The bad, the good and eIF3e/INT6

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1. ABSTRACT

Recent research on translation and protein synthesis in several pathologies, including cancer, peripheral artery disease, and wound healing, demonstrates the key role played by translational factors in tumorigenic and angiogenic processes. This review will focus on one specific translational factor, eIF3e also called INT6, the “e” subunit of the translation initiation factor eIF3. INT6/eIF3e has recently been described as a multifunction protein playing a role in translation, protein degradation, DNA repair, nonsense-mediated mRNA decay, cell cycle and control of cell response to low oxygen (hypoxia or ischemia) through modulation of the Hypoxia Inducible Factors (HIFs). Interestingly, INT6/eIF3e is a double-edged sword that has both oncogenic and tumor suppressive abilities. In addition to its role in tumorigenesis, its silencing has recently been suggested as a potential therapeutic strategy to improve cell survival and function after ischemic injuries. Although a deeper understanding of the molecular mechanisms involved in these pathophysiological functions is essential, particularly to transform the in vitro/in vivo findings into clinical applications, INT6/eIF3e modulation could provide therapeutic benefit for a variety of human diseases such as cancer or vascular diseases.

2. INTRODUCTION

Translation is a fundamental cell mechanism and recent reports clearly establish that critical translation initiation factors play important roles in various cellular processes. In this review, we summarize the current understanding of eIF3e also called INT6, the “e” subunit of the translation initiation factor eIF3. INT6/eIF3e has recently been described as a multifunction protein with complex and diverse roles in translation, protein degradation, DNA damage response, as well as in pathophysiologic conditions such as hypoxia, vascular diseases and cancer. We also discuss the recent findings suggesting that INT6/eIF3e, through the modulation of Hypoxia Inducible Factors (HIFs), could represent a potential therapeutic strategy to either block angiogenesis or tumor growth for the treatment of cancer.
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3. THE “E” SUBUNIT OF THE TRANSLATION INITIATION FACTOR eIF3

3.1. The eukaryotic initiation factor eIF3

Initiation of translation is a sophisticated and highly regulated process involving multiple molecular components. Indeed, at least 6 protein complexes are involved in the first step of protein synthesis. These complexes are named the eukaryotic Initiation Factors (eIFs), and in mammalian cells, the largest of them, eIF3, is composed of 13 subunits from eIF3a to eIF3m (1-3). This protein complex has a molecular mass of approximately 800 KD (4, 5) and has a crucial role in protein synthesis as it recruits other translation initiation factors eIF1, eIF1A, eIF5 and the 40S ribosomal subunit. Once the subunits are assembled, eIF3 can interact with the eIF2 ternary complex (eIF2-GTP-Met-tRNA<sub>Met</sub>) (6, 7) and form the 43S Pre-Initiation Complex (43S PIC) (8) (Figure 1).

In the case of cap-dependent translation, the 43S PIC binds to the “cap” of the mRNA through the interaction of eIF3 and an additional initiation factor, eIF4F (composed of eIF4A, eIF4E and eIF4G) (Figure 1) (4, 9), resulting in the 48S PIC complex (10). Of note, UV-crosslinking and PAR-iCLIP experiments revealed that several eIF3 subunits (eIF3a, eIF3b, eIF3d, eIF3g) can also interact directly with mRNA specific sequences (11-13). After the 48S PIC association, the scanning of the mRNA for the “start” codon (AUG) begins (14). When found and after the release of the different eIFs, the large ribosomal subunit 60S can be recruited onto the mRNA and bind the small 40S subunit to form the 80S ribosome. Formation of the 80S ribosome marks the end of the translation initiation (15, 16). In addition to its role in guiding the initiation of translation process, eIF3 is also known to prevent the binding or enhance angiogenesis, vascular remodeling and therapeutic outcomes in ischemic vascular diseases, such as peripheral artery disease, wound healing, and heart/brain injury.

Figure 1. Initiation of translation. ELF3 is a translation initiation factor composed of 13 different subunits (eIF3a to eIF3m), which allows the initiation of translation with eIF1, eIF2, eIF4 and eIF5. This multi-protein complex helps the recruitment of the 40S/60S ribosomal subunits to the mRNA and starts the elongation process and ultimately leads to protein synthesis.

or enhance angiogenesis, vascular remodeling and therapeutic outcomes in ischemic vascular diseases, such as peripheral artery disease, wound healing, and heart/brain injury.
between the 60S pre-mature ribosomal subunit and the 40S subunit (17). The free 60S subunit will not be recruited onto the mRNA until the elfs are released from the complex. This mechanism helps to prevent any mispairing or binding of the pre-mature 60S subunit to the mRNA. Interestingly, Kolupaeva and colleagues have further demonstrated that the eIF3 complex contributes to this mechanism in collaboration with elf2 and elf1 and it also promotes the dissociation of the 80S ribosome at the end of the translation process.

Structurally, eIF3 is a very interesting complex, and although multiple groups have been working to elucidate the functions of each eIF3 subunit, their exact roles in translation are not completely understood. Currently it is known that eIF3 is a five-lobed complex (4) composed of the three following modules: 1- eIF3a, eIF3b, eIF3g, eIF3i, 2- eIF3c, eIF3d, eIF3e, eIF3k, eIF3l and 3- eIF3f, eIF3h, eIF3m (5). The last eIF3 subunit, eIF3j, is not present in the eIF3 complex but enhances the stabilization of the 43S PIC (18). Recently, it has been suggested that eIF3a and eIF3c are the main subunits that links the different modules (19, 20). Given the importance of eIF3a and eIF3c in the “functional core”, these subunits are also the most conserved, with eIF3b, eIF3g and eIF3i conserved from yeast to mammalian cells (21, 22). Masutani et al. demonstrated that non-conserved subunits, eIF3e, eIF3f and eIF3h are part of the “functional core”, while the presence of conserved subunits, such as eIF3g and eIF3i, is not essential for the function of the eIF3 complex (10).

Another intriguing aspect within the structure of the eIF3 subunits is the presence of two particular domains, the PCI (Proteasome COP Signalosome Initiation of translation) and the MPN (Mpr1 – Pad1 N-terminal) domains. Eight eIF3 subunits actually contain these structures (6 with a PCI domain: eIF3a, eIF3c, eIF3e, eIF3k, eIF3i, eIF3m and 2 with a MPN domain: eIF3f, eIF3h), which allow them to bind other partners from different “Zome” complexes, such as the proteasome or the COP9 signalosome. The proteasome and the COP9 signalosome are major actors in the protein degradation process, and these PCI and MPN domains confer the ability of eIF3 to become a link between protein synthesis and degradation (22-24). Moreover, these domains have been shown as essential for some eIF3 subunits. For instance, loss of the PCI domain for eIF3e can lead to the alteration of the protein function resulting in oncogenic behavior and phenotype (25, 26).

Altogether, these data highlight the scaffolding role of eIF3 in translation initiation complexes. EIF3 allows the recruitment of the different elements contributing to effective translation. However it appears that eIF3, through its individual subunits, plays a much more complex and significant role in cellular processes. Multiples studies have described specific roles for each eIF3 subunits in the translation of specific mRNA. This is an extremely interesting and new concept, which recently led to publication in high impact journals because it suggests that translation of specific mRNAs could be inhibited or enhanced by eIF3 subunits, particularly eIF3a, eIF3b, eIF3d and eIF3g (12). EIF3f has also been described as a negative regulator of translation contributing to ribosomal RNA degradation, which results in inhibition of translation in vitro and in vivo (27, 28). Similarly, some studies (29, 30) have reported that eIF3e was able to positively and negatively regulate the translation of specific mRNA subsets. Therefore, in addition to their structural/scaffolding roles, eIF3 subunits can directly control the translation and fate of certain mRNAs. However, this powerful property can also lead, in case of deregulation, to malignant transformation and cancer (31). This will be further discussed below with a focus on the “e” subunit of eIF3, which shows unexpected characteristics.

3.2. The “e” subunit of eIF3, eIF3e also called INT6

The fifth subunit of the eIF3 complex, eIF3e, also called INT6 for ‘mouse mammary tumor virus (MMTV) Integration site 6’ (32), or p48 has a molecular mass of 52KDa (2, 33). INT6/EIF3e is ubiquitously expressed in human cells and is a gene that has been conserved throughout evolution and has been found in most eukaryotic species, including but not limited to: human, mouse, chicken, Xenopus, Drosophila, and C. elegans cells (32, 34). In mice, expression is ubiquitous in the adult and has been observed as early as day 14 of development. Immunohistochemistry studies have demonstrated that in mouse embryonic tissues, eIF3e is expressed during development in migrating neural crest cells, the notochord, and in condensing cartilage. Further studies localized intracellular expression of eIF3e to the Golgi apparatus in both adult and embryonic mouse tissues, implying essential cellular functions (35). Human Int6/EIF3e gene is found on chromosome 8 and is composed of 13 exons that span 45kb of DNA. It shares 90% homology with the mouse Int6, while the amino acid sequences of the gene products are identical (36), and its expression also begins early in the development, beginning on embryonic day 6.

Similarly to five other eIF3 subunits, eIF3e possesses a PCI domain in its C-terminus, allowing its interaction with other complexes (Figure 2), such as the 26S proteasome and the COP9 Signalosome in mammalian cells (37). EIF3e protein also contains an N-terminal Nuclear Export Sequence (NES) and a bipartite Nuclear Localization Site (NLS) (Figure 2A) (38), allowing translocation between cytoplasmic and nuclear compartments (39). EIF3e was also found in dynamic structures called Nuclear Bodies (NBs) associated with NB proteins, such as Rfp and PML in lymphocytes. In HeLa cells, eIF3e is both cytoplasmic and nuclear...
Interestingly, the role of eIF3e in the initiation of translation is not clearly defined. The original studies characterized the eIF3 core as the set of the most conserved subunits. However, recent works suggest (7, 10, 20) that eIF3e is not fully conserved through evolution, does not have a yeast homolog (Saccharomyces cerevisiae, budding yeast) (3, 5) but is a member of this “functional core”. Indeed, it has been demonstrated that eIF3e deletion in fission yeast (Schizosaccharomyces pombe) is not lethal (41, 42), but eIF3e function still seems essential for the translation process. In 2002, Bandyopadhyay et al., using immunoprecipitation experiments, reported that eIF3e contributes to eIF3 complex stabilization in yeast.

Furthermore, after the 43S PIC formation, the binding between the 43S complex and the cap of the mRNA occurs via the eIF3e/eIF4G1 interaction (9, 43). A recent study showed that eIF3e regulates eIF4E phosphorylation via its interaction with MNK1, which is the eIF4E activating kinase (44). The role of eIF3e in the regulation of translation is also reinforced by recent evidence (45, 46) describing that INT6/eIF3e is responsible for the specific translation of mRNA subsets (29, 47, 48). For instance, eIF3e interaction with MIF4GD and SLIP1 can enhance histone protein translation (49, 50). In addition to its role in cap-dependent translation regulation, another study recently identified a novel eIF3e function in the cap-independent translation process. Indeed, after insertion of the MMTV within the intron 5 of eIF3e gene resulting in eIF3e protein truncation, the interaction between eIF3e and eIF4G
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is altered leading to a decrease in the cap-dependent protein synthesis and a shift in cap-independent mRNA translation (51). The involvement of eIF3e in a cap-independent translation mechanism is also suggested by Gillis and Lewis, who show that translation of Snail1 and Zeb2 mRNAs is maintained when eIF3e is silenced in breast epithelial cells, because these genes, implicated in epithelial-to-mesenchymal transition, contain IRES domains, which could compensate the lack of eIF3e (45). Finally, INT6/eIF3e has also been described to play a role in translation regulation through nonsense-mediated mRNA-decay (NMD), which functions to degrade mRNA with a premature termination codon. Morris et al. demonstrated that eIF3e silencing impairs mRNA-decay and highlighted a physical association with mRNA-decay sensors (52). Yahalom’s group confirmed these data and showed that eIF3e was in competition with Tax, to interact with another mRNA-decay sensor, UPF1, in order to support efficient NMD (53).

According to other studies, it has been reported that eIF3e has additional surprising functions in mechanisms independent of the translation process (Figure 2). Through its PCI domain, eIF3e can bind to multiprotein complexes, other than eIF3 (Figure 2B). For instance, eIF3e can interact with the lid proteasome subunit, RP5 (37, 54), leading to the degradation of specific proteins (see HIF-2alpha section below) (55). Related to this work, it has been shown that eIF3e can participate in proteasome assembly and that eIF3e silencing can result in the accumulation of specific oncoproteins due to loss of proteasomal activity (30). These interactions are of particular interest because they suggest that INT6/eIF3e could control the expression of specific proteins at the mRNA level through the modulation of their translation and degradation, respectively. Additionally, the PCI domain allows eIF3e binding to the nuclear COP9 signalosome (Figure 2) and particularly the subunits, CSN7 in Arabidopsis Thaliana (56) and CSN7, CSN3 and CSN6 in mammalian cells (37). In drosophila, INT6/eIF3e modulates cullin neddylation via the COP9 signalosome (57). Interestingly, in Arabidopsis Thaliana, Yahalom et al. observed that both eIF3e and CSN7 localize to the cytoplasm and nucleus, suggesting that they could interact in the COP9 signalosome complex or outside of it. E1F3e shuttling between cytoplasmic and nuclear compartments is also facilitated through the presence of NES and NLS domains in its sequence (Figure 2A). Nuclear localization may explain another recently reported eIF3e function, which is the surprising eIF3e contribution to DNA damage response (Figure 2B). Morris et al. reported, using immunofluorescence assays after neocarzinostatin treatment or micro-irradiation on HeLa cells that eIF3e is localized to DNA damage sites and contributes to ATM accumulation, enhancing the DNA damage response. The authors also demonstrate a physical interaction between these two proteins via co-immunoprecipitation experiments in 293T cells (58).

Last but not least, eIF3e plays a direct role in the cell cycle. E1F3e association with microtubules during mitosis and eIF3e downregulation impairs spindle formation, chromosome segregation and cytokinesis through a defect in cyclinB-CDK1 activation (59). E1F3e has also been shown to regulate MCM7, a critical actor of S-phase and DNA replication. Bushbaum et al. have demonstrated that eIF3e interacts with the polyubiquitylated form of MCM7 in the nucleus, impairing its proteasomal degradation and leading to increased association with chromatin, and subsequently stabilizing S-phase through a translation-independent mechanism (60). Despite these numerous, surprising, and interesting cellular functions, the role of eIF3e/INT6 in protein degradation and cell cycle remains unclear and the mechanism of eIF3e degradation is unknown, it could be a similar mechanism to eIF3a and its proteasomal degradation (61). Much work remains to be done to determine precisely how eIF3e regulates these processes, to identify specific targets, and to understand what factors govern the presence of eIF3e within the initiation translation complex, eIF3, and the proteasome?


4.1. The “bad” eIF3e, the oncogene

Historically, eIF3e/INT6 was first discovered in 1995 by Marchetti and colleagues studying mouse mammary tumor virus (MMTV) infection of mouse mammary epithelial cells. Integration of MMTV into the Int6/Eif3e gene on chromosome 15, resulted in a truncated protein product (32, 34, 36). MMTV integrates into any of Int6 gene introns in a reverse orientation, opposite of transcriptional output, leading to the insertion of a cryptic stop codon and subsequent expression of a chimeric Int6/Eif3e RNA that terminates early in the MMTV long terminal repeat. These insertional mutations and resulting truncated proteins were found in preneoplastic cells, neoplastic lesions, metastases. Since the integration of MMTV affects one allele of the Int6/Eif3e gene while the other allele remains intact, the truncated protein was postulated to act as a dominant negative. The truncated protein is thought to de-regulate control of mammary epithelial cell growth, creating a premalignant epithelial cell population and opportunity for mammary tumors to develop (32). The role of eIF3e/INT6 in tumorigenesis has been controversial and various reports have implicated differing roles for this protein, both as an oncogene and a tumor suppressor (Figure 3). The role of eIF3e/INT6 in cancer is still not clearly understood and may depend not only on cancer type, but also tumor stage, and the baseline level of expression of eIF3e in the tissue type.

4.1.1. The full length form of eIF3e

Since the discovery of INT6/eIF3e, many studies have implicated an oncogenic role for eIF3e
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**Tumor suppressor**

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<td>- Loss of heterozygosity</td>
<td>- Decreased mRNA expression in tumor stroma</td>
<td>Breast cancer</td>
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<td>- Loss of heterozygosity; decreased mRNA expression</td>
<td>Non-small cell lung cancer</td>
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<td>- Hypermethylation of EIF3e promoter; correlated with overall and disease free survival</td>
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<td>- Expression correlated with tumor grade</td>
<td>- Increased mRNA expression in several human cell lines</td>
<td>Glioblastoma</td>
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<td>- Increased mRNA expression</td>
<td>- Highly expressed and correlated with tumor stage</td>
<td>Colon cancer</td>
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<td>- Expression correlated with overall and disease free survival</td>
<td>- Gene fusions (eIF3e exon 1 and RSP02)</td>
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<td>- Gene amplification in 23% of samples</td>
<td>- Gene amplification in 23% of samples</td>
<td>Oral cancer</td>
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**Oncogene**

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<td>- Increased mRNA expression</td>
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**Figure 3.** EIF3e/INT6, the oncogene and the tumor suppressor. The role of INT6/eIF3e in tumorigenesis is still unclear and controversial. EIF3e exhibits tumor suppressive or oncogenic functions depending on its expression level and/or tumor type.

in tumorigenesis. In an analysis of 81 primary breast cancers, Grzmil and colleagues determined expression levels of eIF3e protein and Eif3e mRNA. The authors found that a high tumor grade was significantly correlated with increased cytoplasmic levels of eIF3e protein in isolated tumor epithelial cells. Strong eIF3e staining was detected in 96% of high-grade tumors, compared to 44% of low-grade tumors, suggesting a potential oncogenic role for eIF3e in breast cancer. When the authors analyzed differential gene expression via microarray in eIF3e-silenced cells, no change in global protein translation was observed, yet 24 genes were found that were either positively or negatively regulated by eIF3e. Included in this group are PLAU, BCL-XL (encoding BCL-XL), and MAD2L1 (MAD2 mitotic arrest deficient-like 1), which are known to play important roles in cell proliferation, invasion and apoptosis. Additionally, eIF3e silencing in the breast carcinoma cells resulted in reduced proliferation and invasion in vitro (29). Similarly, recent characterization of Eif3e mRNA levels in several glioblastoma (GBM) cell lines also attributes an oncogenic role to full-length eIF3e. Int6/Eif3e mRNA was found to be highly expressed in all GBM cell lines tested (LN18, SF767, U87 and U251), with some variability among cell lines. When these cell lines were transfected with Int6/Eif3e specific siRNA, a significant decrease in cell number was observed, indicating the importance of eIF3e for proliferation of these GBM cells (48). Interestingly, Eif3e silencing was found to induce GBM cell apoptosis through both caspase-dependent and caspase-independent mechanisms. No change in overall global translation was observed, contrasting and corroborating other studies (29, 62). Another study in a separate model also suggested that eIF3e has oncogenic properties. In an in vivo zebrafish model, eIF3e has been shown to play a role as a tissue specific modulator of MEK-ERK signalling (63). Loss of eIF3e resulted in the loss of MEK1 specifically at the protein level in human cells and loss of ERK signalling in zebrafish embryos, suggesting a possible role for eIF3e in the regulation of the MAPK signalling pathway, which is a major target in many in cancer therapies.

When 173 colon cancer tissues samples were analysed for eIF3e expression changes via microarray, increased eIF3e expression was found to be elevated in
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colon cancer tissues compared to both normal adjacent mucosa and lymph nodes metastasis. Expression was significantly correlated with tumor size, clinical stage, lymph node involvement, metastasis histopathologic classification and vessel involvement. In vitro silencing of eIF3e resulted in reduced cell proliferation, clonality and increased cell apoptosis in colon cancer cells.

Ultimately, analysis revealed *Eif3e* mRNA levels to be an independent prognostic factor for overall survival and disease free survival in colon cancer patients (64). Recent RNA sequencing studies of additional human colon tumors revealed several gene rearrangements resulting in gene fusions. A fusion involving *Eif3e* exon 1 and *RSPO2*, of the R-spondin family members occurred in 3% of studied samples. This fusion transcript is thought to produce a functional RSPO2 under the control of the *Eif3e* promoter, further implicating *Eif3e* alterations in the development of colon cancer (65). In another study investigating the genetic alterations found in oral cancer, the *Eif3e* gene appears to play a strong oncogenic role.

Multiplex ligation-dependent probe amplification (MLPA) was used to study chromosome 8 abnormalities in 33 oral squamous cell carcinomas. Intriguingly, *Eif3e* was listed as a top gene with the most frequent gains, being amplified in 23% of the carcinomas. In addition, *Eif3e* was found to be co-amplified along with *MYC*, *MYBL1*, and *RECOL4*, at rates of 15%, 12% and 9% respectively. However, *Eif3e* amplification was not found to correlate with lymph node metastasis and did not show any prognostic significance on survival (66).

Finally, Traicoff and colleagues examined levels of *Eif3e* mRNA and protein expression in breast, colon, lung, and ovarian tumor samples (67). They found that eIF3e protein levels varied among tissue types, however breast and colon tumors clustered into distinct groups based on mRNA expression. The authors also sought to analyse the relationship between *Eif3e* and a set of tumor suppressor proteins that are hypothesized to share common pathways with eIF3e. These proteins included TID, Patched, p53, and c-Jun. *Eif3e/Int6* protein was found to positively correlate with protein levels of TID, Patched, p53, c-Jun and phosphorylated-c-Jun in a tissue specific manner, suggesting that these proteins function in common pathways. Specifically, eIF3e expression correlated with TID and Patched in all tumor type tissues tested. Additionally, eIF3e/Int6 and TID levels were detected endogenously and immunoprecipitated as complexes, along with complexes of eIF3e and Patched. This suggests, not only a differential tissue specific function for eIF3e, but also a potential cooperative role between eIF3e and the aforementioned proteins in cell growth and tumorigenesis (67).

4.1.2. The truncated form of eIF3e

Since its discovery, multiple studies have shown that the truncated form of eIF3e could transform cells in vitro. Previous studies indicated that overexpression of other related translation initiation factors, such as eIF4E and eIF4G (68, 69) could induce malignant transformation, but the role of eIF3e had yet to be elucidated (Figure 3). Rasmussen and colleagues transfected both human (MCF-10A) and mouse (HC-11) mammary epithelial cells lines with the truncated *Eif3e* gene and showed that it led to a malignant transformed phenotype despite the presence of 2 fully competent copies of the wild type *Int6/Eif3e* gene (25). Interestingly, it was found that when cells were transfected with full length *Int6/Eif3e*, no transformation or growth rate changes were observed. Implantation of these transformed (with truncated *Int6/Eif3e*) mammary epithelial cells into the scapular and mammary fat pads of nude mice, resulted in nodule formation. This indicates a newly gained capacity for the cells transformed with truncated *Eif3e* to overcome local growth regulatory control in vivo, similar to premalignant epithelial cells (25).

Mayeur et al. expanded on this, showing that stable expression of truncated eIF3e in mouse embryo fibroblasts (NIH3T3) resulted in malignant transformation defined by 4 main criteria: foci formation, anchorage independent growth, accelerated growth, and lack of contact inhibition. Of note, neither this study nor the study by Rasmussen and colleagues was able to successfully identify and isolate truncated eIF3e protein in their transformed cell lines. This was explained by the possible short half-life of the truncated protein. Interestingly, when cells were transfected to overexpress full-length *Eif3e*, this led to a slower growth rate, reduced confluency compared to wild type, no foci formation, and no transformation (70).

Additionally, Chiluiza et al. examined the effect of truncated eIF3e expression on translation in mouse embryo fibroblasts (NIH3T3). Cells were engineered to express the eIF3e protein resulting from truncation at intron 5 of the *Int6/Eif3e* gene, similar to previous studies (25, 70), but expressed from a single gene copy. In this study, the authors found overall translation to be diminished, but observed that cap-independent translation was less affected than cap-dependent translation. These less affected cap-independent mRNAs, include *XIAP*, c-Myc, *CYR61*, and *Pim-1*, genes known to encode proteins that inhibit apoptosis and induce cellular proliferation (51). Further studies led to the development of a transgenic mouse model that expressed the truncated form of eIF3e (*Int6sh*) in differentiating alveolar epithelial cells, under the control of the Whey Acidic Protein (WAP). This model enabled authors to study the effect of mammary-specific expression of truncated eIF3e in vivo. The WAP promoter is targeted to alveolar epithelial cells and is under tight hormonal control, being expressed during late pregnancy and during lactation. 42% of heterozygous transgenic *Int6sh* female mice developed tumors at about 18 months of age after giving birth to several litters. Multiple focal alveolar hyperplasias were also observed in the non-tumor mammary glands of these same mice, frequently showing extensive lymphocytic infiltration. Microarray
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The role of eIF3e in breast cancer has been controversial. Previous studies have shown a reduction in expression of eIF3e in breast cancer tumors, implicating analysis of mammary tissue expressing truncated eIF3e led to the identification of a truncated eIF3e-induced tumorigenic gene expression signature comprised of 59 genes, 22 of which are genes that have previously been implicated in mammary tumorigenesis. Interestingly, these included 2 components of the COP9 signalosome, Cops5 and Cops4. This gene expression pattern was observed in the transgenic mice as early as the first pregnancy and persisted in the alveolar hyperplasias and mammary tumors (26).

Finally, in a yeast model, overexpression of a 13-kDa C-terminal segment of eIF3e led to an increase in the transcriptional activity of Pap1. Of note, this truncated eIF3e differed from the previously mentioned truncated proteins in that it contained only the last 121 codons of Int6/Eif3e. Pap1 is associated with a response to oxidative stress and has been implicated in multidrug resistance. Microarray studies yielded 67 genes that were up-regulated in the setting of this truncated eIF3e, in a profile that matched the expression pattern resulting from the overexpression of Pap1. However, this expression pattern was shown to be the result of a novel, Pap1 independent mechanism, through activation of AP-1, another transcription factor involved in tumor multidrug resistance (71).

4.2. The “good” eIF3e, the tumor suppressor

Though many studies have implicated oncogenic roles for both full-length eIF3e and truncated eIF3e, early studies suggested that eIF3e was a tumor suppressor (Figure 3). Miyazaki and colleagues examined eIF3e/INT6 genetic alterations within the genome of 100 primary breast carcinomas (36). The investigation was done using a polymorphic dinucleotide sequence in intron 7 of the Int6/Eif3e gene to analyze for loss of heterozygosity (LOH). LOH was detected in 28% (11 of 39 informative samples) of tumor samples. Interestingly, LOH had been reported to frequently be associated with chromosome region 8p21.3.-p22 in human breast carcinomas. These findings corroborate the previous findings that LOH represent the most common type of mutation in primary breast carcinomas, affecting many regions of the genome. However, LOH at the Int6/Eif3e locus (8q22-q23) had not previously been shown (36). Subsequent studies have shown decreased Int6/Eif3e at the mRNA level in various cancers. In an examination of 2 of the most prevalent forms of cancer, 62 breast carcinomas and 78 non-small cell lung carcinomas (NSCLC) were analyzed for Eif3e gene status and mRNA expression. LOH was observed in 21% (5 samples) of informative breast carcinomas and 29% (10 samples) of NSCLC. Investigation of mRNA expression revealed reduced expression in 37% (23 samples) of the 62 breast carcinoma samples and 31% (24 samples) of the 78 NSCLC samples. Of note, decreased expression was mainly observed in NSCLC samples classified as adenocarcinomas (72).

Buttitta and colleagues further examined the role of INT6/eIF3e expression in NSCLC and suggested it as a potential new prognostic factor in these patients. In their study, they investigated steady state levels of Eif3e expression, gene methylation status, overall survival and disease free survival (73). Indeed, 101 NSCLC samples were studied along with matching normal lung tissues and it was observed that one third of tumor samples had decreased eIF3e expression compared to control tissue. Upon further investigation, the authors reported that 85% of these tumors displayed hypermethylation of the Eif3e promoter and exon 1. Most importantly, low expression of Int6/Eif3e mRNA was found to be a significant predictor of poor prognosis both for overall survival and disease free survival. In vitro, Suo and colleagues demonstrated that Int6/Eif3e silencing in normal human mammary epithelium cells (MCF-10A) led to abnormal morphogenesis and abnormal acini formation. Interestingly, eIF3e repression in MCF-10AT cells, which harbor a weakly transformed phenotype due to the Ras oncogene, resulted in enhancement of this transformed phenotype. When proteosomal functions were assessed, eIF3e was shown to be necessary for assembly and optimal function of the proteasome and its silencing led to reduced degradation and subsequent accumulation of oncoproteins such as the steroid receptor co-activator 3, providing the potential to transform mammary epithelium (30). Additional studies have also examined the role of the tumor microenvironment, specifically the tumor stroma, in breast carcinoma as eIF3e expression was actually found to be lower in the stroma of both invasive breast carcinomas and premalignant ductal carcinoma in situ. When INT6/eIF3e was silenced in immortalized human mammary fibroblasts, increased cancer-associated fibroblast (CAF) properties were observed, including increased expression of alpha-SMA, a stromal cell derived factor that enhances proliferation of carcinoma cells, and SDF-1. Interestingly, when these cells were cultured with breast cancer cells they were able to enhance colony formation, invasiveness, and ultimately led to a transforming phenotype (46).

Finally, in a recent study searching for protein biomarkers of Tamoxifen therapy resistance, laser capture microdissection was used to isolate breast cancer cells from either tamoxifen therapy-sensitive tumors or tamoxifen-resistant tumors and then comparative proteomics was performed. It was found that high expression of eIF3e was significantly associated with prolonged progression-free survival, implicating eIF3e as a potential predictor for response to Tamoxifen therapy in breast cancer patients (74).

4.3. The “ugly” eIF3e and its role in the epithelial-to-mesenchymal transition

The role of eIF3e in breast cancer has been controversial. Previous studies have shown a reduction in expression of eIF3e in breast cancer tumors, implicating
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a tumor suppressor role. Other studies have shown that eIF3e is not only highly expressed in several cancers, but that it contributes to the translation and stability of several oncoproteins. Interestingly, a recent report demonstrated another unexpected role for eIF3e in the epithelial-to-mesenchymal transition (EMT) process, which is a key mechanism in the motility, invasiveness and malignant transformation of cancer cells. Indeed, silencing of eIF3e with specific short hairpin RNAs in immortalized breast epithelial cells (MCF-10A) resulted in epithelial-to-mesenchymal transition. Upon silencing, the breast cancer cells exhibited: altered cell morphology; decreased acini formation; reduced proliferation associated with increased migration; increased levels of the mesenchymal markers N-cadherin and vimentin; and ultimately increased mRNA and protein expression of the EMT regulators Snail1 and Zeb2. This study not only makes a strong case for eIF3e as a tumor suppressor, but also implicated the loss of eIF3e in EMT in breast cancer (45).

This surprising concept was expanded on in a subsequent study that demonstrated that eIF3e silencing in lung epithelial cells also leads to EMT, characterized by similar phenotypes as seen in the previous study (62). Upon silencing of eIF3e, global translation was decreased. Yet translation of a subset of mRNAs important in the progression of EMT was maintained via cap-independent translation. This correlates well with previous findings that expression of truncated eIF3e causes a shift from cap-dependent to cap-independent translation (51).

Moreover, it was demonstrated that eIF3e silencing in both breast and lung epithelial cells led to the activation of the TGF-beta pathway, and ultimately overproduction and secretion of TGF-beta. Additionally, silencing of TGF-beta reversed the eIF3e associated EMT, indicating a vital role for the TGF-beta pathway in eIF3e associated EMT (62). These results are consistent with the findings of Suo et al. (46) that eIF3e silencing in fibroblasts leads to EMT, increasing protein levels of mesenchymal markers such as vimentin and N-cadherin, and promoting the formation of cancer-associated fibroblasts and a tumorigenic microenvironment.

5. EIF3E, HYPOXIA INDUCIBLE FACTORS, ANGIOGENESIS AND POTENTIAL THERAPEUTIC IMPLICATIONS

5.1. EIF3e and the hypoxia inducible factors, an ambiguous relationship

As described above, in addition to its main role in protein translation, another interesting function of eIF3e is its function in protein degradation via the PCI and MPN domains which allow the interaction with complexes such as the proteasome or the COP9 signalosome, major actors of the protein degradation process (22-24). Indeed, through this unexpected function, eIF3e has been described as a new regulator of the Hypoxia Inducible Factors (HIFs), particularly HIF-2alpha. Recent studies have shown that INT6/eIF3e overexpression or silencing could modulate HIF expression levels suggesting some surprising functions of eIF3e as Hypoxia Inducible Factors are essential transcription factors for cellular response and adaption to low oxygen conditions (48, 55, 75, 76). Indeed, the Hypoxia Inducible Factor (HIF) family regulates a broad array of genes in response to O2 deprivation (77-79). The HIF inducible factor family regulates a broad array of genes in response to O2 deprivation (77-79). The HIF proteins belong to the bHLH-PAS (basic helix loop helix-Per/ARNT/Sim) family and consist of three O2-regulated alpha-subunits, HIF-1alpha, HIF-2alpha and HIF-3alpha, and a constitutively expressed beta-subunit of the Aryl hydrocarbon nuclear translocator (ARNT) family (80). The HIF-alpha subunits are highly homologous containing an N-terminal bHLH domain that mediates DNA binding, specificity through the HRE (Hypoxia Response Elements) consensus sequences within gene promoter regions. The other domains, HLH and PAS mediate heterodimerization with ARNT, while the C-terminal part of HIF-1alpha and HIF-2alpha contains the transactivation domain. Under normal oxygen conditions, the HIF-alpha subunits are rapidly hydroxylated by prolyl hydroxylases (PHD), recognized by the Von Hippel Lindau (VHL) tumor suppressor protein and degraded by the ubiquitin-proteasome pathway (Figure 4). In the setting of hypoxia, stabilized HIF-alpha subunits bind ARNT and induce transcription of downstream target genes involved in cellular responses to hypoxia such as metabolic adaptation, angiogenesis, erythropoiesis, and cell growth and differentiation (Figure 4) (77, 81-84).

Several studies have suggested that in regulating HIFs, eIF3e could potentially have a role in cancer angiogenesis or peripheral arterial diseases (PAD) where the HIFs are critical. Beginning in 2007, Shibasaki and colleagues performed yeast two-hybrid analysis, immunoprecipitation and HRE reporter assays and reported for the first time INT6/eIF3e as a novel target gene product modulating HIF-2alpha expression (55). Interestingly, the authors demonstrate that the N-terminal part of eIF3e (amino acids 4-128) interacts specifically with the C-terminal part of HIF-2alpha (amino acids 571-700), and not with HIF-1alpha or HIF-3alpha. Most importantly, this interaction led to functional consequences under normoxic and hypoxic conditions as eIF3e modulates HIF-2alpha expression at the protein level controlling HIF-2alpha proteasomal degradation process and subsequently its transcriptional activity (Figure 5A). In this study, the specificity of eIF3e binding to HIF-2alpha is traced to its C-terminal region (amino acids 571-828) where the difference between the HIF-alpha subunits is the most important. Regardless of oxygen level, overexpression of eIF3e significantly decreases HIF-2alpha level and cell viability. Consistent with this observation, inhibition of eIF3e, via specific siRNA, enhances HIF-2alpha expression and...
activity (Figure 5A). Additionally, the authors identified HRE sequences within the Int6/eIF3e promoter suggesting a potential negative feedback mechanism to HIF-2alpha activation. Similar studies have been performed on human umbilical vein endothelial cells (HUVECs) and in human aortic endothelial cells (HAECs) and have demonstrated that INT6/eIF3e regulates secretion of the cytokines interleukin-6 (IL-6) and IL-8, through HIF-2alpha and not unlike in HeLa cells, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and epidermal growth factor (EGF) (76).

In contrast to the studies performed with MCF-7 and endothelial cells (55, 76), where INT6/
eIF3e overexpression reduces HIF-2alpha levels and eIF3e silencing promotes HIF-2 alpha expression, in the context of cancer and particularly in glioblastoma (GBM), another study demonstrated that INT6/eIF3e silencing results in a decrease of HIF-1alpha and HIF-2alpha expression leading to inhibition of growth, proliferation, and migration of human GBM cells in vitro (48). This diminished HIF-1alpha and HIF-2alpha correlated with a down-regulation of some HIF target genes such as VEGF, PDGF, AlphaV integrin and Dll4, which are involved in proliferation, migration and cell growth processes (Figure 5B). This study suggests that eIF3e-regulation of the HIFs could be cell type specific due to differences in cellular genetic/metabolic backgrounds. Furthermore, this study suggested that HIF-2alpha levels would not be regulated through proteasomal degradation but instead through specific mRNA translation processes. This result is supported by the work from Grzmil et al. who reported that eIF3e inhibition failed to modify global translation in breast cancer and osteosarcoma cells and that eIF3e positively and negatively regulates specific mRNAs such as PLAU, BCL-XL and MAD2L1 (29) (Figure 5B). The direct or indirect regulation of HIFs and HIF targets by INT6/eIF3e remains an interesting question and although a deeper understanding of the molecular mechanisms involved in this eIF3e-HIFs pathway is necessary, eIF3e could become a new therapeutic option in some cancers. Since downregulation of the HIFs inhibits angiogenesis and decreases tumor growth, regulation of HIF expression and activity, through eIF3e, could evolve as a potential target for modulating tumor angiogenesis and is becoming an attractive approach for the treatment of solid tumors, particularly glioblastoma, which is typically associated with excessive angiogenic activity. In contrast, forced expression or activation of HIFs as suggested by Shibasaki and colleagues, could evolve as a potential effective solution for the improvement of therapeutic angiogenesis and revascularization after critical ischemia in adults affected by ischemic diseases including myocardial infarction, atherosclerosis, and peripheral artery disease.

5.2. EIF3E and its potential therapeutic implications

5.2.1. Cancer angiogenesis

Rapidly proliferating cancer cells can outgrow their vascular network, limiting oxygen diffusion within the tumor itself. Abnormal tumor vasculature can cause hypoxic stress (79) and oxygen concentration is usually below physiological levels in solid tumors (78, 85) with some regions going less than <10 mmHg (78). As a consequence, HIFs are highly expressed in many human cancers compared to the respective normal tissues (80, 86). This strong expression is often correlated with poor clinical prognosis (87). Recently, many studies have shown that HIF-alpha stabilization induces an increase in vascularization leading to tumor growth and metastasis, whereas inhibition of HIF activity has the opposite effect (82, 83). HIFs have a strong impact on endothelial cells in tumor tissues favoring abnormal vascularization and angiogenesis in the hypoxic tumor microenvironment. Interestingly, loss of endothelial HIF-alpha inhibits blood vessel growth in solid tumors (82, 88). Endothelial cells, as well as non-malignant stromal cells, interact with tumor cells, and therefore contribute to tumor angiogenesis in different ways. The selective targeting of the HIFs in distinct tumor sub-compartments might be more effective as an anti-tumor strategy than systemic HIF inhibition. For instance and as previously demonstrated in PHD deficient models, INT6/eIF3e modulation in endothelial cells could potentially normalize tumor vasculature and improve tumor perfusion and oxygenation (89) through the regulation of the HIFs, especially HIF-2alpha (Figure 6). This would render the tumors less malignant and metastatic, making them more vulnerable to the current therapies relying on the presence of oxygen to be fully efficient such as radiation or chemotherapies.

5.2.2. Therapeutic angiogenesis

Occlusive vascular diseases or peripheral artery diseases (PAD) are characterized by obstruction of large arteries leading to vascular dysfunctions in the extremities (90, 91) and are the leading causes of death in industrialized societies (92). Therapies, such as angioplasty or limb amputation (90, 91) remain the only available treatments especially at late stages. Induction of angiogenesis and vascularization in a specific manner would significantly advance the treatment of ischemic vascular diseases. Not surprisingly, the HIF pathways are attractive targets in ischemic diseases and continued study in this area has revealed important insights. First, it is now clear that the HIFs levels (mRNA and protein) are significantly increased following ischemic insult in various animal models (79, 93, 94). Second, impairment of HIF expression, for instance in HIF-1 alpha/HIF-2alpha deficient mice, leads to reduced blood flow recovery and limb perfusion during hindlimb ischemia after ligation of the femoral artery (81, 82). Third, the opposite approach consisting of overexpressing a constitutive form of HIF-1alpha, through a viral vector AdCA5 (95), stimulates reperfusion after femoral artery ligation in mouse and rabbit models (93, 96). Similarly, ectopic expression of the HIFs has been attempted using electroporation of a plasmid encoding HIF-1alpha (VP16) and resulted in increased blood flow recovery and stimulation of angiogenesis in models of ischemia, such as femoral artery ligation and wound healing (94, 97, 98). These complementary findings suggest that modulation of HIF activity is of interest for clinical treatment of ischemic diseases. Manipulation of INT6/eIF3e could also be of special interest in order to modulate HIF expression, particularly HIF-2alpha, which plays essential roles in vascular remodeling, sprouting and stabilization. More specifically, Shibasaki’s group demonstrated that silencing of eIF3e in human umbilical vein endothelial cells (HUVECs) and in human aortic endothelial cells...
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(HAECs) led to a significant HIF-2alpha stabilization resulting in enhanced EC functions through interleukin-8 (IL-8) and interleukin-6 (IL-6) pathways (76). Interestingly, this study highlights cell specificity as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and epidermal growth factor (EGF) were not affected, yet were previously reported as modulated in HeLa cells when eIF3e was inhibited. Additionally, it suggests that these changes in EC functions could potentially re-adapt O2 supply in response to O2 deprivation and improve tumor or tissue perfusion and oxygenation. Inhibition of INT6/eIF3e specifically in ECs would thus provide a conceptually different strategy to improving and or normalizing angiogenesis in a tumor or ischemic setting (Figure 6).

Several groups have already demonstrated that eIF3e could represent a potential new therapeutic target for patients with ischemia diseases such as PAD, hindlimb ischemia, and heart/brain ischemia or injury. Recently, in a rat model used to study mechanisms of brain edema and neuronal apoptosis (cold-induced brain injury), it has been shown that INT6/eIF3e siRNAs reduced cold-induced damage in rats suggesting that eIF3e has a potential therapeutic interest for the treatment of brain ischemia and injury. Using the SHSY5Y in vitro model of human neuroblastoma cells, the authors also demonstrate that eIF3e inhibition leads to increased angiogenic factor (VEGF and PDGF-B) production in an HIF-2alpha-dependent manner. Treatment with INT6/eIF3e siRNAs was sufficient to increase the number of functional vessels and capillaries, and consequently reduce brain injuries in the rats. In another model of wound healing, Chen et al. reinforce the idea that siRNA specific for eIF3e could be of interest clinically in treating ischemic diseases such as skin injury (75). They report in this murine model that inhibition of eIF3e, via intradermal injection of eIF3e siRNA, led to a specific increase of HIF-2alpha, which resulted in a strong stimulation of the angiogenic process with formation of new vessels, arteries and veins, subcutaneously. Histology revealed that subcutaneous fibroblasts were the main targets of the eIF3e siRNAs and were stimulated by ECs through the secretion of VEGF and HGF. Additionally, the eIF3e silencing-induced neovascularization enhanced the wound healing in immunodeficient mice as well as in a genetically engineered diabetic mouse model. HIF-2alpha thoroughly inhibited the neoangiogenic process and confirmed the unique relationship between INT6/eIF3e and HIF-2alpha. Interestingly, in this study, the authors also suggest a negative feedback regulation of HIF-2alpha stability caused by HIF-2alpha-induced transcription of eIF3e via HRE sequences in its promoter.

Finally, in the context of hindlimb ischemia, INT6/eIF3e has been established as an angiogenic
master switch. Indeed, stabilization of HIF-2α by Int6/Eif3e silencing appears to promote neoangiogenesis and recovery of peripheral circulation and ischemic hindlimb functions (Figure 6). The first work on this topic was performed by Okamoto and colleagues using a rat model of intermittent claudication, known to be one of the symptoms in PAD (99). After ligation of the external iliac artery, eIF3e siRNAs were injected into the adductor muscle resulting in a faster recovery of limb function and re-vascularization due to a stronger induction of HIF-2α and consequently VEGF and HGF. Within the same study, angiography analysis showed that blood flow recovery was significantly improved in the eIF3e siRNA-treated rats suggesting that INT6/eIF3e is a promising target in the treatment of PAD. More recently, a similar study came out using a mouse model of hindlimb ischemia. The authors demonstrate that blood flow recovery was improved after femoral artery ligation in mice when eIF3e expression was silenced in the adjacent muscles using injection of plasmid expressing specific INT6/eIF3e siRNAs. In addition to enhanced blood flow recovery, muscle injuries were reduced and limb functions quickly restored in response to eIF3e siRNA treatment. To explain these effects, the authors demonstrate in vitro that eIF3e silencing induced HIF-2α stabilization and upregulation of FGF and PDGF in murine and human primary skeletal muscle myoblasts. These data suggest that manipulation of INT6/eIF3e expression may represent a superior therapeutic approach to enhance vasculization in ischemic diseases due to the multiple pro-angiogenic pathways it regulates through the direct control of HIF-2α compared to administration of simple angiogenic factors such as VEGF and FGFR.

6. CONCLUDING REMARKS

The work reviewed here details the complex and diverse roles of the “e” subunit of the translation initiation factor eIF3. EIF3e, also called INT6, is a critical regulator of the mRNA translation process but clearly plays additional roles in protein degradation, DNA damage response, as well as in pathophysiologic conditions such as hypoxia, vascular diseases and cancer. In this review, we summarized the current knowledge on eIF3e structure, interactions and biology in tumorigenesis. Although future studies will need to define the exact functions and partners of eIF3e in these various mechanisms, the unexpected roles of eIF3e should drive more attention to this protein in the coming years. Additionally, we relate the recent findings suggesting that the eIF3/eIF6-HIF signalling pathway could represent an important therapeutic target for vascular diseases and cancer. The role of eIF3e in models of ischemic vascular diseases is not well understood and future investigations will need to clarify its real clinical applications and potential toxicities. However, given the association between eIF3e and the HIFs in pre-clinical models, HIF-2α manipulation through modulation of eIF3e expression is likely to become an interesting approach for therapeutic interventions in the vascular system. Targeting the HIF pathway through modulation of eIF3e expression in order to block the angiogenic process is of interest for the treatment of cancer (Figure 6) as it may provide increased therapeutic outcomes preventing for instance metastasis. However, enhancing HIF activity may improve angiogenesis, vascular remodeling and therapeutic outcomes in vascular diseases, such as peripheral artery disease, wound healing or heart/brain injury (Figure 6). Of note, the quantity and time of eIF3e/INT6 activity will need to be further studied to determine proper treatment paradigms. To conclude, caution is always required to apply in vitro/in vivo findings in clinical settings, however the molecular targeting of eIF3e/INT6 for the control of the different processes mentioned above appear promising and could potentially be relevant in several human diseases.

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