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Factors interacting with HIF-1 α mRNA: novel therapeutic targets

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Abstract

The heterodimeric transcription factor HIF-1 (hypoxia-inducible factor-1) induces angiogenesis, a process that is aberrantly elevated in cancer. The HIF-1 β subunit is constitutively expressed, but the levels of the HIF-1 α subunit are robustly regulated, increasing under hypoxic conditions and decreasing in normoxia. These changes result from rapid alterations in the rates of HIF-1 α production and degradation. While the regulation of HIF-1 α degradation is understood in significant detail, much less is known about the regulation of HIF-1 α biosynthesis. Here, we review recent evidence that HIF-1 α production is effectively controlled by post-transcriptional mechanisms. We focus on the RNA-binding proteins (RBPs) and the non-coding RNAs that interact with the HIF-1 α mRNA and influence its half-life and translation rate. HIF-1 α mRNA-binding factors are emerging as promising pharmacological targets to control HIF-1 α production selectively and efficiently.

Keywords

post-transcriptional gene regulation; RNA-binding proteins; microRNA; antisense RNA; IRES

INTRODUCTION

Since the discovery of the link between vascularization and cancer progression, and the identification of the hypoxia-inducible factor (HIF) as a key player in this process [1,2], research into HIF function has progressed at a remarkable pace. HIF belongs to the highly conserved basic helix-loop-helix (bHLH) family of transcription factors and consists of two subunits, the oxygen-sensing α subunit and the constitutively expressed β subunit [3]. The complexity of HIF activity is apparent in its structure, with each subunit (α , β) consisting of 3 isoforms (HIF-1, -2, -3) with distinct functions [for an overview, see references 3 and 4].

HIF-1 signaling regulates the cell's response to low oxygen tension (typically <2% O₂) by activating over 100 genes encoding proteins involved in angiogenesis, cell proliferation, cell survival, and glucose metabolism [5]. The heightened levels of HIF-1 α in most cancers and its pivotal role in tumor angiogenesis, expansion, and metastasis, has made it an attractive candidate for targeted cancer therapy. Like other proteins with important cellular functions, the expression levels of HIF-1 α are highly regulated, with evidence that HIF-1 α abundance is controlled through transcriptional, post-transcriptional, and post-translational mechanisms. In order to develop rational HIF-1 α -directed therapies, we must first understand in detail each of these processes. Although this review will focus on the post-transcriptional events, we will describe briefly other levels of HIF-1 α regulation.

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Transcriptional regulation of HIF-1 α expression

Under both normoxia and hypoxia, HIF-1 α mRNA is expressed constitutively in many cell types, including cancer cells and endothelial cells [6-8]. Several putative sites for factors that activate and repress transcription were identified in the HIF1A gene promoter; some transcription factors, like Sp1, were shown to contribute to the constitutive transcription of the HIF-1 α mRNA [6]. Interestingly, several putative hypoxia response elements (HREs), the HIF *cis* acting elements, were identified in the HIF1A promoter, suggesting an autoregulatory loop whereby HIF itself may upregulate the synthesis of HIF-1 α mRNA [6]. Hypoxia and oxidative stress elevated HIF-1 α mRNA levels transcriptionally, likely through the NF- κ B and Egr1 transcription factors acting upon their respective sites on the HIF1A promoter [9,10]. The potential usefulness of HIF-1 α transcriptional regulators in drug development is covered elsewhere in this volume (XX, YY).

Post-transcriptional regulation of HIF-1 α expression

Between transcription of the pre-mRNA in the nucleus and translation of the mRNA by polyribosomes, transcripts can be subject to regulation at the levels of pre-mRNA splicing and maturation, as well as mRNA export to the cytoplasm and cytoplasmic transit, storage, stability, and translation [11]. These processes are governed by specific *cis* elements on the mRNA [in its 5'- and 3'-untranslated regions (UTRs), or in its coding region (CR)] that interact with *trans* binding factors [12]. There are two main classes of *trans* binding factors. One class comprises RNA-binding proteins (RBPs), including 'housekeeping' RBPs that associate universally with mRNAs and RBPs that bind to unique sequences present in subsets of mRNAs [13]. The other class comprises regulatory non-coding RNAs, including microRNAs (miRNAs) and antisense RNAs [14].

As described in the sections below, evidence is emerging that various *trans* binding factors associate various *cis* elements within the ~4 kb-long HIF-1 α mRNA and regulate its post-transcriptional fate (Table 1). For example, a HIF antisense (aHIF) transcript was shown to destabilize the HIF-1 α mRNA, while the RBP human antigen R (HuR) stabilized it [15,16]. However, HIF-1 α biosynthesis appears to be a more prominent mechanism to regulate HIF-1 α levels: by some estimates, translation is expected to account for 40-50% of the upregulation of HIF-1 α levels in response to hypoxia [17,18]. The RBPs HuR, polypyrimidine tract-binding protein (PTB), and cytoplasmic polyadenylation-element-binding protein (CPEB) enhanced HIF-1 α translation, while the miRNAs miR-17-92 and miR-199a repressed HIF-1 α translation [8,19-21].

Post-translational regulation of HIF-1 α expression

Despite mounting evidence of regulation through the HIF-1 α mRNA, the post-translational stabilization of HIF-1 α protein is the best-understood mechanism to increase HIF-1 α levels. Under normoxic conditions, the half-life of HIF-1 α protein is very short, approximately 5 min; its rapid degradation is accomplished by hydroxylation of HIF-1 α at conserved prolyl and asparagyl residues that are targeted for degradation by the von Hippel-Lindau protein (pVHL), the recognition factor of an E3 ubiquitin ligase complex [22,23]. During hypoxia, these hydroxylases are quickly inhibited, in turn triggering a rapid and robust stabilization of HIF-1 α [24].

Besides the control of protein stability, HIF-1 α function is regulated by processes that influence its subcellular localization (cytoplasmic vs nuclear), its dimerization, its ability to recognize DNA, and the interaction of HIF-1 α with transcriptional co-factors that modulate HIF-1 α function. The molecules that regulate these events are also the subject of active investigation as potential therapeutic targets; these processes are described in detail by (XX, YY) in this volume.

REGULATION of HIF-1 α mRNA STABILITY

Exposure to hypoxia stabilizes numerous transcripts, including the vascular endothelial growth factor (VEGF) mRNA, the erythropoietin (EPO) mRNA, the glucose transporter 1 (GLUT1) mRNA [25-27]. These transcripts have long 3'UTRs that are the targets of a family of turnover and translation regulatory (TTR)-RBPs [13]. TTR-RBPs recognize sequences that selectively modulate (stabilize or promote the decay of) the mRNA; some instability determinants are AU-rich sequences that often cluster in AUUUA pentamers [28,29]. The HIF-1 α 3'UTR is highly AU-rich and contains numerous AUUUA motifs, thus constituting a prime candidate for TTR-RBP-regulated mRNA turnover. Indeed, two TTR-RBPs have been described to bind the HIF-1 α mRNA: HuR (a member of the elav/hu family of TTR-RBPs) and PTB. In HeLa cells, HuR associated preferentially with the 5'UTR while in Jurkat cells it was shown to interact with the 3'UTR. The [HuR-HIF-1 α mRNA] ribonucleoprotein (RNP) complex increased in response to treatment with hypoxia, with the hypoxia mimetic CoCl₂ or with androgens [8, 16]. PTB associated with the 5'UTR in kidney cells and preferentially with the 3'UTR in HeLa cells [8,30]. Although HuR and PTB have been extensively implicated in altering the stability of target mRNAs [31,32], there is no evidence as yet that they influence the rate of HIF-1 α mRNA turnover. Moreover, to-date there are no reports linking either the AUUUA/AU-rich sequences or any TTR-RBP to the selective degradation of HIF-1 α mRNA under any condition.

HIF-1 α mRNA degradation: antisense HIF (aHIF)

Instead, HIF-1 α mRNA stability was found to be modulated by an antisense HIF (aHIF) transcript. With rising appreciation for the 'antisense transcriptome' and the development of novel techniques to investigate gene regulation by complementary RNAs [33], several naturally occurring antisense RNA molecules have been described which impact upon the turnover rate of specific mRNAs. Among the best understood antisense RNAs is aHIF. First discovered by Rossignol and coworkers, the aHIF transcript is complementary to the HIF-1 α 3'UTR [34]. Increased aHIF expression after prolonged hypoxia (12 h, 0.5% O₂) or aHIF overexpression triggered the decay of the HIF-1 α mRNA [15]. Interestingly, the expression of aHIF is controlled through a negative feedback mechanism, whereby HIF (including HIF-1 α or HIF-2 α) transcriptionally upregulates aHIF expression through the HRE present within the promoter region of aHIF [15,34]. In turn, increased expression of aHIF after prolonged hypoxia promoted HIF-1 α mRNA decay followed by a reduction in HIF-1 α protein synthesis [15]. The mechanism by which aHIF accelerates HIF-1 α decay has not been elucidated, but it is likely that the double-stranded RNA [aHIF-HIF-1 α 3'UTR] creates sites for the association of decay-promoting TTR-RBP or masks sites for the binding of stabilizing TTR-RBPs.

REGULATION OF HIF-1 α TRANSLATION

The past decade has witnessed much progress in our understanding of the translational control of HIF-1 α . Several regulatory factors have been identified that enhance or reduce HIF-1 α translation. Some participate in universal aspects of translation of cellular mRNAs, while others are specific for HIF-1 α . Although overall translation decreases rapidly and strongly in cells exposed to acute hypoxia (by 60-70% [35]), the translation of HIF-1 α mRNA is selectively upregulated. Both the increase and the decrease of HIF-1 α translation identified to-date is tightly controlled; furthermore, among the levels of translational control (initiation, elongation, termination), all of the regulation of HIF-1 α thus far is elicited by modulating the rate of translational initiation.

Induction of HIF-1 α translation

HIF-1 α translation can be induced under both normoxic and hypoxic conditions. During normoxia, HIF-1 α protein synthesis is largely regulated by *cap-dependent* signaling processes,

mediated mainly through the mammalian target of rapamycin (mTOR) and the mitogen activated protein kinase (MAPK) pathways. During hypoxia and under some normoxic conditions causing cellular damage, cap-dependent translation is inhibited and HIF-1 α translation instead relies on *cap-independent* mechanisms involving specialized HIF-1 α RNA sequences in its untranslated regions, such as the HIF-1 α IRES (internal ribosome entry site), as well as the RBPs (HuR, PTB, CPEB1, CPEB2) that interact with these RNA sequences and promote HIF-1 α translation [36].

Cap-dependent translation—Under normoxic growth conditions, HIF-1 α translation is tightly controlled to ensure low-level expression of HIF-1 α protein in all tissues. Cap-dependent regulation of translation relies on two main control points. The first control point is the assembly of the elongation initiation factor (eIF)4F, consisting of the cap-binding protein eIF4E, the scaffold protein eIF4G, and the RNA helicase eIF4A, at the m⁷GpppN ‘cap’ structure present in the 5′ terminus of the mRNA [37]. In turn, eIF4F promotes the second control point, the recruitment of the 43S preinitiation complex, comprised by the small (40S) ribosomal subunit and the ternary complex (eIF2, GTP, and Met-tRNA). In normoxia, growth factors, vascular hormones, iron chelators, oncoproteins, and cytokines can stimulate constitutive HIF-1 α translation through two main pathways [22,36,38]. One involves activation of the phosphatidylinositol-3 kinase (PI3K)→Akt→mTOR pathways, which controls the assembly of eIF4F at the cap structure by phosphorylating (and thus inactivating) eIF4E-binding proteins that inhibit translational initiation, and by phosphorylating (and thereby activating) S6 kinases (S6K1 and S6K2). Activation of the Ras→MEK→ERK^{MAPK} pathway also culminates in the activation of eIF4F and the stimulation of translation. The contribution of the ERK pathway is less well understood, but under normoxic conditions, activation of mTOR activation increased HIF-1 α translation and inhibition of mTOR potently reduced it [39,40]; moreover, suppressing mTOR inhibited tumor growth, at least in part by reducing tumor vascularization [41]. Although it is unclear if mTOR regulates HIF-1 α translation under low oxygen conditions, under severe hypoxia HIF-1 α partly represses the phosphorylation of ribosomal protein S6, which is believed to represent a negative feedback loop to reduce the cellular translation rate in order to maintain energy homeostasis [40].

IRES—IRESs are highly structured RNA sequences that serve sites for ribosome assembly in the middle of the mRNA, independently of the eIF4F cap-binding complex. Initially described in viruses, IRESs have been identified in the 5′UTRs of numerous cellular mRNAs, including HIF-1 α [30,42–44]. The HIF-1 α IRES was proposed to enhance HIF-1 α translation during hypoxia and serum starvation, two instances in which cap-dependent translation is strongly repressed. However, recent evidence indicates that the HIF-1 α IRES probably does not mediate the hypoxia-inducible translation, at least in several study systems, with cryptic promoters and changes in mRNA abundance accounting for the elevated translation [45,46]. However, other work that evaluated the potential contributions of cryptic promoter activity and changes in mRNA levels showed that the HIF-1 α IRES enhanced HIF-1 α translation constitutively, suggesting that the HIF-1 α IRES ensures a constant basal rate of HIF-1 α synthesis [8]. Further studies to investigate if the HIF-1 α IRES functions as an enhancer of translation during low oxygen tension or other stresses are warranted.

HuR—The ubiquitous member of the elav/Hu family of TTR-RBPs, HuR has been implicated in stabilizing and modulating the translation of target mRNAs bearing AU- and U-rich UTR sequences [31]. In prostate cancer (PC-3) cells, treatment with androgens enhanced HuR binding to the HIF-1 α 3′UTR and was proposed to facilitate its cytoplasmic export [16]. By contrast, in cervical carcinoma HeLa cells, HuR was shown to bind preferentially to the HIF-1 α 5′UTR [8]; in this cell system, exposure to hypoxia or CoCl₂ increased the association of endogenous HuR with endogenous HIF-1 α mRNA. In keeping with the positive influence

of HuR on the translation of other target mRNAs (e.g., PTMA, cytochrome c, MKP-1, p53 [47-50]), HuR was found to promote HIF-1 α translation, particularly in response to CoCl₂ treatment; under these conditions, HuR overexpression promoted HIF-1 α translation, while HuR silencing by using small interfering (si)RNA reduced it. Although the precise mechanisms by which HuR enhances the translation of HIF-1 α or other targets is unknown at present, HuR was shown to modulate the translation of other mRNAs bearing IRES (such as p27, IGF-IR, p53, CAT-1 [47-53]). This finding suggested that HuR binding to an mRNA, even at distal sites from the IRES, can contribute to masking or unmasking IRESs, in turn repressing or enhancing IRES-driven translation, as proposed by some investigators [31,53]. It is important to note, however, that during CoCl₂ stress, the HIF-1 α IRES did not elevate HIF-1 α translation beyond the levels observed in unstressed cells, regardless of the abundance of HuR [8]. By contrast, HuR did cooperate with another protein, PTB, in order to achieve maximal upregulation of HIF-1 α translation during hypoxia, as described below.

PTB—Schepens and coworkers first described that PTB binding to the polypyrimidine tract present in the 5'UTR of the HIF-1 α mRNA promoted its translation during hypoxic treatment of human embryonic kidney cells [30]. Based on these findings, the authors proposed that PTB functioned as an IRES-transacting factor (ITAF), possibly promoting the formation of an IRES that facilitated HIF-1 α translation during hypoxia [30] in a manner akin to the PTB-enhanced translation of p53 through the p53 IRES following exposure to genotoxic agents [54]. Later studies showed that PTB also associated with the endogenous HIF-1 α mRNA in HeLa cells, but bound the HIF-1 α 3'UTR preferentially [8]. Through this interaction, PTB promoted HIF-1 α translation after hypoxia or CoCl₂ treatment independently of IRES activity, but dependent on the presence of HuR. While PTB overexpression promoted HIF-1 α translation after CoCl₂ treatment, PTB binding to HIF-1 α mRNA was severely impaired when HuR was silenced. Conversely, after lowering PTB levels, both HuR binding to the HIF-1 α 3'UTR and HIF-1 α translation were dramatically lower. This collective evidence supports a model whereby PTB and HuR, likely binding on multiple sites of the HIF-1 α mRNA, jointly promote HIF-1 α translation [8,16,30].

CEPB1, CPEB2—Very recently, Hägele and coworkers (2009) showed that the cytoplasmic polyadenylation-element-binding protein (CPEB)1 and 2 were capable of associating with the HIF-1 α 3'UTR [21]. In unstimulated neuroblastoma cells CPEB repressed HIF-1 α translation, but treatment with insulin activated CPEB, elevated HIF-1 α expression levels, and promoted the translation of a heterologous reporter bearing the HIF-1 α 3'UTR. Silencing of CPEB1 or CPEB2 reversed the influence of insulin on HIF-1 α expression and on translation of the chimeric reporter. As further shown by the authors, HIF-1 α translation was upregulated by elongation of the poly(A) tail [21]. This report provides the first evidence that HIF-1 α mRNA translation increases in response to insulin levels.

Inhibition of HIF-1 α translation

HIF-1 α translation can also be repressed under normal and reduced oxygen levels. Several factors have been identified that can associate with the HIF-1 α mRNA and can potentially reduce its translation, including the RBP iron regulatory protein (IRP) and microRNAs.

IRPs—Iron response proteins can bind to target mRNAs bearing specialized stem-loop structures named iron-responsive elements (IREs). IRPs promote the decay of mRNAs with 3' UTR IREs and inhibit the translation of mRNAs with 5'UTR IREs in a cap-independent fashion [55]. Translation of HIF-2 α is strongly repressed by IRP1 and IRP2 acting through a 5'UTR IRE on the HIF-2 α mRNA; during hypoxia, IRP1 is released from the IRE and HIF-2 α protein synthesis is enhanced [56]. Although an IRE has also been identified in the 5'UTR of HIF-1 α [55,56], it has not been demonstrated as yet that IRPs inhibit HIF-1 α translation.

microRNAs—MicroRNAs are ~22-nt long non-coding RNAs that associate with cellular mRNAs and repress gene expression by reducing their half-life, inhibiting their translation, or performing both functions. The miR-17-92 cluster at 13q31.3 was recently shown to target the HIF-1 α mRNA and repressed its translation during normoxia [19]. c-Myc overexpression increased miR-17-92 abundance, which in turn lowered HIF-1 α translation. This inhibition was proposed to contribute to the reduction in HIF-1 α levels in cells with high c-Myc activity [19]. Very recently, miR-199a was found to repress HIF-1 α production under normoxic conditions; exposure to hypoxia rapidly reduced miR-199a levels, thereby relieving its inhibitory influence on HIF-1 α [20]. In addition, miR-519 represses HuR translation, which can impact on HIF-1 α levels [57], although it remains to be studied whether the levels of miR-519 change in response to hypoxia.

DRUG TARGETING OF HIF-1 α mRNA BINDING FACTORS

The rich regulation of HIF-1 α expression, through transcriptional, post-transcriptional, and post-translational events, underscores the importance of modulating the abundance of HIF-1 α both gradually and acutely. Among the growing number of factors that regulate HIF-1 α expression at different levels, the proteins and non-coding RNAs that interact with the HIF-1 α mRNA represent novel targets for highly specific and effective regulation of HIF-1 α production (Figure 1).

HuR Inhibitors

As a protein that stabilizes and modulates the translation of target mRNAs, HuR can elicit rapid and robust changes in protein expression. Therefore, there has been significant interest in identifying drugs that can inhibit HuR function. A recent high-throughput screen identified and characterized three drugs that inhibited HuR function: dehydromutactin, MS-444, and okicenone [58]. These drugs reportedly reduced HuR RNA-binding activity, HuR export to the cytoplasm, and the levels of proteins encoded by HuR target mRNAs (COX-2, IL-1 β , and IL-6); consequently, these drugs lowered T-cell activation [58]. It was not tested whether dehydromutactin, MS-444, or okicenone affected HIF-1 α expression – in T cells or in any other cell type. However, since silencing HuR potently suppressed HIF-1 α expression levels [8], testing the usefulness of these chemical inhibitors is warranted, particularly since at least one of the described inhibitors, MS-444, has relatively low toxicity in animal studies.

The levels and function of HuR can also be inhibited by siRNA-mediated methodologies, although the feasibility of this approach *in vivo* has not yet been tested for HuR. In addition, HuR expression was shown to be strongly repressed by an endogenous microRNA (miR-519) [57]. miR-519 represents a plausible therapeutic target, especially given that it also reduced cellular proliferation and was proposed to function as a tumor-suppressor microRNA [57]. Interventions to target HuR, whether it is through chemical inhibitors or inhibitory small RNAs, hold significant promise to lower HIF-1 α function, since HuR also promotes the expression of HIF-1 α -regulated genes such as VEGF and GLUT1, as well as numerous other cancer-related proteins [59-61].

Inhibition of IRES

The induction of HIF-1 α levels in response to CoCl₂ was potently suppressed by pateamine A [8]. An anti-proliferative, pro-apoptotic, and immunosuppressive compound, pateamine A can inhibit protein translation by preventing cap-dependent and eIF4G-dependent as well as IRES-driven translation at concentrations lower than 0.1 μ M [62-66]. Given the multiplicity of factors that contribute to cap-dependent and cap-independent translation of HIF-1 α , the search for repressors of HIF-1 α translation acting in ways similar to that of pateamine A is likely to increase.

Cap-dependent translational inhibitors

Numerous other drugs have been identified that can block HIF-1 α translation by inhibiting either general regulators of translation or factors as-yet unidentified. This group of compounds includes, e.g., inhibitors of the PI3K pathway (rapamycin, wortmannin, LY294002) and ERK inhibitors (PD98059), as reviewed [67]. Unfortunately, the low specificity of these compounds reduces their usefulness.

IRP1 Inhibitors

The screening of a library of small-molecule inhibitors that repressed HIF function in VHL-deficient cells led to the identification of four compounds that selectively repressed HIF-2 α translation in an mTOR-independent manner [56]. The drugs functioned by enhancing the interaction of IRP and the IRE on the HIF-2 α 5'UTR; since hypoxia caused the release of the IRP from the IRE and thus elevated HIF-2 α translation, the stabilization of IRE-IRP complexes by the four compounds led to a decrease in HIF-2 α protein production under hypoxia. These drugs were also found to reduce HIF-1 α expression; while they did not appear to influence IRP action upon the HIF-1 α IRE, the influence of IRP/IRE upon HIF-1 α translation merits further work.

PX-478

Originally identified in a screen of drugs that would lower HIF-1 α protein levels, the small-molecule inhibitor PX-478 was shown to reduce the transcriptional activity of HIF-1 α , in turn inhibiting angiogenesis and glycolysis. PX-478 was further shown to suppress HIF-1 α levels in multiple ways. Besides reducing HIF-1 α translation, PX-478 inhibited HIF-1 α deubiquitination and lowered HIF-1 α mRNA levels [68]. The translational repressors eIF2 α and 4E-BP appeared not to be affected; more work needs to be done to elucidate the exact mechanism of HIF-1 α by PX-478.

RNA Inhibitors: ASO, siRNA, shRNA, LNA

With deeper appreciation for the action of endogenous RNA molecules as modulators of gene expression, there is growing interest in using similar synthetic molecules to target cancer-related genes, and HIF-1 α in particular. Over the past decade, numerous studies have demonstrated the feasibility of small RNA-based techniques as therapeutic factors against proteins involved in tumor development and progression [69,70]. Because they complement precisely the target mRNAs, these molecules provide unique opportunities to silence the expression of cancer-causing proteins in a highly selective manner. ASOs (antisense oligonucleotides) are synthetic, single-stranded 12-22-nt long RNA molecules that effectively prevent the translation of target mRNAs and trigger their degradation, as reviewed in reference [71]. A similar inhibition of specific mRNAs can be achieved through a process called RNA interference (RNAi), triggered by the administration of synthetic double-stranded RNAs, 21-23 nt in length (*siRNAs* or small interfering RNAs), or longer silencing hairpin RNAs (*shRNAs*) that are processed intracellularly into mature siRNAs; RNAi is elicited by one of the strands of the siRNA, which complements exactly a sequence within the target mRNA. The design of ASOs, siRNAs, and shRNAs has advanced greatly, and modifications to increase their stability have markedly improved their efficacy. At the present time, the delivery of these small RNAs remains the greatest challenge before they can be considered for use in cancer therapy; methods such as siRNA administration to lung tissues via inhalers have been developed and are being assessed in clinical trials [72,73]. Analysis of the efficacy of HIF-1 α silencing by RNAi in a glioma model revealed that HIF-1 α knockdown slowed tumor progression, but this intervention was most effective when used in combination with other antitumor therapies [72]. Promising results have also been achieved with third-generation antisense oligonucleotides termed LNAs (locked nucleic acids). LNAs contain modified nucleotides with a methylene bridge

between the 2'-O and the 4'-C of the ribose which enhances base-pairing to mRNA, increases its stability, and retains optimal target recognition; they bind target mRNAs with very strong affinity and have exceptionally high tissue stability [74,75]. Use of EZN-2968, an LNA designed to target the HIF-1 α mRNA, significantly decreased HIF-1 α expression both in cultured cancerous cells and in animals [76]. After a single injection of EZN-2968, the levels of endogenous HIF-1 α and VEGF mRNAs were reduced for several days. Moreover, EZN-2968 was also highly effective in downregulating HIF-1 α levels and slowing down the growth of human prostate tumor xenografts in nude mice [76]. EZN-2968 is currently being evaluated in phase I clinical trials.

As mentioned above, a major hurdle to overcome before utilizing mRNA-directed RNA molecules for drug therapy is their delivery into the cells to be targeted. Liu et al 2008 [77] devised an elegant approach that involved the complexing of shRNA-expression vectors with transferrin to treat malignant melanoma, which is characterized by high expression levels of transferrin receptor. Transferrin receptor-mediated endocytosis of HIF-1 α shRNA led to the delivery and effective targeting of HIF-1 α in malignant melanoma cells and inhibited their growth in a xenograft model [77].

PERSPECTIVE

The past decade has seen remarkable advances in our understanding of gene regulation at the post-transcriptional level, particularly through changes in mRNA stability and translation. As we gain deeper knowledge of the factors (RBPs and non-coding RNAs) that interact with mRNAs to control these processes, their potential usefulness as therapeutic targets is also becoming increasingly apparent. With its prominent role in carcinogenesis, HIF-1 α has become a valued target of therapeutic development. As highlighted in this review, a growing number of proteins and non-coding RNAs have been identified that directly interact with the HIF-1 α transcript and modulate HIF-1 α biosynthesis (Table 1). Accordingly, the factors that bind the HIF-1 α mRNA represent promising opportunities for therapy. Interventions directed at the RNA-binding proteins that promote HIF-1 α mRNA stability and translation seem particularly beneficial, given their robust effect on HIF-1 α biosynthesis – and in some cases, on the synthesis of other cancer-related proteins. Interventions to deliver non-coding RNAs directed at the HIF-1 α mRNA hold unique potential because they are highly specific and effective. In sum, approaches that target the HIF-1 α mRNA augur an effective suppression of HIF-1 α production and angiogenesis, thus lending strong support to efforts aimed at preventing and halting malignant growth.

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Figure 1. Schematic of the HIF-1 α mRNA and binding factors

Blue line, coding region. The proteins and non-coding RNAs that promote or reduce HIF-1 α levels are indicated (details in box). The compounds that inhibit HIF-1 α mRNA-interacting factors are shown above the factors. The endogenous non-coding RNAs are depicted in italics. The '?' indicates that details of the inhibition of HIF-1 α by these compounds await further study.

Table 1
Factors that interact with the HIF-1 α mRNA

The table includes RNA-binding proteins (*top*) and non-coding RNAs (*bottom*), the HIF-1 α mRNA region(s) with which they interact, the post-transcriptional consequences of their association with the HIF-1 α mRNA, and the conditions under which the factors alter HIF-1 α mRNA stability and/or translation.

Factor	Binding site	influence on mRNA	Conditions of association	References
HIF-1α mRNA-binding Proteins				
HuR	5'UTR	Translation ($\uparrow$)	Hypoxia, CoCl ₂	[8]
	3'UTR	Stability ($\uparrow$)		[16]
PTB	5'UTR, 3'UTR	Translation ($\uparrow$)	Hypoxia, CoCl ₂	[8]
CPEB1/2	3'UTR	Translation ($\uparrow$)	Insulin	[21]
		Translation ($\downarrow$)	Normoxia	[21]
IRP1/2	5'UTR(?)	Translation ($\downarrow$)	Normoxia	[56]
HIF-1α mRNA-binding RNAs				
aHIF	3'UTR	Stability ($\downarrow$)	Prolonged hypoxia	[15]
miR-17-92	3'UTR	Translation ($\downarrow$)	Normoxia, low c-Myc	[19]
miR-199a	3'UTR	Translation ($\downarrow$)	Normoxia	[20]
EZN-296 (ASO)	CR	Stability ($\downarrow$)	Ectopic	[76]