak mRNAs that encode for numerous growth factors and oncogenic proteins.

1.3. Expression of translation initiation factors in cancer

Components of the eIF4F complex are overexpressed in many cancers. Levels of eIF4E are elevated in non-Hodgkin's lymphoma, neuroblastomas, and cancers of breast, bladder, colon, prostate, gastrointestinal tract, and lung. Further, in head and neck as well as in breast cancers [8], levels of eIF4E increase during the progression from normal tissue to invasive carcinomas, and this increase correlates with the risk of recurrence after surgical excision [9] and with the cancer-related mortality [10–12]. Levels of eIF4G are elevated in squamous cell lung carcinomas [13–16], and eIF4A is overexpressed in melanomas and primary hepatocellular carcinomas [17,18]. Experimentally, overexpression of eIF4E in mice leads to malignant transformation. However, ectopic expression of eIF4E-binding protein-1 (4E-BP1), which inhibits eIF4E/eIF4G protein–protein interaction, suppresses translation initiation. This results in partial reversal of transformed phenotype and tumorogenesis. These observations support the notion that pharmacological interventions that reduce the activity of eIF4F may offer a new paradigm for anticancer therapy.

Similarly, overexpression of eIF2, the critical component of the ternary complex, and inactivating mutations eIF2α kinases has been reported in human cancers [19–22]. Both these will increase the abundance of the ternary complex rendering translation initiation unrestricted. Experimentally, forced expression of eIF2α-S51A, a nonphosphorylatable eIF2α mutant [23] or of Met-tRNA$_i$, causes transformation of normal cells into malignant cells [24].

Finally, various subunits of eIF3 are overexpressed in some human cancers, and when that occurs, it usually predicts a poor prognosis [25]. Consistently, overexpression of the five subunits of eIF3, either individually or in combination, causes malignant transformation [26].

1.4. Drug targets in the translation initiation cascade

The availability of a high resolution crystal structure of the cap-binding protein eIF4E, which is considered to be rate limiting for the translation initiation process, and its complexes with 7-Me-GDP [27], as well as with the 17 residues from the consensus sequences of 4E-BP1(51–67) and eIF4GII(621–637) [28], made it the first target for the development of anticancer agents. The developments targeted both the eIF4E/eIF4G interface and the cap-binding site on eIF4E. Another obvious target for inhibitors of initiation is eIF4A, the ATP-dependent RNA DEAD-box helicase. Structural studies [29–31] suggest sites for targeting eIF4A either in the domain interface, the ATP binding sites, or interfaces with the eIF4G middle domain. There are additional potential targets in the translation initiation machinery, such as the eIF5, eIF2, eIF1, and eIF1A proteins. However, the impact of inhibition of these translation initiation factors remains to be studied.

2. STATE OF THE ART

2.1. Inhibitors of eIF4F

Direct targeting of eIF4F complex was accomplished by either disrupting the expression of eIF4E or by blocking the interaction between eIF4E and eIF4G (Figure 2). The former was achieved by either developing eIF4E-specific antisense oligonucleotides (ASOs) that trigger RNase H-mediated RNA digestion [32,33] or by using synthetic eIF4E-specific siRNA that binds and cleaves the cognate eIF4A mRNA [34], while the latter was achieved by high-throughput screening campaign of small molecule libraries employing a fluorogenic eIF4GII-derived eIF4E-binding peptide and a transgenically expressed GB1-eIF4E fusion protein [35].

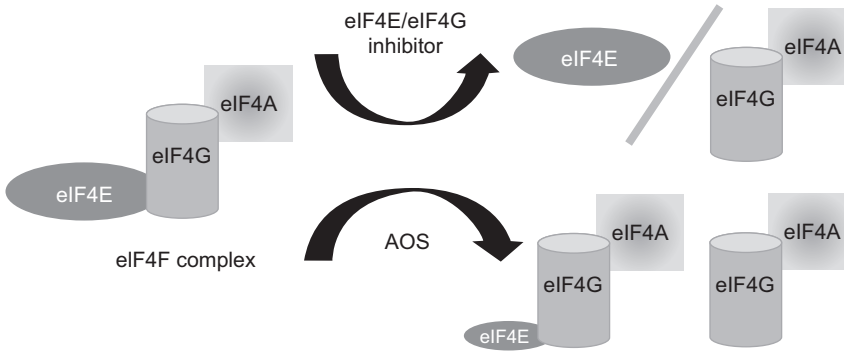


Figure 2 Strategies to inhibit formation of the eIF4F complex.

2.1.1. Targeting eIF4E

2.1.1.1. Antisense Oligonucleotides Early studies demonstrated that many phenotypic changes associated with HeLa cells and *ras*-induced malignant transformation of other cells can be reversed by expression of ASOs complementary to eIF4E mRNA [36,37]. Ectopic expression of eIF4E ASOs in FaDu, a head and neck squamous cell carcinoma (HNSCC) cell line, suppressed tumorigenic and angiogenic properties in a mouse model of human HNSCC [28]. The ASO reduced eIF4E expression in numerous human and murine cell lines and decreased the expression of key malignancy-related proteins such as cyclin D1, VEGF, c-myc, survivin, and BCL-2, but not the expression of housekeeping protein such as β-actin. Moreover, ASO-associated reduction in eIF4E expression leads to apoptosis, reduction in cell viability, and inability of HUVEC to form vessel-like tube structures [38]. Systemic treatment of mice models of human prostate and breast cancers with eIF4E ASO (Figure 3) leads to increased apoptosis, inhibition of tumor growth, and the reduction of eIF4E expression. Immunostaining of VEGF and endothelial specific marker, von Willebrand's factor, suggest that eIF4E ASO treatment reduced tumor vascularity with no apparent toxicity [29]. In a phase I clinical trial with patients who suffer from a variety of cancers, treatment with eIF4E ASO LY2275796 (**1**) induced apoptosis and reduced the tumors' expression of eIF4E and eIF4E-regulated proteins BCL-2 and c-myc [39]. Currently, two phase 1b/2 combination studies of ISIS-EIF4E$_{Rx}$ (LY2275796) with either (a) carboplatin and paclitaxel or (b) docetaxel and prednisone, on non-small cell lung and castrate-resistant prostate cancers, respectively, are underway.

2.1.1.2. Inhibitors of eIF4E/eIF4G Interaction Both eIF4G and 4E-BP1 share a hydrophobic and helical consensus motif, Y(X)$_4$Lϕ, which binds to a conserved and hydrophobic hotspot on the dorsal surface of eIF4E

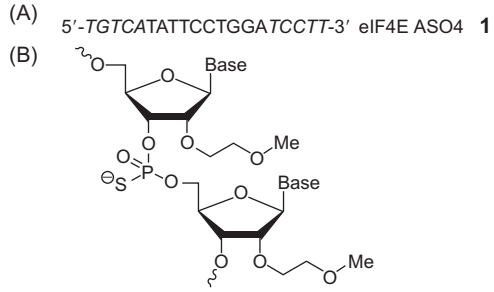


Figure 3 (A) The clinical candidate eIF4E ASO LY2275796 and (B) Schematics of the phosphorothioate backbone and 2′-*O*-(2-methoxyethyl)-RNAs (at the italicized segment in structure A).

(Figure 4) [28]. This motif formed the basis for a high-throughput fluorescence polarization (FP) screening assay, which led to the discovery of a prototypic inhibitor of eIF4E/eIF4G interaction: 4EGI-1 (**2**, Figure 5) [35]. In the cell-free FP assay, 4EGI-1 displayed a $K_d \approx 25$ μM. In rabbit reticulocyte lysate, 4EGI-1 inhibits binding of full-length eIF4G to eIF4E with a K_d of 20 μM and inhibits cap-dependent translation of a dual-luciferase mRNA reporter construct. Interestingly, 4EGI-1 enhances the binding of 4E-BP1 to eIF4E. NMR titration of GB1-eIF4E fusion protein with 4EGI-1 suggests reversible and tight ligand binding to residues on the convex dorsal surface of eIF4E. Importantly, 4EGI-1 inhibited expression of oncogenic proteins such as c-Myc and Bcl-XL at the translational level, with negligible effects on the translation of housekeeping proteins such as

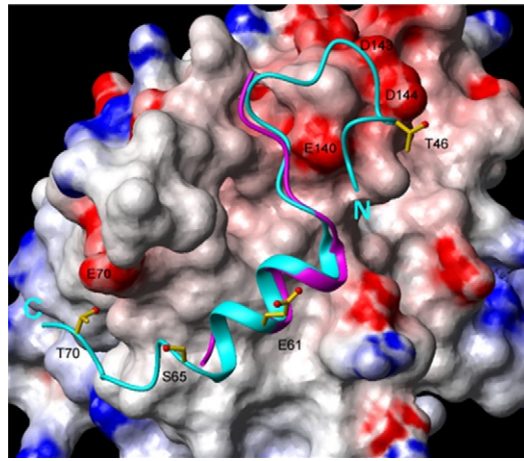


Figure 4 eIF4G- and 4E-BP-derived peptides (cyano and aquamarine, respectively) containing the helical conserved consensus motif, $Y(X)_4L\phi$, bind to the same hydrophobic patch on the convex dorsal surface on eIF4E.

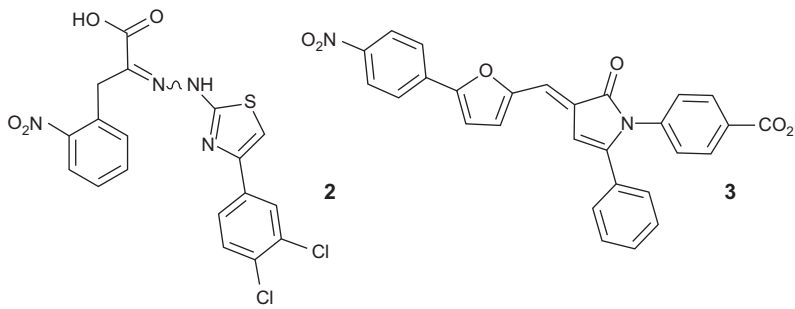


Figure 5 Inhibitors of eIF4E/eIF4G protein–protein interactions: 4EGI-1 (**2**) and 4E1RCat (**3**).

β-actin, tubulin, or ubiquitin. Further, 4EGI-1 inhibits proliferation and/or survival of Jukart cells, various melanoma, breast, lung, and prostate cancer cell lines with IC_{50}s of 1–20 μM, and inhibits preferentially proliferation of p210bcr/abl-transformed Ba/F3 cells compared to vector-transfected immortalized maternal cells (IC_{50} 15 and >40 μM, respectively) [35]. *In vivo*, 4EGI-1 strongly inhibits growth of human CRL-1500 breast and CRL-2813 melanoma cancer xenografts, without any apparent macroscopic- or microscopic-toxicity [40]. As anticipated, in the tumors, treatment with 4EGI-1 led to dissociation of eIF4G from eIF4E and increased the association of 4E-BP1 with eIF4E. The 4EGI-1 treated tumors had a lower expression of proliferating cell nuclear antigen (PCNA) and oncogenic and growth regulatory proteins such as cyclins D1 and E, c-Myc, Bcl-2, and VEGF as compared with the nontreated tumors. Importantly, 4EGI-1 did not increase the fraction of apoptotic cells in the treated tumors as compared with the nontreated ones [40].

The antileukemic potential of 4EGI-1 in acute myeloid leukemia (AML) is demonstrated *ex vivo* by robust reduction of clonogenic growth of AML precursors and a massive induction of blast cell apoptosis [41], with minimal impairment of normal hematopoiesis. Importantly, treatment of AML cells by 4EGI-1, but not the rapalog RAD001, abrogates eIF4F complex formation, reduces translation of c-Myc mRNA, and reduces levels of c-Myc, cyclin D1, and Bcl-xL, proteins implicated in the oncogenic process.

In human lung cancer cells, in addition to the inhibition of cap-dependent translation, 4EGI-1 also augments tumor necrosis factor-related apoptosis-inducing ligand (TRIAL), which mediates apoptosis through induction of death receptor 5 (DR5), and downregulates c-FLIP, which inhibits caspase-8 activation. This pro-apoptotic activity of 4EGI-1 appears to be independent of inhibition of cap-dependent translation [42]. As such, 4EGI-1 contributes in a cooperative manner to the induction of apoptosis by inhibiting cap-dependent translation initiation, while sensitizing human lung cancer cells to TRIAL-induced apoptosis. This could be an exciting demonstration of reinforcing polypharmacology. 4EGI-1 also preferentially suppresses proliferation of malignant pleural mesothelioma cells by inhibiting cap-dependent translation as compared to LP9 normal mesothelial cells and inhibits growth of non-small cell lung cancer by the same mechanism [43,44].

Recently, other screening efforts led to the discovery of 4E1RCat (**3**, Figure 5), which inhibits eIF4E/eIF4G interaction with an $IC_{50} \sim 4$ μM as measured in the time-resolved-FRET assay [45]. It is distinct from **2** because it inhibits not only eIF4E/eIF4G interactions but also the eIF4E/4E-BP1 interaction. In an animal model of Eμ-Myc-driven lymphoma, **3** was effective in reversing chemo-resistance to doxorubicin treatment.

2.1.1.3. Ribavirin The antiviral drug Ribavirin (**4**, Figure 6) is a nucleotide analog that causes hypermutations in the genome of RNA viruses and disrupts replication of DNA viruses [46]. Ribavirin displays cytostatic activity in mammalian cells and is reportedly useful for treatment of patients with acute myeloid leukemia [47,48]. It is currently being evaluated for treatment of breast cancer patients [49]. Although some reports attributed the anticancer activity of Ribavirin to its mimicry of 7-methyl-guanosine (mRNA cap) and inhibition of translation initiation[50,51], others have challenged this view [52,53]. Specifically, ribavirin was two to four orders of magnitude less potent than 7-Me-GTP *in vitro* in inhibiting cap-dependent translation. Further, unlike 7-Me-GTP, Ribavirin did not preferentially inhibit cap-dependent translation compared to cap-independent translation.

2.1.1.4. 7-Methylguanidine (Cap) Analogs The eIF4E interacts with the mRNA cap, forming the 48S initiation complex, which is critical for translation initiation. Therefore, cap (Me^7GTP **5**) analogs (Figure 7) have been sought after as potential inhibitors of translation initiation and novel anticancer drugs. Several attempts were made over the years to synthesize cap analogs that will be cell permeable, bind to eIF4E with high

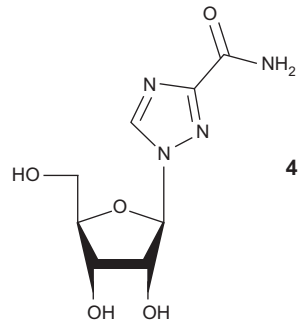


Figure 6 Ribavirin, inhibitor of eIF4E/mRNA cap interaction.

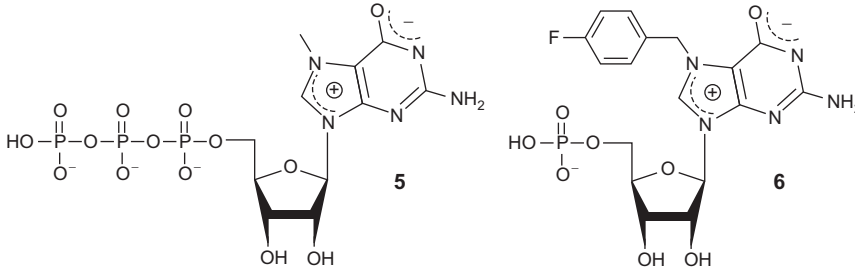


Figure 7 Analogs of the cap, 7-Methylguanidine, Me^7GTP (**5**) and *p*-FBn^7GMP (**6**).

affinity, and inhibit effectively cap-dependent translation [54,55]. An interesting series of Bn^7-GMP derivatives yielded one of the most potent cap analogs *p*-FBn^7GMP (**6**, $K_d = 1.96$ μM) [56]. To date, none of these have been employed as anticancer agents *in vivo*.

2.1.1.5. 4E-BP1 and Related Peptides 4E-BP1 is a natural repressor of translation initiation that disrupts eIF4E/eIF4G interaction. Cell permeable peptides that contain the conserved eIF4E-binding motif present in eIF4G and 4E-BP1 will inhibit eIF4E/eIF4G interaction [28,57]. 4E-BP1-derived peptides fused to cell penetrating peptides, such as penetratin [58] and TAT [59], induce apoptosis in cancer cells [58]. More recently, a helix-stabilized eIF4E-binding peptide derived from eIF4G1 fused to TAT was shown to inhibit translation initiation and induce apoptosis in MCF-7 breast cancer cells [59].

In another approach, 4E-BP1-derived eIF4E-binding sequence was conjugated to an agonist of gonadotropin-releasing hormone [D-Lys^6] GnRH, generating a fusion peptide [D-Lys^6]GnRH–4E-BP1 that can be taken up by GnRH receptor-expressing tumor cells in a tissue-specific manner. GnRH in this context serves the dual purpose of facilitating entry of peptide into the cells, and targeting it only to ovarian cancer cells [60]. In a mouse model of intraperitoneally implanted ovarian cancer, this peptide reduced tumor burden compared to saline treatment [60]. Another approach to this peptide based cancer therapy is the delivery of full-length 4E-BP1 into the cells by adenovirus mediated gene therapy [61]. Adenovirus-mediated transfer of 4E-BP1 by itself failed to inhibit the growth of pancreatic or gastric carcinoma cells because hyperphosphorylation of recombinant protein inhibited its binding to eIF4E. Combined treatment with mTOR inhibitors, on the other hand, reduced the phosphorylation of recombinant 4E-BP1 and inhibited proliferation of cancer cell proliferation *in vitro* and tumor growth *in vivo* [61].

2.1.1.6. MAPK-Interacting Kinases Inhibition of the mitogen-activated protein kinase (MAPK) and interacting kinases (Mnk1), which bind to the C-terminal MA3 domain of human eIF4G and phosphorylates Ser209 of eIF4E, has recently been shown to block eIF4E phosphorylation and suppress outgrowth of experimental lung metastases [62]. Screening has identified antifungal agent cercosporamide (**7**, Figure 8) as a potent, selective, and orally bioavailable Mnk inhibitor. Cercosporamide blocks eIF4E phosphorylation *in vitro* and in normal mouse tissue and xenografted tumors within 30 min after oral administration. Cercosporamide reduces tumor growth in HCT116 tumor bearing animals, and suppresses the outgrowth of B16 melanoma lung metastases [62]. Thus, the Mnk/eIF4G complex may be an attractive target for development of antitumor agents.

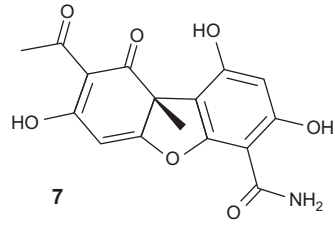


Figure 8 Cercosporamide inhibits Mnk1 and prevents phosphorylation of eIF4E.

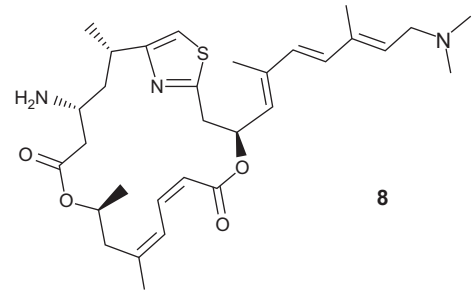


Figure 9 Pateamine A, a thiazole-containing macrolide.

2.1.2. Targeting eIF4A

2.1.2.1. Pateamine A Pateamine A (**8**, Figure 9), a thiazole-containing macrolide isolated from the marine sponge *Mycale* sp., with well-known antineoplastic activity [63,64] interacts with eIF4AI, disrupts the eIF4A/eIF4G interaction, and inhibits translation initiation [65–68]. In biochemical assays, **8** stimulates rather than inhibits the helicase activity of eIF4A by induction of conformational changes in the structure of eIF4A, but because **8**-bound eIF4A cannot associate with eIF4G, it cannot unwind mRNA secondary structures in the 48S preinitiation complex (Figure 10). Treatment of cells by **8** induces formation of stress granules, presumably by inhibiting translation initiation [69,70]. Pateamine A inhibits the nonsense-mediated mRNA decay by directly targeting eIF4AIII, a core component of the exon junction complex (EJC), independent of inhibition of translation initiation [71]. Simplified **8** analogs have been synthesized and shown to possess potent anticancer activity [72]. Pateamine A causes acute inhibition of DNA synthesis presumably by inhibition of DNA polymerases, indicating that polypharmacology, that is, modulation of several unrelated targets by **8** may contribute to its apparent antineoplastic effects.

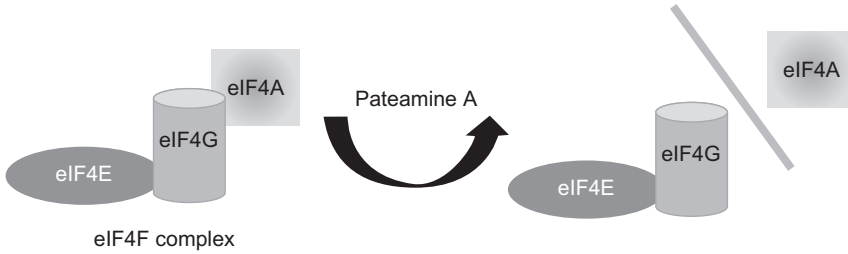


Figure 10 Pateamine A binds to eIF4A and inhibits the assembly of eIF4F complex.

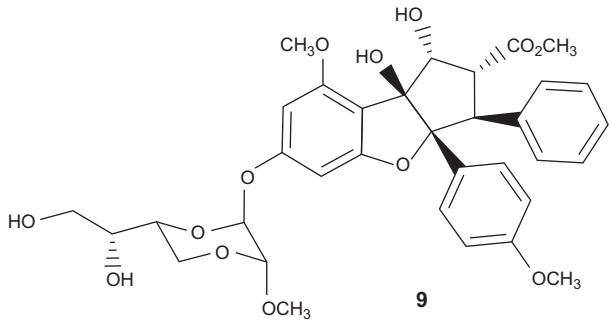


Figure 11 Silvestrol, a natural product and a member of the flavagline family that targets translation initiation.

2.1.2.2. Cyclopenta[b]benzofuran Flavaglines—Silvestrol Cyclopenta[*b*] benzofuran flavaglines (CBFs) are potential anticancer agents originally isolated from the species of *Aglaia* genus of the *Meliaceae* plant family. These agents inhibit protein synthesis and display *in vitro* activity against cancer cells and *in vivo* activity against xenograft tumors [73–78].

Silvestrol (**9**, Figure 11), a CBF isolated from *Aglaia silvestris*, inhibits translation initiation by targeting eIF4A and ribosome loading onto mRNA templates [79]. Silvestrol prevented growth of human Jurkat and LNCaP cells in culture [77,80] and showed weak antitumor potential in xenograft tumor models of human BC1 breast cancer [74]. While ineffective as a single agent against Eμ-myc lymphomas that harbor mutants of tumor-suppressor phosphatase and tensin homolog (PTEN), or overexpress eIF4E [79], Silvestrol inhibited growth of human MDA-MB-231 breast cancer xenografts [81]. Importantly, in combination with doxorubicin, it was effective against *Pten*+/−Eμ-*myc* and Eμ-*myc/eIF4E* tumors, which were refractory to rapamycin/doxorubicin treatment [79]. Silvestrol preferentially inhibits translation of weak mRNAs coding for proteins such as cyclin D1, survivin, Mcl-1, Bcl-2, and c-Myc [81].

2.1.2.3. Hippuristanol Hippuristanol (**10**, Figure 12), purified from the coral *Isis hippuris* extract [82], was discovered in a chemical screen for inhibitors of translation initiation [83]. Hippuristanol interferes with binding of eIF4A to RNA and thereby inhibits the ATPase activity, but does not prevent binding of ATP to eIF4A (Figure 13). As such, **10** inhibits RNA dependent helicase activity, resulting in abrogation of cap-dependent translation [66]. Hippuristanol binds to amino acids adjacent to and overlapping with two conserved motifs present in the carboxy-terminal domain of eIF4A that are implicated in interaction with RNA and ATP, and interdomain contacts [30]. Inhibition of cap-dependent translation is thought to underlie inhibition of adult T-cell xenograft tumor growth [84]. Hippuristanol was synthesized by various synthetic approaches and was

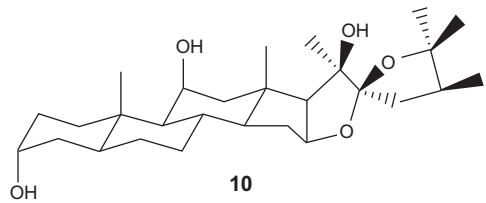


Figure 12 Hippuristanol, a natural product that inhibits RNA dependent helicase activity.

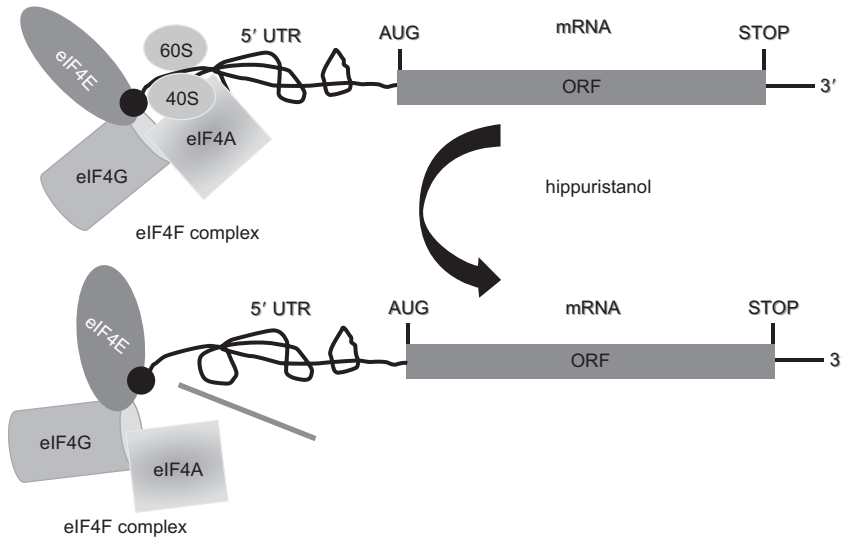


Figure 13 Hippuristanol binds to a subunit of eIF4A and inhibits the helicase activity of eIF4F.

subjected to limited structure activity relationship studies [85–87]. Both the *gem*-dimethyl on the spiro-furan ring F and the size of this ring are important for the antiproliferative activity of **10**.

Overall, these results suggest that inhibition of translation initiation by modulating eIF4A activity is a promising approach to altering drug resistance associated with PI3K/mTOR activation, and for inhibition of tumor growth.

2.2. Inhibitors of ternary complex

2.2.1. Salubrinal

Salubrinal (**11**, Figure 14) was discovered in a screen for agents that protect cells from endoplasmic reticulum stress induced cell death and was shown to cause eIF2α phosphorylation by selectively inhibiting an eIF2α phosphatase [88]. Salubrinal directly interacts with bcl-2 and inhibits induction of apoptosis by DNA damaging agents [89]. Salubrinal inhibits leukemia cell proliferation synergistically with proteasome inhibitors [90] and renders a subpopulation of bortezamib-resistant multiple myeloma cells sensitive to this FDA approved proteasome inhibitor [91].

2.2.2. 15-Deoxyspergualin

15-Deoxyspergualin (**12**, Figure 15), an analog of the immunosuppressive agent spergualin, inhibits cell cycle progression and cytokine production upon naïve T-cell activation [92]. It also displays significant tumoricidal activity [93]. It interacts with the EEVD domain of heat shock protein 70

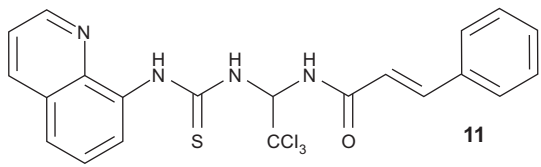


Figure 14 Salubrinal inhibits ternary complex formation by inhibiting eIF2α phosphatase.

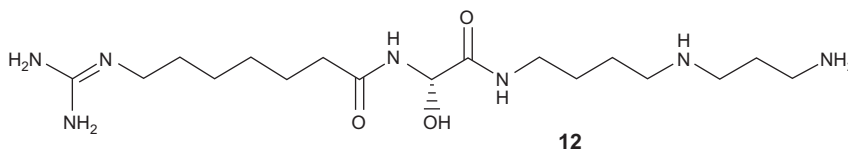


Figure 15 15-Deoxyspergualin inactivates eIF5A by posttranslational hypusination and leads to phosphorylation of eIF2α.

and causes phosphorylation of eIF2α [94]. Activation of eIF5A, an RNA binding protein, involves hypusination of Lys50. Inhibition of eIF5A hypusination by **12** inhibits translation initiation and cell proliferation [95,96]. Consistently, incubation of mouse mammary carcinoma FM3A cells with **12** led to inhibition of cell growth and to the formation of inactive eIF5A.

2.2.3. Thiozolidone-indenones

Thiazolidinediones (TZD) were discovered as peroxisome proliferator activated receptor gamma (PPAR-γ) agonists and developed for the management of lipid and glucose metabolism in type-2 diabetes [97,98]. This class includes drugs such as pioglitazone, rosiglitazone, ciglitazone, and troglitazone (**13**, Figure 16), all of which display some antiproliferative activity against cancer cells *in vitro* and inhibit tumor growth in xenograft models of human cancer [99–102].

Although the activity of TZDs against cancer cell lines or xenograft tumors was thought to be mediated by activation of PPAR-γ, in many instances, it was shown that the antiproliferative activity of these agents is independent of PPAR-γ. These PPAR-γ independent effects are reportedly mediated by: (a) Ca^{2+} store depletion and subsequent phosphorylation of eIF2α and inhibition of translation initiation (Figure 17) [99]; (b) induction of cellular acidosis through inhibition of the Na^{+}/H^{+} exchanger [103]; (c) inhibition of Bcl-xL/Bcl-2 complex [104]; (d) release

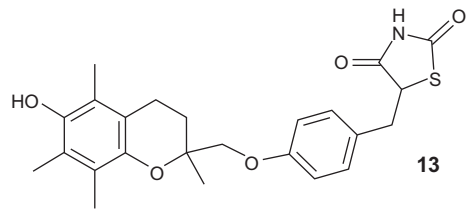


Figure 16 Troglitazone an antiproliferative agent from the TZD family.

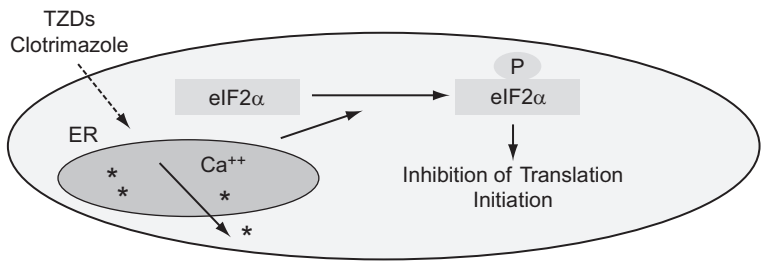


Figure 17 Mechanism of action of antiproliferative agents such as TZDs and clotrimazole that release Ca^{2+} from ER and phosphorylate eIF2α.

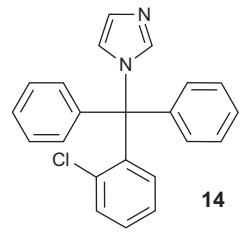
of apoptotic factors from the mitochondria through the production of ROS [105]; (e) upregulation of PTEN expression; (f) AMPK phosphorylation; and (g) downregulation of Akt/mTOR/p70S6 signaling cascades [106]. This multitude of seemingly disparate PPAR-γ independent activities of TZDs suggests either that TZDs are very promiscuous agents or that one of the PPAR-γ independent mechanisms underlies most of these activities. We suggest that eIF2α phosphorylation-dependent inhibition of translation initiation may be the unifying mechanism that underlies the antiproliferative effects of TZDs.

2.2.4. Clotrimazole

Clotrimazole (**14**, Figure 18) was developed in 1970s for the treatment of fungal infections [107]. It was subsequently shown to inhibit Ca^{2+} activated potassium channels, release Ca^{2+} from internal stores, inhibit Ca^{2+} release activated Ca^{2+} influx, and inhibit proliferation of cancer cells *in vitro* and tumor growth and metastasis *in vivo* [108]. Further studies demonstrated that partial depletion of Ca^{2+} stores cause phosphorylation of eIF2α and inhibition of translation initiation. This accounts, at least in part, for the anticancer activity of **14** [109]. Further, **14** inhibits angiogenesis [110] and sensitizes glioblastoma cells to radiation therapy [111]. These affects are likely secondary to the inhibition of translation initiation. Nevertheless, like many first generation translation initiation inhibitors, **14** has polypharmacology that may include inhibition of translation initiation and glycolytic supply of ATP that are required for cancer cells proliferation [112].

2.2.5. 3,3-Diaryl oxindoles

3,3-Diaryl oxindoles (Figure 19) were developed through structure activity relationship studies to identify agents that induce sustained-partial depletion of internal Ca^{2+} stores, thereby inducing eIF2α phosphorylation and inhibiting translation initiation [113,114]. Two lead compounds identified through this effort, #1181 (**15**) and #1430 (**16**), inhibit proliferation of a wide variety of cancer cells with low micromolar potencies. *In vitro*



14

Figure 18 Clotrimazole, an antifungal agents that depletes ternary complex and inhibits cap-dependent translation initiation.

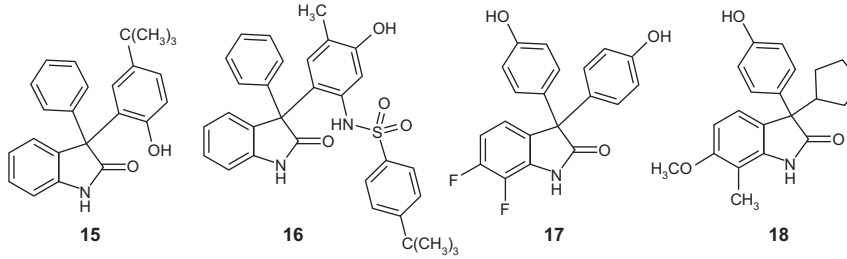


Figure 19 3,3-Diaryl oxindoles with anticancer activity.

studies with compound **15** demonstrate that this agent inhibits translation initiation, and preferentially abrogates the expression of oncogenic and antiapoptotic proteins with negligible effect on the expression of housekeeping proteins. Most, but not all of these effects, are translational. Treatment of mice bearing MCF-7 human breast cancer cell derived tumors ($\sim$150 mm^3) with 140 mg/kg/bid of #1181 sc for 3 weeks resulted in $\sim$30% regression in tumor size [40]. This antitumor activity is associated with phosphorylation of eIF2α and inhibition of the expression of oncogenic proteins. These findings provide proof-of-principle that phosphorylation of eIF2α and reduced formation of the ternary complex are pharmacologically viable targets for cancer therapy.

Screening oxyphenistatin analogs for preferential inhibition of human breast cancer cell (MDA-468) proliferation, as compared to a related noncancer cell, identified 6,7-difluoro-oxyphenistatin (**17**) to have 1000-fold greater antiproliferative activity toward MDA-468 cells ($IC_{50} = 20$ nM) [115]. In addition, oxindole **17** showed potent antitumor activity in xenograft mice models of human prostate and breast cancers following p.o. or i.v. administration [116]. Further optimization led to the development of 3-aryl,3-cycloalkyl-oxindoles that presented a significant departure from the 3,3-diaryloxindole chemotype [117]. Oxindole **18** showed nanomolar antiproliferative activity toward breast and prostate cancer cells and caused complete tumor regression in a rat xenograft model of human prostate cancer [117]. A very recent publication reports on *N*,*N*′-diarylureas that activate heme-regulated inhibitor kinase, induce eIF2α phosphorylation, inhibit translation initiation, and display anticancer activity [118].

3. CONCLUSIONS

Prominent targets for anticancer drugs like phosphoinositide 3-kinase (PI3K), AKT kinase, or mammalian target of rapamycin (mTOR) are all upstream of 4E-BP1 phosphorylation, and they trigger inhibition of translation initiation while modulating other downstream targets. Targeting

cancer pathways downstream of their cellular signaling networks at the level of translation initiation will be more effective and could offer fewer side effects than inhibiting their upstream targets. The location of translation initiation at the apex of many well-defined oncogenic, pro-apoptotic, and tumor-suppressor pathways, and the plethora of distinct targets make it an attractive field for the development of a new generation of anticancer agents. The diverse nature of these targets includes interaction with classical active sites, as well as challenging targets such as protein–protein interactions. Because restoration of translational control down-regulates oncogenic proteins with minimal effect on housekeeping proteins, it will affect predominantly the addicted cancer cells and spare normal ones. Hence, small molecule inhibitors of translation initiation have excellent potential for achieving a wide therapeutic window. Therefore, this emerging field represents a new and highly promising paradigm in cancer therapy.

REFERENCES

[1] T. V. Pestova and V. G. Kolupaeva, *Genes Dev.*, 2002, **16**, 2906.
[2] T. V. Pestova, J. R. Lorsch and C. U. T. Hellen, in *Translational Control in Biology and Medicine*, (eds. M. Mathews, N. Sonenberg, J. W. B. Hershey), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2007, p. 87.
[3] N. Sonenberg and A. G. Hinnebusch, *Cell*, 2009, **136**, 731.
[4] R. Kawaguchi and J. Bailey-Serres, *Nucleic Acids Res.*, 2005, **33**, 955.
[5] H. A. Meijer and A. A. Thomas, *Biochem. J.*, 2002, **367**, 1.
[6] M. Kozak, *Mol. Cell. Biol.*, 1989, **9**, 5134.
[7] M. Kozak, *J. Biol. Chem.*, 1991, **266**, 19867.
[8] M. S. Haydon, J. D. Googe, D. S. Sorrells, G. E. Ghali and B. D. Li, *Cancer*, 2000, **88**, 2803.
[9] C. O. Nathan, N. Amirghahri, C. Rice, F. W. Abreo, R. Shi and F. J. Stucker, *Laryngoscope*, 2002, **112**, 2129.
[10] B. D. Li, J. C. McDonald, R. Nassar and A. De Benedetti, *Ann. Surg.*, 1998, **227**, 756, discussion 761.
[11] B. D. Li, J. S. Gruner, F. Abreo, L. W. Johnson, H. Yu, S. Nawas, J. C. McDonald and A. DeBenedetti, *Ann. Surg.*, 2002, **235**, 732, discussion 738.
[12] Y. Mamane, E. Petroulakis, L. Rong, K. Yoshida, L. W. Ler and N. Sonenberg, *Oncogene*, 2004, **23**, 3172.
[13] R. Pincheira, Q. Chen, Z. Huang, J. T. Zhang and J. Eu, *Eur. J. Cell Biol.*, 2001, **80**, 410.
[14] O. Saramaki, N. Willi, O. Bratt, T. C. Gasser, P. Koivisto, N. N. Nupponen, L. Bubendorf and T. Visakorpi, *Am. J. Pathol.*, 2001, **159**, 2089.
[15] N. N. Nupponen, K. Porkka, L. Kakkola, M. Tanner, K. Persson, A. Borg, J. Isola and T. Visakorpi, *Am. J. Pathol.*, 1999, **154**, 1777.
[16] K. Dua, T. M. Williams and L. Beretta, *Proteomics*, 2001, **1**, 1191.
[17] J. Eberle, K. Krasagakis and C. E. Orfanos, *Int. J. Cancer*, 1997, **71**, 396.
[18] M. Shuda, N. Kondoh, K. Tanaka, A. Ryo, T. Wakatsuki, A. Hada, N. Goseki, T. Igari, K. Hatsuse, T. Aihara, S. Horiuchi, M. Shichita, N. Yamamoto and M. Yamamoto, *Anticancer Res.*, 2000, **20**, 2489.
[19] I. B. Rosenwald, M. J. Hutzler, S. Wang, L. Savas and A. E. Fraire, *Cancer*, 2001, **92**, 2164.

[20] N. Abraham, M. L. Jaramillo, P. I. Duncan, N. Methot, P. L. Icely, D. F. Stojdl, G.N. Barber and J. C. Bell, *Exp. Cell Res.*, 1998, **244**, 394.
[21] I. B. Rosenwald, L. Koifman, L. Savas, J. J. Chen, B. A. Woda and M. E. Kadin, *Hum. Pathol.*, 2008, **39**, 910.
[22] I. B. Rosenwald, S. Wang, L. Savas, B. Woda and J. Pullman, *Cancer*, 2003, **98**, 1080.
[23] O. Donze, R. Jagus, A. E. Koromilas, J. W. Hershey and N. Sonenberg, *EMBO J.*, 1995, **14**, 3828.
[24] L. Marshall, N. S. Kenneth and R. J. White, *Cell*, 2008, **133**, 78.
[25] Z. Dong and J. T. Zhang, *Crit. Rev. Oncol. Hematol.*, 2006, **59**, 169.
[26] L. Zhang, X. Pan and J. W. Hershey, *J. Biol. Chem.*, 2007, **282**, 5790.
[27] J. Marcotrigiano, A. C. Gingras, N. Sonenberg and S. K. Burley, *Cell*, 1997, **89**, 951.
[28] J. Marcotrigiano, A. C. Gingras, N. Sonenberg and S. K. Burley, *Mol. Cell*, 1999, **3**, 707.
[29] J. H. Chang, Y. H. Cho, S. Y. Sohn, J. M. Choi, A. Kim, Y. C. Kim, S. K. Jang and Y. Cho, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 3148.
[30] L. Lindqvist, M. Oberer, M. Reibarkh, R. Cencic, M. E. Bordeleau, E. Vogt, A. Marintchev, J. Tanaka, F. Fagotto, M. Altmann, G. Wagner and J. Pelletier, *PLoS One*, 2008, **3**, e1583.
[31] P. Schutz, M. Bumann, A. E. Oberholzer, C. Bieniossek, H. Trachsel, M. Altmann and U. Baumann, *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 9564.
[32] R. J. DeFatta, C. O. Nathan and A. De Benedetti, *Laryngoscope*, 2000, **110**, 928.
[33] J. R. Graff, B. W. Konicek, T. M. Vincent, R. L. Lynch, D. Monteith, S. N. Weir, P. Schwier, A. Capen, R. L. Goode, M. S. Dowless, Y. Chen, H. Zhang, S. Sissons, K. Cox, A.M. McNulty, S. H. Parsons, T. Wang, L. Sams, S. Geeganage, L. E. Douglass, B.L. Neubauer, N. M. Dean, K. Blanchard, J. Shou, L. F. Stancato, J. H. Carter and E.G. Marcusson, *J. Clin. Invest.*, 2007, **117**, 2638.
[34] N. Oridate, H. J. Kim, X. Xu and R. Lotan, *Cancer Biol. Ther.*, 2005, **4**, 318.
[35] N. J. Moerke, H. Aktas, H. Chen, S. Cantel, M. Y. Reibarkh, A. Fahmy, J. D. Gross, A. Degterev, J. Yuan, M. Chorev, J. A. Halperin and G. Wagner, *Cell*, 2007, **128**, 257.
[36] A. De Benedetti, S. Joshi-Barve, C. Rinker-Schaeffer and R. E. Rhoads, *Mol. Cell. Biol.*, 1991, **11**, 5435.
[37] C. W. Rinker-Schaeffer, J. R. Graff, A. De Benedetti, S. G. Zimmer and R. E. Rhoads, *Int. J. Cancer*, 1993, **55**, 841.
[38] J. R. Graff, B. W. Konicek, J. H. Carter and E. G. Marcusson, *Cancer Res.*, 2008, **68**, 631.
[39] J. R. Graff, B. W. Konicek, R. L. Lynch, C. A. Dumstorf, M. S. Dowless, A. M. McNulty, S. H. Parsons, L. H. Brail, B. M. Colligan, J. W. Koop, B. M. Hurst, J. A. Deddens, B. L. Neubauer, L. F. Stancato, H. W. Carter, L. E. Douglass and J. H. Carter, *Cancer Res.*, 2009, **69**, 3866.
[40] L. Chen, B. H. Aktas, Y. Wang, X. He, R. Sahoo, N. Zhang, S. Denoyelle, E. Kabha, H. Yang, R. Yefidoff-Freedman, J. G. Supko, M. Chorev, G. Wagner, and J. A. Halperin, Submitted for publication.
[41] J. Tamburini, A. S. Green, V. Bardet, N. Chapuis, S. Park, L. Willems, M. Uzunov, N. Ifrah, F. Dreyfus, C. Lacombe, P. Mayeux and D. Bouscary, *Blood*, 2009, **114**, 1618.
[42] S. Fan, Y. Li, P. Yue, F. R. Khuri and S. Y. Sun, *Neoplasia*, 2010, **12**, 346–356.
[43] A. De, B. A. Jacobson, M. S. Peterson, M. G. Kratzke and R. A. Kratzke, American Association for Cancer Research Annual Meeting (San Diego, California), 2008.
[44] A. De, O. Larsson, B. A. Jacobson, M. Peterson, K. Terai, M. G. Kratzke, A. Z. Dudek, P.B. Bitterman and R. A. Kratzke, American Association for Cancer Research Annual Meeting (San Diego, California), 2008.
[45] R. Cencic, D. R. Hall, F. Robert, Y. Du, J. Min, L. Li, M. Qui, I. Lewis, S. Kurtkaya, R. Dingledine, H. Fu, D. Kozakov, S. Vajda and J. Pelletier, *Proc. Natl. Acad. Sci. USA*, 2011, **108**, 1046.
[46] S. Crotty, C. Cameron and R. Andino, *J. Mol. Med.*, 2002, **80**, 86.

[47] S. Assouline, B. Culjkovic, E. Cocolakis, C. Rousseau, N. Beslu, A. Amri, S. Caplan, B. Leber, D. C. Roy, W. H. Miller Jr. and K. L. B. Borden, *Blood*, 2009, **114**, 257.
[48] K. L. Borden and B. Culjkovic-Kraljacic, *Leuk. Lymphoma*, 1805, **2010**, 51.
[49] http://clinicaltrials.gov/ct2/show/NCT01056757, 2010.
[50] A. Kentsis, I. Topisirovic, B. Culjkovic, L. Shao and K. L. Borden, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 18105.
[51] A. Kentsis, L. Volpon, I. Topisirovic, C. E. Soll, B. Culjkovic, L. Shao and K. L. Borden, *RNA*, 2005, **11**, 1762.
[52] B. Westman, L. Beeren, E. Grudzien, J. Stepinski, R. Worch, J. Zuberek, J. Jemielity, R. Stolarski, E. Darzynkiewicz, R. E. Rhoads and T. Preiss, *RNA*, 2005, **11**, 1505.
[53] Y. Yan, Y. Svitkin, J. M. Lee, M. Bisaillon and J. Pelletier, *RNA*, 2005, **11**, 1238.
[54] A. Cai, M. Jankowska-Anyszka, A. Centers, L. Chlebicka, J. Stepinski, R. Stolarski, E. Darzynkiewicz and R. E. Rhoads, *Biochemistry*, 1999, **38**, 8538.
[55] J. Kowalska, M. Lukaszewicz, J. Zuberek, M. Ziemniak, E. Darzynkiewicz and J. Jemielity, *Bioorg. Med. Chem. Lett.*, 1921, **2009**, 19.
[56] C. J. Brown, I. McNae, P. M. Fischer and M. D. Walkinshaw, *J. Mol. Biol.*, 2007, **372**, 7.
[57] H. Matsuo, H. Li, A. M. McGuire, C. M. Fletcher, A. C. Gingras, N. Sonenberg and G. Wagner, *Nat. Struct. Biol.*, 1997, **4**, 717.
[58] T. P. Herbert, R. Fahraeus, A. Prescott, D. P. Lane and C. G. Proud, *Curr. Biol.*, 2000, **10**, 793.
[59] C. J. Brown, J. J. Lim, T. Leonard, H. C. Lim, C. S. Chia, C. S. Verma and D. P. Lane, *J. Mol. Biol.*, 2011, **405**, 736.
[60] S. Y. Ko, H. Guo, N. Barengo and H. Naora, *Clin. Cancer Res.*, 2009, **15**, 4336.
[61] R. Mishra, M. Miyamoto, T. Yoshioka, K. Ishikawa, Y. Matsumura, Y. Shoji, K. Ichinokawa, T. Itoh, T. Shichinohe, S. Hirano and S. Kondo, *Int. J. Oncol.*, 2009, **34**, 1231.
[62] B. Konicek, J. Stephens, A. McNulty, N. Robichaud, R. Peery, C. Dumstorf, M. Dowless, P. Iversen, S. Parsons, K. Ellis, D. J. McCann, J. Pelletier, L. Furic, J. M. Yingling, L. F. Stancato, N. Sonenberg and J. R. Graff, *Cancer Res.*, 1849, **2011**, 71.
[63] K. A. Hood, L. M. West, P. T. Northcote, M. V. Berridge and J. H. Miller, *Apoptosis*, 2001, **6**, 207.
[64] P. T. Northcote, J. W. Blunt and M. H. G. Munro, *Tetrahedron Lett.*, 1991, **32**, 6411.
[65] M. E. Bordeleau, J. Matthews, J. M. Wojnar, L. Lindqvist, O. Novac, E. Jankowsky, N. Sonenberg, P. Northcote, P. Teesdale-Spittle and J. Pelletier, *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 10460.
[66] M. E. Bordeleau, R. Cencic, L. Lindqvist, M. Oberer, P. Northcote, G. Wagner and J. Pelletier, *Chem. Biol.*, 2006, **13**, 1287.
[67] W. K. Low, Y. Dang, T. Schneider-Poetsch, Z. Shi, N. S. Choi, W. C. Merrick, D. Romo and J. O. Liu, *Mol. Cell*, 2005, **20**, 709.
[68] W. K. Low, Y. Dang, S. Bhat, D. Romo and J. O. Liu, *Chem. Biol.*, 2007, **14**, 715–727.
[69] R. Mazroui, R. Sukarieh, M. E. Bordeleau, R. J. Kaufman, P. Northcote, J. Tanaka, I. Gallouzi and J. Pelletier, *Mol. Biol. Cell*, 2006, **17**, 4212.
[70] Y. Dang, N. Kedersha, W. K. Low, D. Romo, M. Gorospe, R. Kaufman, P. Anderson and J.O. Liu, *J. Biol. Chem.*, 2006, **281**, 32870.
[71] Y. Dang, W. K. Low, J. Xu, N. H. Gehring, H. C. Dietz, D. Romo and J. O. Liu, *J. Biol. Chem.*, 2009, **284**, 23613.
[72] G. Kuznetsov, Q. Xu, L. Rudolph-Owen, K. Tendyke, J. Liu, M. Towle, N. Zhao, J. Marsh, S. Agoulnik, N. Twine, L. Parent, Z. Chen, J. L. Shie, Y. Jiang, H. Zhang, H. Du, R. Boivin, Y. Wang, D. Romo and B. A. Littlefield, *Mol. Cancer Ther.*, 2009, **8**, 1250.
[73] T. Ohse, S. Ohba, T. Yamamoto, T. Koyano and K. Umezawa, *J. Nat. Prod.*, 1996, **59**, 650.

[74] S. K. Lee, B. Cui, R. R. Mehta, A. D. Kinghorn and J. M. Pezzuto, *Chem. Biol. Interact.*, 1998, **115**, 215.
[75] S. Kim, A. A. Salim, S. M. Swanson and A. D. Kinghorn, *Anticancer Agents Med. Chem.*, 2006, **6**, 319.
[76] B. Y. Hwang, B. N. Su, H. Chai, Q. Mi, L. B. Kardono, J. J. Afriastini, S. Riswan, B. D. Santarsiero, A. D. Mesecar, R. Wild, C. R. Fairchild, G. D. Vite, W. C. Rose, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, S. M. Swanson and A. D. Kinghorn, *J. Org. Chem.*, 2004, **69**, 3350.
[77] Q. Mi, B. N. Su, H. Chai, G. A. Cordell, N. R. Farnsworth, A. D. Kinghorn and S. M. Swanson, *Anticancer Res.*, 2006, **26**, 947.
[78] B. Hausott, H. Greger and B. Marian, *Int. J. Cancer*, 2004, **109**, 933.
[79] M. E. Bordeleau, F. Robert, B. Gerard, L. Lindqvist, S. M. Chen, H. G. Wendel, B. Brem, H. Greger, S. W. Lowe, J. A. Porco Jr. and J. Pelletier, *J. Clin. Invest.*, 2008, **118**, 2651.
[80] S. Kim, B. Y. Hwang, B. N. Su, H. Chai, Q. Mi, A. D. Kinghorn, R. Wild and S. M. Swanson, *Anticancer Res.*, 2007, **27**, 2175.
[81] R. Cencic, M. Carrier, G. Galicia-Vazquez, M. E. Bordeleau, R. Sukarieh, A. Bourdeau, B. Brem, J. G. Teodoro, H. Greger, M. L. Tremblay, J. A. Porco Jr. and J. Pelletier, *PLoS One*, 2009, **4**, e5223.
[82] J.-H. Sheu, C.-H. Chao, G.-H. Wang, K.-C. Hung, C.-Y. Duh, M. Y. Chiang, Y.-C. Wud and C.-C. Wud, *Tetrahedron Lett.*, 2004, **45**, 6413.
[83] O. Novac, A. S. Guenier and J. Pelletier, *Nucleic Acids Res.*, 2004, **32**, 902.
[84] T. Tsumuraya, C. Ishikawa, Y. Machijima, S. Nakachi, M. Senba, J. Tanaka and N. Mori, *Biochem. Pharmacol.*, 2011, **81**, 713.
[85] W. Li, Y. Dang, J. O. Liu and B. Yu, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3112.
[86] W. Li, Y. Dang, J. O. Liu and B. Yu, *Chemistry*, 2009, **15**, 10356.
[87] K. Ravindar, M. S. Reddy, L. Lindqvist, J. Pelletier and P. Deslongchamps, *J. Org. Chem.*, 2011, **76**, 1269.
[88] M. Boyce, K. F. Bryant, C. Jousse, K. Long, H. P. Harding, D. Scheuner, R. J. Kaufman, D. Ma, D. M. Coen, D. Ron and J. Yuan, *Science*, 2005, **307**, 935.
[89] D. Kessel, *Biochem. Biophys. Res. Commun.*, 2006, **346**, 1320.
[90] H. C. Drexler, *PLoS One*, 2009, **4**, e4161.
[91] D. M. Schewe and J. A. Aguirre-Ghiso, *Cancer Res.*, 2009, **69**, 1545.
[92] H. Holcombe, I. Mellman, C. A. Janeway Jr., K. Bottomly and B. N. Dittel, *J. Immunol.*, 2002, **169**, 4982.
[93] W. E. Muller, N. Weissmann, A. Maidhof, M. Bachmann and H. C. Schroder, *J. Antibiot.*, 1987, **40**, 1028.
[94] T. N. Ramya, N. Surolia and A. Surolia, *Biochem. J.*, 2007, **401**, 411.
[95] K. Nishimura, Y. Ohki, T. Fukuchi-Shimogori, K. Sakata, K. Saiga, T. Beppu, A. Shirahata, K. Kashiwagi and K. Igarashi, *Biochem. J.*, 2002, **363**, 761.
[96] M. H. Park, K. Nishimura, C. F. Zanelli and S. R. Valentini, *Amino Acids*, 2010, **38**, 491.
[97] R. Retnakaran and B. Zinman, *Lancet*, 2009, **373**, 2088.
[98] A. Krishnaswami, S. Ravi-Kumar and J. M. Lewis, *Perm. J.*, 2010, **14**, 64.
[99] S. S. Palakurthi, H. Aktas, L. M. Grubissich, R. M. Mortensen and J. A. Halperin, *Cancer Res.*, 2001, **61**, 6213.
[100] Y. Tsubouchi, H. Sano, Y. Kawahito, S. Mukai, R. Yamada, M. Kohno, K. Inoue, T. Hla and M. Kondo, *Biochem. Biophys. Res. Commun.*, 2000, **270**, 400.
[101] N. Suh, Y. Wang, C. R. Williams, R. Risingsong, T. Gilmer, T. M. Willson and M. B. Sporn, *Cancer Res.*, 1999, **59**, 5671.
[102] M. H. Jarrar and A. Baranova, *J. Cell. Mol. Med.*, 2007, **11**, 71.
[103] F. Turturro, E. Friday, R. Fowler, D. Surie and T. Welbourne, *Clin. Cancer Res*, 2004, **10**, 7022.

[104] C. W. Shiau, C. C. Yang, S. K. Kulp, K. F. Chen, C. S. Chen, J. W. Huang and C. S. Chen, *Cancer Res.*, 2005, **65**, 1561.
[105] I. Inoue, S. Katayama, K. Takahashi, K. Negishi, T. Miyazaki, M. Sonoda and T. Komoda, *Biochem. Biophys. Res. Commun.*, 1997, **235**, 113.
[106] S. Han and J. Roman, *Mol. Cancer Ther.*, 2006, **5**, 430.
[107] M. A. Burgess and G. P. Bodey, *Antimicrob. Agents Chemother.*, 1972, **2**, 423.
[108] L. R. Benzaquen, C. Brugnara, H. R. Byers, S. Gatton-Celli and J. A. Halperin, *Nat. Med.*, 1995, **1**, 534.
[109] H. Aktas, R. Fluckiger, J. A. Acosta, J. M. Savage, S. S. Palakurthi and J. A. Halperin, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 8280.
[110] H. Takahashi, M. Abe, T. Sugawara, K. Tanaka, Y. Saito, S. Fujimura, M. Shibuya and Y. Sato, *Jpn. J. Cancer Res.*, 1998, **89**, 445.
[111] H. Liu, Y. Li and K. P. Raisch, *Anticancer Drugs*, 2010, **21**, 841.
[112] J. Penso and R. Beitner, *Eur. J. Pharmacol.*, 2002, **451**, 227.
[113] A. Natarajan, Y. H. Fan, H. Chen, Y. Guo, J. Iyasere, F. Harbinski, W. J. Christ, H. Aktas and J. A. Halperin, *J. Med. Chem.*, 1882, **2004**, 47.
[114] A. Natarajan, Y. Guo, F. Harbinski, Y. H. Fan, H. Chen, L. Luus, J. Diercks, H. Aktas, M. Chorev and J. A. Halperin, *J. Med. Chem.*, 2004, **47**, 4979–4982.
[115] M. K. Uddin, S. G. Reignier, T. Coulter, C. Montalbetti, C. Granas, S. Butcher, C. Krog-Jensenb and J. Felding, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2854.
[116] J. Felding, H. C. Pedersen, C. Krog-Jensenb, M. Praestegaard, P. Butcher, V. Linde, T.S. Coulter, C. Montalbetti, M. K. Uddin and S. G. Reignier, *Patent Application WO 2005097107 A2 20051020 CAN 143:405798 AN 2005*, 2007.
[117] M. K. Christensen, K. D. Erichsen, C. Trojel-Hansen, J. Tjornelund, S. J. Nielsen, K. Frydenvang, T. N. Johansen, B. Nielsen, M. Sehested, P. B. Jensen, M. Ikaunieks, A. Zaichenko, E. Loza, I. Kalvinsh and F. Bjorkling, *J. Med. Chem.*, 2010, **53**, 7140.
[118] T. Chen, D. Ozel, Y. Qiao, F. Harbinski, L. Chen, S. Denoyelle, X. He, N. Zvereva, J. G. Supko, M. Chorev, J. A. Halperin and B. H. Aktas, *Nat. Chem. Biol.*, 2011, in press.