The Eukaryotic Translation Initiation Factor 4E (eIF4E) as a Therapeutic Target for Cancer

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Contents

Abstract

Cancer cells depend on cap-dependent translation more than normal tissue. This explains the emergence of proteins involved in the cap-dependent translation as targets for potential anticancer drugs. Cap-dependent translation starts when eIF4E binds to mRNA cap domain. This review will present eIF4E's structure and functions. It will also expose the use of eIF4E as a therapeutic target in cancer.
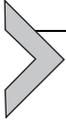
1. INTRODUCTION

When eIF4E was discovered, it was considered as an isolated protein, not belonging to any known protein family. Research of the last decade showed that all eukaryotes have several members of the eIF4E family. Joshi et al. (2005) identified, through sequence analysis, 411 eIF4E family members, in 230 species. Three isoforms (eIFF-1, 4EHP, and eIF4E-3) are present in mammals (Joshi, Cameron, & Jagus, 2004). Not all proteins from eIF4E's family bind to 7 methylguanosine mRNA cap (m7GDP) and to the same ligand (Joshi et al., 2004; Robalino et al., 2004; Rosettani et al., 2007), which give them different physiological functions. Hernandez and Vazquez-Pianzola (2005) suggested that in each organism, there is one member of the eIF4E family expressed that intervenes in translation and that other members have other functions (development, translation repression, specific mRNA nuclear transport). This hypothesis is being confirmed since eIF4E's isoforms are thought to be involved in many functions such as sper- matogenesis, oogenesis, aging, and other functions (Amiri et al., 2001; Dinkova et al., 2005; Evsikov & Marin de Evsikova, 2009; Minshall et al., 2007; Syntichaki, Troulinaki, & Tavernarakis, 2007). Cap-dependent translation starts when eIF4E binds to the mRNA cap domain. Cancer cells depend on cap-dependent translation more than normal tissues (Jia et al., 2012). This review will expose eIF4E's structure and functions and will expose the use of eIF4E as an anticancer target.
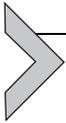
2. eIF4E'S STRUCTURE AND EXPRESSION

2.1 Structure

eIF4E's primary structure (Fig. 1A) is highly conserved in all eukaryotes because of the important role they play in the cell. In the N-terminal end, sequences are variable between different organisms, but this end does not seem to be involved in the initiation to translation function. The tertiary structure was characterized in mice, men, yeast, and wheat (Monzingo et al., 2007; Tomoo et al., 2002). This structure is composed of eight antiparallel β strands and three helices on the convex side (Fig. 1B). eIF4E binds to the m7GDP of the mRNA cap to allow the translation initiation. eIF4E tridi- mensional structures that interact with cap analogs were identified, allowing to identify the interaction site (Gross et al., 2003; Niedzwiecka et al., 2002; Tomoo et al., 2003). The cap interaction happens in a hydrophobic pocket

A

MATVEPETTPTPNPPTTEEEKTESNQEVANPEH
YIKHPLQNRWALWFFKNDKSKTWQANLRLISK
FDTVEDFWALYNHIQLSSNLMPGCDYSLFKDGI
EPMWEDEKNKRGGRWLITLNKQQRRSDLDRF
WLETLLCLIGESFDDYSDDVCGAVVNVRAKGDK
IAIWTTECENREAVTHIGRVYKERLGLPPKIVIGY
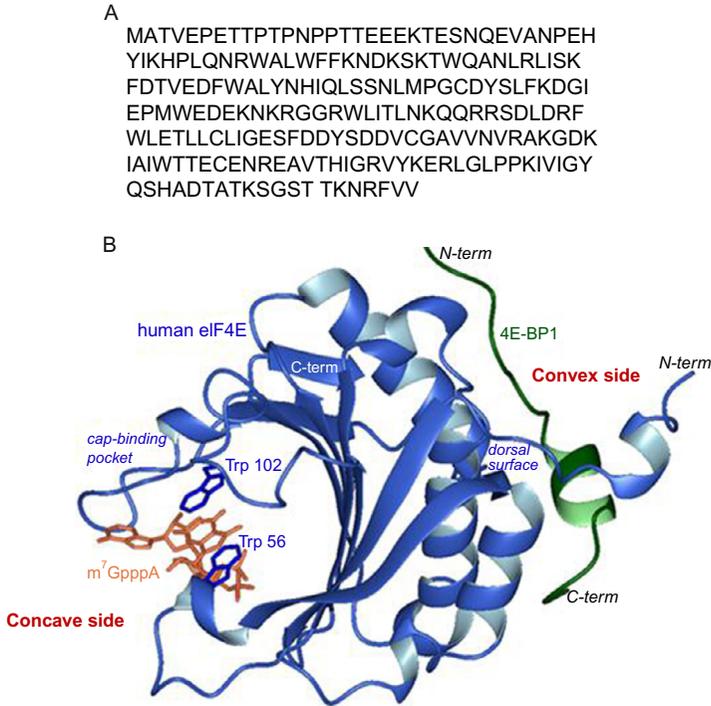QSHADTATKSGST TKNRFVV

Figure 1 (A) Human eIF4E's primary structure. (B) eIF4E's structure. Crystal structure of the human protein eIF4E (blue; dark gray in the print version) linked to the mRNA m7GDP cap (light pink; light gray in the print version) and to its ligand 4E-BP1 (green; gray in the print version) (http://atlasgeneticsoncology.org). The eIF4E interaction with the cap occurs on the concave side and requires two highly conserved tryptophan residues (Trp). The interaction between eIF4E and its ligands 4E-BPs, eIF4G, and PML occurs on the convex side.

on eIF4E's concave side, due to the interaction with two highly conserved tryptophan residues (56 and 102 in mice) (Fig. 1B). This interaction is stabilized by three hydrogen bonds. The interaction with partner proteins involved in translation regulation, such as eIF4G or 4E binding proteins (4E-BP), takes place in a hydrophobic region on the convex side, and it involves two conserved tryptophan residues (43 and 73 in mice) (Fig. 1B). These proteins interact with eIF4E through a bonding pattern, which consensus sequence is: $Y(X)_4L\Phi$, with X being any amino acid and Φ being a hydrophobic residue. The eIF4G or the 4E-BPs' binding to eIF4E causes conformational changes which increases eIF4E's affinity to the cap (Niedzwiecka et al., 2002; von Der Haar, Ball, & McCarthy, 2000). The PML protein (promyelocytic leukemia protein) and the viral

protein Z (VPZ) represent a second class of eIF4E regulators that intervene in the mRNA nuclear export function. These proteins bind to eIF4E's convex side using their RING domain, which, in contrast to the bond to eIF4G and 4E–BP, decreases the affinity of eIF4E to the cap (Cohen et al., 2001; Kentsis et al., 2001; Volpon et al., 2010). Structural studies show that eIF4E has different conformations and different ligand binding affinities depending on whether it is binding to the cap or not (Niedzwiecka et al., 2002; Niedzwiecka, Darzynkiewicz, & Stolarski, 2004; Volpon et al., 2006; Tomoo et al., 2002).

2.2 eIF4E's Expression and Regulation

2.2.1 Expression

Cell and tissue growth depend on protein synthesis. eIF4E's expression is significantly higher in human malignant tissues than in normal tissues. For cells to be viable, it is important for protein translation to be closely regulated to prevent malignant transformation and cancer development. The translation control is rather at initiation, even though there are controls during elongation phase. eIF4E's activity is controlled by several mechanisms described below (Van Der Kelen et al., 2009). Although eIF4E is well studied for its role in the translation initiation and for its involvement in tumorigenesis, little is known about its expression regulation. Surprisingly, eIF4E's overexpression does not lead to a global increase in the proteins' translation, but it leads to a selective increase in the translation of mRNAs that have a structure called "sensible elements to eIF4E" and that are involved in tumorigenesis.

2.2.2 Regulation

Studies show that the eIF4E inhibition can lead to HeLa cancer cell death and its absence is lethal for *Saccharomyces cerevisiae*. When overexpressed, eIF4E can act like an oncogene, by promoting malignant transformation and lymphomagenesis in rodent cells. An overproduction of eIF4E causes uncontrollable cell growth or oncogenesis, which indicates its importance in protein synthesis (Andrieu et al., 2010).

Given the important function of this protein, it is not surprising to find its activity highly regulated.

2.2.3 Transcription Levels

Serum, growth factors, and the immunologic activation of T lymphocyte lead to an increase in the gene transcription (Schmidt, 2004). There are also

consensus binding sites to transcription factors (such as c-Myc and hnRNPK) that are involved in the control of the gene transcription in response to stimuli (Lynch et al., 2005). For example, 4E-BP1 has at least seven phosphorylation sites among which four are known to be regulated by signaling pathways such as mTOR (Gingras, Raught, & Sonenberg, 2001; Heesom et al., 2001; Wang et al., 2005). When c-Myc is over-expressed, due to growth factors, eIF4E's expression rises.

2.2.4 Protein Level

2.2.4.1 Phosphorylation

In mammals, eIF4E is phosphorylated at the 209th serine residue located in a C-terminal motif which is conserved in all species except for plants and *S. cerevisiae*. The Mnk1 and Mnk2 kinases (MAPK-integrating kinases) (Ueda et al., 2004) bind to the C-terminal end of eIF4G, to be close to eIF4E to phosphorylate it. These kinases are themselves activated by phosphorylation realized by the Erk kinase (extracellular signal–regulated kinase) and by the p38 MAP kinase (Fig. 2) (Scheper et al., 2001). Growth factors, phorbol esters, and insulin can activate the Mnk kinases via the Erk pathway (Tschopp et al., 2000). Cytokines and some stress conditions can activate the p38 MAP kinase pathway. Phosphorylation can also be regulated during viral infection. For example, during an adenovirus infection, eIF4E is dephosphorylated because the 100K viral protein binds to eIF4G and moves the Mnk kinases from the eIF4F complex. The same phenomenon was observed during an influenza virus infection (Cuesta, Xi, & Schneider, 2000). However, a coronavirus infection activates Mnk1 and increases eIF4E's phosphorylation via the p38 MaP kinase pathway (Banerjee et al., 2002). Although eIF4E's phosphorylation mechanism is known, the consequences of this phosphorylation on translation initiation are still unclear and depend on the cellular context (Scheper & Proud, 2002). By a modulation of the Mnk–eIF4G interaction, eIF4E's phosphorylation is controlled: eIF4G binding is controlled by MAPK-mediated phosphorylation of the Mnk1 active site. Furthermore in the absence of MAPK signaling, eIF4E phosphorylation is prevented by the C-terminal domain of Mnk1 that restricts its interaction with eIF4G (Shveygert et al., 2010).

2.2.5 4E-BP

The protein family 4E-BP regulates eIF4E capacity to form the cap-binding complex (eIF4F). Currently, three 4E-BPs are known in mammals: 4E-BP1, 4E-BP2, and 4E-BP3. Their interaction strength is regulated by
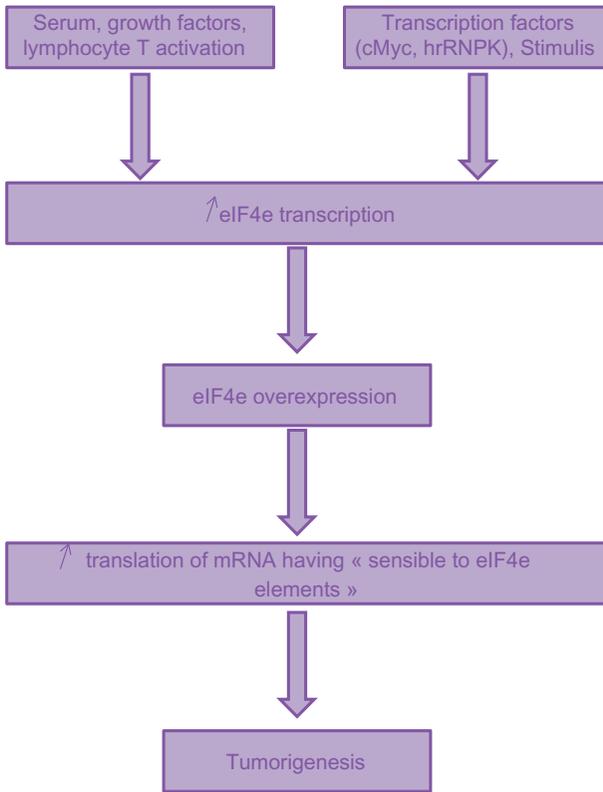
Figure 2 eIF4E's expression regulation and its implication in tumorigenesis. Serum, growth factors, and T-lymphocyte immunologic activation lead to an increase of eIF4E's transcription. There are also consensus binding sites to transcription factors (such as c-Myc and hnRNPK) that are involved in the control of the gene transcription in response to stimuli. When c-Myc is overexpressed, eIF4E's expression rises. eIF4E's over-expression leads to a selective increase in the translation of mRNAs that have a structure called "sensible to eIF4E elements" and that are involved in tumorigenesis.

phosphorylation. The 4E–BPs are phosphorylated in response to growth factors, amino acids, or hormones such as insulin which activates the mTOR pathway (molecular target of rapamycin) (Fig. 3) (Gingras et al., 2001; Gingras, Raught, & Sonenberg, 2004; Kimball, 2001). For example, 4E–BP1 has at least seven phosphorylation sites, among which four are known to be regulated by signaling pathways such as mTOR (Gingras et al., 2001; Heesom et al., 2001; Wang et al., 2005). In contrast, hypoxia induces a phosphorylation decrease in 4E–BP1 (Shenberger et al., 2005). When 4E–BPs are hypophosphorylated, they can sequestrate eIF4E and
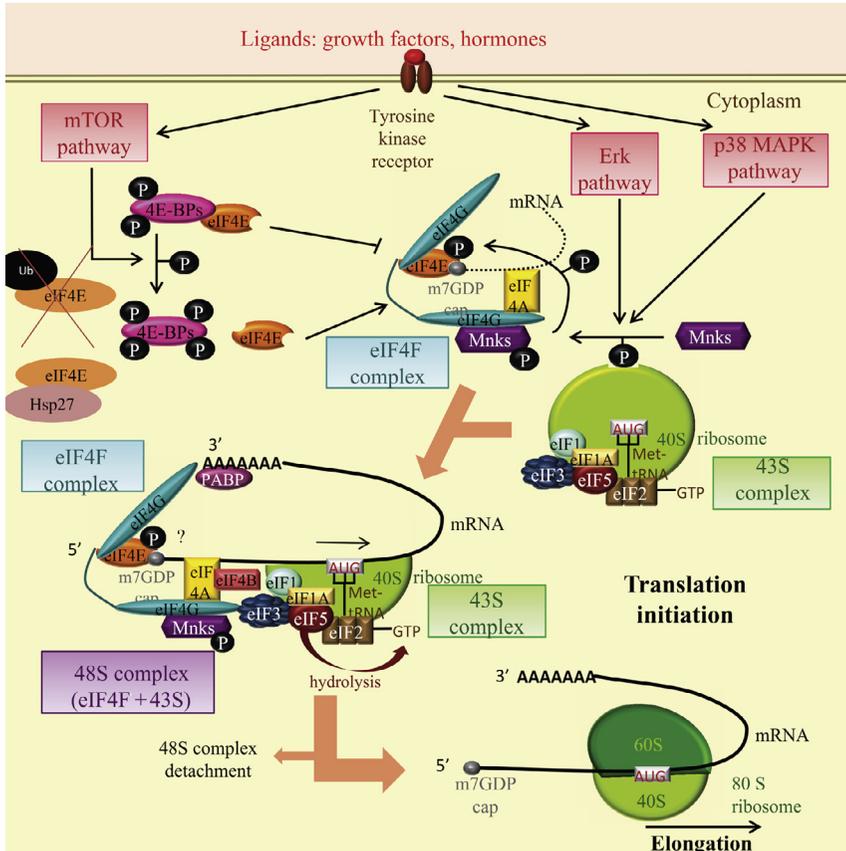
Figure 3 eIF4E's implication in the mRNA translation initiation. The translation initiation of most mRNAs occurs due to a cap-dependent mechanism that involves eIF4E. This mechanism is regulated by eIF4E's phosphorylation by Mnk proteins, as well as by 4E-BP factors. ?=activator or repressor role of eIF4E phosphorylation on translation.

prevent the interaction with eIF4G and inhibit the translation. When they are hyperphosphorylated, they cannot bind to eIF4E, which is then released to participate in the protein translation initiation (Fig. 3) (Gingras et al., 2001). The 4E-BP proteins and eIF4G have the same binding site to eIF4E. So there is a competition between these proteins. On the other hand, the bond between eIF4E and 4E-BP does not prevent its bond to the cap. Otherwise, some viruses can modulate eIF4E's activity by acting on the 4E-BP phosphorylation. For example, the picornaviruses induce 4E-BP's dephosphorylation which inhibits protein synthesis. So the 4E-BPs work as inhibitors of the cap–dependent translation.

2.2.6 Ubiquitination

Another eIF4E's posttranslational modification is ubiquitination. It has been demonstrated that it does not prevent the eIF4E mRNA cap binding but it prevents the eIF4G bond and thus eIF4E phosphorylation is reduced (Murata & Shimotohno, 2006; Othumpangat, Kashon, & Joseph, 2005). However, the ubiquitination consequences on the translation initiation are still unknown.

eIF4E degradation depends on the proteasome and happens principally when ligases such as Chip ubiquitinate the Lys-159 residue (Murata & Shimotohno, 2006; Othumpangat et al., 2005). This ubiquitination does not prevent the bond to the mRNA cap, but the bond with eIF4G and eIF4E's phosphorylation is reduced. Moreover, Hsp27 interacts directly with eIF4E and regulates it. After Hsp27 knockdown, eIF4E is ubiquitinated and degraded through the ubiquitin–proteasome pathway indicating that cytoprotection induced by Hsp27 involves eIF4E. Andrieu et al. showed in castrate-resistant prostate cancers that forced overexpression of Hsp27 increases the protein expression level of eIF4E without affecting its mRNA expression level. They also showed that Hsp27 could exert an effect directly on eIF4E and that the effect of Hsp27 on eIF4E level is independent of 4E-BP1. They showed that a decrease in eIF4E ubiquitination is associated with resistance to androgen withdrawal and paclitaxel, concluding that Hsp27 knockdown reduces eIF4E stability, enhancing its ubiquitination and degradation, thereby reducing cell viability after androgen withdrawal and/or chemotherapy (Andrieu et al., 2010). In pancreatic cancer cells, Baylot et al. demonstrated that the C-terminal part of Hsp27 interacts with eIF4E and that Hsp27 phosphorylation enhances this interaction and eIF4E expression level and gemcitabine resistance. Hsp27 enhances eIF4E protein expression by inducing a decrease of approximately 30% in the amount of ubiquitinated eIF4E, thereby inhibiting its proteasomal degradation (Baylot et al., 2011).

It has also been described that the DIAP1 protein of the IAP family (inhibitor of apoptosis protein) interacts with eIF4E and leads to its ubiquitination (Lee et al., 2007).

2.2.7 Poly-A

A new translation repression mechanism of some specific mRNAs has been described by Richter and Sonenberg (2005). In *Xenopus laevis*, during oocyte development, there is a translation regulation mechanism based on the length of the poly-A tail. Some dormant but stable mRNAs have a short

poly-A tail, unlike the majority of mRNAs who have a long tail. The CPEB protein controls polyadenylation by interacting with the CPE element on the mRNA 3′ extremity. CPEB also binds to the Maskin protein which sequestrates eIF4E and prevents the translation of these specific mRNAs. When the oocytes are stimulated, a signaling cascade takes place and allows the poly-A tail's elongation by CPEB and the Maskin protein's moving. The translation can now start. A similar mechanism is observed in the drosophila with the Bicoid and Cup proteins (Nakamura, Sato, & Hanyu–Nakamura, 2004; Niessing, Blanke, & Jackle, 2002) or during neurogenesis, where the neuroguidin protein binds to eIF4E to prevent the translation (Jung, Lorenz, & Richter, 2006).
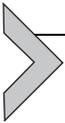
3. eIF4E'S FUNCTIONS

3.1 mRNA Translation Initiation

There are two types of mRNA translation initiation: the cap-dependent translation initiation and the cap-independent translation initiation. Furthermore, there is another mRNA category (10%) that is translated in a cap- and eIF4E–independent manner. These mRNAs have a structure called "IRES" (internal ribosome entry sites) that allows the ribosome's 40S sub-unit to bind directly. Originally identified as a translation mechanism of viral genes, it is now identified as playing an important role during the death cell's process, mitosis, and stress conditions, where cap–dependent protein synthesis is reduced (Stoneley & Willis, 2004).

3.1.1 The CaP-Dependent Translation Initiation Mechanism

The translation initiation of most cellular mRNAs takes place due to a cap-dependent mechanism. The 7-methylguanosine (m7GDP) structure (also called cap) is located on the 5′ extremity of the cytoplasmic mRNAs that process a cap-dependent translational process. It is a posttranscriptional modification introduced by the successive action of several nuclear enzymes. The cap has many roles. It protects the mRNA against degradation by ribonuclease, it intervenes in the nuclear export, and it allows the ribosome recruitment. In fact, this structure is specifically recognized by eIF4E, enabling recruitment of the eIF4F complex to bind to the cap (Fig. 3). This complex is formed by eIF4E associated to eIF4A and eIF4G and allows the recruitment of the ribosome on the mRNAs. The protein eIF4A is a helicase that catalyzes the separation of the paired strands of the RNA, in an ATP-dependent manner. Its activity is slow and requires stimulation by eIF4B/eIF4H and eIF4G

(Rogers, Komar, & Merrick, 2002). The protein eIF4G acts like a scaffolding protein by linking the mRNA to the 40S subunit of the ribosome through its interaction with eIF3, which stabilizes the complex (Gross et al., 2003; Prevot, Darlix, & Ohlmann, 2003). This step leads to the recruitment of the preinitiation complex 43S (40S + eIF3 + eIF1 + eIF1A + eIF5 + eIF2–GTP–Met-tRNAi) on the mRNA cap and to the formation of the initiation complex 48S (ARNm + 43S + eIF4F) (Fig. 3). The mRNA is then scanned in the 5′–3′ direction in order to find the start codon (Kozak, 2002). This is due to the following initiation factors: eIF1, eIF1A, eIF5, and the complex eIF2–GTP–Met-tRNAi. Once the initiation codon is located, eIF5 interacts with eIF2 and promotes the intrinsic hydrolysis of the GTP associated to eIF2. This hydrolysis leads to the detachment of the initiation factor from the ribosome's subunit 40S and to the recruitment of the 60S subunit resulting in the formation of the 80S complex (Fig. 3). The protein synthesis can now begin. The 5′ and 3′ UTR extremities (untranslated region) also play an important role in the translation initiation mechanism. In fact, on the 5′ extremity, the sequence surrounding the start codon plays a role in the initiation site selection by the 48S complex and gives an indication about the translation efficiency that might be weak or strong. In mammals, the Kozak sequence is the best sequence to initiate translation. At the 3′ extremity, the poly–A tail is capable of interacting with the cap in 5′ through the PABPs (polyadenylate-binding proteins) (Fig. 3). This interaction promotes the ribosome's 40S subunit recruitment through direct interaction between PABPs and eIF4G. This interaction gives a circular conformation to the mRNA which improves the translation initiation. So the eIF4E protein plays a major role in mRNA cap-dependent translation regulation (Sonenberg, 2008) and therefore in the cell cycle progression (O'Farrell, 2001).

3.1.2 The CaP-Independent Translation Initiation Mechanism

It has to be noted that other modes of translation initiation are described. The protein eIF4E is, for example, involved in the translation of viral mRNAs that do not have a cap. In fact, it has recently been demonstrated that the calicivirus mRNAs are linked covalently to a viral protein (VPg) that acts like a substitute to the cap to recruit eIF4E (Chaudhry et al., 2006). The VPg-binding site to eIF4E is different from the cap-binding site and from the 4E–BP protein binding site since the complex VPg/eIF4E/4E–BP1 has been isolated (Goodfellow et al., 2005). This interaction is unique within known virus in mammals, but we can find a similar interaction in the potyvirus that infects plants (Dreher & Miller, 2006).

3.2 Nuclear Export

It is described that eIF4E is mainly located into the cytoplasm where it fulfills its role in the translation initiation, but it is also found in the nucleus. Recently, it has been described that eIF4E has a function in the regulation of translation but at a different level than that of the initiation (Strudwick & Borden, 2002). Lejbkowicz et al. were the first one to describe eIF4E's expression in the nucleus' small structures called nuclear bodies (Fig. 4A and B). This was then observed in a variety of mammalian cell lines and would be conserved among eukaryotes. We can find 10–20 nuclear bodies per nucleus, and their size varies between 0.1 and 1 μm (Cohen et al., 2001; Dostie, Lejbkowicz, & Sonenberg, 2000a; Lai & Borden, 2000). These bodies are not affected by RNases or DNases, which indicates that these structures are not formed by nucleic acid (Cohen et al., 2001; Dostie et al., 2000a). eIF4E is exported to the nucleus via importin active pathways involving 4E-T protein (eIF4E-transporter) that binds to eIF4E in a similar region than eiF4G and 4E-BP (Dostie et al., 2000b). About 68% of the eIF4E proteins are found in the nuclear bodies where they are involved in the export of an mRNA category from the nucleus to the cytoplasm (Culjkovic, Topisirovic, & Borden, 2007). This mRNA category has a structure called "sensible to eIF4E elements" 4E-SE that allows eIF4E to recognize these mRNAs (Fig. 4C) (Culjkovic et al., 2005). Normally, mRNAs are prepared for export through a process regulated by the nuclear complex CBC (cap-binding complex), but for this category of mRNAs, eIF4E nuclear bodies are able to regulate their own transport (Cohen et al., 2001; Lai & Borden, 2000). These mRNAs "sensitive to eIF4E" also have a long and complex 5′UTR end that it is hardly decondensed by helicases eIF4A/4B (Zimmer, DeBenedetti, & Graff, 2000). Theoretically, since eIF4E is a limiting factor for the helicase recruitment, an eIF4E increase should rise the helicase activity and thus increase these specific mRNAs' protein synthesis (Zimmer et al., 2000). However, these mRNAs "sensitive to eIF4E" do not show an increase in their translation initiation rate. This mRNA protein synthesis is regulated by their export from the nucleus to the cytoplasm (Lai & Borden, 2000). Indeed, when eIF4E is overexpressed, the cyclin D1 mRNA level does not change, but the level of nuclear mRNA decreases and the level of cytoplasmic mRNA is increased. These results show that an increased in eIF4E expression increases the export of these mRNAs and thus the level of protein (Lai & Borden, 2000). This export mechanism contributes to the oncogenic potential of eIF4E (Cohen et al., 2001). It is therefore possible that there are, in the nucleus, negative
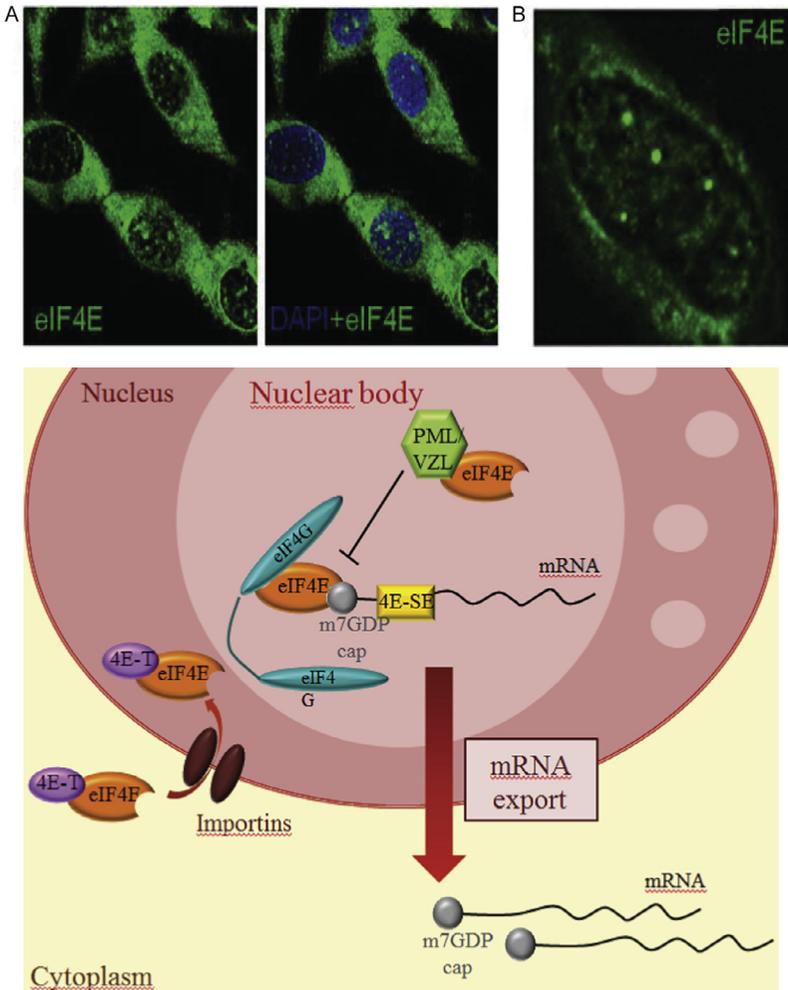
Figure 4 (A) eIF4E's implication in the mRNA nuclear export. (A) eIF4E is localized in the nuclear bodies in the NIH3T3 cells. DAPI=nuclear marker (Culjkovic et al., 2005). (B) U2OS cell's nucleus showing the eIF4E expression in the nuclear bodies (Culjkovic et al., 2006, JCB). (C) eIF4E's implication in the mRNA nuclear export. Diagram representing the eIF4E role in the nuclear export of an mRNA category. This mechanism is regulated by the PML protein.

regulators to this process identical to 4E-BPs in the cytoplasm. Several proteins can associate with eIF4E nuclear bodies such as the ribosomal protein L7 and P, eIF4G (Iborra, Jackson, & Cook, 2001), the PRH protein (proline–rich homeodomain protein) (Topisirovic et al., 2003a), the

homeodomain proteins, the Z protein, and the PML protein which is the most studied (Campbell Dwyer et al., 2000; Cohen et al., 2001; Lai & Borden, 2000). These proteins regulate the eIF4E–cap bond, a bond that is necessary for the mRNA export (Dostie et al., 2000a). The majority of eIF4E nuclear protein colocalizes with the PML protein (Lai & Borden, 2000) as a result of stress, viral infection, or an interferon treatment (Regad & Chelbi-Alix, 2001). This protein interacts on the convex side through its RING domain using the 73th tryptophan residue (Cohen et al., 2001; Lai & Borden, 2000). Even though this interaction site is far from the cap-binding site, this bond can inhibit the eIF4E–cap interaction (Culjkovic et al., 2007). The PML protein binds to eIF4E and lowers its mRNA's cap affinity (100 times), thereby changing its mRNA export function (Fig. 4C) (Cohen et al., 2001; Culjkovic et al., 2007; Kentsis et al., 2001; Lai & Borden, 2000). PML would have an antitumor function. There are approximately 200 homeodomain proteins containing potential binding sites for eIF4E and could therefore regulate it (Culjkovic et al., 2007). So it seems that the ability to modulate eIF4E's activity by acting on its binding to the cap is conserved from the cytoplasm to the nucleus. Finally, it has been suggested that eIF4E contributes to the mRNA translation in the nucleus (Dostie et al., 2000a; Iborra et al., 2001). This nuclear translating phenomenon has already been observed in mammals' cells (Iborra et al., 2001) and an increasing number of proteins from the translation machinery are involved in nuclear processes. This translation may be involved in aberrant transcript elimination, by an mRNA quality control system: NMD (nonsense-mediated decay). Indeed, this system requires an active protein synthesis in order to detect the appearance of premature STOP codons leading to the synthesis of truncated proteins.

All known data on eIF4E's role in translation initiation and nuclear export led to the hypothesis that there is an eIF4E regulon (Culjkovic et al., 2007). The "regulons" are a set of genes regulated by the same protein. The hypothesis has suggested that mRNAs belonging to the eIF4E regulon have a signal that allows its recruitment. The eIF4E protein is considered as regulatory since it allows, on the one hand, the nuclear export through the 4E-SE site recognition and, on the other hand, the protein translation through another unknown signal. In some cases, eIF4E acts on both mechanisms likely due to the presence of both of these signals. The eIF4E protein can thus orchestrate genes' expression and control the cell cycle progression.
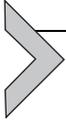
4. eIF4E: A THERAPEUTIC TARGET IN CANCER

4.1 eIF4E in Cancers

Protein synthesis is a highly regulated process that controls mRNA translation. Alterations of this process are associated with the development and progression of cancer. As we described, the components of the translation machinery are regulated by several fundamental signaling pathways that are often disrupted in cancer. Thus, the protein translation process becomes oncogenic. Sonenberg et al. were the first to show the involvement of eIF4E in oncogenesis in 1990. Since then, the oncogenic potential due to eIF4E hyperactivity has been widely described *in vitro* and *in vivo*. The overexpression of eIF4E can induce primary epithelial cells and fibroblast transformation. Similarly, an extended overexpression of eIF4E in NIH 3T3 and CHO cell lines leads to an oncogenic transformation and to a metastatic phenotype (Avdulov et al., 2004; De Benedetti & Graff, 2004; Zimmer et al., 2000). *In vivo*, an eIF4E overexpression leads to lymphoma, angiosarcoma, and lung carcinoma development in transgenic mice (Ruggero et al., 2004). In addition, it is described to be capable to increase cellular proliferation and inhibit apoptosis (Li et al., 2004; Ruggero et al., 2004; Wendel et al., 2004). It can act as a survival factor in serum-deprived cells or cells whose ras and c-Myc oncogene expression is deregulated (Li et al., 2003; Polunovsky et al., 2000; Tan et al., 2000). Upstream signaling pathways that are mutated or amplified in cancers have a direct impact on eIF4E activity. For example, the eIF4E promoter contains two domains that are the oncogene c-Myc's targets. The mTOR pathway's activation, which occurs in many cancers, also allows the 4E-BP1 phosphorylation and consequently eIF4E hyperactivation. The 4E-BP1 hyperphosphorylation is also associated with malignant progression of breast, ovarian, prostate, and colon cancer (Armengol et al., 2007; Coleman et al., 2009; Graff et al., 2009). Finally, an eIF4E level increase was observed in the following human tumors: breast, bladder, colon, lung, skin, head and neck, ovarian, and prostate cancer, compared to healthy tissues (Berkel et al., 2001; Coleman et al., 2009; Crew et al., 2000; Graff et al., 2009; Holm et al., 2008; Matthews-Greer et al., 2005; Nathan et al., 2004; Salehi, Mashayekhi, & Shahosseini, 2007; Thumma & Kratzke, 2007; Wang et al., 2009). Although high eIF4E expression levels seem to correlate with aggressive and metastatic tumors and that this protein is given as a diagnostic marker for cancer (Berkel et al., 2001; De Benedetti & Graff, 2004; DeFatta, Li, & De Benedetti,

2002; Li et al., 2002), it is not found in some aggressive cancers (Yang et al., 2007). In breast cancer, it was shown that patients who, after therapy, have low eIF4E levels have a better survival rate (Hiller et al., 2009). However, those who have high eIF4E levels have a higher risk of recurrence (Holm et al., 2008). eIF4E overexpression also leads to the TLK1B protein over-expression that induces resistance to doxorubicin treatment as well as to radiotherapy (Li et al., 2001; Sillje & Nigg, 2001). In prostate cancer, immu-nohistochemistry studies on 148 tissues showed that eIF4E's and 4E–BP1's phosphorylated form expressions were significantly increased in the advanced prostate cancer compared to benign hyperplasia (Graff et al., 2009). In addition, it has been shown that phosphorylation of eIF4E is required for the translation of several proteins involved in tumorigenesis. Furthermore, phosphorylated eIF4E levels are correlated with pancreas and prostate cancer progression (Baylot et al., 2011; Bianchini et al., 2008; Furic et al., 2010). Moreover, we previously showed that Hsp27 knockdown leads to eIF4E ubiquitination and degradation by the ubiquitin–proteasome pathway and that a decrease in eIF4E ubiquitination and degradation is associated with resistance to androgen withdrawal and paclitaxel in prostate cancer and gemcitabine in pancreatic cancers (Andrieu et al., 2010; Baylot et al., 2011). *In vivo* studies show that blocking eIF4E's hyperactivity by inhibiting the mTOR pathway (PP242) causes an inhibition of tumor growth after its formation in a transgenic mouse model developing thymus lymphomas (Hsieh et al., 2010). All these works dem-onstrate eIF4E's oncogenic potential and the interest of therapeutically targeting this protein's activity.

4.2 EIF4E's Mechanisms in Cancer

The exact mechanism by which eIF4E and the eIF4F complex induce onco-genic transformation is highly debated, but it is described that it may partly be mediated by an mRNA subset's translation increase, rather than an overall increase in the translation rate (Fig. 5). The classification and regression tree (CART) divides the mRNAs according to their 5′UTR end (Davuluri et al., 2000). The vast majority of mRNAs have a short, unstructured 5′UTR end and are strongly translated. These mRNAs encode the "housekeeping" proteins. However, there are also mRNAs whose 5′UTR end is long, structured, and rich in G/C nucleic acids and are poorly trans-lated under normal cellular conditions. This 5′UTR end prevents an effec-tive eIF4F activity and binding to ribosomes. In this second category, the mRNAs encode proteins that have an important role in oncogenesis.
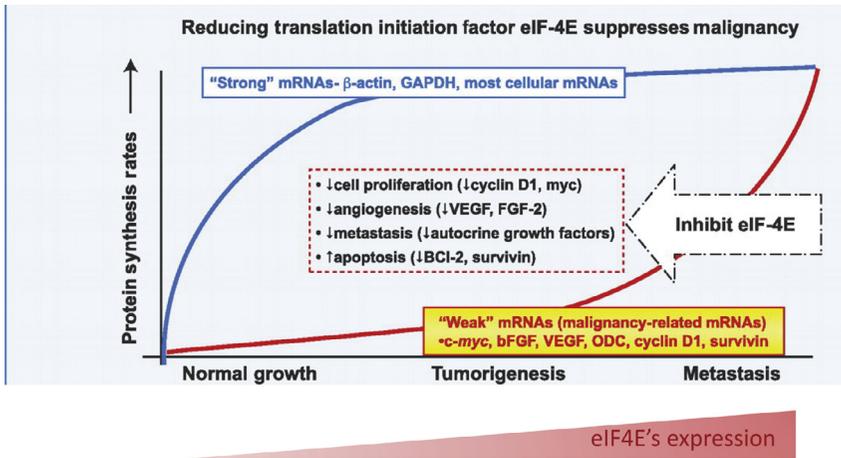
Figure 5 eIF4E's involvement in the cells' oncogenic transformation. Schematic representation of one of eIF4E mechanisms of action inducing the oncogenic transformation. Cellular mRNAs can be divided into two categories: the majority of mRNAs that are "highly translated" even when eIF4E expression is limited and a minority of mRNAs ("weakly translated") which are translated when eIF4E is overexpressed like during cancer development. This second category includes genes involved in tumorigenesis. *Adapted from Graff et al. (2008).*

Thus, there are proteins involved in proliferation (cyclin D1, c-Myc, CDK2), apoptosis (survivin, Bcl–2, Mcl–1), angiogenesis (VEGF, FGF2), and metastasis (MMP9, heparanase) (Mamane et al., 2004; Schmidt, 2004; Zimmer et al., 2000). Given that eIF4E is the limiting factor in the translation initiation mechanism, mRNAs compete in normal cellular conditions. However, if eIF4E's level is increased like in cancers, the mRNAs that are poorly translated are selected and translated disproportionately (Fig. 5) (De Benedetti & Graff, 2004; Graff et al., 2008; Mamane et al., 2004). Thus, the eIF4E factor governs cancer's progression by coordinating certain genes' expression (Avdulov et al., 2004). In addition, it is described that eIF4E overexpression increases specific mRNAs "sensitive to eIF4E" transport and translation (Topisirovic et al., 2003b). Some of these mRNAs encode proteins involved in cell proliferation and tumorigenesis, such as cyclin D1. This transport mechanism would therefore contribute to eIF4E oncogenic potential (Cohen et al., 2001).

4.3 Targeting eIF4E in Cancers

Due to eIF4E's important involvement in the process of tumorigenesis, several inhibitory strategies have been developed to block its functions.

4.3.1 ASOs and siRNAs

The first of these strategies was the development of antisense oligonucleo-
tides (ASOs) that block eIF4E's mRNA translation. Thus, Defatta et al. had
shown that eIF4E translation inhibition through ASOs eliminates tumori-
genic and angiogenic properties in FaDu human squamous carcinoma cell
(DeFatta, Nathan, & De Benedetti, 2000). More recently, a second-
generation ASO (4E–ASO4) was designed by Graff et al. to resist nuclease
(Fig. 6A) (Graff et al., 2007). Nanomolar concentrations of 4E–ASO4 are
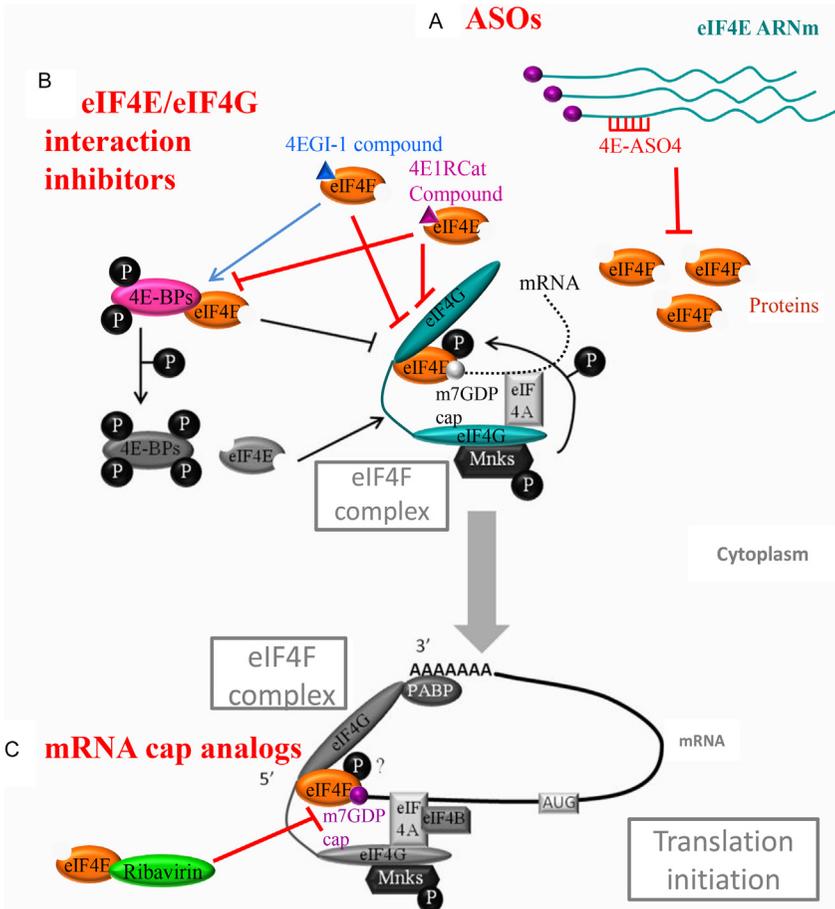

Figure 6 eIF4E's inhibitors. Diagram showing the different strategies to inhibit eIF4E in
cancer therapy: inhibition of eIF4E's production by ASOs (e.g., 4E-ASO4). (A) Inhibition of
eIF4E's interaction with its ligands 4E-BPs and eIF4G through inhibitory molecules (e.g.,
4EGI-1, 4E1RCat) (B) and inhibition of the eIF4E/cap interaction through mRNA's cap
analogs (e.g., the ribavirin) (C).

able to reduce eIF4E level and thus induce apoptosis in several cancer cell lines *in vitro*. *In vivo* models of breast cancer, 4E-ASO4 significantly inhibited tumor growth without side effects or weight loss. eIF4E's expression is reduced by 64% in the observed tissues. Moreover, similar results were observed in prostate cancer xenografts after treatment (Graff et al., 2007). On the other hand, siRNAs targeting eIF4E have recently been described for their ability to inhibit tumor growth, induce apoptosis, and enhance the effect of chemotherapy with cisplatin in breast carcinomas *in vitro* and *in vivo* (Dong et al., 2009). In prostate cancer models, *in vivo*, eIF4E knockdown using siRNA reverses the cytoprotection to androgen withdrawal (serum-free media) and paclitaxel treatment normally conferred by Hsp27 over-expression. Moreover, eIF4E's overexpression confers resistance to combine treatment with paclitaxel and androgen withdrawal in LNCaP cells (Andrieu et al., 2010).

4.3.2 Inhibition of the eIF4E/eIF4G Interaction

Another strategy for inhibition of the eukaryotic factor eIF4E is to target its interaction with eIF4G, which prevents the formation of the eIF4F complex and leads to inhibition of cap-dependent translation. For example, some peptides able to disrupt eIF4E–eIF4G interaction (Hu4G, W4G, 4E-BP2) are developed. These peptides are described to induce apoptosis in MRC5 lung cells in a dose-dependent manner (Herbert et al., 2000). More recently, a high-throughput screening was performed to identify inhibitors of the eIF4E/eIF4G interaction. The compound 4EGI-1 has been identified as a hit by binding to eIF4E and blocking its interaction with eIF4G (Moerke et al., 2007). Although eIF4G and 4E-BPs share the same interaction site on eIF4E, 4E-BPs seem to take a larger space because 4EGI-1 does not block the eIF4E/4E-BP1 interaction. It has even been reported that 4EGI-1 increases the interaction between eIF4E and 4E-BP1, which results in the inhibition of the cap-dependent translation (Fig. 6B). This compound has been shown to reduce the c-Myc and Bcl-2 level, to induce apoptosis, and to inhibit lung cancer cell proliferation (Fan et al., 2010). It would be interesting to know this compound specificity to inhibit the eIF4E/eIF4G interaction by determining all protein–protein interactions and signaling pathways that are blocked. In fact, studies have shown that it can induce apoptosis through an eIF4E/eIF4G interaction-independent mechanism, by degrading the antiapoptotic protein c-FLIP (Fan et al., 2010). More recently, the 4E1RCat compound was characterized as an inhibitor of the interaction of eIF4E with eIF4G and 4E-BP1 (Fig. 6B) (Cencic

et al., 2011a). It has been reported that this compound may partially inhibit the cap-dependent translation and restore the chemosensitivity in a lymphoma mouse model. Another compound from the same screen, 4E2Rcat, inhibits the cap-dependent translation and the coronavirus 229E replication which is dependent on complex eIF4F (Cencic et al., 2011b).

4.3.3 mRNA Cap Analogs

Another strategy is based on inhibition of the synthesis of mRNA cap analogs that would compete with the eIF4FE/cap interaction and block it (Quiocho, Hu, & Gershon, 2000). A series of cap analogs have been developed (Brown et al., 2007; Ghosh et al., 2005, 2009; Kowalska et al., 2009), but only ribavirin is currently used (Fig. 6C). Indeed, using these analogs as drugs is difficult because of the low membrane permeability, due to the nature of the extremely charged phosphate groups, and the metabolic lability, due to the instability of the glycosidic bond. Ribavirin is a broad-spectrum antiviral drug used for the treatment of hepatitis C. The similarities between ribavirin structure and mRNA cap have suggested that this drug can act as an eIF4E inhibitor by mimicking the cap. Later studies showed that ribavirin interacts with eIF4E and prevents it from binding to the mRNA cap. This inhibits the cap-dependent translation and cell transformation (Kentsis et al., 2004, 2005; Tan et al., 2008). However, questions arise as to the specificity of action of ribavirin on eIF4E and studies are controversial (Westman et al., 2005; Yan et al., 2005). Nevertheless, this molecule is currently in a clinical trial phase II in the treatment of acute myeloid leukemia and the first clinical results show that it stabilized or at least partially cured patients (Assouline et al., 2009). This study was the first to show that the cap-dependent translation inhibition has a clinical utility in cancers that over-express eIF4E (Borden & Culjkovic-Kraljacic, 2010).

4.3.4 eIF4E Upstream Pathway Inhibitors

As mentioned earlier, signaling pathway upstream of eIF4E is also involved in tumorigenesis and represent therapeutic targets. Thus, several inhibitors have been developed to target these components and indirectly eIF4E, such as Mnk kinase inhibitors (cercosporamide) and mTOR pathway inhibitors (rapamycin, temsirolimus, etc.) (Choo et al., 2008; Feldman et al., 2009; Garcia-Martinez et al., 2009; Konicek et al., 2011; Yu et al., 2010). In 2007, temsirolimus was approved by the FDA for the treatment of patients with advanced renal–cell cancer, as trials demonstrated that it had significantly outperformed the standard of care in terms of progression-free

survival and overall survival by 2.4 and 3.6 months, respectively. Furthermore, preclinical evaluation of two TORKinibs (second-generation small–molecule inhibitors), PP242 and PP30, demonstrates stronger inhibition of protein synthesis and cell proliferation than sirolimus (Blagden & Willis, 2011).

4.3.5 Inhibition of the eIF4E/Hsp27 Interaction

More recently, targeting Hsp27–eIF4E interaction has been described as an interesting alternative strategy to target eIF4E. We previously found that Hsp27 interacts directly with the eukaryotic translational initiation factor eIF4E. Our work demonstrated that Hsp27 interaction protects eIF4E from its degradation by the ubiquitin–proteasome pathways leading to Hsp27 cytoprotection in pancreas and CRPC (Andrieu et al., 2010; Baylot et al., 2011). Using several Hsp27 deletion mutants, we found that eIF4E interacts with the C-terminal domain of Hsp27. Inhibition of Hsp27–eIF4E interaction using deletion mutants drives resistance to apoptosis induced by gemcitabine in pancreatic cancers (Baylot et al., 2011) and androgen withdrawal and docetaxel in castrate-resistant prostate cancers (unpublished data). This experiment confirmed that this stress-induced cellular pathway is involved in cell death blockade leading to therapy resistance in cancers. Targeting the Hsp27–eIF4E interaction seems to be a promising therapeutic strategy in advanced prostate and pancreatic cancers.
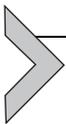
5. CONCLUSION

Tumorigenesis is highly affected by the regulation of the cap-dependent translation. The cap-dependent translation consists of the eukaryotic translation initiation factor 4F complex that can recognize the $5'$ end of cellular mRNAs at the 7-methylguanosine cap structure. eIF4E is a component of this complex which makes it crucial to the cap-dependent translation initiation and regulation of tumor cell apoptosis, proliferation, and, potentially, metastasis. Indeed, since eIF4E's inhibition induces cellular death, we are entitled to ask about this inhibition's consequence on normal cells. It seems however that eIF4E's residual and low levels after drug treatment are tolerated and without adverse effects on normal tissues. In contrast, eIF4E's activity is so important in cancerous cells that its inhibitions have a more visible effect (Graff et al., 2008). Many approaches over the years have been used to try to inhibit eIF4E's function, particularly by using

small–molecule inhibitors that can disrupt the eIF4E–eIF4G interaction, the use of cap analogs to directly target the eIF4E cap-binding site, or ASOs that have been proved to be efficient in reducing the expression level of eIF4E and have advanced to clinical trials in prostate cancer patients. More recently, targeting Hsp27–eIF4E interaction has been described as an interesting alternative strategy to target eIF4E. Taken together, these data seem to show eIF4E to be a promising target for cancer therapy and new approaches of inhibition deserve further studies.

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