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Perspective

Regulatory RNAs: Have mRNA Untranslated Regions Joined the Party?

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In the last decade or so, we have become familiar with the discovery of new classes of nuclear-encoded regulators (see Glossary) as fundamental controllers of gene expression. Each new discovery has again highlighted the incomplete nature of our understanding of the genome and its regulation. First, small interfering RNAs (siRNAs) demonstrated that RNA molecules are not merely components of the cellular machinery (such as tRNAs and rRNAs) or gene-expression intermediates (mRNAs), but can function as potent trans-acting regulators of specific genes. MicroRNAs (miRNAs) continued this theme and now attract much attention among basic scientists and clinicians alike, both as potential regulators of most human genes and as potential diagnostic tools. In this issue of *PLoS Medicine*, a research article by Shigetada Teshima-Kondo and colleagues supports the suggestion that another class of regulatory RNAs exists [1]. Furthermore, this class could be the largest to date, since potential members are contained within every mRNA.

A New Study on the 5' Non-Coding Region of *VEGF* mRNA

The authors show that the 5' untranslated region (UTR) of the *vascular endothelial growth factor (VEGF)* mRNA influences expression of other genes, and thereby cell function, independently of the *VEGF* reading frame. Many UTRs have long been known to function as cis-acting elements on the expression of their own mRNAs [2,3]—but trans-acting regulatory functions are an exciting possibility, which, if commonplace, would be every bit as influential as siRNAs or miRNAs.

The Perspective section is for experts to discuss the clinical practice or public health implications of a published article that is freely available online.

Linked Research Article

This Perspective discusses the following new study published in *PLoS Medicine:*

Masuda K, Teshima-Kondo S, Mukaijo M, Yamagishi N, Nishikawa Y, et al. (2008) A novel tumor-promoting function residing in the 5′ non-coding region of *vascular endothelial growth factor* mRNA. PLoS Med 5(5): e94. doi:10.1371/journal.pmed.0050094

Shigetada Teshima-Kondo and colleagues find that cancer cells have a survival system that is regulated by *vegf* mRNA and that *vegf* mRNA and its protein may synergistically promote the malignancy of tumor cells.

VEGF is a key regulator of cancer development, acting to promote survival of tumour cells and to stimulate angiogenesis; as such, VEGF is well established as a target for cancer therapies [4]. The starting point for this new study was the observation that some uses of therapeutics targeting VEGF protein were less effective than might have been hoped [5]. The authors optimistically (but seemingly correctly) interpreted this observation as a suggestion that *VEGF* transcripts might have cancer-related functions separable from the functions of the VEGF protein, and set about further analyses.

Working initially in tissue culture, the authors showed that VEGF promoted resistance to apoptosis (programmed cell death) induced by the chemotherapeutic 5-fluoro-uracil. This was not surprising given VEGF's familiar anti-apoptotic function [6]. However, in a thorough and detailed set of experiments, the authors also showed that expression of VEGF protein alone was insufficient for the full anti-apoptotic effect seen, and furthermore, that expression of the *VEGF* 5'UTRs in isolation from the *VEGF* reading frame promoted significant resistance.

These experiments are especially complex since the *VEGF* 5'UTR is known to have a variety of cis-acting effects on translation of its reading frame [7]. Notably and unusually, translation can initiate from within the 5'UTR at a number of CUG codons [8]. The authors have established that translation of sequences from within the UTR is not required to specify this apoptotic resistance, and thereby that the activity is truly associated with the RNA itself. Directing their attentions more towards a role for the *VEGF* 5'UTR in carcinogenesis, the authors showed that expression of the UTR, again without its reading frame, induced increased colony-forming activity in vitro, and tumour-forming ability in a mouse xenograft model. Finally, the authors established a link between expression of *VEGF* 5'UTR and a dramatic change in response to interferon alpha using both in vitro and in vivo models. The conclusion is that *VEGF* 5'UTR has independent tumour-promoting activity, acting via mediators

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Abbreviations: miRNA, microRNA; PKR, RNA-activated protein kinase; siRNA, small interfering RNA; uORF, upstream open reading frame; UTR, untranslated region; VEGF, vascular endothelial growth factor

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of interferon alpha signalling, and therefore that the *VEGF* mRNA itself is oncogenic.

Implications of the Study

While the idea that mRNAs might have functions that are independent of their translation may be surprising, there are published precedents for related trans-acting functions of UTRs. Such examples remain few and far between (Teshima-Kondo et al. cite four, and we have found a further two [9,10]) and have not had a significant impact on the general consciousness of researchers. This new work represents an important advance, in that the function takes place in the context of a key player in carcinogenesis that is already used as a cancer therapy target, and therefore the study highlights this class of regulation. What this new study shares with its precedents, however, is the lack of a defined mechanism of action. As such it is difficult to determine the generality of trans-acting functions of UTRs, and it is too early to start considering mRNAs as another class of fundamental gene regulators.

How Do UTRs Exert Trans-Acting Influences?

It is possible to postulate a variety of mechanisms by which UTRs might exert trans-acting influences. First, the RNA-activated protein kinase (PKR) may be activated by binding to double-stranded regions of UTRs. Activated PKR is well known to cause changes in the translational efficiency of certain mRNAs as part of the cellular response to viral infection, but more recently has been implicated in other pathways, including cancer-related mitogen-activated protein kinase signalling [11]. PKR has been shown to bind to one published trans-acting UTR [12], and Teshima-Kondo et al. report preliminary data (although these are not shown) indicating that PKR may bind to the *VEGF* mRNA.

Alternatively, rather than activating a binding protein, trans-acting UTRs may sequester binding proteins away from other targets. In experiments where the UTR sequence has been over-expressed, as is the case for almost all these studies, this over-expression could allow artifactual effects, which is a concern for the physiological relevance of the observations. Some authors have speculated about UTR sequences making direct RNA:RNA interactions with target transcripts and thereby influencing their stability or translation. This mechanism is possible, but whether separate mRNA molecules, each of which would form its own mRNP structure, would have the opportunity to base-pair with each other with any significant frequency seems debatable. Finally, for some 5'UTRs the trans-acting function appears to be dependent on translation of short upstream open reading frames (uORFs) within the UTR. The resultant peptides repress translation of other mRNAs, although seemingly only of those mRNAs arising from the same gene [9, 10]. Whether this peptide-mediated trans-repression is truly this gene-specific remains to be determined. This final proposed mechanism could provide an explanation for the trans-repression seen from the *c-myc* P0 5'UTR [13], which contains a uORF. However, it could not account for the influence of *VEGF* 5'UTRs, since Teshima-Kondo et al. demonstrate, at least in vitro, that their effect is maintained in the absence of significant translation.

Glossary

cis-acting: cis-acting regulation is regulation from within the same molecule. Typical examples are DNA or RNA sequences that influence the function of different sections of the same DNA or RNA molecule.

***c-myc* P0:** *c-myc* is a proto-oncogene that is very frequently over-expressed in cancers. The gene is transcribed from a number of different promoters, one of which is termed P (for promoter) 0.

miRNA: microRNAs are a class of short (~21 nucleotide) *single-stranded* RNAs that can reduce the expression of target mRNAs to which they are *partially* complementary.

mRNA: messenger RNAs are transcribed copies of protein-encoding genes that are exported from the nucleus and translated into proteins by ribosomes in the cytoplasm.

mRNP: mRNPs are mRNAs that are packaged into messenger ribonucleoprotein particles during nuclear export.

Nuclear-encoded regulators: regulators of gene expression that are encoded by the nucleus, the most obvious example being the transcription factors. Non-nuclear-encoded regulators of gene expression include hormones and drugs.

Protein kinases: enzymes capable of phosphorylating specific target proteins, leading to changes in their function.

rRNA: ribosomal RNAs are structural components of ribosomes.

siRNA: short interfering RNAs are a class of short (~21 nucleotide) *double-stranded* RNAs that can reduce the expression of target mRNAs to which they are complementary.

Trans-acting: trans-acting regulatory molecules are those that influence the activity of other *physically separate* molecules.

tRNA: transfer RNAs are essential components of the translational machinery. They bind to individual amino acids and recruit them to extending peptide chains at ribosomes.

Upstream open reading frames: sequences of nucleotides within 5′UTRs that could potentially be translated.

Conclusion

This study has a clear implication for VEGF-targeted therapies, in that strategies to target the mRNA may be required to inhibit the full oncogenic influence of the gene. More generally, the study highlights a little-studied gene regulatory pathway that may yet prove to be far more commonplace than currently appreciated. ■

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