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Editorial

"Going Long": Long Non-Coding RNAs as Biomarkers

Philipp Skroblin, Manuel Mayr

Protein-coding sequences constitute <2% of the human genome. The majority of the remaining 98% was long assumed to be nonfunctional junk DNA but there is increasing evidence that ≤85% of the human genome is transcribed into RNA.[1] Noncoding sequences in the genome increase proportionally with the complexity of the organism, implying a need for additional transcriptional regulation in the evolution of eukaryotic organisms. Thus, the vast majority of the human transcriptome is noncoding RNA, which is divided into short noncoding RNAs (<200 nucleotides: comprising rRNA, miRNA, snRNA, snoRNA, siRNA, and piRNA) and long noncoding RNA (lncRNA, >200 nucleotides). Since their discovery in 2001, miRNAs have been studied extensively and fundamental insights have been obtained about their synthesis, their repression of target genes, and their involvement in disease processes. Indeed, miRNA therapeutics are already tested in clinical trials.

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After the explosion of interest in miRNAs, the attention is now shifting to lncRNAs. lncRNAs have emerged as another important layer of gene regulation. On the basis of their genomic loci, lncRNAs can be classified into 5 categories (Figure): (1) sense lncRNAs are transcribed from a locus overlapping with a protein-coding gene; (2) antisense lncRNAs loci overlap with the antisense strand of protein-coding gene; (3) bidirectional lncRNAs are located on the antisense strand of a protein-coding gene whose start codon is <1000 base pairs away; (4) intronic lncRNAs are transcribed from introns of protein-coding genes; and (5) long intergenic (or intervening) noncoding RNAs loci are not in the proximity of a protein-coding gene at all. Most lncRNAs are located in the nucleus where they can modify transcription by recruiting or sequestering proteins, such as transcription factors or histone-modifying enzymes to and from target loci located in *cis* or *trans*.[2] Other lncRNAs exert their function in the cytoplasm and regulate protein localization, mRNA translation, and stability.[2] The structural and functional diversity of lncRNAs, as well as their sheer number, make their characterization challenging: the NONCODE database, version 4, contains 95 135 human lncRNA transcripts.[3] At present, little is known about the function of most lncRNAs, especially their

role in cardiovascular development, physiology, and disease. Their poor evolutionary conservation may further hamper the translation of findings from animal models to patients. For example, Braveheart, a lncRNA essential for cardiomyocyte differentiation in mice, does not have a known human ortholog.[4] Antisense noncoding RNA in the INK4 locus (ANRIL), which spans the entire region of association with coronary artery disease on the chromosome 9p21 locus, is expressed in humans but not in mouse.[5]

lncRNAs display high organ and cell specificity and are involved in maintaining cell integrity. Previous analyses of cardiovascular lncRNAs were performed in cultured cells,[6–8] animal models,[9,10] and human heart biopsies.[10,11] Circulating lncRNAs have been suggested as potential biomarkers for cancer, but few studies have explored lncRNAs as biomarkers in the context of cardiovascular disease. Recently, Kumarswamy et al[12] reported a screen for lncRNAs in plasma of patients with myocardial infarction (MI). The vast majority of lncRNAs was undetectable. The few lncRNAs that were differentially expressed between patients with and without cardiac remodeling after MI were encoded by mitochondrial DNA. One of these mitochondrial lncRNAs, named long intergenic noncoding RNA predicting cardiac remodeling, was associated with cardiac remodeling and cardiovascular mortality in patients with heart failure. However, the source of long intergenic noncoding RNA predicting cardiac remodeling remained unclear, and the amount of lncRNAs in plasma is low. Unlike short noncoding RNAs, such as miRNAs, most lncRNAs are not detectable in plasma by standard methods, such as microarrays or quantitative polymerase chain reaction.

In this issue of *Circulation Research*, Vausort et al[13] have published the first analysis of lncRNA in full blood of patients with MI. Full blood is a more reliable source of lncRNAs. The underlying hypothesis was that changes in cardiac function ensuing acute MI affect the transcriptome of peripheral blood cells. Inflammation is supposed to play a central role in cardiac remodeling after MI. Five lncRNAs of different types and mechanisms of actions were assessed in 84 healthy volunteers and in 414 patients with MI, selected based on their relevance for cardiovascular disease (Figure): (1) antisense hypoxia inducible factor 1α is induced by hypoxia and upregulated in heart failure; (2) ANRIL is within the most replicated risk allele for coronary heart disease; (3) KCNQ1 overlapping transcript 1 (KCNQ1OT1) regulates the expression of the potassium channel KCNQ1, whose deficiency causes long-QT syndrome; (4) MI-associated transcript (MIAT) contains a single-nucleotide polymorphism in its locus that confers susceptibility to MI; (5) metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is a highly abundant lncRNA implicated in alternative splicing. It is upregulated in endothelial cells under hypoxic conditions and promotes angiogenesis.[14] Other lncRNAs, such as long intergenic noncoding
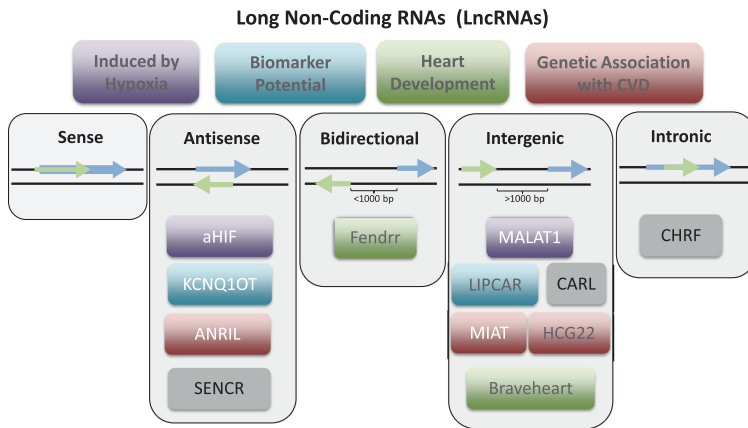
Long Non-Coding RNAs (LncRNAs)

Figure. Cardiovascular long noncoding RNA (lncRNAs). lncRNA genes (green arrows) and their position relative to protein-coding genes (blue arrows) on both DNA strands (black lines) are shown schematically. lncRNAs analyzed by Vausort et al[13] are highlighted in white font. aHIF indicates antisense hypoxia inducible factor 1 alpha; ANRIL, antisense noncoding RNA in the INK4 locus; bp, base pairs; CARL, cardiac apoptosis-related lncRNA; CHRF, cardiac hypertrophy–related factor; CVD, cardiovascular disease; Fendrr, Fetal-lethal noncoding developmental regulatory RNA; HCG22, HLA complex group 22; KCNQ1OT, KCNQ1 overlapping transcript 1; LIPCAR, long intergenic noncoding RNA predicting cardiac remodeling; MALAT1, metastasis associated lung adenocarcinoma transcript 1; MIAT, myocardial infarction–associated transcript; and SENCR, smooth muscle and endothelial cell-enriched migration/differentiation-associated long noncoding RNA.

RNA predicting cardiac remodeling, HLA complex group 22, smooth muscle and endothelial cell-enriched migration/differentiation-associated long noncoding RNA, cardiac hypertrophy–related factor, and cardiac apoptosis-related lncRNA, were only recently implicated in cardiovascular function and were not included in this study.[6–8,12,15]

With the exception of MIAT, all analyzed lncRNAs were differentially expressed in patients with MI: antisense hypoxia inducible factor 1α, KCNQ1OT and MALAT1 were upregulated, whereas ANRIL was downregulated. MALAT1 and antisense hypoxia inducible factor 1α are known to be induced under hypoxia. In agreement with previous observations in healthy donors, lymphocytes were the main source of ANRIL in patients with MI. Comparing non–ST-segment–elevation MI and ST-segment–elevation MI, ANRIL, KCNQ1OT1, MIAT, and MALAT1 were downregulated in patients with MI and ST-segment elevation after adjustment for white blood cell count. This feasibility study demonstrates that acute MI affects the expression of lncRNAs in peripheral blood cells, but none of the selected lncRNAs emerged as particularly promising biomarker. In comparison with established biomarkers (creatine kinase and NT-proBNP [N-terminal pro-brain natriuretic peptide]) and common comorbidities (type II diabetes mellitus), lncRNAs were weak predictors of left ventricular dysfunction. ANRIL and KCNQ1OT improved the prediction of left ventricular dysfunction after MI in univariate but not in multivariate analysis.

The authors should be applauded for measuring lncRNAs in a relatively large patient cohort. The mechanisms how lncRNAs in blood are affected by acute MI and the functional consequences are currently unclear. Importantly, >20 linear and several circular isoforms of ANRIL have been identified.[16] The risk genotype of the lead single-nucleotide polymorphism for coronary artery disease located in the ANRIL locus (rs10757278-G) causes an increased expression of some ANRIL variants but a decrease in others.[16] Similarly, MALAT1 has 11 variants and MIAT has 4. Assessing the levels of particular variants might provide key information, which is missed when the analytic tools do not distinguish between variants. A more detailed analysis as to which splice variants of lncRNAs are differentially expressed in patients with MI will be required. Other drawbacks of the study are the lack of

an independent validation cohort and confounding by inflammatory responses associated with MI. Although KCNQ1OT1 and MIAT did not show significant associations with inflammatory markers, antisense hypoxia inducible factor 1α was positively correlated with inflammation. All selected lncRNAs have been identified in noncardiac tissues. This lack of cardiac specificity questions their usefulness as predictors of cardiac dysfunction. At best, measuring lncRNA expression in full blood might reflect inflammation at the site of MI.

lncRNA research is still in its infancy and a better characterization of the lncRNA transcriptome, advances in the understanding of lncRNA function and improved analytic tools will allow to replicate and extend the findings presented by such early studies.[13] Because of the high number of different lncRNAs, next-generation sequencing methods will be instrumental in exploring lncRNAs in health and disease. Whether lncRNAs are better predictive biomarkers than existing cardiovascular biomarkers or other noncoding RNAs, such as miRNAs,[17] awaits confirmation in future studies.

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