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Article

Long Noncoding RNA: an Emerging Biomarker for Cardiovascular Diseases

Kai-Chien Yang, MD, PhD [1,2]

[1] Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
[2] Graduate Institute and Department of Pharmacology, National Taiwan University, Taipei, Taiwan
* Correspondence: allenmy0920@gmail.com; Tel.: +886-2-23123456 ext. 88327

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Abstract: Although intriguing results have been generated by transcriptional profiling studies, previous works in the cardiovascular system targeted mainly the expression of messenger RNAs (mRNAs) and microRNAs (miRNAs), which in combination account for only 1% of all transcribed RNA species [1]. It is now known that the transcription of the eukaryotic genome is much more pervasive and complex than previously appreciated [1]. It is estimated that up to 90% of the mammalian genome is transcribed [1], and a large proportion of the mammalian genome is transcribed as long noncoding RNAs (lncRNAs), a heterogeneous group of noncoding transcripts longer than 200 nucleotides, encoded from genomic loci within or betweencoding genes [2,3]. LncRNAs have been shown to be functional and involved in specific physiological and pathological processes, including chromatin modification [4,5], cellular responses to DNA damage [6], stem cell pluripotency/differentiation [7], cell cycle control [8], as well as in the pathogenesis of neurologic diseases [9,10] and human cancers [11–13]. Functionally, lncRNAs are known for their roles as modulators of transcription, including epigenetic regulation of chromatin structure [14]. In addition, lncRNAs have been shown to function as regulators of post-transcriptional mechanisms, including transcript splicing [15], mRNA decay [16], and protein translation [17]. In this regard, lncRNAs are unique, functioning not dependent solely on sequence (as with miRNAs) or structure (as for RNA-binding proteins). Instead, lncRNAs seem to function both by sequence homology/complementarity with other nucleic acids, as well as by structure, forming scaffolds for the assembly of macromolecular complexes that regulate biological processes [18]. The potential value of lncRNAs as diagnostic biomarkers has been widely explored. Although lncRNAs are not as abundant as other noncoding RNAs, the cell type- and disease-specific expression patterns make them suitable biomarker candidates. Here, we cover a few examples of the important and well-established lncRNAs as biomarkers for CV diseases.

Keywords: RNAs, Long noncoding RNAs, Cardiovascular diseases

1. ANRIL

Genome-wide association studies (GWAS) revealed a strong association between DNA sequence variants on chromosome 9p21.3 and the risk of coronary artery disease, accounting for 10-15% of disease in non-African populations [19–22]. The 9p21.3 risk locus is adjacent to the last exons of the antisense ncRNA in the INK4 locus (ANRIL) and encompasses multiple single nucleotide polymorphisms (SNPs). This increased CAD risk associated with the single-nucleotide polymorphisms (SNPs) in this region is independent of all known CAD risk factors [21]. Interestingly, the risk alleles for

atherosclerosis-related phenotypes were consistently associated with low expression levels of ANRIL splice variant spanning exons 1–2, but not exon 17-18, of ANRIL, suggesting that different splicing variants of ANRIL might play distinct roles [23]. Indeed, different ANRIL splice variants have distinct expression patterns in peripheral blood mononuclear cells (PBMCs) from carriers of the risk haplotype, which suggests that differential splicing or transcript stability may confer different atherosclerosis susceptibility [24]. In one study enrolling 414 patients with acute myocardial infarction (AMI) treated by primary percutaneous coronary intervention, levels of hypoxia-inducible factor 1A antisense RNA 2, KCNQ1OT1, MALAT1 and ANRIL in peripheral blood cells were significantly altered with AMI. Among them, ANRIL and KCNQ1OT1 improved the prediction of post-MI left ventricular dysfunction in a multivariate, prognostic regression model that includes demographic features, clinical parameters, and cardiac biomarkers [25].

2. CoroMarker

CoroMarker, also named as Aldo-Keto Reductase Family 1 Member B1 Pseudogene 3, was discovered in a cohort study of patients receiving diagnostic coronary angiography for suspected CAD. CoroMarker from PBMCs was found to be a useful biomarker with high sensitivity and specificity for the diagnosis of CAD. The expression levels of CoroMarker showed a positive correlation with genes involved in atherosclerosis. Of note, CoroMarker was stable in plasma [26], and knockdown of CoroMarker decreased the production of pro-inflammatory cytokines from THP-1 monocytes [27]. However, the exact mechanisms via which CoroMarker regulates monocytes or atherosclerosis remain to be determined.

3. LIPCAR

LIPCAR (long intergenic noncoding RNA predicting cardiac remodeling), a mitochondria-derived lncRNA, is highly expressed and consistently detectable in human plasma samples. Plasma LIPCAR has been shown to be an independent predictor for CAD and correlates with the severity of clinical presentation (highest in patients with AMI) [28]. In another study on patients with AMI, LIPCAR was downregulated early after AMI but upregulated during later stages, suggesting its role in chronic heart failure. Consistent with this observation, plasma LIPCAR level is elevated even more in CAD patients with heart failure. In addition, LIPCAR expression level is associated with the future maladaptive cardiac remodeling in patients who experienced an episode of AMI. Of note, LIPCAR is independently associated with cardiovascular mortality in patients with chronic heart failure, regardless of pathogenesis [29]. The mechanism, however, underlying the correlation between LIPCAR and CAD/AMI remains unclear.

4. SENCR

SENCR (Smooth muscle and Endothelial cell-enriched migration/differentiation-associated long NonCoding RNA) is highly expressed in ECs, SMCs, and aortic tissues (vascular-enriched lncRNA) [30]. SENCR, localized mainly in the cytoplasm, stabilizes the contractile state of VSMCs by increasing myocardin expression [30]. Moreover, it was found that SENCR contributes to endothelial commitment in pluripotent cells and the angiogenic capacity of ECs. SENCR expression was diminished in vascular ECs derived from superficial forearm veins of patients with critical limb ischemia and pre-mature coronary artery disease [31]. Using FISH-Flow assay, SENCR is downregulated in circulating ECs, but upregulated in monocytes, in early-onset CAD patients (EOCAD). Moreover, the combination of four molecular markers (intra-circulating EC SENCR, intra-monocyte SENCR, surface/intra-circulating EC CD146 and surface/intra-monocyte CD14) along with diabetes mellitus may serve as the early diagnostic tool for EOCAD [32].

LncRNA	Clinical Application	Physiological/pathological impact	Mechanism involved
ANRIL [33,34]	Adjacent to 9p21.3 CAD risk locus	Risk allele is associated with altered ANRIL expression and splicing	Regulated cell proliferation and senescence of vascular smooth muscle cells either by a scaffold, guiding effector-proteins to chromatin, or by regulating miR-181a/Sirt1
CoroMarker [26,27]	Diagnosis of CAD	Decrease pro-inflammatory cytokine secretion from THP-1 monocytic cells	Unknown
LIPCAR [29]	Prediction of cardiac remodeling	Associated with future development of cardiac remodeling	Unknown
GAS5 [35–37]	↑ in arterial plaques ↓ in plasma of CAD patients	Modulate macrophages and ECs apoptosis after ox-LDL stimulation	Unknown
SENCR [30–32]	Diagnosis of early onset CAD: ↓ in circulating ECs ↑ in monocytes	Regulation of commitment from pluripotent cells and angiogenic capacity of EC	Regulate myocardin gene regulation to stabilize the contractile state of VSMCs
DKFZP434I0714 [38]	Prediction of adverse CV events in uremic patients	Modulate stress-induced EC apoptosis, endothelial dysfunction, and vascular inflammation	Unknown

Table 1. Comparison of baseline characteristics and treatment between Taiwan and other countries according to income inequality

5. LncRNA GAS5

LncRNA GAS5 was significantly increased in the plaque of atherosclerosis patients compared to healthy people [35]. However, the expression level of plasma lncRNA GAS5 was significantly lower in patients with CAD. GAS5 decreased the level of p-mTOR without change of total mTOR in human coronary artery endothelial cells, which is an essential initiator of the pro-inflammatory response of monocytes/macrophages [36]. Furthermore, gain- and loss- of function studies showed that GAS5 modulates macrophages and ECs apoptosis in vitro. Interestingly, these effects of GAS5 on EC apoptosis is mediated by macrophage-derived exomes after oxLDL stimulation, which demonstrated the interplay of macrophage and EC during atherosclerosis development [37].

6. LncRNA DKFZP434I0714

Cardiovascular (CV) diseases are the primary cause of morbidity and mortality in patients with end-stage renal disease (ESRD), accounting for nearly 50% of deaths in this population [39,40]. In a cohort of patients with chronic kidney disease, end-stage renal disease (ESRD) with or without cardiovascular (CV) event, circulating lncRNA expression profiles discriminate between ESRD patients with and without an adverse CV event. Among the differentially expressed lncRNAs, eight plasma lncRNAs were identified as potential predictors of adverse CV outcomes in uremic patients, and lncRNA DKFZP434I0714 was confirmed as an independent predictor of adverse CV outcomes in patients with ESRD. LncRNA DKFZP434I0714 is not dysregulated in failing human heart, but it is shown to regulate endothelial function. Gain- and loss- of function studies showed lncRNA DKFZP434I0714 modulates stress-induced EC apoptosis, endothelial dysfunction, and vascular inflammation, which are hallmarks of vascular complications associated with uremia [38]. Table.1 summarizes the examples of lncRNAs that have been shown to be potential biomarkers for cardiovascular diseases.

Even though the cellular and pathological specificity of lncRNAs make them suitable biomarkers, using lncRNAs as clinical biomarkers is potentially limited by the difficulties in their isolation and quantification. RNA is very difficult to isolate in reasonable quantities from acellular bodily fluids such as plasma or serum. In addition, the high cost and low throughput associated with RNA processing and quantification also limit the application of lncRNA as a biomarker. For example, it seems unlikely that lncRNA could replace cardiac troponins in the diagnosis of AMI, as clinical tests for cardiac troponins are relatively cheap, fast and well-validated. Therefore, the potential usage of lncRNAs as biomarkers is more likely to be prognostic, rather than diagnostic, in cardiovascular diseases.

7. Conclusions

Emerging evidence indicates the critical roles of lncRNAs in the complex regulatory network of cardiovascular development and diseases. It has been well-demonstrated that many of these lncRNAs could be utilized as novel therapeutic targets and/or biomarkers for diagnosis/prognosis for cardiovascular diseases, including cardiac hypertrophy, myocardial infarction, heart failure, and atherosclerosis. It will require extensive efforts, however, to refine the approaches of modulating lncRNA expression in vivo and to improve/standardize the quantitative assays for lncRNA biomarkers to make clinical translation possible.

Conflicts of Interest:
The authors declare no conflict of interest.

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