Targeting the translational machinery as a novel treatment strategy for hematologic malignancies

Patrick R. Hagner,1 Abraham Schneider,1,2 and Ronald B. Gartenhaus1,3

1Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore; 2Department of Oncology and Diagnostic Sciences, University of Maryland Dental School, Baltimore; and 3Veterans Administration Medical Center, Baltimore, MD

The dysregulation of protein synthesis evident in the transformed phenotype has opened up a burgeoning field of research in cancer biology. Translation initiation has recently been shown to be a common downstream target of signal transduction pathways deregulated in cancer and initiated by mutated/overexpressed oncogenes and tumor suppressors. The overexpression and/or activation of proteins involved in translation initiation such as eIF4E, mTOR, and eIF4G have been shown to induce a malignant phenotype. Therefore, understanding the mechanisms that control protein synthesis is emerging as an exciting new research area with significant potential for developing innovative therapies. This review highlights molecules that are activated or dysregulated in hematologic malignancies, and promotes the transformed phenotype through the deregulation of protein synthesis. Targeting these proteins with small molecule inhibitors may constitute a novel therapeutic approach in the treatment of cancer. (Blood. 2010;115:2127-2135)

Introduction

Eukaryotic cells have various mechanisms and levels by which gene expression can be regulated including: transcription, export of mRNA messages, mRNA stability, and posttranslational modifications. Protein synthesis is essential for cell viability, and controlling mRNA translation is a critical step in regulation of gene expression. Translation can be divided between 3 stages: initiation, elongation, and termination. Translational control is principally exerted by regulating the formation of the cap-dependent translation initiation complex. Translation initiation comprises a mechanism in which the eIF4F ternary complex (eIF4E, eIF4A, and eIF4G) recruits a 43S preinitiation complex containing a Met-tRNAi and multiple initiation factors (eIFs 1, 1A, 2, 3, and 5) to the 5′ methyl-7-GTP cap complex on mRNA (reviewed in Sonenberg and Hinnebusch).1 Once the preinitiation complex scans the 5′ untranslated region of the mRNA and reaches the AUG start codon, the large (60S) ribosomal subunit joins the small (40S) ribosomal subunit and begins to synthesize the protein.2

Deregulated protein synthesis plays an important role in human cancer and deregulated translational control has been recognized as an integral part of the malignant state.3 In the past several years it has become clear that the efficiency of expression of key proteins involved in cell-growth regulation, proliferation, and apoptosis may be controlled at the translational level by changes in the activity of components of the protein synthesis machinery.3,5 Various classes of mRNAs differ considerably in their translational efficiency. Typically, mRNAs coding for proteins positively involved in regulating cell growth and survival have a high degree of secondary structure in the 5′ untranslated region (UTR). The translation of such messages is particularly sensitive to the activity of the cap-dependent translation-initiation machinery.6 In view of the fact that translation factors are closely regulated by conditions that affect cell growth, it is not surprising that, experimentally, aberrant expression of some of these factors has been shown to induce malignant transformation of cells.

Translation initiation has recently been shown to be a common downstream target of signal transduction pathways deregulated in cancer and initiated by mutated/overexpressed oncogenes and tumor suppressors.7 Several previous publications indicate that aberrant control of protein synthesis contributes to lymphomagenesis,8 opening up possibilities for innovative therapeutics, that is, targeting the translational machinery. Below, we present an overview of potentially targetable translational machinery components and regulatory signaling pathways that represent a novel approach for the treatment of hematologic malignancies.

eIF4F

A major regulatory step in control of protein synthesis is translation initiation. Translation initiation is modulated by the association of a ternary complex of proteins, eukaryotic translation initiation factor F (eIF4F), composed of eIF4G, eIF4A, and eIF4E.9 The cellular levels of eIF4E molecules are 10- to 30-fold lower than other known initiation factors10,11 and its association with the eIF4F complex is therefore the rate-limiting step in translation initiation (reviewed by Clemens;12 De Benedetti and Graff13); however, the stoichiometry of eIF4F components is still debated by some investigators.14 It was previously hypothesized that an increase in the rate of translation would have an impact on the spectrum of mRNAs synthesized.15 Subsequently, it was shown that overexpression of eIF4E could increase the translation of mRNAs with long highly structured 5′ UTR9 such as chloramphenical acetyl transferase and ornithine decarboxylase.16 Early studies established that eIF4E overexpression resulted in the transformation of immortalized cell lines as exemplified by increased proliferation, anchorage independent growth, and invasiveness.17,18 Recently, the overexpression of eIF4E has been observed in primary human malignancies such as colon,19 breast,20,21 non-Hodgkin lymphoma (NHL),22...
acute myelogenous leukemia (AML), and chronic myelogenous leukemia (CML). These aggregate data prompted several laboratories to investigate the role of eIF4E in neoplastic transformation as well as suggested the feasibility of targeting this molecule as a novel therapeutic approach. Several investigators have examined the impact of overexpressing eIF4E in transgenic mouse models, which can increase the incidence of multiple malignancies including lymphomas.3,24

The disruption of translation initiation through the modulation of either eIF4E protein levels or formation of the eIF4F ternary complex has been examined using RNA interference.25-28 Graff et al demonstrated that intravenous administration of antisense oligonucleotides in a mouse xenograft model could successfully silence the expression of eIF4E in vivo. This decrease in eIF4E levels led to an inhibition of tumor growth and reduced cell viability. While the decrease in eIF4E levels was not restricted to only the tumor cells (80% reduction in normal tissues), the clinical impact on normal tissues was minimal as there were no significant changes in body weight, liver weight, or hepatic enzymes. These data demonstrated that modulating eIF4E protein levels was a useful approach to disrupt tumor growth in vivo without significant off-target effects in normal tissues. The function of eIF4E may also be interrupted by small molecule inhibitors that mimic the 5′ methyl-7-GTP moiety found within the capped structure of mRNA.29,30

The small molecule Ribavirin has been shown to suppress eIF4E-induced transformation through a mechanism in which the inhibitor competes with the endogenous 5′ methyl-7-GTP cap structure for eIF4E. Through this competitive inhibition, Kentsis et al were able to demonstrate a decrease in the translation of oncogenic messages such as ornithine decarboxylase (ODC) as shown by the absence of its mRNA in the polysomal fraction of ribosomes.29 eIF4E has also been shown to regulate translation of mRNA by promoting the transport of oncogenic messages.16 The use of Ribavirin to interfere with eIF4E association to the 5′ methyl-7-GTP cap structure results in the specific disruption of eIF4E-directed transport of messages to the cytoplasm.29,30 The Ribavirin-mediated inhibition of eIF4E has also been successfully demonstrated in a xenograft mouse model with a 6-fold reduction in tumor volume compared with controls further highlighting the critical role of eIF4E in maintaining the malignant phenotype.

In addition, small molecule inhibitors have been investigated targeting the protein-protein domain that governs binding between eIF4E and eIF4G. These inhibitors work by molecular mimicry of the domain on either eIF4G or eIF4E to promote binding of eIF4E. These 4E-BP1 functional mimetic inhibitors have been shown to be effective in promoting apoptosis and impairing cell-cycle progression in both lymphoma and leukemia.31,32 Using a high-throughput approach, Moerke et al were the first to describe an eIF4F-specific inhibitor (4EGI-1) that can disrupt the eIF4F complex and inhibit cap-dependent translation. Importantly, treatment of T-cell acute lymphoblastic leukemia (Jurkat T-ALL) and lung cancer (A549) cell lines with 4EGI-1 was able to induce apoptosis as well as inhibit proliferation.31 The authors also demonstrated that 4EGI-1 preferentially inhibits the growth of cells transformed with the Philadelphia chromosome compared with the nontransformed isogenic Ba/F3 cell line. The use of this inhibitor has also been examined in AML.32 While the macrolide rapamycin has been an effective tool to inhibit the activity of eIF4E and cap-dependent translation in certain cell types,33,34 Tamburini et al demonstrated that AML cells are resistant to inhibition of the mammalian target of rapamycin (mTOR) by treatment with the rapamycin derivative RAD001 or Raptor small inhibitory RNA (siRNA). Nevertheless, treatment of cells with 4EGI-1 significantly reduced the clonogenic growth and induced apoptosis of primary AML cells. Furthermore, 4EGI-1 also proved to be effective in disrupting eIF4F complex formation in primary AML samples lending additional credence to targeting of the eIF4F complex.32 Alternatively, an innovative peptidomimetic approach targeting the association between eIF4G and eIF4E was used in an ovarian cancer mouse model. The authors demonstrated that the peptide could be specifically targeted to the ovary by fusing the peptide to gonadotropin-releasing hormone (GnRH). Interference of eIF4F complex formation with a targeted peptide strongly inhibited proliferation and induced apoptosis in tumor cells compared with both saline and GnRH treated controls, with no toxicity observed in normal tissues.35 Interfering with eIF4F activity and translation initiation has also been shown to restore the chemosensitivity of tumors in preclinical lymphoma models.36,37

The small molecule inhibitor Ribavirin, is currently used in the therapeutic regimen for viral infections such as Lassa fever virus and hepatitis C virus (HCV).38 Treatment of patients with HCV related non-Hodgkin lymphoma39,40 using Ribavirin resulted in a dramatic clinical response in some patients, these responses often correlated with a reduction in HCV viral load. The first clinical trial evaluating the targeting of eIF4E as a monotherapy using Ribavirin was recently conducted in 11 patients with acute myeloid leukemia. The results were striking with complete remission (CR), partial remission (PR), and other hematologic improvements obtained in patients.30 These exciting recent clinical data, along with earlier animal models, together suggest that Ribavirin shows great potential to be an effective new therapeutic agent in the treatment of hematologic malignancies.

mTOR

The evolutionarily conserved target of rapamycin (TOR) is a large serine/threonine protein kinase that belongs to a family of kinases known as the phosphatidylinositol kinase-related kinase family.41 TOR was originally identified in the yeast species, Saccharomyces cerevisiae, because of its sensitivity to the compound rapamycin (sirolimus), a macrocyclic lactone with immunosuppressive properties initially isolated from the bacterial strain Streptomyces hygroscopicus.42 The mammalian homologue, mTOR, is a key component of 2 functionally and structurally distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2. While mTORC1 is responsive to the inhibitory effects of rapamycin, mTORC2 appears to be insensitive in a tissue-specific manner.43 Interestingly, prolonged treatment with rapamycin derivatives reduces mTORC2 signaling in AML cells resulting in a decrease in AKT signaling.44 Compelling evidence demonstrates that mTORC1 is a prime signaling node in many pathways that regulate cell growth and metabolism by transducing signals associated with environmental, nutritional, growth factor, and other bioenergetic cues. mTORC1 is a multimeric protein complex comprising mTOR together with the associated proteins raptor (regulatory associated protein of mTOR), mLST8,45 and PRAS40.46 Upon activation, the catalytic subunit of mTOR directly controls protein synthesis by phosphorylating key regulators of the translational machinery such as the eukaryotic initiation factor 4E-binding protein-1 (4EBP1), an inhibitor of cap-dependent translation, and the ribosomal p70S6 kinase (S6K), a critical factor that may participate in ribosomal biogenesis through activation of ribosomal protein S6 (RPS6).47 In this regard, S6K is phosphorylated by mTOR at threonine
389, which in concert with another phosphorylating event carried out by the serine/threonine kinase phosphoinositide-dependent kinase-1 (PDK1) promotes full activation of 46K through phosphorylation of threonine 229. Once activated, S6K phosphorylates the RPS6 to enhance ribosomal biogenesis and overall protein synthesis (see “RPS6”). While mTOR phosphorylation of p70 S6 kinase results in the activation of the kinase, mTOR controls cap-dependent translation by phosphorylating 4EBP1, which is a suppressor of eIF4E. Because the strong binding of a hypophosphorylated 4EBP1 prevents the assembly of the translation initiation complex to eIF4E, mTOR-mediated phosphorylation of 4EBP1 facilitates the release of eIF4E from its inactive state to form, together with eIF4G and eIF4A, the eIF4F complex. Therefore, by inhibiting mTOR activity, multiple components of protein translation are suppressed, resulting in cell-growth retardation and eventually cell death.48

Cellular processes commonly deregulated in human cancer, such as cell proliferation, cell-cycle progression, survival, and motility are closely related to mTORC1 function.49 In addition, several upstream (PTEN, AKT, TSC1/2) and downstream (eIF4E) mediators within the mTORC1 signaling axis are often mutated or activated in several human malignancies including cancers of the hematopoietic system.50,51 These findings have triggered an intense search for novel therapeutic strategies to target the mTOR signaling pathway in conditions such as leukemia, lymphomas, and multiple myelomas. Indeed, this is evident by the increasing number of preclinical studies and the approximately 20 clinical trials that are either active or about to be initiated on the use of sirolimus, rapamycin analogs “rapalogs,” or second-generation derivatives, such as temsirolimus, everolimus, and deferoxolimus alone or in combination with other agents on hematologic malignancies, as described on www.ClinicalTrials.gov at the time this review was being written.

Early studies using mTOR inhibitors in hematologic conditions provided key positive findings to launch further investigations in the field. Brown et al initially reported on the proapoptotic activity of sirolimus on B-precursor acute lymphoblastic leukemia cell lines. They were also able to demonstrate that by using sirolimus as monotherapy, survival of Eμ-RET transgenic mice, an animal model of leukemia/lymphoma, could be extended compared with littermate controls.52 Later, the same group investigated whether the use of a rapamycin analog, temsirolimus, was effective in preventing the in vitro growth of primary adult acute lymphoblastic leukemia cells. Compared with untreated cells, temsirolimus-treated cells showed a marked decrease in cell proliferation and an increase in apoptosis. They further validated the in vitro data in a nonobese diabetic severe combined immunodeficiency (NOD/ SCID) xenograft model by establishing tumors with the same patient samples. A significant reduction in peripheral blood blasts and splenomegaly was observed in the temsirolimus-treated animals compared with the control group.53 The effectiveness of rapamycin in chronic lymphocytic leukemia (CLL) has been investigated in an Eμ-TCL1 transgenic mouse model, which gives rise to a CD5+ B-cell lymphoproliferative disorder similar to human CLL. The authors demonstrated that rapamycin could significantly extend the life of rapamycin-treated mice compared with controls.54 Xu et al performed the definitive work that provided the rationale for combining rapamycin with chemotherapeutic agents. The authors demonstrated that mTOR was responsible for cell survival after primary AML cells were treated with etoposide and that addition of rapamycin to the treatment could dramatically increase the cytotoxic effects of etoposide.55 Overall, these studies were critical to establishing the promising role mTOR inhibitors may exert against hematologic cancers.

In this regard, the results of a phase 2 clinical trial on the use of deferoxolimus as a single agent in patients with relapsed or refractory hematologic malignancies such as ALL, CML, CLL, and mantle cell lymphoma (MCL) have recently been reported.60 Of the 52 evaluable patients with various types of leukemia/lymphoma who were previously heavily pretreated, 40% responded positively to deferoxolimus treatment through hematologic improvement or stable disease. The effects of deferoxolimus on the translational machinery controlled by mTOR activity was demonstrated by the presence of decreased levels in the phosphorylated status of 4EBP1 in tumor cells from a subset of 9 of 11 patients with acute myelogenous leukemia, confirming the inhibition of mTOR. Similar positive outcomes and biochemical end points relative to the mTOR pathway were obtained in another phase 1/2 clinical study evaluating responses to everolimus, another second-generation mTOR inhibitor, in patients with relapsed or recurrent hematologic cancers. Overall, everolimus was well tolerated at a daily dose of 10 mg. As expected, the phosphorylation status of downstream targets of mTOR, 4EBP1, and p70 S6K was inhibited in 6 of 9 patient samples. The authors concluded that everolimus in combination with other agents targeting components of the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway are warranted.57 Rapamycin has also been shown to be effective in imatinib-resistant chronic myeloid leukemia. A major hematologic response was reported in 2 of 7 patients. The investigators also demonstrated in Ba/F3 cells, which expressed multiple forms of imatinib-resistant BCR-ABL kinases, that rapamycin could inhibit the cell-cycle progression.58

As a matter of fact, more recent preclinical and clinical studies have been addressing the use of combination therapies in hematologic malignancies by adding other targeted agents or cytotoxic chemotherapeutics to protocols that include mTOR inhibitors.50,59-61 Recently, multiple groups have developed and described mTOR kinase-specific small molecule inhibitors Torin1, PP242, and PP3014,62 (Figure 1). Recently, the Gartenhaus laboratory has established a paradigm in which chronic ethanol exposure inhibits mTOR signaling in lymphocytes with a significant repression of cap-dependent translation and reduction in the tumorigenic capacity of NHL in a human lymphoma xenograft system.63 The observed ethanol-mediated repression of mTOR signaling coupled with a decrease in lymphoma growth presented underscore the critical role of mTOR signaling and translation in lymphoma.63

The role of rapamycin and its derivatives and their mechanism of action are still under investigation. A recent report demonstrated that rapamycin treatment increased the cap-dependent translation of cervical carcinoma and embryonic kidney cells lines39 illustrating how rapamycin can have cell type-specific effects. Rapamycin is also reportedly effective in inhibiting mTOR signaling in either 4E-BP1 or p70 S6 kinase 1 null cells. However, another publication demonstrated that RPS6 phosphorylation was still present in MEF cells (p70 S6 kinase 1−/−) and was likely due to a compensatory signal from p70 S6 kinase 2.47 A possible explanation may be that related family member proteins may be differentially inhibited by rapamycin treatments. Certain cell types have been observed to be resistant to rapamycin-mediated mTOR inhibition; the mechanism(s) of resistance are still to be elucidated (reviewed in Kurmasheva et al).64 The earliest resistance mechanism observed was mutations in the FKBP12 gene that prevented the formation of FKBP12-rapamycin complex formation and rendered cells rapamycin resistant.65 Decreased AKT activity has also been shown to
induce rapamycin resistance. This may be due to continued cap-independent protein synthesis of c-MYC and cyclin D1 through internal ribosomal entry sites (IRES) that are negatively regulated by AKT signaling and that this ongoing translation permits cell-cycle progression.66 New strategies are being developed to overcome resistance to small molecule inhibitors including the targeting of multiple proteins within the same pathway (reviewed in Weinstein and Joe).57

The further development and understanding of novel molecules specifically inhibiting mTOR are likely to be even more effective at suppressing tumor growth than rapamycin and rapamycin derivatives. It is anticipated that results obtained from ongoing preclinical and clinical trials will provide valuable information on how to optimize the incorporation of mTOR inhibitors into therapeutic regimens.

**p70 S6 kinase**

As discussed, mTOR plays a major role governing the rate and efficiency of translation by signaling through the p70 S6 kinase (S6K) family members. S6K is generally implicated in ribosomal biogenesis and activity by phosphorylating RPS6, which is thought to be the rate-limiting step in the synthesis of new ribosomal RNA.45 However, the role(s) of p70 S6 kinase and RPS6 has become an area of keen investigation recently as multiple publications have presented conflicting data as to the function of these proteins in ribosomal biogenesis (see “RPS6”). Other S6K substrates have been identified including the eukaryotic elongation factor 2 kinase (eEF2K) and the eukaryotic translation initiation factor 4B (eIF4B). The phosphorylation of eEF2K results in inhibition of kinase activity, which leads to an increase in translation elongation. The phosphorylation of eIF4B by S6K leads to an increase of eIF4B protein levels recruited to eIF4A (reviewed in Ruvinsky and Meyuhas).68 This increased association promotes the helicase function of eIF4A and potentially increases the scanning ability of the ribosome. Because p70 S6 kinase can regulate components of the translational machinery, the targeting of S6K should have a detrimental effect on leukemias and lymphomas that are dependent upon an abnormal rate of protein synthesis. Early indications of a plausible role of S6K in hematologic malignancies, in particular T-cell lymphoma, were first described by Dumont et al nearly 15 years ago. They observed that rapamycin inhibited several biologic responses in the YAC-1 T-cell lymphoma, including proliferation and cell-cycle progression. By generating stable somatic mutants with altered sensitivities to rapamycin, they further demonstrated that the growth inhibitory effects of the drug were in fact associated with the inhibition of S6K activity.69

More recent studies have validated these earlier findings by demonstrating that inhibition of S6K after rapamycin treatment results in both growth inhibition and apoptotic cell death of B-precursor acute lymphoblastic leukemia cell lines in vitro and improved survival in a leukemia/lymphoma transgenic mouse model.52 S6K hyperactivation has also been suggested as a critical mediator underlying the pathogenesis of the malignant Hodgkin/Reed-Sternberg cells of Hodgkin lymphoma (HL). Dutton et al showed that constitutive activation of the PI3K/AKT pathway was common in HL primary tumors and cell lines. As a consequence of this activation, the mTOR substrates 4E-BP1 and S6K were also hyperphosphorylated. This relationship was demonstrated not only by the clear inhibitory actions of rapamycin on these proteins but also by the use of LY294002, a commonly used inhibitor that targets PI3K family members, including mTOR (reviewed in Liu et al).70,71 These results highlight the critical role of the PI3K/AKT/mTOR pathway in sustaining the growth of leukemia/lymphoma cells. The inhibition of p70 S6 kinase has been shown to activate other multiple signaling pathways through a S6K/PI3K feedback mechanism. Therefore, the targeting of p70 S6 kinase with small molecule inhibitors would optimally be incorporated with other
therapies to disrupt signaling pathways known to be affected by this feedback such as mitogen-activated protein kinase (MAPK), MAPK signal-integrating kinase (MNK), and AKT.72-74

RPS6

Ribosomes in higher eukaryotic cells are large multimeric ribonucleoprotein complexes consisting of 4 ribosomal RNA (rRNA) molecules and approximately 80 ribosomal proteins which make up a large (60S) and small (40S) subunit.75,76 In view of the fact that a significant proportion of the cell’s energy is spent in ribosomal biogenesis,77 it is reasonable to expect that the proteins and rRNA that are the constituents of ribosomes be tightly regulated. Ribosomal biogenesis and assembly is extremely complex with ribosomal proteins themselves being involved in the regulation of other ribosomal proteins. Experimental evidence has recently demonstrated that the lack of a specific ribosomal protein, RPS6, can be catastrophic for the formation of a nascent ribosomal subunit. Due to the interdependency of ribosomal protein regulation, the loss of RPS6 can lead to an overabundance of other ribosomal proteins and the activation of extraribosomal functions of these proteins secondary to loss of regulation.78 Of all the essential ribosomal proteins, RPS6 has attracted the most attention since it was the first ribosomal protein shown to be phosphorylated in response to stimuli.79-81 The significant body of research conducted on RPS6 over the past few decades has implicated RPS6 in the regulation of protein synthesis (through the regulation of 5’ terminal oligopyrimidine [5’ TOP] RNA synthesis) and cell size. These messages containing an oligopyrimidine tract are important for ribosomal biogenesis as they encode most of the translational apparatus.82,83 A selective increase in the translation of these messages can be induced in mitogen-stimulated cells80,81,84 and can be selectively decreased through treatment with rapamycin or its derivatives,85 suggesting that this group of mRNAs may have a important role in the control of cellular growth. However, the role of RPS6 and its upstream regulatory molecules have come into question in recent years with publications demonstrating that 5’ TOP mRNA are still regulated in mouse embryonic fibroblasts (MEF) in which all 5 possible phosphorylation sites on RPS6 had been mutated to alanines86 or in p70 S6 kinase 1 null mice.87 Conversely, another report demonstrated that RPS6 phosphorylation was still present in MEF cells (p70 S6 kinase 1−/−) due to a compensatory signal from p70 S6 kinase 2.87 To resolve these discrepancies a more thorough molecular understanding of the phosphorylation status of RPS6 is needed.

The haploinsufficiency of RPS6 has been linked to multiple different phenotypes including cancer,88,89 and specifically cancers of the hematopoietic system.90 In contrast, the Gartenhaus laboratory has recently found that RPS6 is highly expressed in primary diffuse large B-cell lymphoma (DLBCL) samples compared with indolent lymphoid malignancies when examined by tissue microarray (TMA) analysis.91 The overexpression of MCT-1 has been previously implicated in cellular transformation through its ability to stimulate cell proliferation, suppress apoptosis, and enhance signaling pathways involved in cell survival.92,93 Knockdown MCT-1 protein levels in DLBCL significantly reduced cell viability through an apoptotic mechanism,97 providing the first direct genetic evidence that interfering with MCT-1 function was able to induce apoptosis in lymphoma cells with high endogenous levels of MCT-1 protein.

MCT-1

The oncogene MCT-1 (multiple copies in T-cell lymphoma-1) is found on chromosome Xq22-24 and has been shown to be overexpressed in several B-cell and T-cell lymphoma lines and a small subset of diffuse large B-cell lymphoma (DLBCL), the most common form of non-Hodgkin lymphoma.95,96 Recent data demonstrate that MCT-1 protein levels are highly elevated in the majority of primary DLBCL samples compared with indolent lymphoid malignancies when examined by tissue microarray (TMA) analysis.97,98 The overexpression of MCT-1 has been previously implicated in cellular transformation through its ability to stimulate cell proliferation, suppress apoptosis, and enhance signaling pathways involved in cell survival.92,93 Knockdown MCT-1 protein levels in DLBCL significantly reduced cell viability through an apoptotic mechanism,97 providing the first direct genetic evidence that interfering with MCT-1 function was able to induce apoptosis in lymphoma cells with high endogenous levels of MCT-1 protein.

Earlier work demonstrated that a MCT-1 deletion mutant containing only the PUA domain still retained the ability to interact with RNA,99,100 thereby allowing the cell to translate the message. A recent report demonstrated that the PUA domain-containing DENR/DRP protein to the cap complex. Currently, there are no specific small molecule inhibitors that can directly inhibit suppression of RPS6 function, leads to an inhibition in proliferation and an increase in cell apoptosis through a p53-dependent pathway.78,91,92 Because the inactivation of p53 function in non-Hodgkin lymphoma is relatively low,93 targeting RPS6 function in non-Hodgkin lymphoma may represent a potential therapeutic paradigm.
Table 1. Small molecule inhibitors involved in the suppression of translation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Binding Site</th>
<th>Mechanism of action</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>Ribavirin</td>
<td>5′ methyl-7-GTP cap</td>
<td>Competitive inhibitor of eIF4E binding to capped mRNA</td>
<td>29,30</td>
</tr>
<tr>
<td>4EGI-1</td>
<td>eIF4G</td>
<td>Competitive inhibitor of eIF4E binding to eIF4G</td>
<td>31,32</td>
</tr>
<tr>
<td>GrnRH-4EBP fusion peptide</td>
<td>eIF4E</td>
<td>Peptidomimetic inhibitor of eIF4E binding to eIF4G</td>
<td>35</td>
</tr>
<tr>
<td>Rapamycin, analogs and derivatives</td>
<td>PKB-rapamycin-binding (FRB) domain of mTOR</td>
<td>Inhibits recruitment of PKBP12 protein to mTOR</td>
<td>Reviewed in 45</td>
</tr>
<tr>
<td>Torin1</td>
<td>mTOR kinase domain</td>
<td>Inhibition of ATP binding to kinase domain</td>
<td>34</td>
</tr>
<tr>
<td>PP242</td>
<td>mTOR kinase domain</td>
<td>Inhibition of ATP binding to kinase domain</td>
<td>62</td>
</tr>
<tr>
<td>PP30</td>
<td>mTOR kinase domain</td>
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<td>62</td>
</tr>
<tr>
<td>CGP57380</td>
<td>MNK kinase domain</td>
<td>Inhibition of ATP binding to kinase domain</td>
<td>113-116</td>
</tr>
</tbody>
</table>

MCT-1 protein function. Nandi et al previously demonstrated that phosphorylation of MCT-1 protein on threonine 81 by ERK1/2 is critical for protein stability and for its ability to promote cell proliferation. Taking advantage of a recently identified ERK1/2 small molecule inhibitor, MCT-1 function was disrupted by targeting its upstream kinase with significant impact on lymphoma cell viability and clonogenic capacity. Importantly, using a human lymphoma xenograft model Dai et al were able to demonstrate significant antilymphoma activity in vivo using this approach, thereby supporting the development of novel small inhibitory molecules directed toward MCT-1 as a promising approach in DLBCL.

These exciting findings support both the clinical relevance of potentially targeting the MCT-1 oncogene and provide additional evidence linking MCT-1 with lymphoma development and/or progression. The current challenge is to identify the complete repertoire of translated mRNAs regulated by MCT-1 and how they participate in lymphomagenesis.

**MNK**

In addition to the availability of eIF4E protein to form the eIF4F translation initiation complex, eIF4E activity is also regulated by phosphorylation events. Phosphorylation of eIF4E is known to be enhanced when cells are treated with specific stimuli including; serum, phorbol esters and growth factors. The signals from these stimuli were determined to be transduced through the MAPK pathway, specifically MNK.

The exact mechanism as to how the phosphorylation of eIF4E regulates protein translation is still unclear. Prior data suggested that the phosphorylation of eIF4E enhanced the 5′ methyl-7-GTP cap binding potential of the protein. However, more recent experimental evidence has shown that the phosphorylation of eIF4E decreases its affinity for a capped substrate. In contrast, the physiologic role of MNK phosphorylation of eIF4E may be better defined. Wendel et al have demonstrated that the insertion of a constitutively activated MNK1 into an Eμ-Myc mouse model produced lymphomas of a mature B-cell phenotype. Ueda et al created a MNK1/2 double knockout mice and demonstrated that there was no phosphorylated eIF4E levels in all tissues examined. These results demonstrated that eIF4E phosphorylation is not necessary for general protein synthesis and cap-dependent translation.

With the advent of a novel small molecule inhibitor specific to the MNK kinases, CGP57380, these data may become more easily reconciled. Treatment of prostate cancer cell lines with CGP57380 reduced the levels of phospho-eIF4E and reduced the amount of mRNA in the polysomal fraction of translating ribosomes and inhibited proliferation. CGP57380 has also been shown to be effective in inhibiting polysome associations with mRNA in chronic myelogenous leukemia cells and that treatment with this inhibitor enhances the activity of imatinib and induces apoptosis in imatinib-resistant CML cells. The inhibition of MNK has also been shown to affect the ability of eIF4E to transport oncogenic messages from the nucleus to the cytoplasm. Topisirovic et al convincingly demonstrated that phosphorylation of eIF4E contributes to the oncogenic potential of eIF4E by mutating the residues which can be phosphorylated. These mutants were less able to form anchorage-dependent foci compared with the wild-type eIF4E. A theory proposed by Morley and Naegle could explain the molecular mechanism and the phenotypic evidence presented here, they propose that phosphorylation of eIF4E and its subsequent release from the 5′ methyl-7-GTP cap complex could allow for a second molecule of eIF4E to interact with the cap and prime another round of translation initiation seen in polysomes.

The overexpression of ERK1/2 and p38 kinases, which are responsible for the activation of MNK, has been demonstrated in follicular lymphoma and DLBCL. In addition, the absence of microRNAs (miRNA) that regulate the MAPK pathway has also been observed in primary DLBCL samples while still present in matched normal B-cell lymphocytes from the same patients. Dai et al also found increased levels of ERK protein in a large percentage of human DLBCL samples. These observations in conjunction with the experimental evidence of using small molecule inhibitors in CML cells demonstrate that the MNK kinases are viable targets for future treatment strategies in hematologic malignancies.

**Future directions**

Although significant data have been accumulated to date, the potential of targeting components of the translational machinery in hematologic malignancies has yet to be fully realized. The seminal work accomplished in targeting the eIF4F complex demonstrates that inhibition of translation initiation should be given high priority for future development of new and more effective small molecule inhibitors. While the work on rapamycin and its analogs is broad and has finally reached the clinical settings in several cases, we are excited to see work from laboratories that are working to develop molecules that target the mTOR kinase domain directly. We have shown that specific targeting of MCT-1 has significant preclinical activity and experiments are in progress designed to disrupt the MCT-1/DENR association observed at the cap complex during translation initiation. Finally, the improved understanding and discovery of novel molecules involved in translation will...

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provide innovative clinical approaches toward the treatment of lymphoma and leukemia.

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Correspondence: Ronald B. Gartenhaus, Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201; e-mail: rgartenhaus@som.umaryland.edu.


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