

Review Article

Understanding and Targeting the Eukaryotic Translation Initiation Factor eIF4E in Head and Neck Cancer

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The eukaryotic translation initiation factor eIF4E is elevated in about 30% of human malignancies including HNSCC where its levels correlate with poor prognosis. Here, we discuss the biochemical and molecular underpinnings of the oncogenic potential of eIF4E. Studies in human leukemia specimens, and later in a mouse model of prostate cancer, strongly suggest that cells with elevated eIF4E develop an oncogene dependency to it, making them more sensitive to targeting eIF4E than normal cells. We describe several strategies that have been suggested for eIF4E targeting in the clinic: the use of a small molecule antagonist of eIF4E (ribavirin), siRNA or antisense oligonucleotide strategies, suicide gene therapy, and the use of a tissue-targeting 4EBP fusion peptide. The first clinical trial targeting eIF4E indicates that ribavirin effectively targets eIF4E in poor prognosis leukemia patients and more importantly leads to striking clinical responses including complete and partial remissions. Finally, we discuss the relevance of these findings to HNSCC.

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1. Generalized Role for eIF4E in Cancer

The eukaryotic translation initiation factor 4E (eIF4E) is a protein that plays a central role in the regulation of gene expression at the posttranscriptional level. eIF4E binds the 7-methyl guanosine “m⁷G cap” structure found on the 5′ end of mRNAs. In the cytoplasm, eIF4E catalyses cap-dependent protein synthesis [1, 2]. Importantly, eIF4E effects the translation of some mRNAs, known as eIF4E sensitive, more than other transcripts. For instance, elevated eIF4E levels selectively increase translation of mRNAs coding for a variety of potent growth stimulatory proteins such as VEGF, Pim-1, and ornithine decarboxylase (ODC) [3–5]. In the nucleus, eIF4E mediates in the export of another subset of mRNAs (such as cyclin D1 and ODC mRNAs) to the cytoplasm [5–7]. Thus eIF4E can modulate gene expression at two levels: by exporting mRNAs to the cytoplasm increasing their concentration therein and by enhancing the translational efficiency of transcripts that are already in the cytoplasm. Not all transcripts are affected at both levels. Importantly, eIF4E requires its m⁷G cap binding function in order to act

in either of these functions. Clearly, dysregulation of eIF4E will profoundly affect the cellular proteome.

The process of malignant transformation requires multiple molecular events involving activation of proto-oncogene products that stimulate growth and inactivation of suppressor genes that inhibit cellular proliferation. Together, these events result in selective dysregulation of cellular metabolism and growth. Critical control points in the cell cycle, DNA replication, and protein synthesis are just a few of many potential sites where alterations of normal functions may result in tumorigenesis. Because the overexpression of eIF4E results in the upregulation of multiple gene products that play critical roles in cycle progression and survival, it is not surprising that the elevation of eIF4E has been detected in various malignancies [3].

eIF4E is overexpressed in many epithelial cell cancers, including breast [8–12], colon [13, 14], bladder [15–19], cervix [20, 21], lung [22–24], and squamous cell carcinoma of the head and neck [25–32]. Some studies report that eIF4E is overexpressed in almost 100% of tumors of the breast, head and neck, and colon [8, 27, 32]. Several retrospective

studies indicate that eIF4E elevation is correlated with poor prognosis. As discussed below, high eIF4E levels in the HNSCC correlated with higher incidence of relapse [26–29, 32]. eIF4E overexpression was detected at a range of 3–30 fold in breast carcinomas, compared to normal breast tissue [8, 10], and eIF4E levels were significantly increased in vascularized malignant ductules of invasive carcinomas [33]. Breast cancer patients with high eIF4E expression (>7-fold to normal) experienced a statistically significant poorer clinical outcome with a higher risk for recurrence and cancer related death [11]. Further, increased levels of eIF4E are observed in non-Hodgkin's lymphomas but not in benign lesions [34, 35]. Here, eIF4E levels correlated with the aggressiveness of these lesions [34, 35]. In prostate cancer, eIF4E levels were also correlated with worse prognosis [36]. In acute myeloid leukemia (AML), elevated eIF4E levels are characteristic of the poor prognosis M4 and M5 AML subtypes [37].

Given that both normal and cancer cells express eIF4E, it is important to develop therapeutic strategies that target cancer cells without harming normal cells. There is evidence that cancer cells have developed an oncogene addiction to, or dependency on, eIF4E. In studies in primary human leukemia specimens, subtypes of leukemias with elevated levels of eIF4E are sensitive to inhibition of eIF4E by antagonists at levels 100-fold less than those that effect normal bone marrow or other leukemic subtypes [38]. More recent studies suggest a similar case in a prostate cancer mouse model [39].

In animal models, eIF4E overexpression is correlated with not only increased numbers of tumors but also increased invasion, metastases, and angiogenesis [3, 15, 40]. Mice with transgene overexpression of eIF4E developed a variety of cancers of distinct histological origin [41]. These cancers develop despite the fact that the level of eIF4E overexpression in these mice is much less than the corresponding levels of eIF4E overexpression found in patients [32, 33, 37]. Further, a lymphoma mouse model showed that eIF4E overexpressing mice developed more lymphomas [42].

2. Dysregulation of eIF4E in HNSCC

eIF4E is found to be elevated in the vast majority (in some studies even 100% of cases) of HNSCC specimens, with levels being 3 to 24 fold elevated relative to normal controls [26–30, 32]. High eIF4E levels in surgical margins are predictive of increased risk of recurrence in HNSCC [26–29]. Overexpression of eIF4E in >5% of the basal layer of histologically tumor-free surgical margins of HNSCC patients predicted a significantly increased risk of recurrence [27]. This prediction is important for patient outcome as most HNSCC patients will succumb due to local recurrence [26, 28, 29]. It has been demonstrated that eIF4E overexpression is associated with eIF4E gene amplification in both HNSCC and in breast carcinomas [30, 43–45]. An increased level of eIF4E gene amplification was observed when benign tumors and invasive carcinomas of the head and neck were compared. Benign tumors only had moderate evidence for gene amplification, while malignant tumors had a 4–15 fold

level of amplification [43]. eIF4E protein levels were elevated in premalignant lesions in the larynx, but to a lesser extent than observed in HNSCC [25]. These studies suggest that progression to the malignant phenotype paralleled eIF4E gene amplification and overexpression [43]. Also, there was a progressive increase in the degree of eIF4E gene amplification and protein expression when comparisons were made among samples from tumor free margins of resected carcinoma specimens, tumor free regions adjacent to tumor core and tumor core samples [44]. This suggests that molecular events such as eIF4E gene amplification may precede cellular morphological changes, and that surgical margins which appear tumor free microscopically, may have elevated eIF4E protein levels. Thus, eIF4E levels could be used as a marker for prediction of early recurrence. It has been postulated that somewhere in the multistep pathway of carcinogenesis, elevation of eIF4E is a necessary event in progression of most solid tumors, and that eIF4E does not only reflect the proliferative status of cells but also their malignant properties [28, 46].

Consistent with their derivation from hypopharyngeal squamous carcinoma, FaDu cells [48] have elevated eIF4E [49], and as seen in many cell types, eIF4E is found in both the nucleus and cytoplasm (Figure 1). Further, eIF4E levels are elevated in FaDu cells due to both gene amplification, and increased mRNA stability [50]. Thus, there appears to be multiple ways to elevate eIF4E levels (see below).

3. Biochemical Underpinnings of eIF4E's Biological Effects

eIF4E overexpression profoundly alters the cellular proteome. However, experiments as early as 1980 [51] and more recent studies using knockdown strategies indicate that alterations in eIF4E expression do not uniformly alter the proteome [3, 5, 52–57]. In other words, the expression of some genes is more affected by modulation of eIF4E levels. These genes are referred to as eIF4E sensitive. In the cytoplasm, eIF4E recruits the transcript to the ribosome thereby increasing its translational efficiency. When eIF4E is overexpressed, sensitive transcripts have a higher ribosomes/mRNA ratio enabling more efficient translation without modulating mRNA levels in the cytoplasm. Notably, sensitive mRNAs have more highly structured 5' UTRs versus insensitive housekeeping mRNAs such as GAPDH or β -actin which contain short, unstructured 5' UTRs [3, 52, 58]. Transcripts controlled at this levels often code for proteins involved in proliferation such as c-Myc, Pim 1, VEGF, and ODC [4, 5, 55, 59].

Up to 68% of eIF4E is found in the nucleus of cells from a wide variety of species ranging from yeast to humans [7, 60–63]. These include FaDu cells, which have high eIF4E levels relative to normal cells and have eIF4E in both the nucleus and cytoplasm (Figure 1). As in the cytoplasm, only a subset of transcripts is sensitive to eIF4E dependant mRNA export [5]. These mRNAs contain a discrete 50 nucleotide element in their 3'UTR known as the eIF4E sensitivity element (4E-SE) [6, 64, 65]. Removal of the 4E-SE

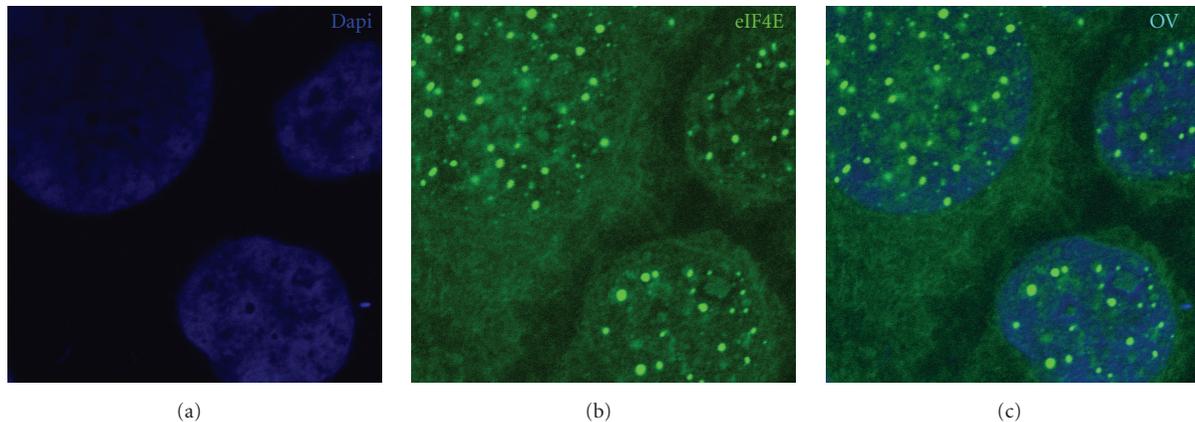


FIGURE 1: FaDu cells immunostained for eIF4E showing cytoplasmic and nuclear localization. Cells were stained using eIF4E mAb conjugated directly to FITC (green) and nuclear marker DAPI (blue) as described [37, 47]. Micrographs were collected on laser scanning confocal microscope using 100X objective and 2x digital zoom.

ablates eIF4E sensitivity [65]. Many mRNAs sensitive to eIF4E at the export level code for proteins that promote proliferation and survival. Increased export of the transcripts leads to increased levels of the mRNA available to the translation machinery, without altering translation efficiency [5]. In the nucleus, eIF4E is found in the nucleoplasm, in nuclear bodies co-localising with 4E-SE containing mRNAs or colocalising with promyelocytic leukaemia (PML) nuclear bodies (with no RNA). PML is a potent inhibitor of its mRNA export function and a potent inhibitor of eIF4E mediated transformation [47, 60, 66, 67].

Regulation of transcripts by eIF4E can occur at the mRNA export level, the translation level, or both (Figure 2). For instance, cyclin D1 transcripts are only sensitive to eIF4E at the mRNA export level [5, 60, 61, 65]. VEGF transcripts are only sensitive to eIF4E at the level of translation [5, 58]. In contrast, ODC transcripts are sensitive to eIF4E at both the mRNA export and translation levels [5]. ODC is regulated at both levels because it contains both the complex 5'UTR sensitising it to translation and the 4E-SE in its 3'UTR sensitising it to eIF4E dependent mRNA export. Importantly, 4E-SE containing mRNAs is exported through a pathway that is distinct from bulk mRNA export [64]. Unlike bulk mRNA export which is TAP/NXF1 dependent, eIF4E dependent mRNA export is CRM1 dependent and requires the 4E-SE and the mRNA export factor LRPPRC [64, 68].

The combinatorial effects that eIF4E have on gene expression position it as a central node in an RNA regulon governing proliferation and cell survival [64, 65]. The RNA regulon is a theoretical construct that outlines a means by which posttranscriptional gene expression can be coordinated [69, 70]. In this model, elements in the UTRs of transcripts sensitise groups of transcripts to the same level of regulation. Transcripts with the same combination of elements, known as USER codes, will be coregulated. In this way, transcripts coding for proteins acting in the same biochemical pathway can have their production coordinated and thus the biochemical output of the pathway optimised. In the case of eIF4E, the complex 5'UTR and the 4E-SE in the

3'UTR can be considered to be USER codes for translation and export, respectively [6].

An example of the RNA regulon is the ability of eIF4E to modulate Akt signalling. eIF4E overexpression, via its mRNA export function, upregulates the expression of an activator of Akt, NBS1 [71, 72]. Furthermore, it enhances the expression of several downstream effectors of Akt including c-myc, cyclin D1, and cyclin E1 [5, 64]. eIF4E rescues serum-starved fibroblasts from serum-induced apoptosis. However, eIF4E loses this activity in Akt1^{-/-} cells whereas reintroduction of Akt1 enables eIF4E to rescue the cells again [71]. Thus, through the coordinated regulation of genes involved in the Akt pathway, eIF4E can promote cellular survival. These observations are particularly interesting in the context of HNSCC progression. In a study of HNSCC tumors and surgical margins, elevated levels of eIF4E correlated with elevated Akt activation [73].

In summary, eIF4E modulates gene expression at two levels: mRNA export and translation. These functions are coordinated through the RNA regulon. In many cases, eIF4E sensitive mRNAs act in the same biochemical pathways such as cell cycle progression or survival pathways. This coordination potently drives the oncogenic potential of eIF4E [6].

4. Molecular Basis for eIF4E Mediated Transformation

eIF4E overexpression leads to transformation in cell culture, as well as in animal models, as described above. Specifically, eIF4E overexpression leads to loss of contact inhibition of fibroblasts, growth in soft agar and increased proliferation [47, 58, 61, 74–76]. eIF4E overexpression rescues cells from certain types of apoptotic stimuli [77–81]. In fibroblasts, serum deprivation induced apoptotic rescue of eIF4E is Akt1 dependent [82]. Both the nuclear and cytoplasmic functions of eIF4E contribute to its oncogenic potential [37, 47, 58, 60, 61]. For instance, a mutant of eIF4E, W73A, which acts in

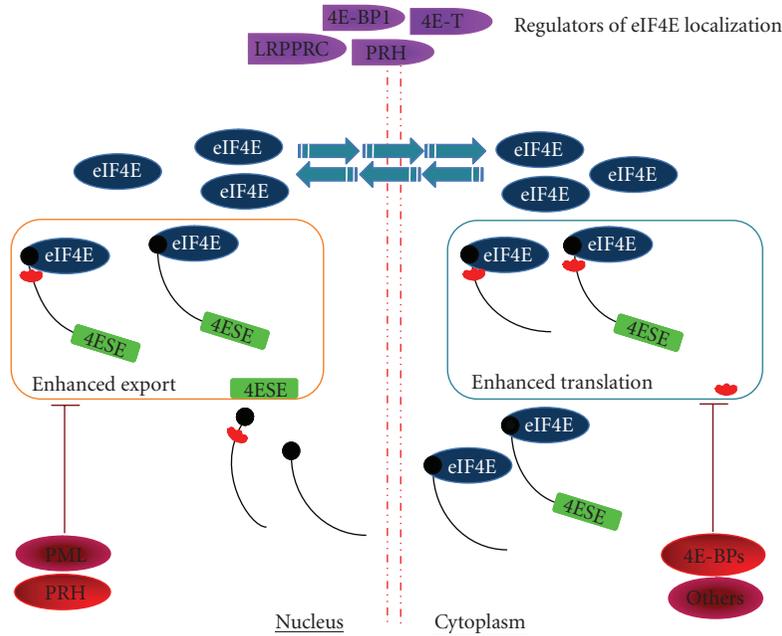


FIGURE 2: A diagram summarizing the nuclear and cytoplasmic functions of eIF4E. Some factors that directly regulate eIF4E functions and proteins involved in regulation of eIF4E subcellular distribution are shown. Not all regulators are shown for the sake of clarity. mRNAs are depicted as black lines with black balls denoting the 5' m⁷cap and with/without complex 5'UTRs shown in red and 4ESE element shown in green.

mRNA export but is deficient in promotion of translation, acts in both transformation and survival to the same extent as wild-type eIF4E [47, 60, 76].

5. Redundant Regulation of eIF4E

Regulators of eIF4E functions are positioned to modulate the eIF4E regulon, co-ordinately modulating cell cycle progression, and cell survival (Figure 2). One of the best-characterized regulators of eIF4E is eIF4E binding protein 1 (BP1) [58, 83]. This protein uses a conserved eIF4E binding site to associate with eIF4E, and thereby precludes access of eIF4E to eIF4G and the rest of the translation machinery [58]. This binding site is defined as follows: YXXXXL ϕ (where X is any residue and ϕ is a hydrophobic residue). Studies suggest that BP1 increases cap affinity and thereby sequesters both eIF4E and the RNA in question from the translational machinery [1]. The association of BP1 with eIF4E is modulated by phosphorylation of BP1 [58, 83]. Phosphorylation of BP1 leads to a reduction in its interaction with eIF4E and thereby results in increased translational activity of eIF4E. Phosphorylation is mTOR dependent and thus rapamycin treatment leads to reduced phosphorylation of BP1, increasing its association with eIF4E and thereby reducing translation of eIF4E sensitive mRNAs [84]. In contrast to eIF4E, BP1 overexpression sensitizes Ras transformed cells to apoptosis when treated with certain cytostatic drugs [85]. In addition, BP1 overexpression represses eIF4E mediated transformation of NIH 3T3 cells [58]. However, BP1^{-/-} and BP1^{-/-} BP2^{-/-} mice

do not develop cancers more readily than controls [86–89], highlighting the importance of redundancy of regulators in the control of eIF4E. Studies on BP1 in the literature focus on BP1 as a regulator of the cytoplasmic functions of eIF4E [58, 84]. However, endogenous BP1 associates with eIF4E in both the nuclear and cytoplasmic compartments and thus likely modulates eIF4E activity at both the level of translation and mRNA export (see [90] and our unpublished observations).

Counter-intuitively, BP1 levels are elevated in prostate and breast cancer and these levels correlate with a more advanced stage [91]. In esophageal cancers, there are more BP1-eIF4E complexes than in normal tissues, further complicating the accepted model of BP1 regulation of eIF4E [92]. Clearly, there is much more to be understood about BP1 and its implications for eIF4E activity.

There are many other regulators of eIF4E. The vast majority of these regulators contain the YXXXXL ϕ motif like eIF4G and the BPs. These regulators include a set of over 200 homeodomain proteins that contain this motif. Some of these members are negative regulators of eIF4E, such as PRH/Hex. PRH is a nuclear protein that impedes eIF4E's mRNA export function [76]. PRH overexpression leads to the cytoplasmic redistribution of eIF4E [37, 76]. Other members of this group of homeodomain containing regulators include Emx2, Otx, Engrailed 2, Hox11, Bicoid, and HoxA9 [93]. HoxA9 can stimulate both the nuclear and cytoplasmic functions of eIF4E [94]. Emx2 travels from one neuron to another through the synapse enabling localized translational control of eIF4E via signals to the adjacent neuron. In this way, Emx2 controls eIF4E activity remotely

[93]. Thus, eIF4E function can be regulated in a tissue and context dependent manner.

There is also a discrete class of eIF4E regulators that utilize a RING domain to impede eIF4E function. These regulators include the promyelocytic leukemia protein PML, HHARI, and arenaviral Z proteins from LCMV and Lassa viruses [47, 60, 95]. PML and the Z proteins use their RING motifs to associate with eIF4E and inhibit eIF4E function by reducing the affinity of eIF4E for the m⁷G cap by up to 100-fold [28, 37, 96]. These were the first proteins reported to reduce the affinity of eIF4E for the m⁷G cap. PML is a key cellular inhibitor of the oncogenic activities of eIF4E. The ability to inhibit eIF4E function is closely tied with the ability of PML to impair cap binding, and thus the mRNA export activity of eIF4E [47, 64, 65, 71, 97]. Similarly, Z also impairs eIF4E cap binding and function [97]. Notably, PML and Z do not alter eIF4E levels, and therefore do not appear to act directly or indirectly in its protein stability, unlike other RINGs [97].

Clearly, the regulation of eIF4E activity is redundant and multifactorial. There are tissue specific regulators such as the homeodomain proteins and more ubiquitous regulators such as PML and BP1 (Figure 2). Redundancy of regulators is seen for both the nuclear and cytoplasmic arms of eIF4E activity.

6. Controlling eIF4E Localization—a Key Step in the Regulation of eIF4E

Clearly, modulating the subcellular distribution of eIF4E will have profound impacts on the sets of genes it regulates and thus on its biological effects. For instance, eIF4E localization is substantially altered during *Xenopus* gastrulation [7]. Furthermore, eIF4E nuclear-cytoplasmic localization changes dramatically during differentiation of mouse embryonic stem cells to macrophages (KLBB, unpublished observation).

As discussed above, eIF4E is found in both the nuclear and cytoplasmic compartments. Recent studies indicate that within the cytoplasm, eIF4E is found not only associated with actively translating transcripts, but also with cytoplasmic structures known as processing bodies (P-bodies) [98, 99]. These structures contain a variety of factors including many associated with RNA degradation as well as eIF4E [100]. RNAs associated with these structures are sequestered from the translational machinery. It is thought that P-bodies are a temporary storage depot for these RNAs while their fate (in terms of degradation, sequestration, translation, etc.) is being decided [100]. Thus, in the cytoplasm, eIF4E is associated with both the translation machinery and in some cases with mRNAs that are being sequestered from this machinery (e.g., in P bodies), perhaps left there until the time is right for these mRNAs to be translated.

Ultimately, the biochemical pathways in which eIF4E functions (mRNA export, mRNA translation, or mRNA sequestration) depend on the subcellular distribution of eIF4E. Little is known about what regulates nuclear entry of eIF4E and what determines which cytoplasmic compartments in which eIF4E will be found. To date, the only factor known to directly modulate the subcellular distribution of

eIF4E is the eIF4E transporter protein (4E-T) [98, 101]. 4E-T uses its conserved eIF4E binding site to interact directly with the dorsal surface of eIF4E. The original study suggested that 4E-T transported eIF4E protein into the nucleus [101]. However, several other studies, including subsequent studies by the Sonenberg group [98], indicate that overexpression of 4E-T leads to relocalization of the majority of nuclear eIF4E to the cytoplasm, where a subset is found in P-bodies. The molecular mechanism for this redistribution is not yet known.

Other factors also modulate the subcellular distribution of eIF4E, including BP1 [90], the proline rich homeodomain protein PRH [37, 76], and the leucine rich protein LRPPRC [68] (Figure 2). PRH is a potent inhibitor of the mRNA export function of eIF4E [76]. PRH overexpression leads to redistribution of nuclear eIF4E to the cytoplasm [37, 76]. LRPPRC overexpression leads to re-distribution of eIF4E within the nucleus. Here, upon LRPPRC overexpression, LRPPRC competes for PML leading to reduced PML-eIF4E co-localization. This redistribution correlates with increased eIF4E dependent mRNA export [68]. In summary, these factors are positioned to impact the nuclear and cytoplasmic arms of eIF4E activity and thus alter the effects of eIF4E on the proteome.

There are other means to modulate the subcellular distribution of eIF4E. Interestingly, transduction of primary leukemia specimens (M4/M5 AML) with the inhibitor of NF κ B activity, I κ B-SR, leads to a substantial re-organization of eIF4E, reducing the amount of eIF4E found in the nuclear fraction and increasing the amount in the cytoplasm, and reorganization of the remaining eIF4E nuclear bodies into structures which are morphologically indistinguishable from normal cells [37, 93]. Thus, the subcellular distribution of eIF4E appears linked to NF κ B activity. As expected, transduction of I κ B-SR leads to reduced eIF4E dependent mRNA export in these specimens [37, 93]. In this way, eIF4E localization is linked to NF κ B activity.

In addition, the subcellular distribution of eIF4E can be modulated by small molecules [38, 47, 102]. Treatment of cells with the m⁷G cap analogue (m⁷GpppG) leads to disruption of eIF4E nuclear bodies and re-distribution of eIF4E to the cytoplasm [47, 102]. Treatment with a physical mimic of the m⁷G cap, ribavirin, has a similar effect where it leads to an increased fraction of eIF4E in the cytoplasm [38]. Consistently, ribavirin treatment leads to reduction in eIF4E dependent mRNA export. Note that ribavirin or m⁷GpppG, under these conditions, does not alter the levels of eIF4E [38, 47, 102].

In summary, factors such as 4E-T that so drastically affect the subcellular localization of eIF4E, are positioned to affect eIF4E's physiological activities in proliferation and oncogenic transformation (Figure 2). In addition, given that eIF4E modulates the expression of some transcripts only at one level (such as cyclin D1 at the export level or VEGF at the translation level), modulation of its subcellular distribution is likely to lead to differential effects on eIF4E sensitive transcripts. In this way, these eIF4E traffickers could modulate gene expression differentially favouring/disfavouring subsets of genes (e.g., export versus translation) and thereby

modulate the biological effects of eIF4E. This level of modulation would allow a more tailored response to cellular stresses and stimuli.

7. How Does eIF4E Become Elevated in Cancer?

Given that elevated eIF4E levels are found in many human cancers and are associated with poor prognosis [28, 30, 43, 103], it is critical to understand how eIF4E levels become elevated. There are likely multiple mechanisms that could account for elevated eIF4E mRNA levels in these primary patient specimens, for example, gene amplification, transcriptional dysregulation, and alterations in mRNA stability. In fact, elevated eIF4E levels may result from any combination of these. For instance, eIF4E levels are elevated, at least in part, in breast cancer and head and neck squamous cell carcinomas due to amplification of the eIF4E gene [13, 104, 105]. Studies in cell culture indicate that the eIF4E promoter contains an E-box, and that its expression is regulated by c-myc [5, 6, 64]. Interestingly, c-myc is a downstream mRNA export and mRNA translation target of eIF4E which suggests existence of a potential feedback loop [106, 107]. As eIF4E is made in c-myc null mice, there must be other means by which it is induced [37]. Further, eIF4E mRNA levels are substantially reduced in primary leukemia specimens transduced with the I κ B-SR [3, 108]. In addition, some studies have found increased eIF4E expression during hypoxic conditions by IHC analysis of confined breast cancer biopsies [50]. In this way, the hypoxia that accompanies tumor growth may stimulate eIF4E expression.

Another mechanism that appears to be involved in the elevation of eIF4E in HNSCC is HuR dependent stabilization of eIF4E transcripts. Specifically, in FaDu cells, both HuR and eIF4E levels are elevated relative to control cells. Here, the mRNA stability factor, HuR, associates with eIF4E mRNA and enhances its stability [109]. HuR is a member of a family of proteins which modulate the stability of mRNAs by associating with U or AU rich elements (denoted AREs) typically in the 3'UTR of these messages [109]. Hu/ELAV family members are primarily neuronal with the exception of HuR, which is ubiquitously expressed. HuR modulates the expression of many proliferative mRNAs which contain AREs including (but not limited to): cyclin D1, cyclin B1, c-myc, VEGF, and so forth [109, 110]. Interestingly, many of these target mRNAs are also export and /or translational targets of eIF4E (e.g., all of the ones listed above). HuR has been implicated in oncogenesis. Its overexpression is correlated with the formation of tumors in mouse xenograft models [111, 112]. Microarray data of normal and cancer tissues indicated that HuR is elevated in human breast and lung cancer [96, 113, 114]. Further, HuR promotes angiogenesis, as does eIF4E [37]. The overlap in mRNA targets coupled to the fact that both eIF4E and HuR are involved in transformation and elevated in human cancers, suggests that eIF4E could be a downstream effector of HuR activity. Thus HuR is positioned to modulate the eIF4E regulon by both altering its expression and the expression

of eIF4E's downstream effectors. Future studies that monitor HuR levels in HNSCC could be very interesting and may suggest HuR as another prognostic marker.

8. Targeting eIF4E in HNSCC- from Cells to Patients

To date, targeting of eIF4E in HNSCC remains in the preclinical stage. Three main pre-clinical strategies have been described: knockdown of eIF4E levels through the use of antisense oligonucleotides or RNA interference, suicide gene therapy, hormone analog—4EBP fusion peptide, and targeting eIF4E activity with ribavirin.

Inhibition of eIF4E using antisense oligonucleotides to eIF4E was first performed in HeLa cells and Ras-transformed mouse fibroblasts, and resulted in the reversal of the malignant phenotype [115–117]. Decreasing levels of eIF4E in the human breast cancer cell line MDA-MB-435 and human prostate cancer cell line PC-3 diminished their angiogenic and tumorigenic properties [33, 39]. The first laboratory to find eIF4E levels elevated in both breast and HNSCC, the De Benedetti lab, was also the first to target eIF4E in HNSCC cells [33, 49]. Using antisense RNA to eIF4E, they demonstrated that they lowered both eIF4E levels and the levels of its downstream targets, VEGF and FGF-2. FaDu cells treated with antisense oligonucleotides also show reduced oncogenic properties of these cells including displaying increased contact inhibition, reduced growth in soft agar, and reduced tumorigenicity in xenograft mouse models [49]. A related strategy used small interfering RNAs targeting eIF4E either alone or in combination with cisplatin in the UMSCC22B HNSCC cell line [118]. As expected, siRNA to eIF4E lowered eIF4E levels and reduced the oncogenicity of this cell line. The addition of cis-platin increased the effects of the knockdown of eIF4E alone. This same strategy, combining siRNA to eIF4E with cisplatin, was also used in breast carcinoma cells with success [119].

Antisense oligonucleotides (ASOs) were also used by the Graff lab in a human prostate cancer xenograft mouse model [39]. Here, mice that intravenously received antisense oligonucleotides showed significant reduction of eIF4E expression and suppressed tumor growth. No toxicity was observed. The ASOs used also target murine eIF4E, leading to an 80% reduction of eIF4E in mouse liver; however there was no affect on body weight, organ weight, or liver transaminase levels. Eli Lilly is currently pursuing clinical trials using this strategy.

Suicide gene therapy is a method of introducing a gene, the expression of which will make a tumor cell uniquely susceptible to attack and destruction [120]. This strategy utilizes delivery of herpes simplex virus-thymidine kinase (HSV-Tk) by nonreplicative adenovirus vectors to the cells and subsequent ganciclovir (GCV) treatment [121, 122]. The HSV-Tk has the ability to phosphorylate and activate prodrug GCV to its cytotoxic triphosphate form with 1000-fold higher efficiency than its mammalian homologues. As a consequence, cells transfected with HSV-Tk can be

targeted for death by treatment with ganciclovir, while normal cells would remain mainly unaffected [121, 122]. Although this strategy gained wide popularity as potential treatment for HNSCC, this strategy had two principal challenges: acceptable cytotoxic specificity to tumor cell targets and adequate delivery of the suicide gene. In order to specifically target eIF4E overexpressing cells, a long 5'UTR (from FGF-2) was fused to the thymidine kinase gene (5'UTR-Tk) to preferentially sensitize expression of this gene to eIF4E levels [123, 124]. This system was reported highly efficient in a broad spectrum of breast cancer cell lines [124]. Using a mouse minimal residual disease soft-tissue metastasis model for HNSCC, the Li group examined the efficacy of this strategy to target solid tumors cells that are overexpressing eIF4E [123]. In this study, mice that received Ad-HSV- 5'UTR-Tk fusion and GCV treatment showed longer disease free survival than the control group [123].

In order to inhibit eIF4E in ovarian cancers, Ko et al. [125] designed 4EBP-based peptide fused to an analog of gonadotropin-releasing hormone (GnRH) to specifically target ovarian and other endocrine cancer cells, that are widely overexpressing the GnRH receptor. This fusion peptide inhibited growth of the GnRH receptor expressing tumor cells and showed potent antitumor effect in a mouse xenograft model of epithelial ovarian cancer, without significant cytotoxic effects in other tissue.

9. Successful Targeting of eIF4E in the Clinic

To date, eIF4E has been successfully targeted only in a particularly aggressive form of acute myeloid leukemia French American British (FAB) subtype M4/M5 AML. These poor-prognosis leukemias are characterized by elevated eIF4E levels [37]. In these studies, ribavirin, a competitive inhibitor of the natural ligand of eIF4E the m⁷G cap, was used to target its biochemical and oncogenic activities [38]. In a phase II proof-of-principle clinical trial of refractory, relapsed or patients who cannot undergo induction chemotherapy were treated with ribavirin [126]. eIF4E inhibition led to striking clinical improvement including complete remission, partial remission, and blast response. Ribavirin was originally used as an antiviral drug and was well tolerated with no therapy related toxicities observed. Note that in these studies, ribavirin was the only cytotoxic chemotherapy permitted.

Clinical response correlated with inhibition of eIF4E activity and redistribution of the eIF4E protein from the nucleus to the cytoplasm [126]. In these AML patients, eIF4E was both highly upregulated and found mainly in the nucleus [38, 82]. The mRNA export activity of eIF4E is also upregulated in these specimens [37, 126]. After 28 days of treatment with ribavirin, eIF4E was markedly re-distributed from the nucleus to the cytoplasm [126]. Surprisingly, eIF4E protein levels were also downregulated, which is the first time this downregulation has been reported postribavirin treatment (note that in the previous experiments, ribavirin treatment was followed in cell culture

for up to 48 hours, not 28 days as used for patients [127–130]). It is possible that this downregulation occurs via a negative feedback loop due to prolonged inhibition of eIF4E. Alternatively, decreased eIF4E levels could be a result of differential sensitivity within a heterogeneous cell population to ribavirin. At the same time, the production of eIF4E mRNA export targets such as cyclin D1 and NBS1 mRNA is repressed in patients. Further, eIF4E dependent Akt activation is reduced, which is consistent with its requirement for NBS1.

Durability of clinical response is key to success. In the treatment of M4 and M5 AML, a regimen of chemotherapy typically combines Ara-C with danurubicin or idarubicin [127–130]. This regimen, named 7+3, induces remission in most patients. However, in the absence of consolidation therapy (typically with Ara-C), remissions only last 2–4 months [127–130]. Like many targeted monotherapies such as ATRA in APL or flt3 inhibitors in AML [131–134], after 2–4 months of ribavirin treatment, development of drug resistance was observed [126]. In these cases, although eIF4E levels remain low, eIF4E relocalizes to the nucleus, which generally correlated with relapse of the disease. The molecular events underpinning the return of eIF4E to the nucleus are not known, but clearly these events play a critical role in the response of these cells to ribavirin. To try to overcome resistance, ribavirin will be combined with other chemotherapy regimens. Although these studies are in AML patients, the ability to target eIF4E has clear implications for the development of treatments for HNSCC and other cancers with elevated eIF4E.

10. From Leukemia to HNSCC

Several previous studies indicate that ribavirin is an effective inhibitor of growth in FaDu cells [38, 82]. In xenograft mouse models, studies had shown that genetically reducing the levels of eIF4E protein by antisense RNA substantially impaired tumour growth [39, 119]. Similarly, addition of oral ribavirin to mice after FaDu xenograft led to significantly smaller tumours than for control animals [38]. Further, ribavirin inhibited anchorage dependent growth in FaDu cells in culture and significantly reduced levels of cyclin D1 and NBS1 proteins, and decreased Akt activation [82]. These findings in cell culture as well as results from treatment of AML patients suggest that targeting eIF4E with ribavirin in HNSCC may yield promising clinical results.

11. Conclusions

In this paper, we have described the biochemical function and biological effects of eIF4E. We summarize the roles and regulation of eIF4E in gene expression (Figure 2). We discussed the dysregulation of eIF4E in multiple cancers and the current strategies being considered to target its activity. eIF4E is an indicator of poor prognosis in HNSCC and hopefully, therapeutic approaches targeting eIF4E will benefit these patients.

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