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Mini-review

CFIm25 and alternative polyadenylation: Conflicting roles in cancer

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ABSTRACT

Alternative polyadenylation (APA) is now widely recognized to regulate gene expression. APA is an RNA-processing mechanism that generates distinct 3′ termini on mRNAs, producing mRNA isoforms. Different factors influence the initiation and development of this process. CFIm25 (among others) is a cleavage and polyadenylation factor that plays a key role in the regulation of APA. Shortening of the 3′UTRs on mRNAs leads to enhanced cellular proliferation and tumorigenicity. One reason may be the up-regulation of growth promoting factors, such as Cyclin D1. Different studies have reported a dual role of CFIm25 in cancer (both oncogenic and tumor suppressor). microRNAs (miRNAs) may be involved in CFIm25 function as well as competing endogenous RNAs (ceRNAs). The present review focuses on the role of CFIm25 in cancer, cancer treatment, and possible involvement in other human diseases. We highlight the involvement of miRNAs and ceRNAs in the function of CFIm25 to affect gene expression. The lack of understanding of the mechanisms and regulation of CFIm25 and APA has underscored the need for further research regarding their role in cancer and other diseases.

1. Introduction

It is known that dynamic regulation mechanisms under diverse physiological conditions affects the processing and maturation of mRNAs in eukaryotic cells. Polyadenylation involves the attachment of a poly(A) tail to the 3′untranslated regions (UTR) of messenger RNAs (mRNA) promoting stability, enabling efficient nuclear export and translation [13,23,44,82]. Polyadenylation in mammals includes six primary protein complexes: (a) cleavage and polyadenylation specificity factor (CPSF); (b) cleavage stimulatory factor (CSTF); (c) cleavage factors I and II (CFIm and CFIIm); (d) poly(A) polymerase; (e) poly(A) binding protein; (f) RNA polymerase II and additional proteins [49,67].

Alternative polyadenylation (APA) is a process that produces mRNA isoforms that differ only in their 3′-UTRs [74]. APA can result from the presence of multiple polyadenylation sites or mutually exclusive terminal exons. APA can cause differential expression of mRNA transcripts by influencing their stability, export to the cytoplasm, and translation efficiency. One of the major factors that governs APA was found to be CF1m [47]. CFIm is a heterodimer consisting of CFIm25 and either of two closely related subunits, CFIm68 or CFIm59.

CFIm is one of the strongly conserved components of eukaryotic mRNA 3′ processing machinery, which acts in a sequence-specific poly (A) location via two collaborating protein sub-units: a 25 kDa sub-unit consisting of a nudix domain (CFIm25), and a larger sub-unit which can

Abbreviations: APA, Alternative polyadenylation; AP1, activating protein-1; AREs, AU-rich elements; ceRNA, competing endogenous RNAs; CF, cleavage factor; CFIm and CFIIm, cleavage factor I and II; CPSF, cleavage and polyadenylation specificity factor; *CstF*, cleavage stimulatory factor; CLP1, cleavage and polyadenylation factor I subunit 1; DSE, downstream sequence element; IPF, Idiopathic fibrosis; PCF11, PCF11 cleavage and polyadenylation factor subunit; FIP1L1, factor interacting with PAPOLA and CPSF1; TAA, termination codon; MCL, B-cell malignancy mantle cell lymphoma; MRE, miRNA response element; NFKBIZ, NF-kB inhibitor zeta; PAS, polyadenylation signal; PH, Pulmonaryhypertention; RBPs, RNA-binding proteins; TSS, transcription start site; 3′-UTRs, 3′ untranslated regions; WDR33, WD repeat domain 33

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be 59, 68, or 72 kDa (CFIm59, CFIm68, or CFIm72) with a RNA recognition motif (RRM). Prior research has shown CFIm25 is necessary and sufficient to bind sequences specific for the poly(A)-site up-stream component UGUA [87]. Yang and colleagues demonstrated the crystalline structures of CFIm25 complexed with RRM domains of RNA and CFIm68 [86]. Binding in the CFIm25 dimer can take place at opposite sides through two CFIm68 RRM domains. All CFIm25 sub-units specifically bind a single UGUA component. Biochemical analysis indicates that stronger RNA binding and easier RNA looping are provided by the CFIm68 RRMs. The inherent property of CFIm to direct RNA looping can offer an explanation for its ability to regulate the choice of alternative poly(A) attachment sites [86].

CFIm25 can function as a tumor inhibitor or as an oncogene under various conditions [54].

In glioblastoma, down-regulation of CFIm25 led to the enhancement of tumorigenic properties and increased tumor size. On the other hand, over-expression of CFIm25 led to reduction in these properties and impeded tumor growth [47]. A subset of CFIm25-regulated genes with shortened 3′UTRs due to APA were found in various tumors with reduced expression of CFIm25. Despite the connection between CFIm25 and tumorigenicity found in this study, more research is needed to fully elucidate the role of CFIm25 in cancer. Some other studies have suggested that the over-expression of CFIm25 is associated with tumorigenesis.

Lou et al., found that there was a significant up-regulation CFIm25 in human glioma tissues and also that CFIm25 seems to induce proliferation of glioma cells, likely via the NF-κB signaling pathway [42]. Their results revealed that CFIm25 is an upstream modulator of the NF-κB pathway and could be a potential molecular marker for the mesenchymal subtype of GBM [42].

In another study, Zhang et al., found there was a significant increase in the expression of CFIm25 in subjects with primary chronic myelocytic leukemia, and in K562 leukemic cells at the mRNA level in comparison to a healthy group and in PBMCs [89]. Their results suggested that down-regulation of CFIm25 could inhibit the proliferation, and induce apoptosis in K562 cells. Pathway analysis showed that the MAPK/ERK pathway is a crucial molecular mechanism underlying the effects of CFIm25 knockdown in K562 cells [89].

The dual functions of CFIm25 may be due to the utilization of samples from different sources or tumors at different stages, thus making the true role of this gene uncertain.

2. Alternative polyadenylation

Studies have investigated the impact of the dynamic adjustment in different physiological situations in which APA takes place at the 3′UTRs of mRNAs [44,48]. More than 50% of the genes in humans, possess several poly-adenylation sites that result in an increased variety of mRNA transcript lengths [73]. Through such different poly(A) sites (PAS), APA may be used to produce mRNA isoforms. Various uses of alternative PAS situated in the same terminal exon is the commonest form of APA [9]. Negative regulation components, which decrease stability of mRNA or impair the translation efficacy, including AU-rich elements (AREs), and miRNAs may be inhibited via processing on the most proximal PAS [64,84]. Expression of mRNAs containing shorter 3′UTRs is up-regulated in rapidly proliferating cells and in transformed cells [5,11,68]. However, there are still unknown mechanisms, which are concerned with the shifting of PAS from distal to proximal sites that is found in proliferating and or transformed cells, the cause and effect relationships, and important target genes that are dependent on such regulation (Fig. 1).

One of the classes of these post-transcriptional regulatory molecules is RNA binding proteins (RBPs). Increasing evidence shows that RBPs may be dysfunctional in cancer genomes [21]. Findings have demonstrated that RBPs are crucial players in APA. For instance, the modified expression of RBPs in the brain of Huntington's disease (HD) patients can result in alterations in the expression of mRNA 3′UTR iso-forms [62]. There can be interactions between RBPs and nascent mRNA during the processes of APA and transcription that lead to modifications in the choice of polyA location, and the isoforms produced. For example, aberrant expression of the 3′ end regulatory factors can result in multiple alterations in isoforms [15]. Romo and colleagues reported that distinct expression of three 3′-end processing proteins was found in HD grade 1 motor cortex: *PCBP2*, *THOC5, CPSF2* [62]. It is possible that RBPs bind post-transcriptionally to the isoforms, which thus influences isoform stability and decay, resulting in modification of the isoform abundance. Researchers have found a relationship between lower CNOT6 deadenylase and less *HTT* long isoform; however, short or mid-length isoforms did not change. Additional research is crucial to determine the mechanisms of 3′UTR isoform alterations in HD. One study demonstrated that the amounts of *HTT* and *SECISBP2L* isoform responded to modifications in the levels of *CNOT6* mRNA. Nonetheless, not all the isoform changes discussed in the present study can be explained by the modified expression of *CNOT6* in HD. The rest of the RNA binding proteins, including 3′ end processing factors probably influence the isoform abundance in HD. New treatment approaches for HD may be achieved by discovering reasons for the isoform modifications in the HD motor cortex [62].

The consequences of APA for gene expression and cell function are becoming steadily clearer, as this is an active area of research. The understanding of the mechanisms that regulate APA is of high importance, and an increasing number of APA-regulatory factors have been identified and characterized [44].

3. CFIm25 as a tumor suppressor in cancer

CFIm25 has been shown to have tumor suppressive properties. Several studies have investigated the effects of CFIm25 protein and the *NUDT21* gene (which encodes CFIm25) in tumor cells, and various molecules and signaling pathways have been explored. The MAPK pathway is an important signaling pathway involved in proliferation and metastasis of tumors [54]. A recent study reported that CFIm25 knockdown led to an increase in phosphorylated JNK, p53, and c-Jun in hepatocellular carcinoma cells (HCCs). It was shown that the c-Jun-driven activating protein-1 (AP-1), a downstream effector of c-Jun, was activated by inhibition of CFIm25. In addition, regulation of the p38 and JNK/c-Jun pathways by CFIm25 resulted in increased expression of E-cadherin in these cells [78]. According to recent findings, CFIm25 may be involved in the process of tumor metastasis. EMT is an important stage required for tumor metastasis, and E-cadherin is the main mediator of cell-to-cell adhesion junctions in epithelial tissues [34]. Research has shown that inhibition of E-cadherin leads to adoption of mesenchymal cell morphology, and increased invasion and metastasis [25]. EMT is a dynamic and reversible process, which switches between the epithelial and mesenchymal phenotypes during tissue growth and healing [75]. The EMT leads to switching of epithelial cells with tight junctions into fibroblast-like cells with looser junctions [65]. Results indicated that re-expression of E-cadherin in cell lines, in which it has been depleted, can reverse the poorly-differentiated malignant phenotype with fibroblastic morphology. This reduces the invasiveness, and leads to a more differentiated epithelioid phenotype with tighter cell-to-cell junctions [61]. Depleting E-cadherin activates oncogenic signaling pathways such as MAPK, rat sarcoma viral oncogene (Ras), Ras-related C3 botulinum toxin substrate (Rac1), and disrupts the Hippo signaling pathway all of which are involved in cancer cell migration and invasion [25]. Findings have demonstrated that the expression of E-cadherin is reduced with higher expression of CFIm25 and *vice versa*. Interestingly, changes in the expression of N-cadherin move in opposite directions to those of E-cadherin. Results have suggested that metastasis of tumors such as HCC could be prevented by CFIm25 through suppression of the EMT [25].

Sheng Tan et al. [71] showed that the expression of CFIm25 gene

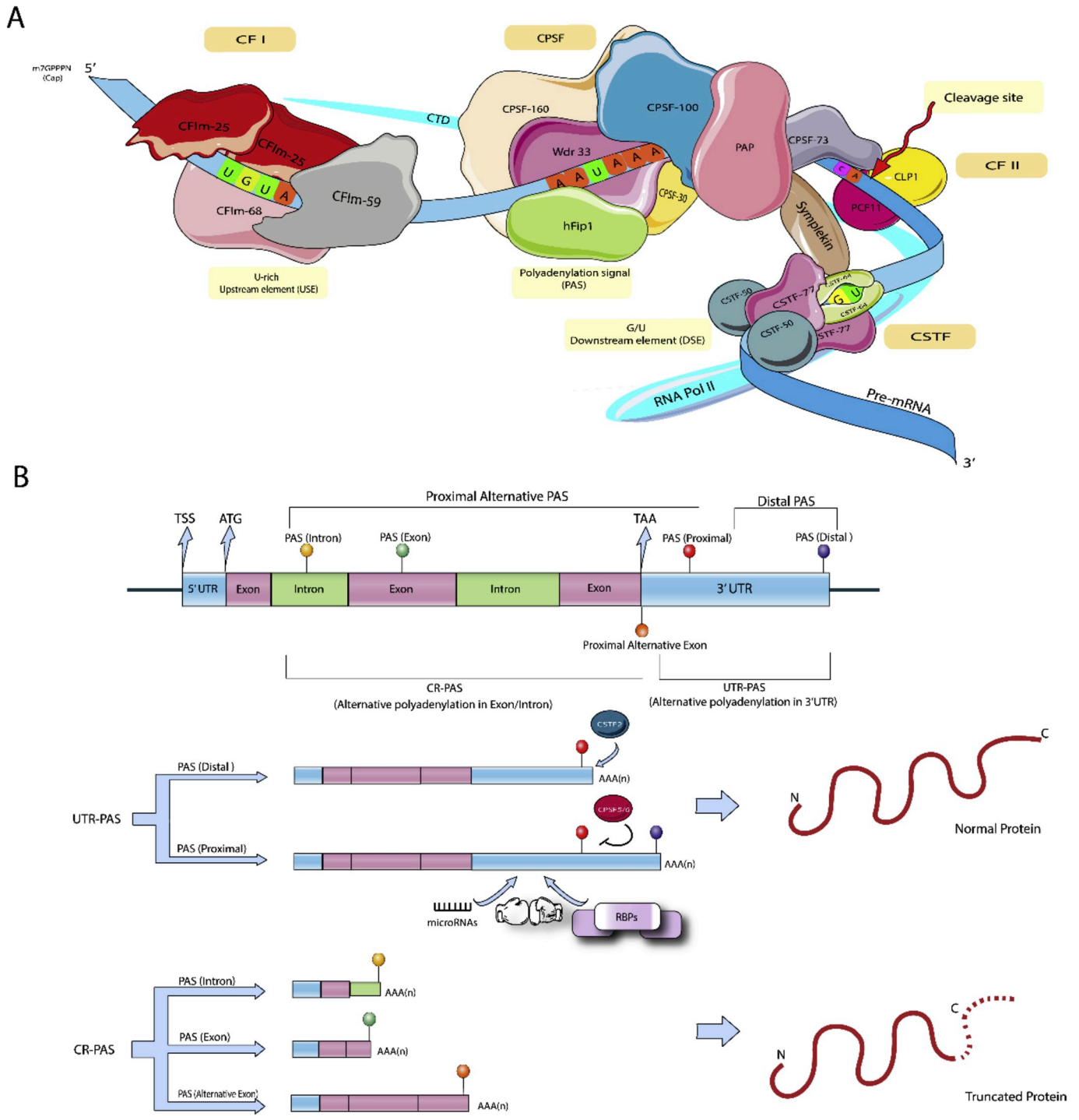

Fig. 1. Alternative polyadenylation. (A) 3′-End processing complexes and cis-acting elements of the 3′-terminal exon for polyadenylation. There is an association between RNA polymerase II C-terminal domain (CTD) with 3′-end processing proteins. To determine the cleavage site of a transcript during termination of transcription, a combination of upstream sequence elements (USE), downstream sequence elements (DSE), and polyadenylation sites (PAS) are used. (B) Alternative polyadenylation (APA) and its impact on protein production. In 3′-untranslated region (UTR) APA, an increase in proximal PAS is possible by cleavage stimulation factor 2 (CSFT2) and a decrease in proximal PAS is possible by cleavage and polyadenylation specificity factor 5/6 (CPSF5/6). The interaction with miRNAs or RNA-binding proteins (RBPs) and modulation of protein synthesis could be influenced by the length of 3′-UTR. In intron regions, the coding capacity of protein and thus truncated protein production could be influenced by APA. CF, cleavage factor; PCF11, PCF11 cleavage and polyadenylation factor subunit; CLP1, cleavage and polyadenylation factor I subunit 1; WDR33, WD repeat domain 33; FIP1L1, factor interacting with PAPOLA and CPSF1; ATG, initiation codon; TAA, termination codon; TSS, transcription start site.

was decreased in HCC tissue, as well as that lower CFIm25 expression was related to shorter survival-time in patients with HCC after surgery. They also reported that knockdown of CFIm25 led to HCC cellular proliferation, HCC development and metastasis. They suggested that the tumor suppressive effects of CFIm25 may be related to its regulatory function on the PSMB2 and CXXC5 genes. PSMB2 is one subunit of a multi-catalytic proteinase complex that contains a well-ordered ring-like 20S core structure [7]. Even though proteasomes are considered to

Table 1
CFIm25 as tumor suppressor in different cancers.

Ref	Sample size/cell line	Model (In vitro, In vivo, human tissue)	Effect(s)	Expression in cancer	Type of cancer
[78]	62	Human	Suppress the p38 and JNK/c-Jun signaling pathways by targeting AP-1	Down regulation	HCC
[71]	59	Human	HCC cell proliferation and invasion is suppressed by knockdown of PSMB2 or CXXC5.	Down regulation	HCC
[22]	A549	In vitro	CFIm25 knockdown decreased the dPAS usage of several oncogenes (IGF1R, CCND1 and GSK3β)	Down regulation	Lung cancer
[90]	MG63	In vitro	3′UTR of CFIm25 mRNA was targeted by miR-181a and the expression of CFIm25 was downregulated in osteosarcoma cells	Down regulation	Osteosarcoma
[35]	Flp-In-T-REx-293, 29	In vitro, in vivo, Human	Significant down-regulation of the RHAMM protein (positive control) was due to silencing CFIm25	Down regulation	Seminoma
[17,47]	HeLa, U251 and LN229	In vitro, Human	Tumorigenic properties and increased tumor size by down-regulation of CFIm25 expression in glioblastoma cells	Down regulation	Glioblastoma
[6]	LN229, U251	In vitro	Regulation of Pak1 expression	Down regulation	
[43]	15, U87 and U251	In vitro, Human	Promote glioma cell proliferation, probably through the NF-κB signaling pathway and NFKBIZ	Up regulation	
[69,70]	56	Human	Significant correlation between gene expression profile and resistance to etoposide	Up regulation	Acute leukemia, ALL
[88]	K562 cells	In vitro	Modulate the expression of p-ERK promoted K562 proliferation	Up regulation	CML
[46]	HeLa, 293T	In vitro, In vivo	Glutaminase alternative terminal exon ability to modulate miR-23 function was regulated by CFIm25.	Down regulation	Ovarian cancer

be treatment targets in several types of cancer, the contribution of PSMB2 has not been confirmed. In addition, reports have indicated that over-expression of CXXC5 is an undesirable prognostic factor in solid tumors including breast cancer [27]. However, there have been few studies on the contribution of CXXC5 to HCC. Knockdown of CFIm25 resulted in a significantly increased expression of these genes. In addition, knockdown of PSMB2 and CXXC5 inhibited HCC cell invasion and proliferation.

Another study investigated the role of CFIm25 expression in glioma cell proliferation. This study showed that increased expression of CFIm25 induced glioma cell proliferation by regulation of NF-kB signaling pathway. CFIm25 regulated NF-kB inhibitor zeta (NFKBIZ) which is a downstream target for this gene [43]. Further evidence has shown that decreased expression of CFIm25 in glioblastoma cells promoted tumorigenic features as well as increased tumor size [47]. These findings suggested that CFIm25 could inhibit glioblastoma growth, possibly by mediating the repression of APA-dependent shortening of mRNA 3′UTRs. This study identified CFIm25 (among fifteen different poly-adenylation and cleavage factors) to be one of the most important factors that governs the broad regulation of APA. It was important to provide insight into how APA modulates genes by large-scale shortening of 3′UTRs, resulting in increased cell growth and tumorigenicity by enhancing growth factors, including cyclin D1. Conversely, increased expression of CFIm25 inhibited these oncogenic traits. Thus, these findings clarified the suppressive role of CFIm25 in the initiation and progression of glioblastoma tumors [17,47]. Moreover, the findings illustrated the significance of 3′UTRs in controlling the growth of cells, and emphasized the necessity of additional studies on APA and its potential relationships with other human diseases [47].

In the testis, planar division of undifferentiated germ cells is an important event, which when disrupted leads to testicular atrophy and low fertility, as well as the development of testicular germ cell tumors. Planar cell division of germ cells is regulated by the spindle-associated RHAMM. The expression of RHAMM is also regulated by CFIm25. Huaibiao Li et al. investigated the role of CFIm25 in regulation of RHAMM expression. They observed that CFIm25 up-regulated RHAMM expression. Therefore, they concluded that impairment in expression of CFIm25 led to decreased expression of RHAMM creating testicular atrophy and low fertility, which are related to initiation and promotion of testicular germ cell tumors [37]. Recent studies have investigated the relationship between gene expression profiles and chemotherapy drug resistance. In leukemic cells, transcriptome profiling showed that the CFIm25 gene had the best correlation with resistance to etoposide. These findings suggested that this gene could be useful to evaluate the sensitivity to some drugs, which are used in chemotherapy [69,70]. The possible mechanism of the effect of CFIm25 on leukemia cells was assessed in another study [89]. In this study silencing of the CFIm25 gene led to inhibition of the proliferation and induction of apoptosis in K562 cells. It was also observed that CFIm25 regulated p-ERK expression, and subsequently enhanced K562 proliferation. These results showed that the MAPK/ERK signaling pathway may be a mechanism affected by CFIm25. Reports have suggested there is a strong relationship between the ERK pathway and rapid growth and apoptosis of cells. The rapid growth of K562 cells and reduction of apoptosis in vitro, have been confirmed using agonists of the ERK pathway. PTEN is a classic inhibitor of the ERK pathway. qRT-PCR and Western blot analysis revealed there was significant up-regulation of PTEN expression after NUDT21 knockdown. It should be noted that PTEN contributes to the phosphorylation of ERK1/2 and tumorigenesis. Results confirmed that rapid growth and reduced apoptosis of leukemia cells were caused by NUDT21 knockdown, via up-regulation of PTEN. Nonetheless, it is possible that other pathways besides PTEN and ERK are also engaged. These results emphasized the necessity of additional studies to explore the mechanisms of suppression of proliferation and increased apoptosis in K562 cells after NUDT21 knockdown. Collectively, these findings suggest that CFIm25 and its encoding gene, *NUDT21*, have tumor-

Table 2
Effects of CFIm25 in non-cancerous diseases.

Ref	Function (s)	Target (s)	Disease
[52,53,80]	Increase the levels of fibronectin and collagen I in pulmonary fibrosis	Fibronection, collagen 1	Idiopathic Pulmonary Fibrosis
[45]	Induce proliferation	*CCND1*	B-cell malignancy mantle cell lymphoma (MCL)
[28,29]	Up-regulation of miR-203	Profibric and matrix gene	Idiopathic fibrosis (IPF)
[8,79]	Remodeling of pulmonary arteries	ECM component, TGF-β and the Wnt pathways	Pulmonaryhypertention (PH)
[14]	Increase usage of the distal polyadenylation site in the *MECP2* 3′ UTR which results in an enrichment in inefficiently translated long mRNA isoforms	*MECP2*	Neuropsychiatric disease

suppressive properties, which may provide a novel therapeutic strategy for cancer [89]. Table 1 lists studies of CFIm25 as a tumor suppressor in different malignancies (see Table 2).

4. CFIm25 and microRNAs

Only a small part (~1.5%) of the genetic material in the human genome is involved in coding proteins, yet most non-coding genomic DNA is still involved in the regulation of gene expression [32]. In particular, gene expression has two levels of regulation: transcriptional regulation controls gene transcription and its extent, while post-transcriptional regulation affects the transcribed RNA molecules, including their stability, translation efficiency and subcellular localization [50]. Several multi-component cellular mechanisms are involved in regulating each level. Each one of these mechanisms carries out a separate step in gene expression regulation. Gene expression regulators, rather than working as a simple linear assembly line, couple together to form a complex network. These networks coordinate their activities, thus maximizing the efficiency and specificity of each step in gene expression [31]. In recent years, post-transcriptional gene regulation has attracted more attention in mammalian organisms. Using processing steps such as alternative splicing or APA, the same primary transcript can generate a number of different mRNA isoforms [73,77]. Today, new classes of non-coding RNA genes, including the abundant and conserved family of miRNAs, have been identified [41,60]. APA often creates mRNA 3′ UTRs with different lengths, and miRNAs, by binding to specific mRNA 3′ UTRs, regulate gene expression (Fig. 2). The association between APA and nonsense-mediated RNA decay (NMD) has been discussed in previous studies. NMD is a mRNA surveillance mechanisms that eliminates mRNAs containing a premature stop codon [10,36,40,55,76]. It is logical that there should be an interplay between APA and miRNA-mediated mRNA decay. The *COX2* gene in colorectal cancer cells shows this property. The 2.6-kb isoform of COX-2 mRNA lacking the miR-101/miR-199a target of the 3′ UTR, rather than 4.5-kb isoform, was selectively stabilized in growing cells. Accordingly, it can be concluded that in cancer cells, COX2 mRNA may escape regulation through the usage of alternative PAS. A shift in expression of proximal PAS isoforms may lead to the avoidance of regulation by miRNAs, causing enhanced proliferation and unrestricted cell cycle progression (40). This is consistent with the fact that miRNAs influence many aspects of cellular proliferation and the cell cycle progression. Accordingly, down-regulation of miRNAs expression increases cellular transformation and tumorigenesis. This phenomenon has been observed in many different cancer cells [2,12,24] (Fig. 2). Over the course of evolutionary selection, changes in the length of the 3′ UTRs has led to many genes reducing their miRNA binding sites [1]. An increased level of COX2 protein has been observed both in human and animal colorectal tumors. Nevertheless, the normal intestinal mucosa displays low to undetectable levels of COX2 expression [30]. Similarly, an increased level of COX2 resulting from defects in its regulation, has been reported in many other solid tumors, including breast, lung, prostate, pancreas, bladder, stomach, esophagus and head and neck cancers [4,59,83]. Therefore, it is reasonable to suppose that an interplay between APA in COX2 and regulation by miR-101/miR-199a, at least to some extent, may contribute to the development of cancer.

Physiological conditions finely regulate the expression of the PTEN (phosphatase and tensin homolog) gene by transcriptional and post-transcriptional mechanisms. The *PTEN* gene is one of the most frequently mutated genes in cancers [39]. The changes in PTEN expression, even if modest, have a significant influence on tumorigenesis and tumor progression in mouse models [3]. Loss of a single copy of *PTEN* in some body organs, like prostate, lung, breast and colon can lead to oncogenic consequences. The remaining copy of PTEN is typically retained until the development of more aggressive and metastatic tumors [38,57]. Thivierge et al. showed that APA leads to an important diversity of co-expressed *PTEN* 3′-UTR mRNA isoforms. They also suggested that the relative expression of PTEN 3′-UTR mRNA isoforms is dynamically controlled by the cell density [72]. The findings of their study showed that, despite encoding a diversity of validated miRNA-binding sites, longer 3′-UTRs were largely refractory to miRNA-mediated silencing, and accounted for the bulk of PTEN protein expression. Four lines of evidence support this finding. Firstly, no response to inhibition or over-expression of miRNAs, whether endogenous or expressed from reporter constructs, was observed in long PTEN 3′-UTR isoforms. In addition, a potent response was observed in reporters encoding 3X miRNA-binding sites. Secondly, the long 3′-UTR isoforms, in comparison with the shorter 300 nt isoform, were significantly more stable. Accordingly, they encode fewer miRNA-binding sites. Thirdly, despite the co-expression of the shorter 3′-UTR isoforms, the rate of knockdown of longer *PTEN* 3′-UTR mRNA isoforms and the changes in PTEN protein were closely correlated. Fourthly, upon specific knockdown of longer PTEN 3′-UTR isoforms, activation of PI3K/Akt/mTOR signaling was observed. This occurred while the shorter *PTEN* 3′-UTR isoforms were still expressed. Therefore, it can be concluded that shorter *PTEN* 3′-UTR isoforms cannot compensate for the loss of longer 3′-UTR isoform mRNAs, whether functionally or affecting the overall PTEN protein load [72].

According to Hinske et al., APA is a possible mechanism that governs the negative feedback exerted by intronic miRNA on their host genes [18]. The findings of their study also showed that the host genes that contain matching sites for their intronic miRNAs will have longer 3′UTRs with more polyadenylation sites. In addition, a significant difference was observed between the expression of these host genes and the host genes of miRNAs lacking potential miRNA binding sites. Then, in order to investigate the relationship between ZFR (zinc finger RNA binding protein) and its intronic miRNA miR-579, they carried out in-silico modeling, and used the U87 cell line as a biological model. They showed that ZFR was targeted by its intronic miRNA, which allowed differential targeting due to APA.

In this study, bioinformatics analysis and RNA-Seq were used to evaluate the potential cross-talk between intronic miRNAs and APA. The findings showed that CPSF2, a gene previously associated with APA signal recognition, might alter the polyadenylation signal which is associated with negative feedback by intronic miRNA [18].

Previous studies showed that miR-181a was increased in osteosarcoma, and enhanced the proliferation and suppressed apoptosis in

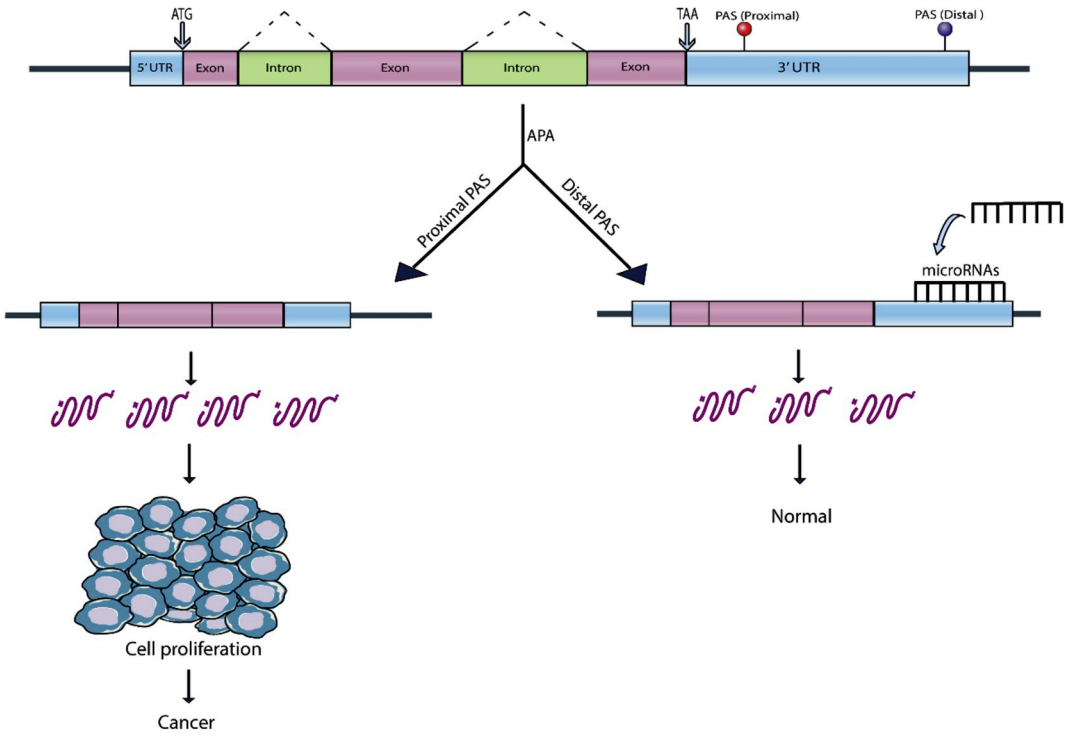

Fig. 2. Different regulatory impacts of miRNAs on longer and shorter isoforms of mRNA which cause cellular proliferation and cancer.

osteosarcoma cells. Accordingly, a recent study evaluated the relationship between miR-181a and CFIm25 expression. The results of this study showed that miR-181a and down-regulated CFIm25 expression led to increased cell proliferation and inhibition of apoptosis in these cells [90]. Over-expression of CFIm25 inhibited the rapid growth, enhanced apoptosis, and suppressed the expression of cyclin D1 in osteosarcoma cells. Moreover, they showed that silencing of miR-181a in osteosarcoma cells was induced by expression of CFIm25 protein [90]. The molecular mechanism responsible for growth inhibition and apoptosis of osteosarcoma may involve silencing miR-181a via restoration of CFIm25. It is possible that the inhibition of miR-181a could be a treatment strategy for restoration of CFIm25-mediated function. However it will be necessary to confirm the connection between CFIm25 and miR-181a using in-vivo experiments in animal models.

5. miRNA as an important mediator in CFIm25 function

APA sites are found in most mammalian genes, and lead to variation in the 3′UTR lengths [20,56,85]. CFIm25 apparently prevents breakdown of the proximal PAS by CPSF, because lack of this protein inside the cell leads to shortening of the 3′-UTR, as well as the lack of several miRNA regulatory elements (MRE) (58). The fate of the mRNA is associated with changes in the length of 3′ UTRs. The reason could be altered binding between RBPs and miRNAs. Proliferating cells have more APA sites, which in turn results in shorter 3′UTRs. Such shortening prevents mRNAs from being targeted by miRNAs whose binding sites are eliminated (Fig. 3). For example, proliferating T cells have significantly shorter 3′ UTRs compared to resting T-cells, which could be due to having fewer binding sites for miR-155 and miR-17-92. Cancer cells express mRNAs with overall shorter 3′ UTRs. Due to the escape from miRNA regulation, this shortening has been linked to the activation of oncogenes [51]. The same mechanism, although with different details, accounts for the shortening of 3′UTRs in oncogenes and tumor suppressor genes. miR-23 can down-regulate the shortened KGA (kidney glutaminase) 3′UTR, although it only slightly affects the full-length KGA 3′UTR, GAC (C-terminal truncated splice variant, or glutaminase C) is subject to repression by miR-23 [46].

The ceRNA network is another mechanism for regulation of gene expression. According to the theory of ceRNA regulation, pseudo-genes and non-coding RNAs share MREs with coding genes. Hence, miRNAs and MREs are considered the main operators and regulators of ceRNA, respectively [63]. Therefore, the greater the number of pseudo-genes for a single gene, that gene will also have a greater number of MRE. Also, the competition between miRNAs to target transcripts with a common MRE leads to an increase in overall gene expression. The effect of the ceRNA network on the 3′UTR shortened mRNAs coding for tumor suppressor genes (a total decrease in the number of MREs), reduces the number of copies of the tumor suppressor gene by an increased silencing by miRNA. For example, in EPS15 (epidermal growth factor receptor substrate 15), the overlap between 4 miRNA binding sites and the tumor suppressor PTEN, combined with 3′UTR shortening resulted in loss of the miRNA sites, and consequently prevented it from competing with PTEN miRNAs, silencing the PTEN. For tumor suppressor genes, but not oncogenes, 3′ UTR shortening ceRNA hub genes were enriched, showing that 3′ UTR shortening represses tumor suppressor genes *in trans*. CFIm25 repressed tumor suppressor genes including PHF6 (plant homeodomain-like finger 6) and LARP1 (la ribonucleoprotein domain 1) in trans in a miRNA-dependent manner [58]. In the miR17-92 cluster, the binding sites which are up-regulated in rapidly proliferating cells, are particularly enriched just upstream to the APA sites. This probably provides them with stronger inhibitory activity upon shortening. Therefore, 3′UTR shortening both enables escape from inhibition of growth promoting genes, and potentiates repression of anti-proliferative genes [19].

6. Role of CFIm25 in non-cancerous diseases

Several roles of CFIm25 have also been investigated in other diseases and pathological states. APA is an important process in the expression of fibrotic proteins and extra cellular matrix components. It has been shown that CFIm25, (a key factor of APA), is decreased in the lungs of patients and in mice with pulmonary fibrosis, leading to elevation of expression of fibronectin, alpha smooth muscle cell actin and collagen I (all markers for pulmonary fibrosis) and therefore CFIm25

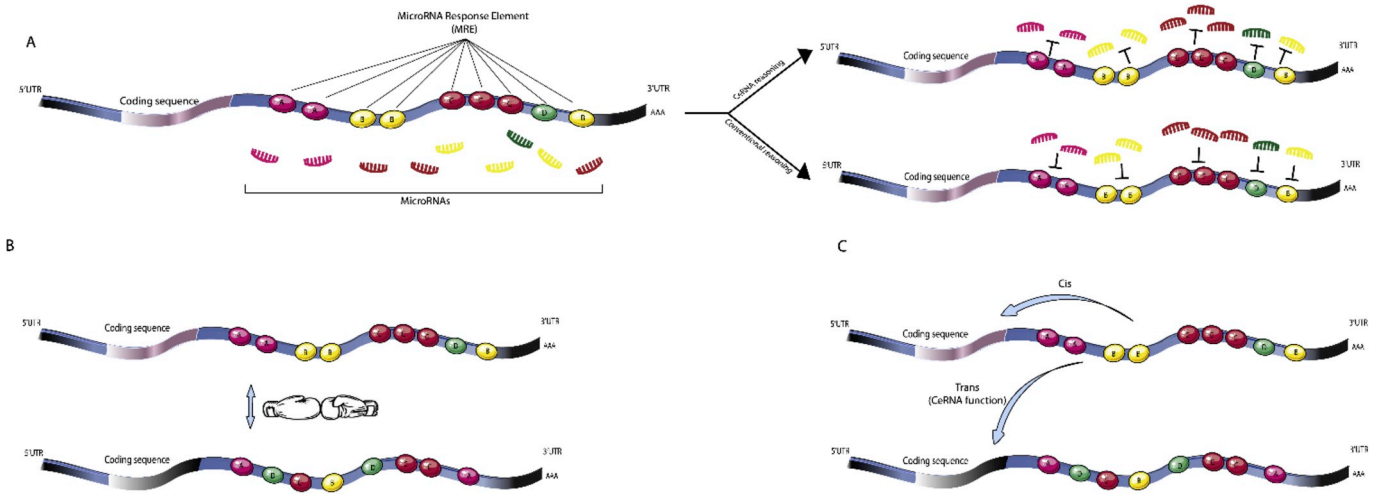

Fig. 3. The operation of the ceRNA network. How mRNAs impact miRNAs is less well understood than how miRNAs impact mRNAs. (A) The association between mRNAs and miRNAs can be reciprocal [66], so that the level of one mRNA can affect the level and activity of another mRNA. (B) Hence, RNA molecules can communicate with each other via miRNA and miRNA MREs. The larger the number of shared MREs, the greater the level of "communication" and therefore co-regulation. (C) The 3′ UTRs of RNA molecules contain MREs, which could function *in cis* to modulate the RNA molecule itself, but also probably *in trans* to modulate levels of miRNAs and subsequently other RNAs.

may be involved in the development of this disease. These findings suggest that CFIm25 and APA may combine to result in the production of excessive amounts of ECM proteins [52,53,80].

Some studies have demonstrated that the down-regulation of CFIm25 in fibroblasts of pulmonary fibrosis patients and in mice is related to the TGF-β signaling pathway. It has been found that expression of TGF-β1 (a pro-fibrotic cytokine) reduced the expression of CFIm25 in lung fibroblasts via activation of the mTOR pathway, therefore, leading to the development and progression of idiopathic pulmonary fibrosis (IPF). Prevention of the reduction of CFIm25 by rapamycin (a mTOR inhibitor) supported the previous results [28,29].

Weng and colleagues investigated whether APA was involved in IPF. The researchers demonstrated reduced levels of CFIm components (CPSF59, CFIm25, & CPSF68) in myo-fibroblasts isolated from the lungs of patients with IPF, and also in mice with pulmonary fibrosis. This finding indicated that 3′-UTR shortening was involved in the differentiation process of fibroblasts into myofibroblasts [81]. Additionally, downregulation of CFIm25 can lead to shortening of the 3′-UTRs of profibrotic genes, including *Tgfbr1* and *Col1a1*, and enhancement of the major intermediates in numerous pro-fibrotic pathways. Furthermore, the authors produced pulmonary fibrosis in mice by exposing them to bleomycin, and found that CFIm25 was depleted in lung fibroblasts. This showed that the down-regulation of CFIm25 and increased APA played a role in the pathogenesis of pulmonary fibrosis. It should be noted that when CFIm25 was knocked out in *Col1a1*-expressing cells before treatment with bleomycin, pulmonary inflammation was further enhanced in mice with CFIm25-depleted fibroblasts. This possibly occurs by activation of pathways, including JAK/STAT3, which are engaged in inflammation [81]. IL-6, via its receptor IL-6R, is known to activate STAT3 [33]. Moreover, it is possible that Wnt5A binds to the FZD2 receptor, and thus phosphorylates STAT3, which regulates the EMT in cancer [16]. *IL6* and *JAK2* and together with *WNT5A* and *FZD2* all cause 3′-UTR shortening in CFIm25-knockdown cells, implicating the STAT3 pathway in CFIm25-depleted cells. Furthermore, *Col1a1-CreER-CFIm25*$^{fl/fl}$ probably triggers the depletion of CFIm25 in *Col1a1*-expressing cells, including osteoblasts and odontoblasts, which can influence inflammation [26]. The observation that elimination of CFIm25 in Foxd1-expressing pericytes increases pulmonary fibrosis without any impact on inflammation, suggests that depleting CFIm25 may have different eff3cts in different cells [81].

Another study investigated the role of CFIm25 in vascular remodeling in pulmonary hypertension (PH). The results showed that decreased CFIm25 in pulmonary arterial smooth muscle cells resulted in production of some ECM components including fibronectin, collagen, laminin, syndecan and thrombospondin. This alteration in ECM components through CFIm25 and the APA process, led to vascular remodeling as well as the enlarged right ventricles seen in PH [8,79].

Another function of CFIm25 is in psychiatric disease by changing the expression of MeCP2 (methyl CpG binding protein 2). A relatively small alteration in the amount of MeCP2 in the brain may result in neuropsychiatric disease. It is reported that increased CFIm25 led to elevated MeCP2 production, whereas normalization of CFIm25 levels returned MeCP2 back to normal levels [14]. These results show that although CFIm25 is one of the major mediators in the process of shortening the transcripts of genes, other influential factors are miRNAs, the distribution of MREs on transcripts, and the network of the ceRNAs. However, among these factors, the key role is played by miRNAs.

7. Conclusion

Global shortening of mRNAs through APA is an important mechanism that regulates gene expression. This process, which occurs during enhanced cellular proliferation, needs to be further elucidated. CFIm25 is a broad repressor of proximal poly (A) site usage and in case of CFIm25 depletion, cell proliferation increases. Significant increases in the expression of several known oncogenes, such as cyclin D1 have been reported as a consequence of CFIm25 depletion. Certain genes can escape the regulation of miRNA and other RBPs by truncating their 3′ UTRs, which leads to cell proliferation. Research shows that this pattern of polyA tail truncation contributes to the development of diseases including cancer. A significant number of all of genes have different isoforms caused by variation in the 3′ UTRs. 3′ UTRs are involved in nonsense mediated decay, AU-rich element mediated decay and mRNA surveillance, miRNA-mediated decay, to name just a few. Regarding the distribution of MREs, since the shortening of mRNA transcripts of tumor suppressor genes causes the MREs to become more active, as well as the shortening of their ceRNA partners. Eventually shortening the transcripts of these competitors would increase the miRNA-mediated silencing of tumor suppressor genes in cancer cells. Both the oncogenes and the tumor suppressor genes will undergo shortening of the 3′UTR to the benefit of cancer progression. This mechanism shows how the

shortening of the 3′UTR of two different categories of genes in the tumor leads to tumor progression.

Elucidating the mechanism by which restoration of CFIm25 influences proliferation and apoptosis in cancer cells requires further study. The suppression of specific miRNAs (such as miR-181) may represent a promising therapeutic strategy to restore the CFIm25-mediated regulation of proliferation and apoptosis. Moreover, the complex interplay between alternative splicing, APA, and mRNA decay and the regulation of metabolic enzymes (such as glutaminase) should be further addressed. Also, since it has been shown that CFIm25 disrupts the EMT and thereby inhibits cancer cell migration and invasion, enhancing CFIm25 may be a potential clinical approach for cancer treatment. CFIm25 leads to extensive shortening of UTRs, and causally leads to enhanced cellular proliferation and tumorigenicity, probably through the up-regulation of growth promoting factors, such as cyclin D1. The significance of UTRs in control of cell growth underlines the need for additional research into the mechanism and regulation of APA, and its potential links to other human diseases.

Collectively, further research is needed to investigate the interplay between APA and the regulation of various CFIm25 targets (such as miRNA) in clinical settings. New research will improve our understanding of cancer etiology and help us to work out new ways to prevent and treat it.

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Conflicts of interest

Michael R Hamblin is on the following Scientific Advisory Boards.
Transdermal Cap Inc, Cleveland, OH.
BeWell Global Inc, Wan Chai, Hong Kong.
Hologenix Inc. Santa Monica, CA.
LumiThera Inc, Poulsbo, WA.
Vielight, Toronto, Canada.
Bright Photomedicine, Sao Paulo, Brazil.
Quantum Dynamics LLC, Cambridge, MA.
Global Photon Inc, Bee Cave, TX.
Medical Coherence, Boston MA.
NeuroThera, Newark DE.
JOOVV Inc, Minneapolis-St. Paul MN.
AIRx Medical, Pleasanton CA.
FIR Industries, Inc. Ramsey, NJ.
UVLRx Therapeutics, Oldsmar, FL.
Ultralux UV Inc, Lansing MI.
Illumiheal & Petthera, Shoreline, WA.
MB Lasertherapy, Houston, TX.
ARRC LED, San Clemente, CA.
Varuna Biomedical Corp. Incline Village, NV.
Niraxx Light Therapeutics, Inc, Boston, MA.
Dr Hamblin has been a consultant for.
Lexington Int, Boca Raton, FL.
USHIO Corp, Japan.
Merck KGaA, Darmstadt, Germany.
Philips Electronics Nederland B.V.
Johnson & Johnson Inc, Philadelphia, PA.
Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany.
Dr Hamblin is a stockholder in.
Global Photon Inc, Bee Cave, TX.
Mitonix, Newark, DE.

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