Circulating long non-coding RNAs as biomarkers of acute myocardial infarction

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: For the past decades, acute myocardial infarction (AMI) has been the most common cause of death in both genders. The invention of percutaneous intervention (PCI) has saved thousands of lives. The most important prognostic factor for AMI is the time between the onset of AMI and revascularization. Heart centers around the world have devoted themselves to improving the evaluation tools for AMI. Among these evaluation tools, MI biomarkers have undergone the most evolution over the past decade. Traditionally used protein biomarkers like CK-MB and troponin are limited by serum instability and low sensitivity, especially if patient is admitted right after chest pain. Because both biomarkers require 4–6 hours to be detectable. Recently, the researches of one type of noncoding RNA namely long non-coding RNA (lncRNA) in the MI field has become popular. Even not coding any protein in most cases, lncRNA has an astounding effect on protein expression and modification. Expressions of lncRNA in the peripheral blood have been found to change during AMI. Both basic and clinical studies suggest that circulating lncRNA can be potentially used as a biomarker for myocardial injury.

Keywords: Circulating long non-coding RNA; acute myocardial infarction (AMI); biomarker

Introduction

Acute myocardial infarction (AMI) is recognized as the number one killer worldwide. Epidemiology studies suggested no significant changes in the incidence rate of hospitalized AMI from the 1980s to 1990s among the general population. However, 28-day case mortality remains high, especially in community surveillance studies (1). Gender differences exist in the data, as well. Since 1984, more women suffered from cardiovascular disease than men. Every year, 6.6 million American women are affected by coronary heart disease, with an estimated 262,000 of those women developing acute coronary artery syndrome (ACS) (2). Social status is another significant variant that may obscure the incidence. In a study investigating disparities and trends in AMI between 1999 and 2013, similar declination in AMI hospitalizations and 1-year mortality were observed in patients of different income levels (3).

Early detection of AMI is crucial since timely treatment improves survival. For now, troponin is the most commonly used biomarker to diagnose AMI and predict mortality. However, it has its limitations as it takes hours for troponin levels to be detectable. Furthermore, troponin is less reliable in conditions complicated by renal failure, pulmonary disease, atrial fibrillation or myocarditis (4). Nowadays, researchers have turned their attention to the RNA field. With the prevalent use of microarray and RNA-sequencing, increasing evidence show noncoding RNAs contribute to disease development. Circulating noncoding RNAs could potentially serve as an indicator for disease progress and prognosis. Unlike protein markers, these RNAs are more

stable in the peripheral blood and organ-specific. Thus far, noncoding RNA has been well-studied in cancer (5).

Only 2% of genomic transcripts are in charge of coding protein. The majority of RNAs are not expressed and are grouped as non-coding RNAs. Despite this, non-coding RNAs are extremely important because they are involved in protein modification, promotion, and silencing. Non-coding RNAs consist of microRNAs (miRNAs), snoRNAs, siRNAs, snRNAs, piRNAs and long non-coding RNAs (lncRNAs). Apart from the crucial roles they have in cancer development, emerging evidence has proven that they also have a close relationship to the pathophysiology of the human heart (6-8). While miRNAs are well studied, the overall understanding of lncRNAs remain uncertain. In this review, we will discuss circulating lncRNA and its use as a biomarker for AMI.

Functions of lncRNA

lncRNAs are RNAs longer than 200 nucleotides. They are expressed constantly throughout the genome.

In general, lncRNAs can be classified into housekeeping noncoding RNAs and regulatory noncoding RNAs. The former is expressed consistently, while the latter is expressed when modifications are required. In terms of function, lncRNAs can also be divided into five major categories: sense, antisense, bidirectional, intronic and intergenic (9). A well-known generic model for X chromosome dosage compensation in female mammals demonstrated the contribution of lncRNAs in epigenetic regulation. The lncRNA: H19, was one of the first identified genes involved in X chromosome imprinting (10). lncRNAs influence gene expression through: (I) imprinting XIST, such as Airn, H19, and KCNQ1OT1; (II) guiding molecules through various biological activity; (III) enhancing transcription of proteins; (IV) acting as a molecular sponge and creating alternative splicing activity to generate different protein isoforms (11); (V) serving as precursors for small RNAs and affecting the process of transcription by switching their pre-mRNA splicing patterns; and (VI) acting as structural RNAs in the maintenance of the cellular cytoskeleton (12). Most lncRNAs are located in the nucleus to modify protein transcription. Other lncRNAs which are detectable in the peripheral blood functions by recruiting or sequestering proteins. These circulating lncRNAs have been found to be related to different clinical conditions, especially cancer and cardiovascular diseases (13).

Circulating lncRNAs identified to be related to cardiovascular disease

Table 1 summarizes circulating lncRNAs which are proven to be related to cardiovascular disease. Among these lncRNAs, LIPCAR, ANRIL and MIAT are the most well-studied in cardiovascular disease.

LIPCAR was one of the most well-known lncRNAs that have been studied. Taking advantage of genome-wide screen (GWS), a good amount of lncRNAs were found to present differentially in plasma of patients with or without severe left ventricular remodeling after AMI. Among them, LIPCAR has the strongest association with ventricular remodeling. Furthermore, the study also demonstrated that higher levels of LIPCAR is closely related to increased cardiovascular mortality (18).

ANRIL is one of the lncRNAs which could be found both in tissue and blood. It is encoded in the chromosome 9q21 region which was found to be closely associated with coronary artery disease (CAD) susceptibility. This correlation is independent of previously established risk factors like hyperlipidemia, hypertension, obesity and diabetes (24). ANRIL is transcribed by RNA polymerase II and has multiple isoforms. After binding polycomb proteins like CBX7 and SUZ12, it controls cell pathophysiology by regulating histone modification in the CDKN2A/B locus. CDKN2A/B manages cell proliferation. Overexpression of ANRIL leads to cell proliferation and tumor formation. In contrast, depressed ANRIL will lead to suppressed cell growth. These mechanisms are reported as key factors in the formation of atherosclerosis lesions (25).

Myocardial infarction associated transcript (MIAT) was another group of important circulating lncRNAs identified by studying a large group of haplotype-based single nucleotide polymorphism (SNP) markers. In vitro study demonstrated that a variant of its SNPs will change the transcription level of its expression, which may contribute to some extent of the pathogenesis of complications like cardiac fibrosis after AMI (15). In an animal study, MIAT showed its ability in controlling progress of cardiac fibrosis in the fibrosis controlling system: MIAT ↑ → miR-24 ↓ → Furin/TGF-β1 ↑ → cardiac fibrosis ↑ (26).

Other circulating lncRNAs found to be related to CAD includes H19, CHAST, CDKN2B-AS1, MALAT1, NRF, etc. These lncRNAs have been found to participate in cardiac remodeling, regulation of thrombosis, and cardiometabolism.

Table 1 Summary of the lncRNAs related to cardiovascular diseases

Candidate gene	Diseases association	Results of studies
ANRIL	Atherosclerosis	Transcripts EU741058 and NR_003529 of ANRIL were significantly increased in carriers of the risk haplotype ($P=2.1\times10^{-12}$ and $P=1.6\times10^{-5}$, respectively); expression of ANRIL transcripts was directly correlated with severity of atherosclerosis (EU741058 and NR_003529; P=0.02 and P=0.001, respectively) (14)
MIAT	MI	MIAT was isolated from the SNPs that showed markedly significant association with MI (chi^2=25.2, P=0.0000005; comparison of allele frequency, 3,435 affected individuals versus 3,774 controls, in the case of intron 15,338 C > T, rs2331291) (15)
H19	Heart failure, CAD	H19 increases in heart failure patients, both end-stage and non-hyperhomocysteinemia produces tissue-specific changes in H19 differentially methylated domain methylation and increased vascular expression of H19 (16)
CHAST	Cardiomyocyte hypertrophy	CHAST is up-regulated in cardiomyocytes in vivo in transverse aortic constriction-operated mice. In accordance, CHAST homolog in humans was significantly up-regulated in hypertrophic heart tissue from aortic stenosis patients and in human embryonic stem cell-derived cardiomyocytes upon hypertrophic stimuli (17)
LIPICAR	Cardiac remodeling	Level of LIPCAR 1 year after MI in patients with significant LV remodeling is shown as reference. P<0.0001 vs. LV remodeling 1 year after MI. Level of LIPCAR in patients with future cardiovascular death vs patients without cardiovascular death in the case-control study. P<0.0001 vs. no cardiovascular death (18)
CDKN2B-AS1	Hypertension	SNPs rs10757274, rs2383207, rs10757278, and rs1333049 within CDKN2B-AS1 are detected at significantly higher frequencies in hypertension patients (P=0.001) (19)
MALAT1	Endothelial cells modification	Genetic deletion of MALAT1 inhibits proliferation of endothelial cells; pharmacological inhibition of MALAT1 reduces recovery of capillary density after ischemic events (20)
Necrosis-related factor (NRF)	Cardiomyocytes necrosis	NRF regulates cardiomyocytes necrosis by directly binding to miR-873 and modulates RIPK1/RIPK3 expression (21)
Noncoding repressor of NRAT (NRON) and myosin heavy-chain-associated RNA transcripts (MHRT)	Heart failure	NRON and MHRT were both elevated in plasma levels in HF patients. The predictive power of NRON is comparable to that of NT-proBNP: AUC is 0.844 for NT-proBNP and the value for NRON was 0865. AUC value for MHRT is 0.702 (22)
aHIF	Heart failure	The HIF pathway is observed to remain activated in chronic human heart failure. The level of aHIF was nearly twofold higher (P<0.01) in failing myocardia compared to control group (23)

CAD, coronary artery disease.

Circulating lncRNA as biomarker for AMI

Traditional biomarkers for AMI

In an effort to minimize the complications post MI, sensitive protein markers like CK-MB, CK and troponin are majorly used. However, in the very early phase of AMI, these markers could be undetectable since they need time to enter the peripheral blood after injury.

Troponin consists of troponin T (TnT), troponin I (TnI), and troponin C (TnC). These isoforms sit on the thin filament of the striated muscle contractile apparatus. Once the muscle is exposed to ischemic injury, release of TnT occurs in a biphasic pattern. Comparatively, TnI has a smaller store so its release pattern tends to be monophasic. Both TnI and TnT levels begin to rise in the first 4-8 hours, followed by a peak level at 12–24 hours (27). These characteristics limit the use of troponin in the very acute phase of myocardial injury. A new high-sensitivity troponin assays were developed in the hope to solve this problem. The assay is able to detect a troponin concentration below the 99th percentile above its limit of detection for greater than 50% of healthy people (28). A prospective cohort was carried out to explore the sensitivity of a 1-hour diagnostic algorithm to diagnose AMI by using high-sensitivity troponin I assay. Results suggested that the algorithm showed significant improvement in mortality compared to a 3-hour approach (29). This study revealed the advantages of the new assay over their conventional counterparts and showed that the new assay can be used for quick diagnosis and decision making. However, debate exists in the specificity of the new assay. After all, AMI is not the only cause of elevation in troponin. Myocardial ischemia, cardiomyocyte apoptosis, and increased cell wall permeability can also cause elevation in troponin levels. In addition, the existence of individual-specific biological variability may obscure the results of the new assay. These pre-analytic variations change over time among different people. Factors like circadian rhythm, seasonal differences, and random biological fluctuations may trigger the changes. Thus, either the criteria of the high-sensitivity troponin assay should be adjusted or more critical interpretation of the results should be performed (18).

Before high-sensitivity troponin assay was available, another low weight protein, heart-type fatty acid binding protein (H-FABP), was studied for early precise detection of AMI. H-FABP has a low plasma concentration ranging from 1.0 to 11.4 µg/L, making it possible to catch even slight elevations in concentration levels as early as half an hour after the onset of myocardial injury. Furthermore, H-FABP peaks at 4 hours and normalizes within 24 hours, which makes it ideal for evaluating re-infarction (30). Some clinical studies appreciate the value of H-FABP by comparing it to traditional biomarkers, such as CK-MB and troponin (31,32). In patients with non-ST segment elevation acute coronary artery syndrome (NSTE-ACS), H-FABP has shown to be more sensitive than troponin T (33). Unfortunately, other studies failed to come up with supportive conclusions. A meta-analysis involving 3,709 patients argued that H-FABP is not qualified for quick diagnosis of AMI when used alone (34).

Apart from the markers for necrosis during AMI, markers for neutrophilic activation like myeloperoxidase and matrix metalloproteinase, inflammation like CRP and PaPPA, endothelial activation like endothelin and adrenomedullin, biomechanical stress like BNP, ANP, GDF-15 and ST2 have all been explored during the past decade (35). Most involved studies used death or major cardiovascular events to measure the effectiveness of these biomarkers. Lack of stratification of other morbidities influences the usefulness of these biomarkers.

Circulating lncRNAs that has already identified to be biomarker for AMI

Encouraged by the previous study done on lncRNAs and cardiovascular disease, later studies explored whether circulating lncRNAs could be used as biomarker for AMI.

Research based on a group of Chinese Uyghur patients were carried out to explore the lncRNAs expression pattern in peripheral blood during AMI on GWS. There were 3,624 up-regulated lncRNAs and 1,637 down-regulated lncRNAs were caught between the AMI patients and non-AMI patients. These differences serve as rationale for using lncRNAs as markers for AMI (36).

Sequential studies have found several specific lncRNAs which are more sensitive to be caught and more closely related to disease progress. A group of lncRNAs named urothelial carcinoma-associated 1 (UCA1), initially found in bladder and lung cancers, is proven to be a candidate marker for AMI. In this study, blood was drawn from patients with and without AMI. qRT-PCR was used to measure the concentration of lncRNA from these samples. The level of UCA1 was found increased in the first 2 hours followed by a decrease in the serum level. The lowest level observed were 6–12 hours after an AMI (P<0.05). Levels of UCA1 returned to control levels in 48–72 hours. Even 72–96 hours

after an AMI, UCA1 levels in AMI patients remained higher than that of non-AMI patients (37). The study also found that there were no significant differences in UCA1 levels of AMI patients with or without hypertension and/or diabetes, suggesting that expression of UCA1 is independent of these two most common co-morbidities of AMI (38).

After adjusting for precipitating factors like hypertension and diabetes, another study was performed with 138 AMI patients and 149 non-AMI control patients. By comparing the other known cardiac-specific lncRNA, such as SRA, DIO3OS, SAF, NESPAS, MIAT, NRON, ANRIL, CARL, HCG22, SENCER, FENDRR, MHRT and aHIF, circulating lncRNAs called zinc finger antisense 1 (ZFAS1) and Cdr1 antisense (CDR1AS) showed significant differences of expression in the blood samples. ZFAS1 was lower in AMI (0.74±0.07) than in non-AMI individuals (1.0±0.05, P<0.0001) and healthy individuals (1.22±0.08, P<0.0001). In contrast, results of CDR1AS revealed a markedly increased level in the AMI group (2.18±0.24) compared to the non-AMI group (1.0±0.05, P<0.0001) (37). Both univariate analysis and logistic regression suggested ZFAS1 and CDR1AS were diagnostic for AMI. Like UCA1, these two lncRNAs were independent of other cardiac risk factors.

Similar studies on AMI animal model showed that more than 700 of lncRNAs were affected by MI. Most of these were inflammatory-related, suggesting an early inflammatory change. Among them, MIRT1 and MIRT2 had the most significant changes in levels between MI mice and sham-operated mice. Both were positively related to MI and ejection fraction (EF), but negatively correlated with infarct size (39). Related study isolated 168 differentially expressed lncRNAs during MI period. LncRNAs such as ENSMUST00000124047 was down-regulated in the MI group while AK166279 was up-regulated (40).

Other candidate circulating lncRNAs are summarized in *Table 2*.

LncRNAs and risk factors for AMI

LncRNAs affect the risk factors for cardiovascular disease. It has been well-established that hypertension and metabolic disease are the main risk factors contributing to AMI (45). Started with the finding of chromosome 9p21.3 which is a major locus for cardiovascular disease independent of conventional risk factors, more and more studies have been done to verify the power of lncRNAs (19). Among 30,586 lncRNAs, about 1.9% of them were differently expressed

(DE) (P<0.05) in human aortic smooth muscle cells after mechanical stretch, providing insight into their role in vasculature remodeling (46). lncRNAs like LncRNA-XR007793 and growth arrest-specific 5 (GAS5) are found to contribute to hypertension development by controlling this process (47,48). A traditional Chinese herbal: Lycium barbarum L (or Goji) can soften blood pressure by targeting the expression of a lncRNA named sONE (49). On the other hand, linc-NFE2L3-1 has been proven to promote waist-hip ratio and increase risk for metabolic disease (50). Thanks to the GWS, a decent number of lncRNAs variants have been discovered to modulate metabolic reactions involving lipid transfer, glucose homeostasis and inflammation (51).

Circulating lncRNAs and prognosis after AMI

Circulating lncRNAs could also be used to evaluate prognosis after AMI. The most widely used score system used to predict outcomes after AMI is the thrombolysis in myocardial infarction (TIMI) score. It mainly includes the age, diabetic history, baseline blood pressure, BMI, ECG changes and previous history of CAD. lncRNAs enrich the predictors for mortality and morbidity after AMI. One of the well-known lncRNAs is uc022bqs.1, also named as LIPCAR, was first identified as potential marker for LV remodeling. Its expression was found to be associated with the severity of coronary heart disease and could predict mortality in patients with heart failure (41). Following researches on other circulating lncRNAs claimed ZFAS1, CDR1AS to be candidate to predict mortality by comparing to traditional prognostic markers like AST, LDH, and CK. Both correlated with survival independent of diabetes, hypertension, and smoking history (49). Evaluation of the 5 circulating lncRNAs: aHIF, ANRIL, KCNQ1OT1, MIAT and MALAT1 proves their ability to predict LV dysfunction at 4-month follow-up by EF ≤40%. However, they failed to find any strong correlation between the expression of lncRNAs and the severity of infarction (52).

Perspective

Circulating lncRNAs have emerged as a promising new biomarker for cardiac injury. It is more tissue specific and correlates to the extent of the injury. What is more, compared to protein markers, lncRNAs are less affected by other metabolic factors and morbidities. Several lncRNAs have been identified as candidates for markers, specifically UCA1, ZFAS1, CDR1AS, MIRT1, and MIRT2. In

Table 2 Summary of lncRNAs involved in AMI

Candidate gene	AMI relation	Results of studies
ANRIL	Epigenetic regulation of ANRIL promoter methylation predicts later coronary heart disease in children.	CpG5 was associated with increased childhood pulse wave velocity [β=0.066 m/s/10% methylation increase (95% CI, 0.004 to 0.128), P=0.037]; 10% decreases in methylation at CpG1 and CpG2 were associated with increased heart rate [CpG1 β=1.93 (0.07 to 3.8) beats/min, P=0.041; CpG2 β=2.30 (0.18 to 4.41) beats/min, P=0.033] (38)
Haplotype G-A-A-G of lincRNA-p21	Associated with decreased risk of CAD and MI	Haplotype G-A-A-G is associated with decrease in risk for CAD (OR =0.78, 95% CI: 0.63–0.97, P=0.023); it also has a significantly lower risk for MI (OR =0.68, 95% CI: 0.51–0.91, P=0.010); the benefit effect is more pronounced in premature CAD and MI (P=0.017, OR =0.67 for premature CAD, and P=0.041, OR =0.65 for premature MI) (37)
UCA1	Marker for AMI	UCA1 level in AMI patients were decreased in 2–6, 6–12, 12–24 and 24–48 h after AMI (P<0.05). The level is the lowest in 6–12 h, then recover back to control value in 48–72 h (41)
ZFAS1 and *CDR1AS*	Marker for AMI	ROC analysis: AMI and non-AMI, the area under ROC curve was 0.664 (95% CI: 0.594–0.733) for ZFAS1 alone, 0.671 (95% CI: 0.600–0.742) for CDR1AS alone, and 0.691 (95% CI: 0.622–0.760) for combination of both (42)
MIAT	Altered expression of MIAT by the SNP confers risk of MI	6 SNPs in 22q12.1 showed markedly significant association with MI (chi²=25.27, P=0.0000005; comparison of allele frequency), MIAT was identified among them (27)
Myosin heavy chain associated RNA transcripts (*MHRT*)	Marker for AMI	MHRT is significantly elevated in the blood from AMI patients compared with the healthy control (P<0.05); mouse study showed that deletion of MHRT gene leads to significant more apoptotic cells in H$_2$O$_2$-induced cardiac injury (43)
Uc022bqs.1 (*LIPICAR*)	Early diagnosis of CAD	Serum uc022bqs.1 is found to be up-regulated in coronary heart disease patients and more sensitive to be detected. In addition, circulating uc022bqs.1 is higher than those in patients with unstable angina (0.26±0.29), stable angina (0.03±0.33) and normal control (−0.29±0.39) (44)

CAD, coronary artery disease; AMI, acute myocardial infarction.

addition, changing levels of circulating lncRNA imply MI/RI, which is a common, but often neglected problem, in AMI.

Despite all the encouraging evidence about lncRNAs, challenges still exist. The mechanism of how different lncRNAs are released into circulation remains unclear, which makes it difficult to determine whether fluctuating levels of lncRNAs are due to pathologic destruction or physiologic injury (53). Also, only limited numbers of specific lncRNAs have been tested. Nevertheless, lncRNAs keeps being a hot topic and have a promising future biomarkers. Multi-center studies need to be performed to identify more lncRNAs and their association with AMI and other cardiovascular diseases.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/10.21037/ncri.2017.08.03). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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