Decoding Noncoding RNA
Da Vinci Redux?

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The discovery by Crick, Franklin, Watson, and Wilkins of the molecule that encodes our genetic information enabled Holley, Khorana, and Nirenberg to break the 4-letter alphabet of DNA and translate it into the language of amino acids by deciphering protein-coding RNA. Recent advances in RNA profiling have revealed that 75% of the human genome is capable of being transcribed into RNA, yet only a small percentage of transcribed RNA codes for proteins. Therefore, the next step in our quest to understand the mysteries of life is to decode the secrets hidden in nonprotein-coding RNA.

Noncoding RNA is divided into 2 major classes: short (<200 nucleotides) and long (≥200 nucleotides). Short RNAs include microRNAs (miRNAs), endogenous short-interfering RNAs, and germline-restricted RNAs that interact with P-element induced wimpy testis proteins (piRNAs), which act to prevent the invasion of transposable elements into the genome. Of these short RNAs, the miRNAs are the best understood and play important roles in cell behavior, including proliferation, differentiation, contractility, inflammation, and fibrosis. miRNAs typically act by repressing gene expression post-transcriptionally.

Long noncoding RNA (lncRNA) includes antisense, intronic, intergenic, and overlapping bidirectional transcripts. Many lncRNAs bind to chromatin-modifying proteins and recruit their catalytic activity to specific sites in the genome to alter chromatin states and impact gene expression and, as such, are part of a broad epigenetic regulatory network through histone modifications and DNA methylation. LncRNAs also serve post office–like functions by enabling genes and protein complexes to arrive at the appropriate locus, allele, or cellular address where they can perform their nuclear-, cell-, and tissue-specific functions. Thus, lncRNAs could be ideal drug targets, given their attributes of temporal and spatial specificity that are not possible to achieve with proteins and small RNAs. We have a much poorer understanding of their role in pathophysiology, although lncRNAs have been implicated in some human disorders caused by chromosomal deletions and translocations and in cardiac pathophysiology.

In this issue, Leung et al present a comprehensive study of genes in rat vascular smooth muscle cells (VSMC) that are differentially regulated by the peptide angiotensin II (Ang II). Of the 498 genes identified, novel transcribed RNAs, including 14 protein-coding and 24 nonprotein-coding RNAs, were discovered. Evidence in support of lncRNAs was achieved by analyzing the transcriptome and epigenome associated with the activity of histone methylation. One of these nonprotein-coding RNAs, Lnc-Ang362, is located proximal to 2 microRNAs, miR-221 and miR-222. Lnc-Ang362 is upregulated in VSMC by Ang II similar to the 2 microRNAs. The authors also show that inhibiting the expression of Lnc-Ang362 with small interfering RNA results in reduced expression of miR-221 and miR-222, as well as reduced VSMC proliferation. Several other lncRNAs were shown to be of interest because Ang II was able to upregulate (lnc-Ang112, lnc-Ang162) and downregulate (lnc-Ang219, lnc-Ang249) them.

This research is important for several reasons. First, this is the first report of an lncRNA that is upregulated by Ang II and which serves as a host transcript for miRNAs, miR-221 and miR-222. Previously, Liu et al showed that platelet-derived growth factor increased miR-221 and miR-222 expression in VSMC. Furthermore, small interfering RNA knockdown of these 2 miRNAs decreased VSMC proliferation in culture and inhibited the development of neointimal lesions in rat carotid arteries. Thus, the authors may have discovered a novel mechanism by which Ang II regulates the hyperproliferation and hypertrophy of VSMC associated with diffuse and diverse vascular diseases, including coronary artery disease, ischemic stroke, and arterial aneurysms, because both these miRNAs are implicated in VSMC proliferation.

Second, the identification of novel RNAs that are regulated by Ang II is a critical first step in our efforts to fully understand the critically important and fundamental actions of this pleiotropic hormone in mammalian physiology. Not only does Ang II control blood pressure and fluid homeostasis, this peptide is implicated in development, growth, and aging. Furthermore, inhibitors of Ang II action are being explored for the treatment of various diseases, including cancer, stroke, and inflammatory brain disorders, including anxiety, depression, and cognitive decline.

Third, this article illustrates the power of RNA-seq in identifying genes that are differentially expressed under various conditions since 38 novel protein-coding and nonprotein-coding RNAs were discovered. There are many advantages of RNA-seq over other technologies that deduce and quantify the transcriptome, including hybridization- or sequence-based approaches and genomic tiling microarrays that represent the genome at high density. The RNA-seq technique is more
sensitive for less abundant transcripts and has a greater dynamic range in measuring transcripts. This technique also enables discovery of allele-specific (single-nucleotide polymorphism) expression, quantification of splicing, and detection of novel alternative splice variants. Furthermore, combining this technique with chromatin immunoprecipitation sequencing enables confirmation of the RNA-seq findings.

What remains unknown is how lnc-Ang362 hosting of miR-221 and miR-222 is coordinately regulated during VSMC proliferation and whether lnc-Ang362 has any effects that are independent of these miRNAs. The finding that other identified lncRNAs are proximal to transcripts known to be regulated by Ang II suggests that they may function as enhancers of gene expression, and lncRNAs are known to play this role; however, much research needs to be done to fully understand lnc-Ang362 and the other identified lncRNAs regulated by Ang II in VSMC and other tissues (eg, cardiomyocytes, adipocytes). For example, is lnc-Ang362 hosting of miR-221 and miR-222 an important event in other Ang II–responsive tissues? Is lnc-Ang362 regulated under physiological conditions known to modulate the renin–angiotensin system, such as alterations in dietary sodium? What happens to this lncRNA under pathological conditions involving dysregulation of the renin–angiotensin system–like hypertension and atherosclerosis? Is lnc-Ang362 regulated in a tissue-specific manner? Are there sex differences in its regulation? Does it operate in cis and thus is restricted to acting at the site of synthesis on 1 or several linked contiguous genes on the same chromosome or does it widely diffuse from the site of synthesis and act in trans on distant genes, including other chromosomes?

One of the challenges to answering these questions lies in the tight link among RNA, chromatin, and transcriptional activity. As the DNA duplex is unwound by Pol II, chromatin structure is altered, which could independently induce epigenetic changes in the nascent transcript. Furthermore, nuclear lncRNAs are difficult to knock down, leading to residual lncRNAs that could complicate interpretation of data.

Although RNA-seq is superior to older technologies for transcriptome profiling, a newer method called reverse transcriptase polymerase chain reaction (RT-PCR)-seq has been developed, which has additional advantages. Rather than having to validate RNA-seq data by RT-PCR amplification followed by DNA sequencing, RT-PCR-seq combines traditional RT-PCR with high-throughput sequencing. It is more sensitive and more unbiased than RNA-seq for categorizing protein-coding genes, pseudogenes, and noncoding transcribed loci and is especially useful for confirming exon–exon junctions. Howald et al showed that RT-PCR-seq was 5x more accurate in identifying true exon–exon junctions in the Encyclopedia of Genes and Gene Variants data set (ie, the most complete human lncRNA annotation to date) than large human transcriptome profiling. Using this method, they estimated that ≥18% of known loci have yet unannotated exons. Thus, to catalog the full spectrum of genic elements regulated by Ang II in VSMC and in other tissues, the dual use of RNA-seq and RT-PCR-Seq is warranted. Nonetheless, the lncRNAs identified by Leung et al have provided the field with exciting new clues into Ang II actions in VSMC and move us closer to decoding the encrypted message within noncoding RNA. This study also provides a glimpse into the future, when harnessing the power of IncRNA for therapeutic use in diseases involving Ang II dysregulation will become possible.

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Disclosures
None.

References
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