LIPCAR
A Mitochondrial Inc in the Noncoding RNA Chain?

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Only one link of the chain of destiny can be handled at a time.

—Winston Churchill

All proteins are translated from RNA, but only a fraction of RNAs seem to be translated into proteins. Advanced genomics technologies have uncovered noncoding regulatory RNAs exhibiting extraordinary diversity in size, structure, and molecular function. We know a great deal about small (≈20 nucleotides) microRNAs that post-transcriptionally regulate translation of protein from target mRNAs having complementary 3′ sequences. These linear single-stranded nucleotides are transcribed either as independent gene products or as passengers within so-called miRtrons (microRNA-containing introns) of protein-coding genes and then processed and exported from the nucleus where they incorporate into RNA-induced silencing complexes.1 Because some microRNAs exhibit altered expression or differing circulating levels, they are being assessed as diagnostic biomarkers in cardiac disease. As yet, we know far less about the larger (≈200 nucleotides) long noncoding RNAs (lncRNAs). lncRNAs are generated as antisense transcripts of protein-coding genes, as sense transcripts from within protein-coding gene introns, or as independent transcripts originating from intergenic regions.2 Unlike short microRNAs, lncRNAs are sufficiently long to develop multiple intramolecular RNA–RNA interactions conferring on them complex 3-dimensional structures. The unique physical configurations of different lncRNAs enable many of them to bind specific sets of proteins. Likewise, openly configured single-strand RNA sequences within lncRNA structures permit them to bind to complementary sequences in the genome. The combination of these 2 characteristics, protein binding and recognition of specific DNA sequences, evokes a characteristic lncRNA functionality: chaperoning transcriptional modulators or chromatin modifiers to specific genomic locations. In this manner, lncRNAs can epigenetically regulate gene expression.3

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Our nascent but growing understanding of IncRNA biology generally assumes that they are produced by, and act on, the nuclear genome. In this issue of Circulation Research, Kumarswamy et al4 provide evidence that late circulating levels of the IncRNA uc022bq.s.1, which they have named LIPCAR, are associated with adverse outcomes after myocardial infarction (ventricular dilatation) or in chronic heart failure (cardiovascular mortality). Intriguingly, this IncRNA is described as mitochondrial derived. Indeed, approximately three fourths of IncRNAs reported as “highly abundant” in plasma samples assayed from the REVE-2 study cohort were classified as originating from the mitochondrial genome. Likewise, the 7 IncRNAs Kumarswamy et al4 consistently detected in plasma samples were all categorized as mitochondrial. Although mitochondrial dysfunction5 and mitochondrial-derived factors6,7 have previously been nominated as biomarkers of cardiac disease, the observation that mitochondrial-derived IncRNAs are so numerous and abundant has implications far beyond their detection in disease.

Modern animal mitochondrial (and plant chloroplast) genomes are fragmentary remnants of the primordial bacterial genomes that immigrated into our eukaryotic single-cell ancestors through endosymbiotic partnering.8,9 Like their bacterial predecessors, mitochondrial genomes comprised circular double-stranded DNA that lacks chromatin structure. Compared with nuclear DNA, mitochondrial DNA has a different genetic alphabet for encoding amino acids. Human mitochondrial DNA is ≈16.5 kb and encodes the 16S and 12S mitochondrial ribosomal RNAs and 22 transfer RNAs necessary for mitochondrial protein synthesis, as well as 13 proteins of the electron transport chain that drives ATP synthesis. The primordial mitochondrial DNA that encoded the other >1000 mitochondrial protein-coding genes has, over evolutionary time, been exported to the nucleus.10

The 2 strands of the circular mitochondrial genome are designated “heavy” (H) and “light” (L) based on their GC content and sedimentation characteristics (Figure). The H strand is genetically congested, containing both ribosomal RNA genes, 14 of the tRNAs, and 12 of the 13 protein-coding genes. By comparison, the L strand has only 1 protein-coding gene and the remaining 8 tRNAs. Mitochondrial protein-coding genes are intronless and, with 3 exceptions, are each separated by tRNAs. Both strands are transcribed as large polycistronic precursor transcripts that are processed into individual functional units.

Evidence has been accumulating in support of a mitochondrial origin for some noncoding RNAs. Numerous putative mitochondrial small RNAs were first detected after small RNA deep sequencing data mapped to human and murine
mitochondrial genomes; many of these noncoding RNAs were validated as originating from mitochondria in studies comparing normal and mitochondria-depleted cells. Recently, strand-specific RNA sequencing uncovered 3 lncRNAs generated from the human mitochondrial genome. Because each lncRNA is the antisense counterpart to a mitochondrial protein-coding gene, they were designated lncND5 RNA, lncND6 RNA, and lncCytb RNA (Figure). The ND5 and CytB protein-coding genes are on the H strand, so their lncRNA gene counterparts are on the L strand; conversely, the lncND6 gene is located on the H strand. These mitochondrial lncRNAs can form RNAse-resistant duplexes with their respective complementary mRNAs, which is postulated to modulate mRNA expression or stability. These interesting data support the existence of functional mitochondrial lncRNAs and provide a strong impetus for efforts to discover and characterize others.

As shown by the research that uncovered mitochondrial lncND5 RNA, lncND6 RNA, and lncCytb RNA, establishing the genomic position and sequence complementarity of an lncRNA can provide clues to its biological function. Mapping the complete 781 nucleotide LIPCAR sequence (including the UCSC Genome Browser: http://genome.ucsc.edu) to the human mitochondrial genome provides an unexpected result: the 5′ half (nucleotides 1–392) maps to antisense of the mitochondrial Cytb gene (nucleotides 15887–15496 of NC_012920.1), but the 3′ half (nucleotides 385–781) maps to antisense of the mitochondrial COX2 gene (nucleotides 7982–7586; Figure and Online Figure I). Indeed, the 5′ half of LIPCAR is wholly contained within the previously described mitochondrial lncCytb gene. Thus, the 2 halves of LIPCAR are half a mitochondrial genome apart. Given that mitochondrial genes lack introns and are not known to undergo splicing, discontinuity of the LIPCAR lncRNA seems incongruous.

As noted, mitochondria have exported the vast majority of their ancestral genomes to the nucleus. What is sometimes overlooked is that the current mitochondrial genome has also been copied to the nuclear genome. Because differences between the amino acid codes of nuclear and mitochondrial genomes prevent nuclear-integrated copies of modern-day mitochondrial DNA from producing their encoded proteins, they have been considered to be nonfunctional and therefore commonly referred to as pseudogenes. Nevertheless, human mitochondrial DNA-derived nuclear insertions are abundant, comprising ≥500,000 base pairs (or 0.016% of the 3 billion base pair nuclear genome), and are present on all 24 nuclear chromosomes. Indeed, the entire mitochondrial genome, including all protein-coding, rRNA, tRNA, and noncoding sequence, is replicated many times over within the nuclear genome. An early report described almost 300 nuclear inserts of mitochondrial DNA ranging from nearly complete 10- to 14-kb inserts on chromosomes 1, 2, 4, and 9 to dozens of >2-kb fragments randomly distributed throughout the genome. As recent evidence indicates that pseudogenes can generate functional lncRNAs, the question arises as to whether nuclear-integrated mitochondrial pseudogenes also function as real genes that express noncoding RNAs. A BLAST search of the LIPCAR nucleotide sequence to the human nuclear genome shows >90% identity of the 385 to 781 nucleotide sequence to chromosome 1 and of the entire 1 to 781 nucleotide sequence to chromosome 5 (Online Figure I). The 385 to 781 half of LIPCAR also has ≥75% identity to COX2 pseudogene sequences on chromosomes 2, 4, 7, 8, 9, 10, 17, and X. Because the quantitative polymerase chain reaction primers Kumarswamy et al used to validate LIPCAR regulation in the post–myocardial infarction left ventricular remodeling study and assess its relationship to heart failure outcome are internal to the 385 to 781 nucleotide half (Figure), this polymerase chain reaction assay will not confidently distinguish between mitochondrial-derived and nuclear-derived transcripts. Likewise, it is unclear what sequence tags for LIPCAR are present on the microarrays used by Kumarswamy et al for their initial screening tests. Therefore, the conservative interpretation is that the circulating RNA that predicts ventricular remodeling (and the other circulating lncRNAs the authors designated as mitochondrial derived) may originate in the nucleus, mitochondria, or both. Because the various nuclear pseudogenes for mitochondrial COX2 have acquired subtle but site-specific nucleotide changes, RNA sequencing of unamplified plasma lncRNA might resolve ambiguities about LIPCAR biogenesis. Such information could also propel efforts to define the cell of origin and potential DNA targets of LIPCAR, which are currently indeterminate.

The confounding influence of nuclear-entrapped mitochondrial genomic fragments is not new. Furthermore, whether the LIPCAR lncRNA (or its polymerase chain reaction–amplified fragment) is mitochondrial or nuclear does not alter its potential value as a cardiac biomarker. Indeed, the issues of biological function and potential diagnostic usefulness seem separate. A new biomarker will be useful if it shows a better sensitivity and specificity profile, or enhanced predictive
value, than standard clinical diagnostics. As heretical as it may first seem, the molecular mechanism (or even existence) of biological activity for a biomarker is not important. Future prospective studies to assess whether circulating LIPCAR specifically predicts post–myocardial infarction ventricular remodeling, and to define its reliability in identifying patients who are at greater risk for adverse outcomes in heart failure, will ultimately determine its usefulness as a biomarker. Meanwhile, discovering what noncoding RNAs are generated within mitochondria, and uncovering how they affect normal metabolic homeostasis and the response to injury or stress, will require an approach focused on basic mechanisms.

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