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News and Commentary

eIF4A1 is a promising new therapeutic target in ER-negative breast cancer

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It is well accepted that post-transcriptional control makes a significant contribution to the overall regulation of gene expression.[1] One of the major ways in which translation can be regulated is by controlling the formation of the eukaryotic initiation factor (eIF)4F complex. This heterotrimeric complex is composed of the scaffold protein eIF4G, the cap-binding protein eIF4E and a DEAD-box helicase eIF4A1, which is required to unwind the structured regions of RNA that would otherwise be inhibitory to the scanning ribosome. Other proteins that also interact with this complex include poly(A)-binding protein (PABP) (which interacts with both the poly(A) tail and eIF4G), eIF4B, which stimulates eIF4A1 and helps circularization of the complex via its interaction with PABP, and eIF3, which interacts with the 40S ribosomal subunit. Thus, via the interaction of eIF4F with 7-methylguanylate (m7G) cap at the 5' end of the mRNA and eIF3, this complex is able to coordinate the binding of the mRNA with the 40S ribosomal subunit (Figure 1). Signalling through the PI3K/mTOR pathway activates translation by phosphorylating 4EBP1, a negative regulator of eIF4E. In its hypophosphorylated state 4EBP1 binds and limits the bioavailability of eIF4E and therefore eIF4F complex formation.

Given the role of protein synthesis control in gene expression regulation, it is unsurprising that aberrant control of translation is associated with tumorigenesis.[2] This can occur by enhanced signalling through to translation, releasing protein required for eIF4F complex formation (e.g., eIF4E) from their negative regulators.[1] Several chemotherapeutic agents target pathways that activate protein synthesis are in development (reviewed in Blagden and Willis[3]) and in particular there has been much interest in inhibitors of the PI3K/mTOR pathway, several of which are in clinical use as cancer treatments.

A number of recent studies have also shown that the helicase component of the eIF4F complex, eIF4A1 and the proteins that are known to modulate its function, including eIF4B, eIF4H, PDCD4 and eIF4E,[4] have a crucial role in the progression and development of tumours.

Thus, it has been shown that in diffuse large B-cell lymphoma overexpression of eIF4B is associated with poor prognosis by overcoming the inhibitory effects imposed by the highly structured 5' untranslated regions (UTRs) of mRNAs that encode anti-apoptotic and DNA repair proteins.[5] eIF4E, an established oncogene,[6] is known to stimulate the translation of mRNAs that contain long highly structured 5' UTRs such as c-*myc* and cyclin D1,[6] whereas PDCD4, which inhibits the helicase function of eIF4A by direct binding, is a tumour suppressor protein.[7]

There are a number of inhibitors that directly target eIF4A, although their role as anticancer agents still remains under-explored. The rocaglamides (including silvestrol) act as eIF4A inhibitors and have been shown to exert antitumour activity both *in vitro* and *in vivo*.[8–10] More recent studies have shown that the natural product inhibitor hippuristanol (isolated from the coral *Isis hippuris*[8] is capable of reversing drug resistance in tumours engineered to be dependent on PI3K/AKT/mTOR signalling.[11] Moreover, it has been shown in cell lines and in xenographs derived from patients with malignant melanoma that formation of eIF4F complex is associated with resistance to anti-BRAF(V600) inhibitors. Inhibition of eIF4A function with hippuristanol synergized with anti-BRAF or anti-MEK inhibitors and the data suggested that combinations of drugs targeting BRAF and eIF4A (as part of eIF4F) would be sufficient to overcome most resistance mechanisms in BRAF (V600)-mutant cancers and provide new treatment options for patients with this aggressive cancer.[12]

However, despite these studies, the frequency and role of increased eIF4A1 activity in tumorigenesis has not been extensively investigated. Modelska *et al.* carried out an important large-scale study to determine the clinical significance of expression of eIF4A1 and its modulators in breast cancer by scoring tissue microarrays derived from ~4000 patients.[13] The data showed that expression of eIF4A1, and its stimulators eIF4B and eIF4E, predicted poor outcome in ER-negative breast cancers in a way that was statistically independent of other known prognostic factors. This observation is impressive because, apart from involvement of regional lymph nodes, there are very few prognostic factors known to predict survival in this aggressive disease. Thus, the markers of eIF4A activity may have clinical use as the new prognostic markers in these difficult cancers.

To examine the mRNAs that were particularly dependent on eIF4A1 expression, Modelska *et al.* decreased the expression of this protein using siRNA in the ER-positive breast

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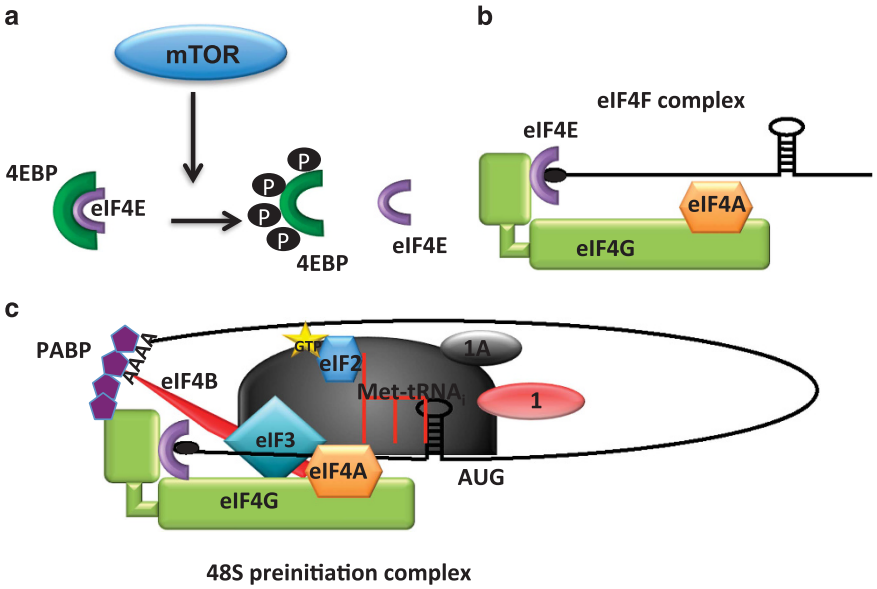

Figure 1 Schematic to illustrate the formation of 48S preinitiation complex. (**a**) Phosphorylation of 4EBP1 by mTOR releases eIF4E. (**b**) This allows the eIF4F complex formation and eIF4E interacts with the m7G cap structure at the 5' end of the mRNA. eIF4A, which is also bound by eIF4G, is required to unwind the regions of secondary structure. (**c**) Via the interaction of eIF3 with eIF4G, the 40S ribosome is recruited in conjunction with ternary complex ($tRNA_{imet}$, eIF2 and GTP). The entire complex is further stabilized by the interaction of PABP with eIF4G and eIF4B (which also stimulates eIF4A activity)

carcinoma cell line MCF7 and carried out polysome profiling.[13] This technique couples sucrose density gradient isolation of translationally active mRNAs with RNA sequencing. In total, 175 mRNAs were identified that showed a decrease in their polysomal association following eIF4A1 knockdown, whereas 49 mRNAs showed an upregulation. The group of upregulated transcripts was enriched for three structural protein families including G-protein *α*-subunits, cyclin N-terminal domains and serine threonine protein kinases. In addition, several genes involved in oncogene signalling including SMAD2, TGFB1 and CBC25B were identified as requiring eIF4A for their efficient translation. In a related study, ribosome footprinting was used to assess the translational status of mRNAs after inhibition of eIF4A activity with silvestrol in a murine model of T-cell acute lymphoblastic leukaemia.[14] Again a number of known oncogenes were identified as particularly dependent on eIF4A including transcription factors such as c-*myc*, c-*myb* and NOTCH1.[14]

When the mRNAs that were identified as eIF4A dependent were analysed in detail, Modelska *et al.* found that they contained 5' UTRs with a higher GC content and a predicted greater degree of secondary structure. In contrast, those mRNAs that contained short 5' UTRs with a low potential for structure formation, including mRNAs containing terminal oligopyrimidine tracts that encode ribosomal proteins, were found in the upregulated group. What is particularly interesting is that both studies (identified an enrichment for G-quadruplex-forming sequences in mRNAs that were eIF4A dependent. Thus, in the paper from Modelska *et al.*[13] there was a prevalence of (GGC)*n* motifs and a GGAGG-containing element, which they suggested could form novel structures. In contrast, in Wolfe *et al.*[14] the 5' UTRs on the eIF4A1-dependent list were shown to be enriched for the 12-nucleotide quartet (CGG)4 that again forms RNA G-quadruplex structures. Clearly, the precise role of G-quadruplexes in controlling the translation of these eIF4A-dependent mRNAs will be of considerable interest.

In summary, these new data from Modelska *et al.*[13] illustrate the clinical relevance of increased expression of eIF4A1 and, when taken in context with other research in this area, highlight that this protein is a good therapeutic target. Therefore, in the future it is likely that novel anticancer agents that directly inhibit eIF4A1 or alter the activity of its modulators will provide promising treatments for a range of cancers either as direct agents or in combination therapies.

Conflict of Interest

The authors declare no conflict of interest.

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