Lost in translation: Dysregulation of cap-dependent translation and cancer

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Activation of the phosphatidylinositol 3′ kinase-Akt pathway has long been associated with malignant transformation and antiapoptotic signaling. Mutations downstream of Akt that activate the TOR kinase are found in tumor-prone syndromes, while overexpression of translation initiation complex components, such as eIF4E, occurs frequently in human cancer. However, direct roles for TOR signaling or eIF4E overexpression, in the genesis of cancer, have been lacking. Recent papers, including one by Avdulov et al. (2004) in this issue of *Cancer Cell*, clearly establish that dysregulation of cap-dependent translation confers malignant characteristics and induces cancer by suppressing apoptosis, underscoring the potential of therapeutics that selectively target the Akt-TOR-eIF4E pathway.

The TOR kinase has emerged as a central regulator of cellular responses to such diverse environmental stress as amino acid starvation, hypoxia, and growth factor deprivation (Bjornsti and Houghton, 2004; Schmelzle and Hall, 2000). Under stress conditions, TOR signaling is suppressed, which leads to cell cycle arrest. Thus, TOR acts as a gatekeeper, preventing cells from progressing from G1 phase and initiating DNA replication under suboptimal growth conditions. Indeed, TOR may be in a pathway parallel to p53 in controlling cell cycle transit and replication. However, if TOR plays a role analogous to p53, is this restricted to cell cycle regulation, or, like p53, does the TOR pathway also regulate apoptosis? Several recent findings (Ruggero et al., 2004; Wendel et al., 2004), including the Avdulov et al. (2004) paper in this issue, establish that TOR signaling also regulates apoptosis and plays an important role in tumorigenesis.

In the yeast *Saccharomyces cerevisiae*, the TOR signaling pathway directly or indirectly controls many aspects of cellular metabolism, including translation initiation, protein turnover, and transcription. Many of these pathways have been maintained in mammalian cells (Bjornsti and Houghton, 2004). Increasing evidence places TOR as a central regulator of cell growth (size), proliferation, and, more recently, survival (Figure 1). In response to mitogen stimulation, mammalian TOR regulates translation initiation through two distinct pathways: (1) phosphorylation and activation of ribosomal p70 S6 kinase (S6K1), and (2) cap-dependent translation via eukaryotic initiation factor 4E (eIF4E), which binds the ^{7}me GpppN cap of mRNA and directs the correct positioning of ribosomal subunits to initiate translation. In the latter case (Figure 2), TOR directly phosphorylates the 4E binding protein (4E-BP1, the suppressor of eIF4E), causing its dissociation from eIF4E. eIF4E then binds eIF4G, promoting the assembly of the eIF4F initiation complex.

It has been recognized for some time that dysregulation of this pathway, either proximal or distal to TOR, occurs in many human cancers, implicating aberrant signaling in the genesis and maintenance of the transformed phenotype (reviewed by Bjornsti and Houghton, 2004; Harris and Lawrence, 2003; Luo et al., 2003; Vivanco and Sawyers, 2002). For example, oncogenes, such as Ras or activated growth factor receptors, upregulate PI3K signaling (Figure 1). Human cancers frequently harbor activating mutations in the p110 catalytic subunit of PI3K or genomic amplification of sequences encoding the downstream kinase, Akt2. Normally, the dual function phosphatase PTEN negatively regulates Akt. However, PTEN activity is attenuated in many tumors through deletion, silencing, or mutation, resulting in constitutive activation of TOR-dependent pathways. Activated Akt also promotes survival under conditions of cellular stress. This antiapoptotic effect is, in part, mediated by direct phosphorylation or alterations in the translation of apoptotic regulators and decreased transcription of proapoptotic genes,

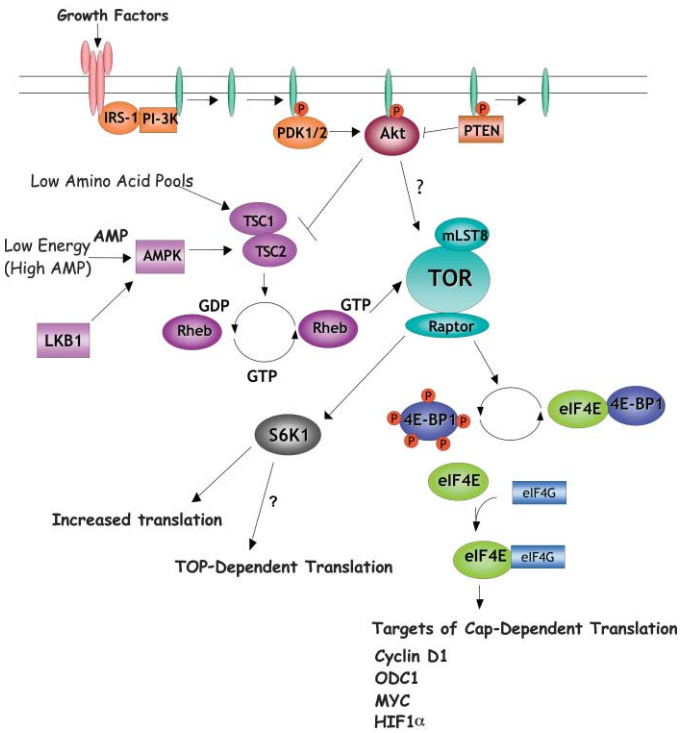

Figure 1. Regulation of TOR signaling and translation initiation by TOR

Activation of TOR, by PI-3K-Akt signaling, regulates translation through two independent pathways, S6K1 and eIF4E.

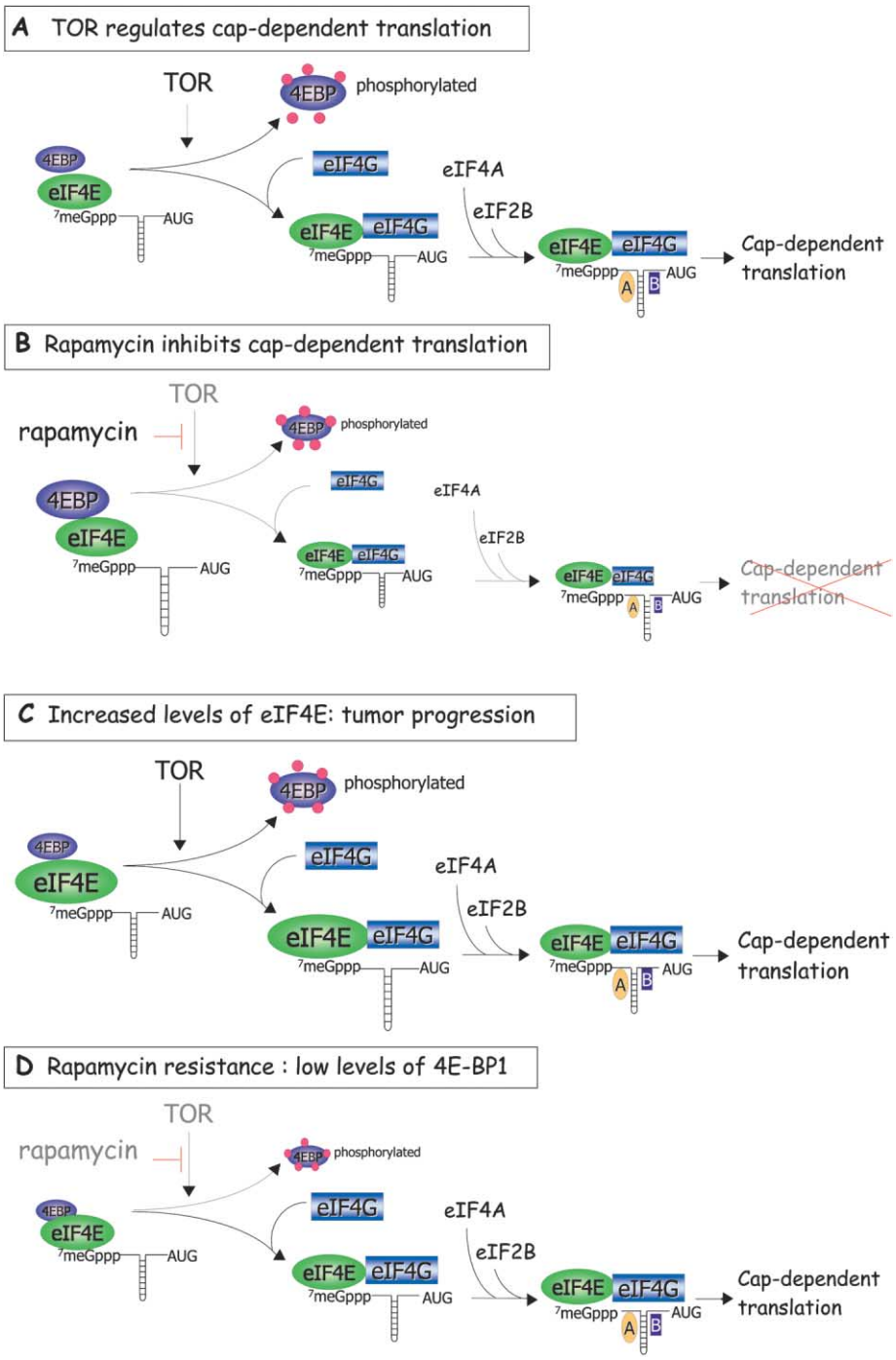

Figure 2. TOR control of cap-dependent translation

A: TOR phosphorylates 4E-BP, allowing the RNA cap binding protein eIF4E to associate with the scaffold protein eIF4G and establish the eIF4F initiation complex.

B: Rapamycin inhibits cap-dependent translation by inhibiting mTOR-dependent phosphorylation of 4E-BP. Hypophosphorylated 4E-BP binds eIF4E, thereby preventing eIF4E binding to eIF4G.

C: Increased eIF4E, with or without decreased 4E-BP, is associated with oncogenesis, progression in many human malignancies, and rapamycin resistance.

D: Intrinsic and acquired resistance to rapamycin is associated with decreased expression of 4E-BP1, the suppressor of eIF4E, and increased anchorage-independent proliferation.

possibly via inhibition of forkhead transcription factors.

Mutations downstream of Akt that lead to activation of TOR are also associated with cancer-prone syndromes such as tuberous sclerosis (TSC) and Peutz-Jeghers syndrome (Cheadle et al., 2000; Woods et al., 2003). The TSC complex negatively regulates TOR by inhibiting a small GTP binding protein, Rheb, considered to be the direct activator of TOR (Stocker et al., 2003; Tee et al., 2003; Zhang et al., 2003). Mutation or loss of heterozygosity in the components of the TSC complex gives rise to TSC syndrome (Cheadle et al., 2000). While inactivation of TSC may not lead to malignancy per se, deregulation of this pathway is associated with increased risk of developing malignant renal carcinoma of clear cell histology and appears critical for angiogenesis in developing malignancies. The latter is consistent with the observation that TSC2 regulates vascular endothelial growth factor (VEGF) through TOR-dependent and -independent pathways (Brugarolas et al., 2003). The overexpression or activating mutation of TOR in human cancer has not been reported. Nevertheless, the observations that TSC mutations predispose to malignant transformation, while Rheb is overexpressed in transformed cells (Gromov et al., 1995), suggest that activation of Akt per se is not the whole story. Rather, events downstream of Akt may also be critical for development of malignancies.

Each of the alterations in signaling, discussed above, activates TOR, which in turn controls initiation of translation through two independent pathways, S6K1 and 4E-BP-eIF4E. However, a large body of data indicates that the dysregulation of cap-dependent translation via alterations in 4E-BP-eIF4E pathway, rather than activation of S6K1, is associated with human cancer (Bjornsti and Houghton, 2004). For instance, the RNA cap binding protein (eIF4E), considered to be the rate-limiting step in formation of the eIF4F initiation complex, is overexpressed in multiple human cancers, and advanced stage disease is often characterized by genomic amplification (Bjornsti and Houghton, 2004; De Benedetti and Harris, 1999). Moreover, several lines of evidence suggest that increased expression of eIF4E is oncogenic. Overexpression of eIF4E alone, or in combination with v-myc and E1A, transforms rat embryo fibroblasts (Lazaris-Karatzas and Sonenberg, 1992), while microinjection of eIF4E is mitogenic in NIH 3T3 cells and is dependent upon Ras signaling (Lazaris-Karatzas et al., 1992). Cells transformed by chemical mutagenesis, onco-

genes, or viruses frequently exhibit increased transcription of eIF4E and eIF2α, another component of eIF4F (Graff et al., 1995; Miyagi et al., 1995; Rinker-Schaeffer et al., 1993; Rosenwald, 1996). In squamous cell carcinoma of the lung, the eIF4G scaffold protein is overexpressed (Bauer et al., 2002). In contrast, alterations in S6K1 in human cancer are rare (Bjornsti and Houghton, 2004).

Thus, dysregulation of translation initiation may result from activation of upstream effectors of TOR signaling and/or overexpression of components of the translation machinery, such that cap-dependent translation is no longer regulated in a TOR-dependent manner. A role for TOR signaling in maintaining the transformed phenotype has previously been suggested (Nomura et al., 2003); however, a causal relationship between constitutive activation of TOR signaling and transformation is more clearly established in several recent papers. The study by Avdulov et al. (2004) implicates TOR signaling in maintaining cap-dependent translation as a critical component in suppressing apoptosis and in maintaining the tumorigenic phenotype. They show that in normal human mammary epithelial cells (HMECs), cap-dependent translation is suppressed. eIF4E-Cap-bound complexes contained predominantly 4E-BP1, whereas those isolated from carcinoma cells contained predominantly eIF4G, indicating formation of an active eIF4F initiation complex only in cancer cells. Importantly, under serum-free conditions, increased eIF4F was associated with decreased apoptosis, thus implying that TOR signaling to eIF4E functions as a survival pathway, in agreement with previous studies (Hosoi et al., 1999; Huang et al., 2003). Of note is that tumor cells most resistant to serum-withdrawal-induced apoptosis maintained 4E-BP1 phosphorylation (as would be anticipated for cells with activating mutations upstream of TOR).

Forced overexpression of eIF4E in HMECs increased clonogenic potential, conferred anchorage-independent growth in soft agar, and reduced apoptosis. All of these effects were reversed by overexpression of 4E-BP1, confirming that malignant characteristics are induced by dysregulated cap-dependent translation. As diagrammed in Figure 2, whether these phenotypes are a consequence of increased eIF4E, or result from changes in the stoichiometry of eIF4E:4E-BP1, which would abrogate TOR control of cap-dependent translation, remains to be ascertained. Along these lines, downregulation of 4E-BP1, which is characteristic of normal or malignant myogenic cells selected for acquired resistance to rapamycin (a specific inhibitor of TOR signaling), is accompanied by increased c-MYC levels and increased anchorage-independent growth (Dilling et al., 2002).

Avdulov et al. (2004) provide additional evidence consistent with the idea that restoring control of cap-dependent translation in cancer cells leads to apoptosis. In these studies, overexpressing phosphorylation site mutants of 4E-BP1 (which constitutively bind eIF4E and thereby preclude formation of the eIF4F initiation complex) induced apoptosis of both MDA-MB-231 and MDA-MB-468 breast carcinoma cells. Expression of these 4E-BP1 mutants decreased cell cycle transit, consistent with previous studies (Jiang et al., 2003), and reduced anchorage-independent growth. These data clearly implicate dysregulation of eIF4F-dependent translation in conferring the malignant phenotype in normal epithelial cells and in the maintenance of such phenotypes in cancer cells in culture. Importantly, these results also extended to in vivo models, where overexpression of wild-type and phosphorylation-defective mutant forms of 4E-BP1 inhibited the tumorigenicity of breast carcinoma cell lines in athymic nude mice. Notably, in tumor cells overexpressing wild-type 4E-BP1, apoptosis increased from 2% to a maximum of 14%, and the S phase fraction decreased slightly. These findings indicate that modest changes in cell proliferation and cell death suffice to prevent tumor formation. Of interest is that expression of a 4E-BP1 phosphorylation mutant led to early apoptosis. In those tumors that developed, the loss of mutant protein expression is consistent with strong negative selection to allow cell proliferation. Although increased signaling through TOR and overexpression of eIF4E have been associated with transformation and progression in human cancer (Bjornsti and Houghton, 2004; De Benedetti and Harris, 1999), the study presented by Avdulov et al. points to a mechanism in which dysregulation of cap-dependent translation confers resistance to apoptosis under conditions of stress (i.e., serum starvation in vitro or tumor formation in vivo).

A role for eIF4E in tumorigenesis was also supported by two recent papers using well-defined genetic models of cancer (Ruggero et al., 2004; Wendel et al., 2004). However, there are significant differences between the results of Avdulov et al. (2004) and those derived from transgenic experiments. In particular, eIF4E-overexpressing HMECs were not tumorigenic in mice, which contrasts with recent studies by Ruggero et al. (2004), where overexpression of eIF4E leads to a high incidence of cancers in mice. Transgenic mice were engineered to overexpress eIF4E from the β actin promoter. Compared with wild-type littermates, there was a marked increase in tumorigenesis (notably lymphoma, lung adenocarcinoma, angiosarcoma, and hepatoma) in transgenic βT-Eif4e mice. It is important to note that the tumors developed late (beginning at 16 months of age), suggesting that either eIF4E is an oncogene per se, as concluded in the study, or alternatively, that dysregulation of translation initiation results in genomic instability and the accumulation of mutations that in concert with increased translation result in tumorigenesis. The latter possibility may explain the discrepancies between the two studies and is supported by observations that increased expression of eIF4E has been shown to increase levels of the ribonucleotide reductase 2 subunit, which has been linked with neoplastic transformation in mammalian cells and increased rates of DNA mutations in yeast (Abid et al., 1999; Chabes et al., 2003). In addition, nuclear eIF4E is reported to associate with PML bodies and to mediate nucleocytoplasmic transport of specific transcripts, which may contribute to its transforming activity (Topisirovic et al., 2003).

Both Ruggero et al. (2004) and Wendel et al. (2004) report similar effects of eIF4E overexpression in the context of an Eμ-Myc transgenic model of B cell lymphoma, where Myc overexpression is driven by the immunoglobulin heavy-chain enhancer transcription element. In this genetic model, modest overexpression of murine Eif4e (∼2.5-fold over control) markedly accelerated lymphomagenesis, and was associated with a decreased rate of apoptosis in tumors. Thus, Eif4e suppresses Myc-driven apoptosis, leading to more rapid tumor development. In the study of Wendel et al. (2004), the effect of Eif4e overexpression was similar to that induced by constitutive activation of Akt, i.e., suppression of apoptosis and accelerated lymphomagenesis. Significantly, both studies show that, in a p53 heterozygous host, tumors that rapidly develop in Eμ-Myc/Eif4e mice retain the wild-type p53 allele. Thus, relatively low-level overexpression of Eif4e, through suppression of apop-

tosis, abrogates the requirement for loss of p53-induced apoptosis for B cell lymphoma development in this model. In addition, Ruggero et al. (2004) propose that overexpression of Eif4e in mouse embryonic fibroblasts and Eμ-Myc B cells leads to cellular senescence, and that Eif4E-driven senescence is abrogated by Myc. This Eif4e-driven senescence is in contrast to results obtained by Avdulov et al. in human mammary epithelial cells (HMECs) and human cancer cells (Dilling et al., 2002), where overexpression increased growth rate and anchorage-independent growth.

Together, these three studies support a causal role for dysregulation of cap-dependent translation initiation in both the genesis of cancer and in its progression. How can this information be used to develop improved treatments for human cancer? The studies of Wendel et al. (2004) shed some light. In their work, the transgenic Akt/Eμ-Myc and Eμ-Myc/Eif4e B cell lymphoma models were used to investigate the effect of Akt or Eif4e on tumor chemosensitivity. The results are intriguing. Compared to control lymphomas, which are highly chemosensitive, Akt/Eμ-Myc tumors were resistant to the bifunctional alkylating agent cyclophosphamide and the DNA topoisomerase II poison doxorubicin. Importantly, treatment with the TOR inhibitor rapamycin restored sensitivity to both cytotoxic agents, implying TOR function in the Akt-activated antiapoptotic pathway. As anticipated, overexpression of eIF4E abrogated the effect of rapamycin, supporting the conjecture that small changes in the stoichiometry of the suppressor 4E-BP1 and eIF4E are one determinant of rapamycin sensitivity. In contrast, rapamycin alone had no significant effect on tumor progression, leading one to conclude that the effect of Akt in suppressing Myc-driven apoptosis is independent of TOR. However, this interpretation should be taken with some caution. In human cells, the translation of c-Myc is cap-dependent, and is suppressed by rapamycin (Carter et al., 1999; Gera et al., 2003; Hosoi et al., 1998). Indeed, in human cells in which the Akt pathway is activated, rapamycin causes a significant shift of c-Myc RNA from the polysomal fraction to the translationally inactive pool (Gera et al., 2003). Thus, it would be important to assess whether, in the Akt/ Eμ-Myc lymphomas, Myc translation is still TOR-dependent before extrapolating this result to other tumor models.

Taken together, these three studies support the conclusion that dysregulation of cap-dependent translation through activation of the TOR pathway, or overexpression of eIF4E, plays a role in transformation and tumor progression. Importantly, they provide insight into the mechanism by which dysregulated translation suppresses apoptosis, which may counter the pro-apoptotic activities of p53. These studies further highlight the potential for development of therapeutic agents that target the Akt-TOR-eIF4E pathway, which may have significant antitumor and chemopreventive activities.

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