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Review

Aberrant regulation of messenger RNA 3′-untranslated region in human cancer

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Abstract. The messenger RNA 3′-untranslated region (3′UTR) is emerging as critically important in regulating gene expression at posttranscriptional levels. The 3′UTR governs gene expression via orchestrated interactions between mRNA structural components (*cis*-elements) and specific *trans*-acting factors (RNA-binding proteins and non-coding RNAs). Alterations in any of these components can lead to disease. Here, we review the mutations in 3′UTR regulatory sequences as well as the aberrant levels, subcellular localization, and posttranslational modifications of *trans*-acting factors that can promote or enhance the malignant phenotype of cancer cells. A thorough understanding of these alterations and their impact upon 3′UTR-directed posttranscriptional gene regulation will uncover promising new targets for therapeutic intervention.

Keywords: Post-transcriptional gene regulation, RNA-binding proteins, non-coding RNAs, miRNAs, AU-rich elements (ARE), ribonomics, *cis*-elements, *trans*-acting factors

1. Introduction

Cancer genes are recognized by their altered gene expression and/or activity leading to an abnormal phenotype. These changes provide the cell with a competitive growth advantage that is realized *via* at least five cancer-cell phenotypes: enhanced cell division, resistance to apoptosis, maintenance of angiogenesis, invasion of tissues and metastasis, and evasion of antitumor immune responses [47]. Traditionally, only mutated genes have been considered as candidate cancer genes. However, clearly many more genes present altered gene expression in cancer cells than are mutated [92] and thus there is mounting interest in studying alterations in the numbers of chromosomes or parts of chromosomes [31], and aberrant epigenetic events [37]. In addition, alterations in posttranscriptional regulatory processes are increasingly recognized as contributing to abnormal levels of gene products in the absence of apparent mutations in growing types of cancers. Protein content in the cell is determined by several levels of control: gene dosage, gene transcription, posttranscriptional control of the mRNA, and regulated proteolysis. Posttranscriptional gene regulation is emerging as a fundamental and effective cellular tool to regulate gene expression. Posttranscriptional events comprise pre-mRNA processing, nucleo-cytoplasmic export, mRNA localization, mRNA stabilization and translational regulation [77] (Fig. 1). Due to the impact that each of these steps can have on gene expression, each of them is tightly regulated. The mechanisms underlying this regulation, still poorly understood, involve mRNA structural components (*cis* elements) and *trans*-acting factors [primarily RNA-binding proteins and non-coding RNAs (ncRNAs)]. Therefore, alterations in these processes can cause numerous pathologies including developmental defects, immunological disease, and neurodegeneration [7,73,79,101]. Abnormalities in posttranscriptional processes have been also described in cancer [4].

The posttranscriptional processes that affect the mRNA are regulated by the orchestrated interactions between mRNA structural components (*cis* elements) and specific trans-acting factors. Well-characterized RNA sequence elements can be found throughout the body of mRNAs including the 5′-untranslated region

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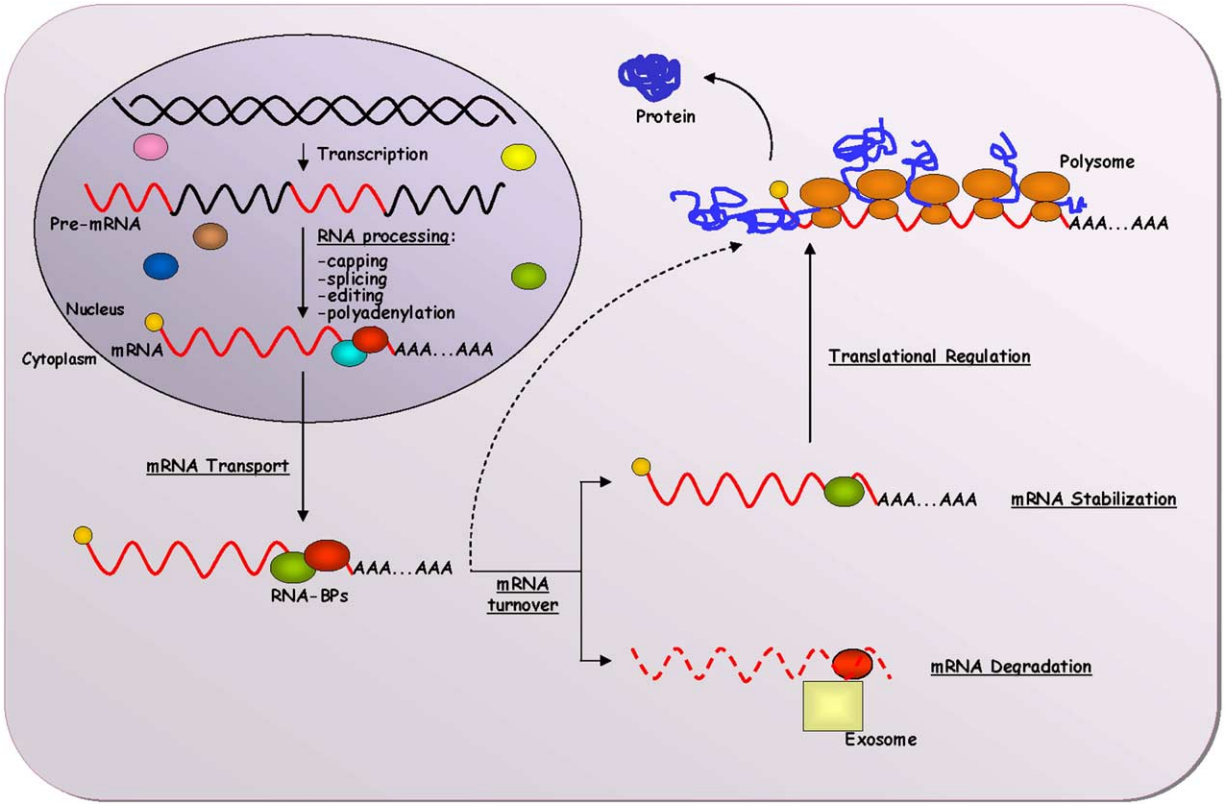

Fig. 1. Schematic of posttranscriptional gene regulation. During and after transcription, immature transcripts (pre-mRNA) are processed into the mature messenger (mRNA) within the nucleus. The maturation process consists of several steps including the addition of a 5′ cap and a 3′ poly-A tail, splicing to remove intronic regions and RNA editing. Then, the mature mRNA is transported into the cytoplasm where it is the subject of mRNA turnover processes (mRNA stabilization or mRNA degradation); if the mRNA remains undegraded, it is then recruited to the polysomes for translation.

(5′UTR), the coding region, and the 3′UTR (Fig. 2A). Most RNA sequence elements (e.g., the 5′-cap structure and the 3′ poly(A) tail) are universally present in all mRNAs and direct constitutive processes without apparent selectivity of one mRNA relative to another. However, specific RNA elements have been identified which affect the stability and/or translation of given subsets of mRNAs. A number of specific *cis* elements can be found in the three main parts of the transcript. In the 5′UTR, the iron-response elements (IREs), JNK-response elements (JRE) and turnover determinants such as that found in the KC mRNA. These elements govern the activity of *trans* factors which elicit influence on processes such as translation, turnover, storage and transport. In the coding region of mRNAs like c-fos, c-myc, and β-tubulin, mRNA decay elements such as the CRD-1 have been described. Within the 3′UTR, the most commonly found *cis* elements are turnover and translation determinants such as the AU-rich elements (AREs) and IREs. Cell cycle-regulated histone mRNA stem-loop determinants are also found in the 3′UTR of histone mRNAs. Interested readers are referred to an excellent review by Guhaniyogi and Brewer [46]. The vast majority of such specific RNA sequences are present in the 3′UTR; among them, the best characterized are regions rich in adenine and uridine residues known as AU-rich elements (ARE). mRNAs encoding oncogenes, cytokines, different interleukins, TNF-α, and cell-cycle regulators such as c-fos, c-myc, and cyclins A, B1 and D1 contain ARE-elements in their 3′UTR. Many of them are overexpressed in cancer during cellular transformation due to mRNA stabilization processes or enhanced translation. It is crucial, therefore, to understand the regulation of these ‘cancer genes’ *via* these elements because of their demonstrated involvement in cancer. Other elements that can be found in the 3′UTR are the iron response element (IRE) and specific secondary structures like stem loops present in the mRNAs of certain types of histones [46] (Fig. 2A). Together, these

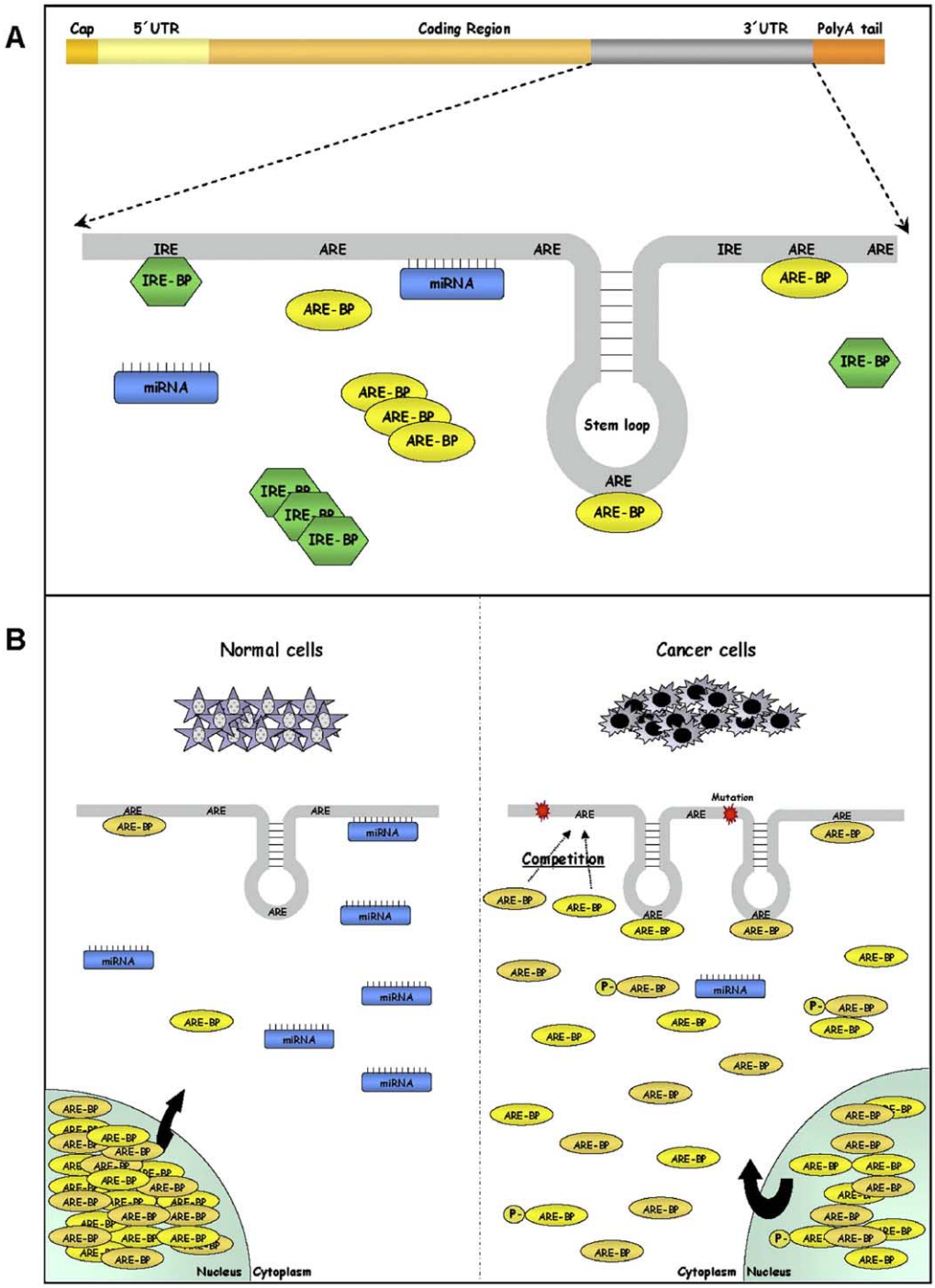

Fig. 2. 3′UTR determinants and their alterations in cancer. (A) Schematic of the different regions in the mRNA. The 3′UTR is enlarged to show the *cis*-elements (AREs, IREs and secondary structures) that are found in this region and the *trans*-acting factors (RNA-binding proteins and non-coding RNAs) that associate to them. (B) In cancer cells, two main groups of aberrant 3′UTR regulation can be found: (1) Genetic alterations in *cis*-elements such as mutations, deletions, translocations and polymorphisms and (2) Altered levels of *trans*-acting factors (e.g. increase of ARE-BPs and decrease miRNAs levels), changes in their subcellular localization (e.g. increased cytoplasmic levels of ARE-BPs) or their posttranslational modifications (e.g. phosphorylation of ARE-BPs). ARE-BPs: AU-rich binding proteins. miRNAs: microRNAs. ARE: AU-rich elements.

elements serve as binding sites for a variety of RNA-binding proteins that modulate the stability and the efficiency of translation. We will pay special attention to the family of RNA-binding proteins that associate to the ARE-determinants (ARE-BPs). While ARE-BPs regulate numerous posttranscriptional aspects of the mRNA (such as splicing, mRNA localization, and mRNA storage), this review will focus on the literature describing their influence on mRNA stability and translation [6,22,42,54] (Fig. 2A and Table 1).

In recent years, a distinct class of novel regulators is receiving increasing attention. They are RNA molecules known as non-coding RNAs (ncRNAs) that function directly as structural, catalytic or regulatory RNAs, rather than expressing mRNAs that encode proteins (Fig. 2A). Although many different subclasses of ncRNAs have been described, we will focus here on microRNAs (miRNAs), small RNA molecules that associate with the 3′UTR of target mRNAs and negatively regulate their expression at the posttranscriptional level by promoting mRNA degradation or translational repression [48]. Deregulation of miRNA levels is a frequent occurrence in diverse types of cancers where they are expressed at significantly different levels in tumors compared with normal tissues [69]. We will also address the contribution of other longer ncRNAs such as antisense transcripts (only those spanning the 3′UTR region) and Riboregulators (3′UTR subfragments that act in *trans*) to cancer progression [20,71].

Deregulation of any of the aforementioned elements/factors can be an underlying cause for the acquisition or enhancement of malignant phenotypes in the cell. Therefore, the purpose of this review is to examine, by using specific examples, the altered 3′UTR gene regulation that can be found in the cancer process. Identifying such alterations and studying how they modify cancer cell biology will help to better understand the mechanisms involved in tumorigenesis and will facilitate the development of novel therapeutic modalities.

2. Defective 3′UTR regulation during cancer

Given the aforementioned involvement of 3′UTR elements and *trans*-acting factors (Fig. 2A) in dictating mRNA stability and translation, it is easy to envision how alterations in any of these components can have a major impact on mRNA half-life and/or translation. In turn, defective mRNA turnover can cause abnormal stabilization or decay of mRNAs, while disregulated translation can elevate or lower translation rates. Together, these anomalous processes will result in aberrant levels of expressed protein and hence metabolic changes leading to disease. Defective mRNA half-life and translation can arise from (1) Mutations in regulatory *cis*-elements, and (2) Aberrant expression and/or subcellular localization of *trans*-acting factors (RNA-binding proteins and ncRNAs) (Fig. 2B). In cancer, alterations in both *cis*-elements and *trans*-acting factors have been described. In the next sections, specifics examples will be given to illustrate both aspects.

2.1. Mutations in 3′UTR cis-regulatory elements

To-date the best characterized 3′UTR *cis*-element are the adenine- and uridine-rich elements (AREs). AREs frequently, though not always, contain a variable number of AUUUA pentamers, sometimes harbored within a U-rich region. An attempt to classify different types of AREs has been reported [5,24] but the canonical ARE structures recognized by each individual *trans*-factors remain largely unknown. The canonical motifs identified for RNA-biding proteins HuR and TIA-1 [67,68] consist of a combination of primary sequence and secondary structure in the 3′UTR. AREs are well known to influence stability and are increasingly recognized to affect translation [35, 46]. AREs are bound by a group of proteins known as ARE-binding proteins (ARE-BP). Depending on which RNA-binding protein is associated, the mRNA is the subject of stabilization (as described for HuR), degradation (AUF1, TTP), enhanced translation (HuR) or reduced translation (TIA-1, TIAR). Sometimes, several ARE-BPs can bind to different areas within the same transcript or compete for the same binding site. The final fate of the mRNA depends on the target mRNA itself, the RNA-binding protein abundance, the subcellular localization of the ribonucleoprotein complex and the cellular environment. A compilation of the major ARE-BPs and their functions can be found in Table 1.

Other regulatory determinants can be found in the 3′UTR region include the IRE and stem-loop motifs. The IRE consists of a stem-loop structure with a 23- to 27-bp stem with a mismatched C and a 6-nucleotide loop with a C at its 5′end. The 5′UTR of the transferring receptor (TfR) mRNA contains one IRE that regulates translation initiation and five IREs in the 3′UTR that modulate the stability of the TfR mRNA. Two multifunctional IRE-binding proteins have been de-

Table 1
List of AU-rich binding proteins (AU-BPs)

RNA-binding protein	Official name	Gene Symbol & (Ref Seq)	Subcellular localization	RNA-binding domain	Function	Target mRNAs (examples)
HuR	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	ELAVL1 (NM_001419)	Mainly nuclear (ubiquitous)	3 RRM	mRNA stabilization (1), translational enhancer (2)	c-fos (1), c-myc (1), p21(1), COX-2 (1), TNF-α (1), cyclin A, B1, D1 (1), iNOS(1), IL-3(1), MyoD(1), p53 (2)
HuB	ELAV (embryonic lethal, abnormal vision, Drosophila) like 2 (Hu antigen B)	ELAVL2 (NM_004432)	Mainly nuclear (neuronal and sex glands)	3 RRM	mRNA stabilization (1), translational enhancer (2)	GLUT1 (1 and 2), NF-M (2)
HuC	ELAV (embryonic lethal, abnormal vision, Drosophila) like 3 (Hu antigen C)	ELAVL3 (2 isoforms: NM_001420; NM_032281)	Mainly nuclear (neuronal)	3 RRM	mRNA stabilization	VEGF, c-myc
HuD	ELAV (embryonic lethal, abnormal vision, Drosophila) like 4 (Hu antigen D)	ELAVL4 (NM_021952)	Mainly nuclear (neuronal)	3 RRM	mRNA stabilization (1), translational enhancer (2)	GAP-43 (1), MARCKS (1), Msi-1 (1 and 2)
AUF1	heterogeneous nuclear ribonucleoprotein D	hnRNPD (4 isoforms: NM_031370; NM_031369; NM_002138; NM_001003810)	Isoforms p42 and p45 are nuclear; p37 and p40, nucleo-cytoplasmic	2 RRM	mRNA destabilizing	c-myc, GM-CSF, cyclin D1, GADD45, bcl-2, cyclin D1
TTP	Tristetraprolin	TTP (NM_003407)	Nuclear (20%) and cytoplasmic (80%)	2 C3H zinc fingers	mRNA destabilizing (1), decapping (2)	PAI-2 (1), TNF-α(1), COX-2 (1), GM-CSF (2)

Table 1
Continued

RNA-binding protein	Official name	Gene Symbol & (Ref Seq)	Subcellular localization	RNA-binding domain	Function	Target mRNAs (examples)
TIA-1	cytotoxic granule-associated RNA binding protein	TIA1 (NM_022173)	Mainly nuclear	3 RRM	Translational represor (1), alternative RNA processing (2)	COX-2 (1), TNF-α(1), Fos (2)
TIAR	TIA1 cytotoxic granule-associated RNA binding protein-like 1	TIAL1 (NM_003252)	Mainly nuclear	3 RRM	Translational represor (1), alternative RNA processing (2)	IL-8 (1), iNOS (1), β2-AR (1), GADD45 (1), calcitonin/CGRP (2)
KSRP	KH-type splicing regulatory protein	KHSRP (NM_003685)	Mostly nuclear	4 KH-domains	mRNA destabilizing (1), RNA splicing (2)	c-fos (1), c-jun (1), IL-2 (1), TNF-α (1), iNOS (2)
BRF1	zinc finger protein 36, C3H type-like 1	ZFP36L1 (NM_004926)	Nuclear and cytoplasmic	C3H zinc finger	mRNA destabilizing	cIAP2, IL-3
NF90	interleukin enhancer binding factor 3, 90 kDa	ILF3 (3 isoforms: NM_004516; NM_012218; NM_153464)	Mainly nuclear	2 dsRBM	mRNA stabilization (1), Translational represor (2)	IL-2 (1), acid beta-glucosidase (2)
CUG-BP2	CUG triplet repeat, RNA binding protein 2	CUGBP2 (3 isoforms: M_001025076; NM_001025077; NM_006561)	Mainly nuclear	3 RRM	RNA editing (1), mRNA stabilization (2) and translational silencer (3)	Apolipoprotein B (1), COX-2 (2 and 3)

Numbers in brackets of each individual target mRNAs refer to the matched function shown for each specific RNA-binding protein. RRM: RNA recognition motif. C3H zinc finger: Cys-Cys-Cys-His zinc finger. KH-domain: K-homology domain. dsRBM: double stranded RNA-binding motif.

scribed to bind to IRE regions: IRP1 and IRP2. They bind to TfR mRNA when the intracellular iron concentration is low, provoking the stabilization of the messenger and allowing iron uptake [90]. Stem-loops that regulate mRNA stability, as found in the 3′UTR of granulocyte-colony stimulating factor (G-CSF) and histone H4 mRNA, promote rapid deadenylation of the transcript and cell cycle-dependent decay, respectively [13,84,89].

Genetic alterations in 3′UTR sequences can modify the binding properties of *trans*-acting factors and lead to deregulation in protein production. Still, most studies have sought to identify mutations in the coding region and very few naturally occurring mutations in noncoding areas have been described to date. The examples below illustrate the role of AREs in causing aberrant levels of oncogenic proteins which are directly implicated in malignancy. The first example described was for the oncogene c-fos. C-fos dimerizes with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, it has been implicated as regulator of cell proliferation, differentiation, and transformation. The viral counterpart of c-fos, v-fos is the transforming gene of the FBJ-murine osteosarcoma retrovirus. The coding region of both c-fos and v-fos is identical differing only in a missing 67-bp (that contains a ARE) in the v-fos 3′UTR. Consequently, v-fos mRNA is more stable than c-fos mRNA and this may account in part for its higher oncogenic potential [75]. Human papillomavirus type 16 (HPV-16) DNA genomes are found integrated into the host chromosome of many cervical cancers. The 3′UTR early viral region of the HPV-16 encodes an ARE that causes instability of transcripts encoding oncogenes E6 and E7. During the integration of HPV-16 genome, an increased steady-state levels was observed for E6 and E7 mRNAs. This was due to a disrupted integration of the 3′UTR of the viral early region. Therefore, the integration of HPV-16 DNA can result in the increased expression of the viral E6 and E7 oncogenes through altered mRNA stability and lead to malignant transformation [56]. Another gene that presents altered regulation by 3′UTR elements during cancer is Cyclin D1. Cyclin D1 forms a complex with and functions as a regulatory subunit of CDK4 and CDK6, whose activity is required for cell cycle G1/S transition. This protein has been shown to interact with the tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb. Mutations, amplifications and overexpression of cyclin D1 alter cell cycle progression, have been observed in a variety of tumors and contribute to tumorigenesis. The cyclin D1 3′UTR is rearranged in patients with mantle cell lymphomas or truncated in the human cancer cell line MBD MB-453 [64,87]. In both cases, missing regions of cyclin D1 3′UTR caused increased mRNA stability compared to the full length mRNA. This deregulation perturbs the G1/S transition of the cell cycle and thereby contributes to tumor development. HER-2/neu is a potent oncogene that predicts poor outcome when overexpressed in ovarian cancer. In addition to the well-characterized 4.5-kb HER-2 transcript, a longer 8-kb transcript was found in the SKOV-3 ovarian carcinoma cell line, one of the only models for HER-2-driven ovarian cancer. Doherty et al., found that the 8-kb transcript had a half-life of 13 h compared to the 5.5 h for the 4.5-kb transcript. The increased stability of the longer transcript may confer a selective advantage for SKOV-3 cells by providing enhanced HER-2 expression [30]. C-myc is a proto-oncogene involved in the control of cellular proliferation, differentiation and apoptosis. Like many other early-response genes, c-myc expression is largely controlled at the level of mRNA stability. The sequences responsible for the c-myc mRNA short half-life are localized in a region of 140-bp in the 3′UTR [14,57], although other stability determinants have been found in the 5′end of the transcript [85] and coding regions [8]. C-myc mRNA is constitutively stabilized in both a human plasma cell myeloma and a derivative cell line with a 3′UTR translocation [51] and in a human T-cell leukaemia line missing a 61-nt ARE [1], suggesting that loss of this region stabilizes c-myc mRNA in cancer cells. Nonetheless, other studies show that artificially-generated deletions of the c-myc 3′UTR do not affect its mRNA steady state level [61,63].

The half-life of cancer-related, ARE-bearing mRNAs is not only influenced by large deletions or insertions, 3′UTR sequences as short as 1 base in length can also influence ARE-driven processes. For instance, three polymorphisms in the thymidylate synthase (TYMS) gene have been shown to influence TYMS expression, one of them present in the 3′UTR. TYMS catalyses the conversion of deoxy-uridylate to deoxy-thymidylate which is essential for DNA synthesis. As the sole *de novo* source of thymidylate in the cell, TYMS is an important target for chemotherapy drugs, such as 5-fluorouracil (5FU), methotrexate and other novel folate-based drugs. Over-expression of TYMS is linked to resistance to TYMS-targeted chemotherapy drugs. The polymorphism in the 3′UTR consists of the deletion (D)/insertion (I) of a 6-bp

stretch (TTAAAG), 447 bp downstream from the stop codon. This polymorphism varies greatly within different ethnic populations being 41% in non-Hispanic whites, 26% in Hispanic whites, 52% in African-Americans and 76% in Singapore Chinese. The D allele showed decreased message stability compared to the I allele due to increased binding to decay-promoting protein AUF1 [83]. In agreement with this finding, colorectal tumors from D-allele carriers have decreased intratumoral TYMS mRNA levels [70] suggesting that the 3′UTR polymorphism can have an impact on the efficiency of TYMS-targeted chemotherapy treatment. Another relevant example is a novel single-nucleotide polymorphism (SNP) in the human dihydrofolate reductase (DHFR) gene. The 829C → T polymorphism in the 3′UTR of the DHFR was identified in Japanese patients with childhood leukaemias/lymphomas. This enzyme catalyzes the reduction of dihydrofolate to tetrahydrofolate, which rejoins the pool of active folate cofactors. It is one of the most commonly used drug targets in the treatment of many different types of cancer. Although no significant differences were found in genotype frequencies between cases and controls, a higher expression of the DHFR transcript was demonstrated in samples with DHFR 829T/T polymorphism [44], suggesting that the DHFR 829 polymorphism influences mRNA expression levels. In another example, Ruggiero and colleagues identified a 1-bp deletion in a microsatellite region embedded in the long 3′UTR of CEACAM1 gene, which encodes a protein that is thought to be involved in tumor onset and progression. The authors showed that the wild-type but not the mutated CEACAM1 3′UTR greatly decreased transgene expression at both the mRNA and protein levels [91], reinforcing the idea that a single mutation in a 3′UTR might strongly affect gene expression.

2.2. *Trans-acting factors*

The regulation of mRNA stability and translation via the *cis*-elements mentioned above requires their interaction with *trans*-acting factors, which in turn target the mRNA for rapid degradation or protect it from nuclease access and can regulate translational efficiency. Two types of trans-acting factors are known to regulate the transcript half-life via its 3′UTR: specific RNA-binding proteins and non-coding RNAs (ncRNAs). *Trans*-factors frequently share target mRNAs, can bind cooperatively or competitively, and are capable of influencing stability and translation both positively and negatively. Their net influence upon the mRNA will ultimately dictate the levels of expressed protein. Within this complex regulatory paradigm, several *trans*-acting factors, both RNA-binding proteins and ncRNAs, are emerging as pivotal regulators of the expression of cancer-associated genes [9,62].

2.2.1. *Altered regulation of RNA-binding proteins in cancer*

In the human genome there are at least 500 proteins containing known RNA-binding domains [3]. Among them, the well-characterized family of RNA-binding proteins that bind to ARE-determinants (ARE-BPs) is the interest for this review. ARE-BPs regulate the stability and translation of mRNAs encoding critical cell-cycle regulatory proteins, oncoproteins, tumor suppressors, and cytokines. At least 14 ARE-BPs (see Table 1) have been identified so far. As detailed below, different types of alterations on ARE-BPs have been described in cancer: (1) changes in the levels of ARE-BPs, (2) abnormal subcellular localization, (3) altered pattern of posttranslational modifications, and (4) aberrant competition among ARE-BPs which will ultimately influence their net influence upon the fate of the mRNA (stabilisation, translation).

Generally speaking, the levels of RNA-binding proteins are frequently elevated in cancer. One of the most extensively studied RNA-binding proteins in cancer is HuR, a member of the embryonic abnormal vision (ELAV)/Hu family. ELAV/Hu proteins were initially identified as specific tumor antigens in cancers of individuals with paraneoplastic neurological disorder [26,98]. The ubiquitously expressed HuR was subsequently found to regulate the expression of labile mRNAs bearing AU- and U-rich sequences by enhancing their stability, translation or both processes [12]. Different studies have linked an active role of HuR in different types of cancer. Tissue array analysis revealed that the abundance of HuR protein was significantly greater in malignant tumors than in benign tumors or normal tissues [10,67]. In the same study, subcutaneously injection of HuR-overexpressing RKO colon cancer cells into nude mice produced significantly larger tumors than those arising from control populations, whereas RKO cells expressing reduced HuR gave rise to significantly smaller and slower-growing tumors [67]. Tumor suppression can be achieved by interfering with mRNA turnover as the next example illustrates. Tristetraprolin (TTP), an mRNA-binding protein that promotes mRNA decay, acts as a potent tumor suppressor in a v-H-ras-dependent mast cell tumor model in which cells express abnormally stable

interleukin-3 (IL-3) mRNA as part of an oncogenic autocrine loop. TTP obstructs this autocrine loop by enhancing the degradation of IL-3 mRNA and, therefore, reducing the secretion of IL-3 [95]. AUF1, also known as heterogenous nuclear ribonucleoprotein (hnRNP) D, is another RNA-binding protein involved in mRNA degradation which has been directly linked to cancer. AUF1 is a family of four isoforms with both nuclear and cytoplasmic functions. AUF1 also binds to ARE-elements contained in the 3′UTRs of many short-lived mRNAs. Transgenic mice which overexpress one isoform of AUF1, p37(AUF1), exhibit altered levels of expression of several targets mRNAs, such as c-myc, c-jun, cjun, c-fos, granulocyte macrophage colony-stimulated factor, and tumor necrosis factor alpha. Moreover, the transgenic line with the highest amount of the p37(AUF1) developed sarcomas and expressed high levels of cyclin D1 [45]. Together, these studies suggest that ARE-BPs play a central role in cancer by binding to mRNAs encoding proteins involved in malignant transformation, and inducing or repressing their expression by altering mRNA stability and/or translation rates.

Most ARE-BPs, including HuR, AUF1, and TIA-1 are predominantly nuclear proteins that shuttle between the nucleus and the cytoplasm. Their cytoplasmic presence appears to be intimately linked to their influence upon target mRNAs and hence ARE-BP localization has been the subject of cancer-related gene expression studies. For instance, immunohistochemical analysis on tissue arrays shows that the expression and cytoplasmic abundance of HuR increased with malignancy, particularly in colon carcinomas [67]. Furthermore, it seems to be associated with a poor histologic differentiation, large tumor size, and poor survival in ductal breast carcinoma [50]. In different types of cancer, overproduction of interleukin-10 (IL-10), a cytokine which inhibits both immune surveillance and tumor rejection, resulted from increased half-life of IL-10 mRNA due to reduced binding of the destabilizing protein AUF1. In this regard, cytoplasmic extracts of normal melanocytes possessed higher AUF1 levels than those from MNT1 melanoma cells, where AUF1 appeared to be restricted to the nuclear fraction [15]. Conversely, there was a strong correlation between increased cytoplasmic expression of both AUF1 and HuR with urethane-induced neoplasia and with butylated hydroxytoluene-induced compensatory hyperplasia in mouse lung tissue [10]. Importantly, activation of the Wnt/β-catenin pathway can trigger profound changes in mRNA turnover by altering the subcellular localization of ARE-BPs. Wnt activation induced changes in the cytoplasmic distribution of KSRP, TTP, and HuR leading to changes in Pitx2 mRNA-ARE BPs interactions that, in turn could be responsible for Pitx2 mRNA stabilization in pituitary cells; this finding was particularly relevant given that sustained *in vivo* overexpression of Pitx2 caused hyperplasia in the anterior pituitary [17]. These data indicate that the subcellular location of the RNA-binding proteins can have important consequences on cancer phenotypes. What triggers the ARE-BP nucleo-cytoplasmic shuttling? It was shown, for example, that the tumor suppressor protein von Hippel Lindau (VHL) enhanced the translation of p53 by promoting the cytoplasmic abundance of HuR and consequently the association of p53 mRNA with polysomes [41]. Elevated activity of the AMP-activated protein kinase (AMPK), an enzyme that participates in the cellular response to metabolic stresses and inhibits cell growth, was found to modulate the nucleo-cytoplasmic shuttling of HuR by reducing its cytoplasmic abundance [103].

Posttranslational modifications of RNA-binding proteins can affect their ability to bind to target mRNAs as well as their subcellular location. For instance, TPA treatment of THP-1 monocytic leukaemia cells showed translational modifications of the major cytoplasmic isoform, p40AUF1, concomitant with changes in RNA-binding activity and stabilization of ARE-containing mRNAs encoding IL-1 beta and tumor necrosis factor alpha. P40AUF1 recovered from polysomes was phosphorylated on Ser83 and Ser87 in untreated cells but lost these modifications following TPA treatment [104]. PMA treatment also causes the destabilization of the sarco(endo)plasmic reticulum calcium ATPase 2a (SERCA2a) mRNA by increasing its binding of AUF1. The binding activity was predominantly found in the nuclear fraction and was associated with increased threonine phosphorylation of AUF1 [11]. KSRP promotes rapid mRNA decay by recruiting the degradation machinery to ARE-containing mRNAs. KSRP undergoes p38-dependent phosphorylation, which in turn compromises the binding of KSRP to its ARE-containing targets and fails to promote their rapid decay, although it retains the ability to interact with the mRNA degradation machinery [16]. Cell signaling events may also alter mRNA stability, translation and ARE-BP abundance. For instance, the MAPK pathway affects mRNA stability and translations through the differential phosphorylation of RNA-binding proteins [16,96]. Also, JNK activation alters the pattern of expression of protein phosphatase PP2A.

The loss of protein abundance of the PP2A subunit B56alpha is accompanied by a decrease of mRNA levels and a 4-fold increase of the RNA-binding protein AUF1 [43]. These studies provide evidence of the critical role that posttranslational modifications of ARE-BPs or the activation of signaling pathways have in understanding the alterations in gene expression during cancer.

Increasing evidence supports the notion that several RNA-binding proteins can bind to a common ARE-containing target mRNA on both distict, nonoverlapping sites, and on common sites in a competitive fashion. Thus, HuR and AUF1 were found together in the nucleus within stable ribonucleoprotein complexes whereas in the cytoplasm HuR and AUF1 bound target mRNAs individually, HuR colocalizing with the translational apparatus and AUF1 with the exosome-bound fraction [62]. This study suggests that the composition and fate (stability, translation) of ribonucleoprotein complexes depend on the target mRNA of interest, RNA-binding protein abundance, stress conditions, and subcellular compartment. For instance, IL-8 plays an integral role in promoting the malignant phenotype in breast cancer and its production is directly influenced by inflammatory cytokines in the tumor microenvironment. In keeping with this notion, activation of the IL-1 receptor on malignant breast cancer cells strongly induced IL-8 mRNA levels. HuR, KSRP and TIAR were found to bind one or more locations within the IL-8 3′UTR although the association of the stabilizing factor HuR was 20-fold greater than that of the destabilizing factor KSRP [97].

The posttranscriptional regulation of cyclooxygenase 2 (COX-2) expression has received much attention given its elevated levels in cancer. Many different ARE-BPs have been implicated in its regulation in response to mitogen stimulation. Together with Cox-1 (the constitutively expressed isoenzyme), Cox-2 catalyzes the conversion of arachidonic acid to prostaglandin H_2. The levels of COX-2 are increased in human colorectal adenocarcinomas as well as in other types of cancers. There are two major transcripts of COX-2, the full-length mRNA and a short polyadenylation variant lacking part of the 3′UTR. TTP binds to the 3′UTR of the full-length COX-2 mRNA reducing its transcripts levels whereas the truncated transcript is refractory to TTP binding and TTP-mediated down-regulation. This polyadenylated variant is prominent in a colon cancer cell line [93]. Besides the destabilizing RNA-binding protein TTP, the translational repressor TIA-1 also binds the COX-2 mRNA. TIA-1 null fibroblasts produced significantly more COX-2 protein than wild-type fibroblasts and this was not accompanied by differences in transcript levels, supporting the view that TIA-1 contributed to maintaining low levels of COX-2 expression by reducing protein production [29]. HuR also binds to the COX-2 mRNA, and HuR cytoplasmic presence is critically linked to the elevated levels of COX-2 in different type of cancers including ovarian, breast, and gastric malignancies [28, 33,34,78].

2.2.2. Alterations of non-coding RNA in cancer

Although it was generally assumed that most of the genetic information is transcribed into protein-coding mRNA, recent evidence suggests that a significant proportion of transcripts in complex organisms, including mammals, are non-coding RNAs (ncRNAs). ncRNAs include small regulatory RNAs (e.g. miRNAs) as well as longer transcripts. It is increasingly apparent that ncRNAs are potent and versatile regulators of gene expression both in *cis* and in *trans*, affecting a wide repertoire of biological functions [72]. Altered expression of certain ncRNAs has been associated with some forms of cancer. Although ncRNAs participate in a wide range of functions, including the control of chromosome remodeling, splicing, RNA editing, translational inhibition and mRNA destruction, through their association to different DNA and RNA regions, we will focus on those ncRNAs that affect the regulation of the 3′UTR mRNA region and are altered in cancer. We will first provide examples of altered expression of miRNAs found in many types of cancer and later we will discuss the contribution of longer RNAs such as antisense transcripts and Riboregulators in the tumoral process.

MicroRNAs (miRNA) are a family of small ncRNAs that negatively regulate gene expression at the posttranscriptional level. It is estimated that approximately one-third of human protein-coding genes are controlled by miRNAs. The precise molecular mechanisms that underlie posttranscriptional repression by miRNA remain largely unknown. Translational inhibition by non-perfect base pairing to the 3′UTR of target genes seems to be the predominant mechanism by which miRNAs negatively regulate target mRNAs throughout the animal kingdom. Although most animal miRNAs repress target translation, one miRNA, miR-196, was found recently to direct mRNA cleavage of its target, Hoxb8 [106]. The translational fate of the target mRNA is guided by miRNAs but miRNAs also need the association of protein factors for translational repression to occur [82]. Moreover, miR-

NAs act in conjunction with RNA-binding proteins to be able to respond rapidly to specific cellular needs. Thus, a recent report shows that the translational repression of CAT-1 mRNA mediated by miR-122 is relieved after different stress conditions and is linked to the binding of the RNA-binding protein HuR to the 3′UTR of CAT-1. These events lead to the release of the CAT-1 mRNA from cytoplasmic P-bodies and its recruitment to polysomes [9]. Few details are as-yet available regarding the functional roles of miRNAs but a picture is emerging wherein miRNAs have the potential to regulate virtually all aspects of cell physiology and pathology. Deregulation of miRNA levels is a frequent occurrence in diverse types of cancer such as Burkitt's lymphoma [74], colorectal cancer [2], lung cancer [99], breast cancer [52], and glioblastoma [23]. Recently, Golub and co-workers found that half of the 217 mammalian miRNAs examined were expressed at significant lower levels in tumors compared with normal tissues [69]. Those miRNAs associated with cancer are called 'oncomirs'. Northern blot analysis and miRNA microarrays have been useful in determining tissue-specific 'signatures' of miRNA genes in humans. These miRNAs signatures are being used to classify cancers and to define miRNA markers that might predict favorable prognosis [69].

miRNAs are thought to function as both tumor suppressor genes and oncogenes. The first evidence that miRNAs could function as tumor suppressor was found in B-cell chronic lymphocytic leukaemia (B-CLL), where the most frequent chromosomal abnormality is the deletion of 13q14, the locus of miR-15a and miR-16-1, two miRNAs whose levels are frequently absent or downregulated in CLL patients [18] and is associated with prognostic factors and disease progression in CLL [19]. Interestingly, miR-15a and miR-16-1 negatively regulate BCL2, an anti-apoptotic gene that is often overexpressed in many type of cancers. The downregulation of miR-15a and miR-16-1 results in increased expression of BCL2, which in turn promotes leukaemogenesis and lymphomagenesis in haematopoietic cells [21]. The cluster miR-17-92 exemplifies miRNAs functioning as oncogenes. The 13q31 locus is preferentially amplified in different types of lymphomas. However, the only gene that has been found to be upregulated within this region is the ncRNA, *C13orf25*, which encodes the mir-17-92 cluster comprising seven miRNAs: miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19-b1, and miR92-1. He and colleagues showed that five members of the miR-17-92 cluster had increased expression on those cell lines carrying an amplification of 13q31 [49]. Furthermore, the miR-17-92 cluster functions cooperatively with MYC since overexpression of mir-19b-1 (the vertebrate-specific portion of the mir-17-92 cluster) and MYC in haematopoietic cells injected in mice develop malignant lymphomas faster than those animals that received cells expressing MYC alone [49]. Further studies identified the 3′UTR of the transcription factor E2F1 as the target region of the MYC-regulated mir-17-92 cluster and show that MYC induces the transcription of both the mir-17-92 cluster and the transcription factor E2F1, and, in turn, these miRNAs cluster negatively regulated the translation of E2F1 [81]. Therefore, the mir-17-92 cluster might also function as a tumor suppressor, in contrast to the findings of He and colleagues, underscoring the intricacies of miRNA-mediated gene regulation. Further examples about miRNAs as tumor-suppressor genes and/or oncogenes are described in a recent comprehensive review [36].

Regarding the contribution of longer transcripts spanning the 3′UTR, the expression of bcl-2 in human follicular lymphoma t(14;18) cell lines serves to illustrate the regulatory influence of antisense transcripts. The fusion of the bcl-2 gene with the IgH locus creating a bcl-2/IgH hybrid gene characterizes this type of lymphomas. A bcl-2 antisense transcript was identified which encompasses the t(14;18) fusion site and spans the complete 3′UTR region of the bcl-2 mRNA bcl-2/IgH fusion. This antisense transcript is thought to mediate the upregulation of bcl-2 mRNA levels by masking AU-rich motifs present in the 3′UTR of the bcl-2 mRNA. The resulting overproduction of antiapoptotic bcl-2 protein contributes to the neoplastic transformation of follicular B cell Lymphoma [20, 94]. Functional and mutational approaches have identified a distinct class of novel RNA regulatory molecules called *Riboregulators*. Whether they can be considered as ncRNAs remains in question. Riboregulators consist in large 3′UTR subfragments that act in *trans* controlling key processes such as cell proliferation, differentiation and tumor suppression. This was the case for *prohibitin*, a protein which, in association with retinoblastoma protein, induces growth suppression and repress E2F-mediated transcription [102]. Not only does the protein prohibitin possess antiproliferative activity but the 3′UTR of the encoding mRNA (in the absence of the coding region) also has an antiproliferative activity, as described in normal fibroblast and in cancer cell lines. A search for mutations that affect the non-coding areas identified the prohibitin 3′UTR in

a number of human cancer cell lines; in this investigation, cell cycle progression was shown to be inhibited by the introduction of prohibitin 3′UTR [59]. One of the mutations was identified as a germ-line polymorphism with potential clinical significance [58]. Later on, it was demonstrated that this RNA molecule functions as a tumor suppressor in human breast cancer [71]. Other riboregulators have been described in the 3′UTR of α-tropomyosin and were likewise found to regulate cellular growth and differentiation [86], and in the 3′UTR of ribonucleotide reductase (a key rate-limiting enzyme in DNA synthesis) which also functions as a suppressor of tumorigenic and metastatic phenotypes in cancer cells [38,39]. The precise mechanism by which these *riboregulators* exert their biological function is not clear and seems to be dependent on the type of RNA molecule. The α-tropomyosin riboregulator binds to and thereby activates a protein kinase that causes inhibition of translation, whereas other riboregulators bind to promoter regions resulting in repression of gene transcription or activate RNA-dependent protein kinase (PKR) [27,88].

3. Discussion and future perspectives

Deregulation of gene expression is a common hallmark in cancer. The above studies emphasize the notion that control exerted via the 3′UTR mRNA is crucial for the correct regulation of gene expression and that alterations in this control can lead to cancer. Much more work is needed to dissect the molecular mechanism and signal transduction pathways responsible for the deregulation of the 3′UTR in cancer since, in the past, virtually all efforts were focused on the coding region. Additionally, the optimization and development of new methods *in vitro* and *in vivo* to assess the interaction between *cis*-elements and t*rans*-acting factors as well as their kinetics will shed light on the complexity that surrounds gene regulation in cancer. The intricate regulatory events that accompany cancer-associated gene expression are well illustrated by studies in which HuR levels were modulated. We assessed, by using cDNA array platforms, gene expression profiles in three systems of varying complexity: tumors with different HuR abundance, the colon cancer cells that generated the tumors and lysates from the same cells immunoprecipitated with HuR following isolation of mRNA targets. Comparison of the transcript sets identified in each system revealed a strikingly limited overlap in HuR-regulated mRNAs [66]. This report underscores also the broad usefulness of integrated approaches to comprehensively elucidate the gene regulatory events that underlie the cancer process.

To investigate the *in vitro* interaction of RNA-binding proteins and its mRNA targets on a transcript-by-transcript basis, several methodologies are available, such as biotin pulldown and RNA electrophoretic mobility shift assays (REMSA) [66,67]. More importantly, genome-wide *in vivo* binding assays were described by Tenenbaum and colleagues. This procedure is based on the immunoprecipitation of an endogenous RNA-binding protein using specific antibodies under conditions in which the interaction between the endogenous target mRNAs and the RNA-binding protein is maintained. Following the isolation of the target mRNAs, their identities are elucidated *en masse* after reverse transcription and hybridization of the resulting cDNAs using microarrays [100]. Determining possible functional linkages among the gene products of the mRNA targets identified by this method is the last and most challenging strategic goal of the *Ribonomic* approach [100]. It is also important to develop more precise *in vitro* and *in vivo* methods to assess the local RNA structures that will determine the binding kinetics of *trans*-acting factors. mRNA folding predictions can be made using a variety of algorithms, tested by nuclease mapping, and their thermodynamic stability quantitatively assessed *in vitro* using fluorescence resonance energy transfer (FRET) [40]. Given that *trans*-acting factors can compete for similar binding sites, being able to quantify the *in vivo* kinetics of binding and the competition events will help to gain knowledge about regulatory events implicating the 3′UTR. *In vitro* protein–RNA binding affinity can be quantified by fluorescence anisotropy and, by this means, it was shown that a hairpin-like structure within an AU-rich mRNA-destabilizing element regulated *trans*-factor binding selectivity and mRNA decay kinetics [40]. Additionally, abnormal messenger RNA decay in cancer could be detected by pharmacological inhibition of the translation machinery followed by microarray analysis [80,105].

For systematically identifying new ncRNAs, three sets of tools are available. First, computational comparative genome analysis that uses BLASTN screens, identification of regions than conserve some particular type of RNA structure, and ncRNA gene-finding programs. Second, cDNA cloning strategies followed by sequencing, a technology that clearly favors highly expressed ncRNAs. And third, the possibility of using high-density oligonucleotide microarrays [32,48].

More than 100 novel miRNAs were identified by cloning and sequencing endogenous small RNAs of 21–25 bp from worms, flies and mammals. In addition to the continued cloning efforts, novel miRNAs have been isolated through their association with polysomes and ribonucleoprotein complexes. In addition to experimental approaches, improved bioinformatic predictions have helped to identify novel miRNAs in various organisms, mostly on the basis of pre-miRNA hairpin structures and sequence conservation throughout evolution (see review [48]). Since miRNAs regulate multiple gene targets, the current challenge is to accurately identify the group of targets that are regulated by a given miRNA. Because incomplete complementarity also has biological relevance, simple BLAST searches do not provide sufficient information. However, current bioinformatics approaches have taken advantage of the fact that miRNAs within families have highest homology at the 5′end of the mature miRNA (first 2–8 bases). These studies have revealed that a single miRNA might bind to as many as 200 gene targets. Additionally, the use of antisense inhibition of human miRNA will help to functionally screen miRNA genes that specifically control cancer-related processes such as cell proliferation and apoptosis [25].

By gaining a more detailed knowledge of the 3′UTR regulatory sequences and the *trans*-acting factors specifically binding to them, it will be possible to design effective therapies. As noted earlier, a 6-bp polymorphism in the 3′UTR of thymidylate synthase decreases mRNA levels [70] and, thereby could be useful in predicting the efficacy of TYMS-targeted chemotherapy treatment. TNF-alpha is effective in the treatment of advanced solid tumors such as melanoma and soft tissue sarcoma. When analyzing mRNA levels of 22 genes in tumor biopsies from patients treated with doxorubicin alone or combined with TNF-alpha, TIA-1 was the only gene differentially expressed between the two groups. When TNF-alpha effects were tested *in vitro* in endothelial cells, fibroblasts, CTLs and NK cells, TIA-1 became upregulated only in endothelial and NK cells. These findings could indicate that TNF-alpha-induced TIA-1 overexpression might sensitize endothelial cells to proapoptotic stimuli present in the tumor microenvironment and enhance NK cell cytotoxic activity against cancer cells [76]. The chemotherapeutic agent Prostaglandin A_2 (PGA_2) causes growth arrest associated with decreased cyclin D1 in several cell lines. PGA_2 leads to the destabilization of cyclin D1 mRNA via a 3′UTR element that binds the ARE-BP AUF1 [65]. These studies also underscore the potential importance of understanding 3′UTR regulation in cancer therapy. Furthermore, a number of approaches that exploit RNA's structural dynamics and sequence-specific binding abilities (RNA interference, antisense RNA) are already in place to modulate gene expression. However, there is increasing need for developing synthetic riboregulators that can be integrated into biological networks to function with a wide array of genes and yield insights into RNA-based cellular processes. Isaacs and colleagues were able to engineer riboregulators that both repress and activate translation *in vivo*, enabling precise control of gene expression through highly specific RNA–RNA interactions [53]. Moreover, the administration of the above mentioned riboregulator, prohibitin RNA, effectively controlled tumor cellular proliferation *in vivo* and induced systemic antitumor immunity in this rat model [71]. The emergence of miRNAs as important players in cancer is likely to have a growing influence on the development of gene-based therapies. Synthetic antisense oligonucleotides that encode sequences complementary to mature oncogenic miRNAs – termed anti-miRNA oligonucleotides (AMOs) – are being designed with the specific goal of blocking tumor progression. To this end, antagomirs (AMOs conjugated with cholesterol) have been shown to be effective tools to inhibit miRNA activity in mice, and are being explored as therapeutic agents [60]. Viral or liposomal delivery tools for miRNAs that function as tumor-suppressor genes are also under study, although the immune response can limit the effectiveness of these RNA delivery methods [55].

In summary, in this review we have described the *cis*-elements and *trans*-acting factors known to date to regulate the mRNA 3′UTR and how their dysregulation can lead to cancer. Genetic alterations in *cis*-elements and abnormal levels and subcellular localization of *trans*-acting factor can profoundly impact on malignant development. A more detailed understanding of 3′UTR regulatory events through increased efforts directed towards the study of this region and the development of adequate analytical methods will aid in the design of novel chemotherapeutic venues.

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