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Circulating non-coding RNAs as biomarkers in coronary artery disease

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Abstract

Coronary artery disease (CAD) is a leading cause of mortality worldwide. Atherosclerosis involves an interplay of different pathological mechanisms, such as progressive inflammation, abnormal lipid metabolism, and oxidative stress, and as such represents the basic pathological phenomenon underlying CAD. Atherosclerotic plaque narrows the lumen of coronary arteries, creating an ischemic environment for the heart muscle, which finally leads to clinical complications, such as acute myocardial infarction. Currently, there are no biomarkers that could predict plaque stability or major adverse cardiovascular events (MACE). Numerous functional non-coding RNA (ncRNA) species influence basic cellular functions, and as such play a role in the development and progression of CAD. Of these ncRNAs, micro RNAs (miRNAs) and long non-coding RNAs (lncRNAs) are the most investigated. Considering that ncRNAs detected in extracellular fluids can originate from different cells, circulating ncRNAs are being intensively investigated as potential biomarkers in the diagnosis and prognosis of CAD. In the following paper, we provide current insights into potential molecular mechanisms by which miRNAs and lncRNAs contribute to the pathology of CAD and discuss their potential role as biomarkers in diagnosis and prognosis of disease.

Keywords: coronary artery disease, non-coding RNA, miRNA, lncRNA, biomarkers

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Introduction:

Coronary artery disease (CAD) is a leading cause of mortality worldwide. According to the latest reports by World Health Organization, CAD now accounts for 16% of total deaths from all causes (1). The latest official report of the Institute of Public Health of Serbia from 2019., showed that 51.8% of all deaths was due to some form of cardiovascular disease, with 17.7% of those deaths being the consequence of CAD (2). The basic pathological phenomenon which underlies CAD is the formation of atherosclerotic lesion, most often at an early age, which gradually, over the course of decades, can progress into atherosclerotic plaque (3). This plaque narrows the lumen of coronary arteries, creating an ischemic environment for the heart muscle, which finally leads to the clinical complications, such as angina pectoris, acute myocardial infarction (AMI) with or without ST elevation (STEMI, NSTEMI), or sudden cardiac death (SCD) (4). Many studies suggested that development of acute coronary events, such as AMI, was actually influenced by the composition and vulnerability of the plaque. Moreover, the stability of these plaques may also be influenced by calcification and neovascularization (3). Atherogenesis is a complex multifactorial process which involves an interplay of different pathological mechanisms, such as the infiltration of immunogenic cells in tunica intima of coronary arteries, progressive inflammation, abnormal lipid metabolism (3), oxidative stress (5). To this day, there are no non-invasive methods that could predict the behavioral nature of plaques and predict acute coronary events or major adverse cardiovascular events (MACE). The latest trends in modern diagnosis and prognosis of CAD should be focused on the search for novel biomarkers that could reliably and efficiently asses this disease.

Given that the protein coding region makes up less than 2% of whole genome (6-8), questions arose about non-coding regions of DNA molecule and their potential roles in the pathology of CAD. Recent studies have shown that 80% of the non-coding region is transcribed into functional non-coding RNA (ncRNA) molecules (9). There are numerous species of ncRNA molecules, of which micro RNAs (miRNAs) and long non-coding RNAs (lncRNAs) are the most investigated (10). In almost every cell, these molecules influence basic cellular functions, such as development and differentiation, through epigenetic regulation (11). Recent studies have also shown that ncRNAs have substantial roles in the development and progression of CAD, marking them as potential novel biomarkers and pharmaceutical targets. With the advances in personalized medicine which are contributing to the treatment of complex diseases, increasing evidence suggests that understanding of complete ncRNA expression profile and its involvement in the pathology of CAD is becoming very important. However, we are far from fully understanding how these molecules can be utilized as biomarkers in the assessment of CAD. Even though traditional biomarkers have been proven to be sensitive and specific in later stages of CAD (12,13), there are still no biomarkers which can assess CAD in the early stages of its development or predict MACEs. Therefore, the search and validation of these promising novel ncRNA biomarkers in different CAD settings has been ongoing.

The aim of this review is to provide current insights into potential molecular mechanisms by which miRNAs and lncRNAs contribute to the pathology of CAD and to discuss their potential role as future biomarkers in diagnosis and prognosis of disease.

Biogenesis and functions of miRNAs

MicroRNAs (miRNAs) are a class of ncRNAs that regulate gene expression post-transcriptionally (14). These small ncRNAs are single-stranded RNAs, 19-24 nucleotides in length (15). So far, the miRBase data set (version 22) counts 1917 miRNA precursors and 2656 mature miRNAs in humans (16). It is estimated that 60% of protein-encoding genes in mammals are regulated by miRNAs (17).

The formation of mature miRNA requires two ribonucleases, Drosha and Dicer, with the former localized in the nucleus, and the latter in the cytosol. Drosha cleaves the nascent miRNA transcript (primary miRNA, pri-miRNA) to 65-75 nucleotide long miRNA precursor (pre-miRNA), thereafter Dicer cleaves pre-miRNA and a mature miRNA duplex is formed (18). One strand of miRNA duplex, called the guide strand, is incorporated into the RNA-induced silencing complex (RISC), which leads the action of miRNAs and downregulates gene transcripts through a process called RNA interference (18,19). MiRNA-guided RISC binds to the 3'-untranslated region (3'-UTR) of a target molecule – a complementary messenger RNA (mRNA), leading either to its translation inhibition or its degradation (14). Since binding of miRNA to the target mRNA does not require perfect base pairing, one miRNA has multiple target mRNAs, but one mRNA is also the target molecule for a larger number of miRNAs (20,21).

Although primarily localized intracellularly, miRNAs are also detected in extracellular fluids (plasma, serum, cerebrospinal fluid, urine, saliva, breast milk) originating from damaged or living cells (14,15). Considering the availability of blood as a sample, circulating miRNAs are being intensively investigated as potential biomarkers in the diagnosis and prognosis of cardiovascular and other diseases (22). Mitchell et al. (23) demonstrated high plasma miRNA stability, despite high RNase levels. What gives circulating miRNA stability is packaging in microparticles (microvesicles (MV), exosomes and apoptotic bodies) and association with proteins (Argonaute 2) or lipoprotein particles (high-density lipoprotein, HDL) (22). Today, circulating miRNAs are known to be fairly stable even in adverse environmental conditions, such as high temperature, base/acidic environment, prolonged room temperature stays or repeated freeze-thawing. MiRNA that are part of microparticles could act as intercellular signalling molecules with autocrine, paracrine, and endocrine functions (14). These functions imply the regulation of various essential biological and cellular processes, such as differentiation, proliferation, and apoptosis (17).

Potential roles of miRNAs in CAD

MiRNAs are now recognized as important players in almost every step of atherogenesis, including endothelial dysfunction, leukocyte invasion and activation, foam cell formation, vascular smooth muscle cell (SMCs) migration, angiogenesis and

degradation of extracellular matrix and plaque stability (17). Angiogenesis contributes to the development of atherosclerotic plaque, which becomes more unstable because of the accumulation of erythrocytes and inflammatory cells and can easily rupture. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) play a key role in the process of angiogenesis. VEGF and FGF exert their pro-angiogenic effect through their receptors, VEGFR2 and FGFR1 (24). Muscle-specific miR-133a (myomiRNA) suppresses angiogenesis by inhibiting gene transcription for these receptors (24,25). The fibrous cap of atherosclerotic plaque is thinned by the action of matrix metalloproteinase-9 (MMP-9), which has endopeptidase activity and breaks down extracellular matrix proteins. MMP-9, secreted from macrophages, is more present in unstable than in stable plaques (26). Reversion-inducing-cysteine-rich protein inhibitor with Kazal motifs (RECK) regulates the action of this enzyme either by inhibiting MMP-9 secretion, or by inhibiting its activity (27). MiR-21 inhibits the transcription of the gene-encoding RECK, allowing higher MMP-9 activity and consequently unstable plaque formation (28). This miRNA, one of the most present ncRNAs in most mammalian cells, is increased in inflammation, and simulates an anti-inflammatory response (29). The SMCs present in the tunica media of the arterial wall are responsible for artery contraction and ECM protein production. They have two phenotypes: contractile, which is a characteristic of healthy blood vessels and expresses more contractile proteins, and synthetic, which is dominant in atherosclerosis and is characterized by higher expression of ECM components and poorer differentiation. When SMCs are activated and migrate from the tunica media to the tunica intima, phenotype switching occurs and SMCs predominantly produce fibrous cap proteins (30). MiR-143 and miR-145, whose expression is down-regulated in atherosclerotic plaques, target transcription factors Kruppel-like factor 4 (Klf4), myocardin, and Elk-1, which are stimulators of SMCs differentiation and inhibitors of SMCs proliferation (31). HMB box-containing protein 1 (HBP1) is a transcription repressor that reduces the expression of p47 phagocyte oxidase (p47phox) and migration inhibitor factor (MIF), leading to reduced production of reactive oxygen species (ROS) and reduced cholesterol entry into the damaged blood vessel wall (32). MiR-155 promotes atherosclerosis by reducing the expression of HBP1, but also another transcriptional suppressor - B-cell lymphoma-2 (BCL6), which reduces the C-C motif chemokine ligand 2 (CCL2) expression (32,33). Increased miR-155 expression decreases BCL6 expression, resulting in increased CCL2 expression and stimulation of monocyte recruitment into plaque (33). Previous studies have given conflicting results regarding the role of miR-126 in the development of atherosclerosis. miR-126 is highly expressed in endothelial cells and exerts its action by paracrine mechanism, which means it stimulates the proliferation of SMCs that come into close contact with endothelial cells in plaque. Forkhead box O3 (FoxO3), BCL2 and insulin receptor substrate-1 (IRS1) are miR-126 target genes involved in this process (34). On the other hand, miR-126 acts in an anti-atherogenic manner by indirectly increasing the expression of the chemokine C-X-C motif ligand 12 (CXCL12), which stimulates the recruitment of progenitor cells (35). MiR-126 also reduces the expression of Noth1 delta-like 1 homolog inhibitor (Dlk1),

which directly affects endothelial cells and stimulates their proliferation (36). Through the inhibition of vascular cell adhesion molecule 1 (VCAM-1), miR-126 reduces leukocyte adhesion (37).

MiRNAs as potential diagnostic biomarkers in CAD

Creatine kinase-MB (CKMB) mass concentration and troponin-I (TnI) are traditional cardiac biomarkers for detecting AMI with sensitivities >92% at 8–24 h post-infarct (38). The gold standard for diagnosing AMI is cardiac TnI (cTnI); however, its elevated plasma concentrations may be a consequence of other conditions, such as atrial fibrillation, chronic kidney disease, sepsis, and severe heart failure (39). Wang et al. (39) reported a significant increase in plasma levels of miR-133a in patients with AMI, with peak concentrations circa 21 h after the first symptoms appeared. cTnI was determined in the same samples, and a similar trend of concentrations was observed, with complete normalization after 3 days in both markers. miR-133a expression positively correlated with cTnI in plasma, but also with the severity of coronary artery stenosis. Receiver operating characteristic (ROC) analysis showed that circulating miR-133a had high diagnostic accuracy for AMI with an AUC of 0.918. Diagnostic accuracy increased by adding cTnI and clinical model (age, sex, smoking status, presence of hypertension, diabetes and hyperlipidemia) to the miR-133a (an AUC of 0.947) (39). Cardio-specific miR-208a showed even greater sensitivity than cTnI in AMI detection. An increase in miR-208a was observed in all study patients in the first 4 hours, while this was not the case with cTnI. Moreover, plasma miR-208a levels did not change under the influence of chronic renal disease, making miR-208a a more specific biomarker for detecting AMI than cTnI. The same study demonstrated significantly higher levels of miR-1, miR-133a and miR-499 in patients with AMI compared to healthy controls (HC) (40). miR-499 is detectable in plasma 1 h after the chest pain appears, which is earlier in comparison with CK-MB and cTnI (2 h and 2-4 h respectively). Despite this, the diagnostic accuracy of miR-499 is lower than cTnI (an AUC of 0.86 vs 0.90). The level of circulating miR-499 is also independent of renal function (41). Significantly higher plasma levels of miR-1, miR-133a, and miR-208b were observed in patients with AMI compared with patients with unstable angina pectoris (UAP), while levels of miR-133b, miR-208a, and miR-499 did not differ significantly (42). The expressions of miR-320b and miR-125 were down-regulated in patients with AMI compared to HC (43). Different studies have also addressed differences in the expression of individual miRNAs between STEMI and non-STEMI. Levels of miR-133a, miR-208b, miR-451 and miR-499 were significantly higher in patients with STEMI compared to non-STEMI (42,44,45), while levels of miR-145 and miR-208a were significantly lower (42,46).

MiRNAs as potential prognostic biomarkers in CAD

In their review from 2019, Melak and Baynes listed miRNAs with potential prognostic significance in CAD (17). The cohort, which included a large number of CAD patients (ACS and SAP), examined the prognostic significance of 3 serum miRNAs. The follow-up period was around 4 years and the number of cardiovascular deaths was

recorded. The results showed that miR-197 and miR-223, involved in the processes of endovascular inflammation and platelets activation, can predict cardiovascular death (47). Expressions of 10 miRNAs with vascular functions: miR-126, miR-222, let-7d, miR-21, miR-20a, miR-27a, miR-92a, miR-17, miR-130 and miR-199a were examined in circulating MV and plasma in patients with SAP. Although there was no correlation in plasma between these miRNA levels and cardiovascular adverse events, the expression of miR-126 and miR-199a in circulating MVs correlated with a lower percentage of cardiovascular adverse events. This study also showed that of all the plasma compartments, miR-126 and miR-199 are the most present in MV, originating from endothelial cells and platelets (48). Although miR-208b expression was higher in ACS patients who died during the first 30 days of follow-up, this miRNA could not predict long-term mortality (44), as with miR-145 in the serum of AMI patients one year after percutaneous coronary intervention (49). MiR-155, miR-380 (50), miR-328 and miR-134 (51) are associated with an increased risk of mortality in post-AMI patients.

Circulating miRNAs as blood-based biomarkers in CAD have a lot of promise for early diagnosis, severity assessment, and prognosis. They have the potential to classify patients with or without CAD, as well as patients with stable CAD or unstable CAD. Since single miRNAs are not disease-specific enough, implementing a miRNAs panel test could help increase their diagnostic accuracy.

Biogenesis and functions of lncRNAs

LncRNAs are described as RNA transcripts with a length of more than 200 nucleotides (11). Various DNA regions can be transcribed into lncRNAs, and in accordance with those regions we can divide lncRNAs by their transcriptional origins into: whole or partial natural antisense lncRNA; enhancer associated lncRNAs; promoter associated lncRNAs; Exonic/Sense lncRNAs; long intergenic lncRNAs; and others (52). Different expression profiles of lncRNAs are a direct consequence of specific states in which different cells can present themselves at any given time, depending on specific conditions, such as changed cell morphology due to illness. Furthermore, it was shown that transcription of lncRNAs follows a pathway similar to that of mRNA synthesis (53). Molecular mechanisms by which lncRNAs express diverse functions are numerous and they usually act through pattern interactions with DNA, RNA and different proteins (54). In the nucleus, they usually lead to the regulation of gene expression in cis or in trans. Cis-acting lncRNAs can regulate gene transcription by recruitment or displacement of transcriptional factors and elements responsible for remodeling of chromatin which leads to the activation or repression of nearby promoter regions, thus affecting the transcription of neighboring genes. Trans-acting lncRNAs can regulate gene transcription by modulating recruitment of transcription factors and chromatin remodeling, but these actions are observed at genes distant from the original place of lncRNAs synthesis (54,55). In cytoplasm, lncRNAs demonstrate a variety of functions, such as: post-transcriptional gene regulation; regulation of mRNA turnover and translation efficiency; modulation of miRNA expression where they act as sponges to

sequester miRNA from their targets (56). Recent studies have shown remarkable connections between specific expression profiles of lncRNAs in different diseases, such as diabetes (57), neurodegenerative disorders (58), but also CAD, which only raises new questions about their overall potential functions in different diseases.

Potential roles of lncRNAs in CAD

A great number of lncRNAs have shown potential involvement in atherogenesis and rupture of plaques, with most of them being up or down regulated. Long antisense non-coding RNA in INK4 locus (ANRIL) of chromosome 9p21 is one of the up-regulated lncRNAs in CAD (59, 60). ANRIL greatly influences the viability, apoptosis and survival chances of myocardial cells by attenuating the expression of miR-181b and increasing the expression of p50/p65 (59). These changes in regulation of different messengers can lead to loosened vascular remodeling. Furthermore, the capability of ANRIL to induce inflammatory processes by inducing gene expression of inflammatory cytokines, such as IL-8 and IL-6 was demonstrated. In addition, it was shown that different single nucleotide polymorphisms (SNPs) of ANRIL (rs1004638, rs1333048, and rs1333050) were significantly associated with CAD (60). However, the effects of different ANRIL polymorphisms and isoforms in atherosclerosis or plaque instability remain controversial (60,61). The aforementioned SNPs in homozygote carriers were associated with higher atherosclerotic risk, while heterozygote carriers showed an intermediate risk compared to wild-type and homozygous carriers (60). Furthermore, although linked to CAD, these SNPs were not significantly associated with AMI, suggesting limited effects or an absence of their effects on plaque stability. In contrast to other ANRIL isoforms, circANRIL isoform have positive effects on vascular protection (61). Myocardial infarction associated transcription (MIAT) lncRNA is another up-regulated lncRNA associated with CAD. A recent study has demonstrated a positive correlation between overexpression of MIAT and pro-inflammatory cytokines IL-6, IL-8 and TNFα, while there was a negative correlation between the expression of MIAT and anti-inflammatory cytokine IL-10 (62). This study has also shown a potential influence of MIAT in down- regulation of mir-29b-3p, which was shown as a protective factor against plaque formation. Gao Y et al. have shown that H19 lncRNA can also influence vascular remodeling and inflammation (63). They have shown that H19 binds miRNAs, especially those of let-7 family miRNAs, thus cancelling the positive effects of these miRNAs on shielding smooth muscle cells of blood vessels from oxidative stress. Overexpression of H19 also leads to overexpression of transforming growth factor β1 (TGF-β1), which acts as an inflammatory mediator (64). HOXA transcript at the distal tip (HOTTIP) is another up-regulated lncRNA associated with CAD. In the proliferating endothelial cells, upregulation of HOTTIP is induced by pro-inflammatory cytokines TNFα and platelet-derived growth factor (PDFG). Furthermore, HOTTIP might be implicated in the endothelial cell proliferation and migration via activation of the Wnt/β-catenin pathway (65). DYNLRB2-2 is a lncRNA which has shown protective effects against atherogenesis. Upregulation of DYNLRB2-2 is stimulated by ox-LDL

particles and its effects lead to raised ATP-binding cassette transporter A1 (ABCA1) cholesterol efflux and also to suppression of inflammation in foam cells (66). Upregulation of lncRNA LEF1- AS1 might promote migration and proliferation of smooth muscle cells of blood vessels by targeting miR- 544a/PTEN axis (67). Upregulation of IFNG-AS1 enhances the severity of disease by enhancing synthesis of pro- inflammatory cytokines, such as TNF-α and IL-6 (68). Also, a positive correlation between the expression of IFNG-AS1 and hsCRP was demonstrated. Upregulation of lncRNA FAL1 could be involved in atherosclerosis progression in diabetic patients by promoting endothelial cell proliferation through the PTEN/AKT pathway (69).

Downregulation of certain lncRNAs might have a great influence on overall pathology mechanisms of CAD. Downregulation of one lncRNA called NEXN-AS1 leads to the promotion of atherosclerosis through downregulation of Nexilin F-actin–binding protein (NEXN), which was shown to be a great modulator of cell adhesion and migration (70).

LncRNAs as potential diagnostic biomarkers in CAD

LncRNA H19 is one of the most studied lncRNAs in a different disease. In one study, H19 was elevated in peripheral blood mononuclear cells (PBMCs) of AIM patients (71). Furthermore, ROC analysis for a combination of 3 lncRNAs (H19, MALAT1 and MIAT) showed 68.1% sensitivity, and 76% specificity. These results suggest diagnostic values of these lncRNAs in AIM patients. H19 was found to be more elevated in patients with AIM compared to other forms of CAD, but overall levels of H19 were much higher in patients with CAD compared to control (72). Multiple ANRIL transcriptional isoforms are also correlated with a greater risk of CAD, marking this lncRNA as a promising circulating diagnostic biomarker (61). Fang et al. have shown that the expression of lncANRIL (exon4-6) in CAD patients was up-regulated, while circANRIL (exon14-4) expression was down-regulated. The results of ROC analysis showed that the AUC of circANRIL (exon14) reached 0.730, with a sensitivity of 94.74% and specificity of 42.11%, while the AUC for lncANRIL (exon1) was 0.709, with a sensitivity 41.18% and specificity of 82.35%. Pearson correlation analyses between the expression of ANRIL transcripts and the severity of CAD showed slightly better results for circANRIL (exon14-4), so they validated the diagnostic value of circANRIL (exon14-4) in clinical performance. In this second independent cohort, ROC curve analysis of circANRIL (exon14-4) demonstrated an AUC of 0.713, with sensitivity and specificity being 94.6% and 35.9%, respectively. To improve the diagnostic value of circANRIL (exon14-4), they introduced the ratio of lncANRIL (exon-1) and circANRIL (exon14-4) in their ROC analysis. This combination of lncRNAs with potentially opposing effects resulted in a higher AUC of 0.730, with a sensitivity of 61.29% and specificity of 75.22%. More so, they have shown that the introduction of highly-sensitive CRP into theirs ROC analysis increased the sensitivity to 88.17%, with specificity being 50.43%, suggesting that a combination of these markers could be a great diagnostic tool. LncRNA CoroMarker was studied in a larger cohort that included 221 CAD patients and 187 control individuals

(73). The stability of CoroMarker in plasma was demonstrated, which is proposed to be the result of its localization in extracellular vesicles. It was also demonstrated that CoroMarker can be used as stable diagnostic biomarker with high sensitivity and specificity of 78.05% and 86.49%, respectively, after the correction with traditional risk factors.

LncRNAs as potential prognostic biomarkers in CAD

Up-regulated lncRNA LIPCAR demonstrated more prominent prognostic effects in CAD settings for younger subjects, diabetic patients and non-smokers (72). In a study by Meng et al., expression levels of LIPCAR were studied alongside 8 other lncRNAs in patients with STEMI (74). Overexpression of three lncRNAs in patients with STEMI: aHIF, KCNQ10T1, and LIPCAR was demonstrated. The results of ROC analysis showed that LIPCAR had highest predictive values, with an AUC of 0.782 (95% CI: 0.707–0.894, sensitivity 82% and specificity 75%). Furthermore, it was shown that peak expression levels of LIPCAR are expected around the 24^{th} hour after the onset of symptoms of STEMI. Moreover, it was demonstrated that occurrences of MACEs over the period of 12 months are more probable in patients with higher expression levels of LIPCAR compared to a group with lower expression levels. Lv et al. have shown that an lncRNA called MALAT1 was upregulated in CAD and independently associated with a greater risk of CAD (75). MALAT1 acts as endogenous sponge for mir125-b. Lv et al. have shown that overexpression of MALAT1 and downregulation of mir-125b was correlated with coronary stenosis, hyperlipidemia, and systemic inflammation. MACE occurrences were also highly correlated with the overexpression of circulating MALAT1 and downregulation of circulating mir-125b. The study conducted by Lv et al. strongly suggests the potential role of these two independent factors in their clinical application as biomarkers for screening and assessment of CAD and MACEs.

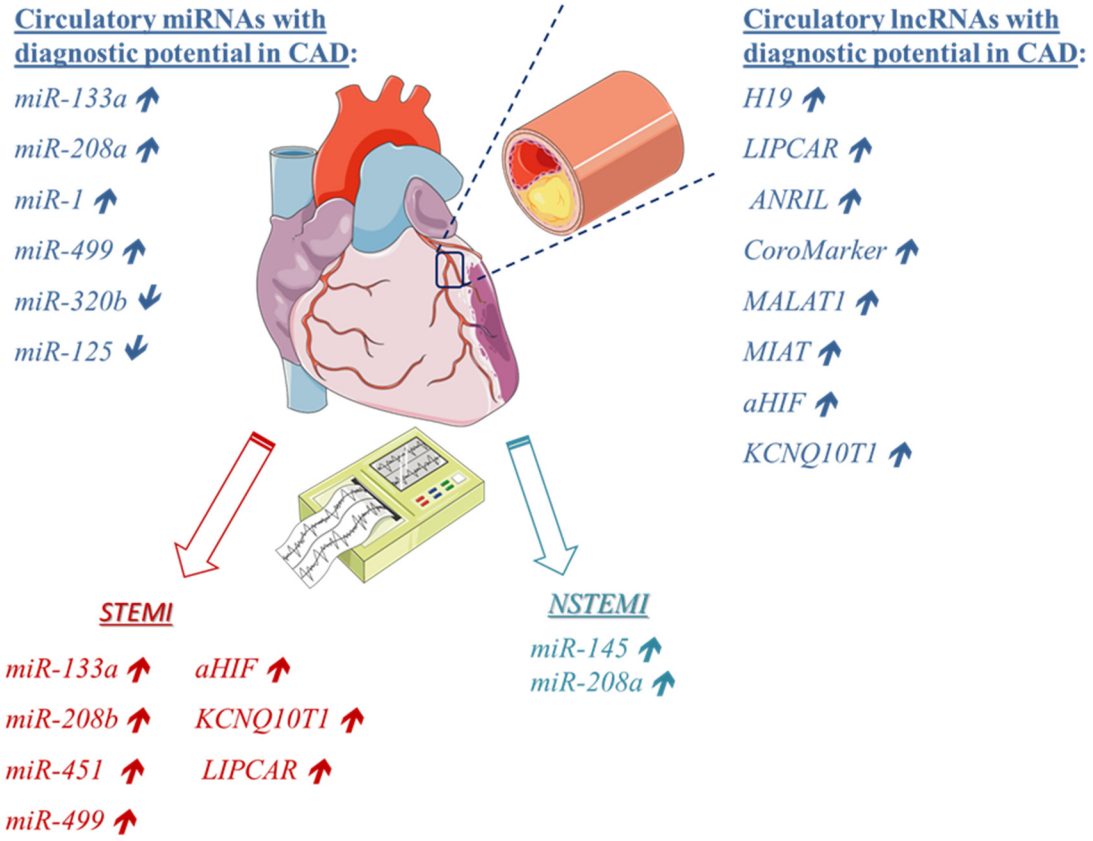

Figure 1. **Summary of ncRNAs with diagnostic potential in CAD (created using https://smart.servier.com/)**

Slika 1. **Pregled nekodirajućih RNK koji imaju potencijal zadijagnozu KSB (slika je napravljena uz pomoćhttps://smart.servier.com/)**

Future perspectives

It is evident that, despite advances in clinical protocols and treatments for CAD, there is still an unmet need for better diagnostic tools that will enable timely diagnosis and prediction of adverse cardiovascular events. So far, four mechanisms have been identified as triggers of acute ischemic episodes: plaque rupture with and without inflammation, arterial spasm, and plaque erosion. Relatively good control of traditional risk factors, such as hypercholesterolemia, hypertension, and smoking, largely contributes to the overall good treatment and prognosis in case of plaque rupture with inflammation. However, the other three categories may account for an increasing proportion of adverse events. Current clinical strategies that asses the risk of adverse events are based on the use of traditional risk factors and conclusions drawn from population studies (i.e., SCORE). However, most CV events occur in patients with 1 or few traditional risk factors, whereas individuals classified as high-risk may never

experience clinical events. Thus, to move forward in the management of CAD, we need to develop and validate novel soluble biomarkers that reflect the underlying mechanism of acute ischemia.

Circulating, cell-free ncRNAs have shown a promising potential as therapeutic targets and biomarkers. With the latest advancements in the development of RNA-based vaccines and drugs such as Inclisiran for the treatment of heterozygous familial hypercholesterolemia, it is expected that the RNA field will progress even more rapidly. With the new tools around the corner, such as the use of machine learning and artificial intelligence algorithms, ncRNAs could be combined with other genomic, proteomic, metabolomic and imaging data into multi-marker models that could outperform traditional risk assessment and give deeper insights into pathophysiological mechanisms behind CAD.

One of the main current challenges that prevent the use of ncRNAs for the diagnostic purposes is related to the contradictory results regarding the diagnostic potential of many ncRNAs, probably due to a lack of consensus for the best laboratory practices regarding ncRNAs quantification (such as: choice of biological sample, RNA extraction methods and quantification protocols). Therefore, the critical step is to perform extensive standardization and inter-laboratory harmonization of preanalytical (sample handling, sample type) and analytical issues (use of various quantification approaches with different sensitivity and specificity, normalization), regarding miRNAs and lncRNA quantification, prior to translation of these markers from bench to bedside.

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Cirkulišuće nekodirajuće RNK kao biomarkeri u koronarnoj arterijskoj bolesti

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Kratak sadržaj

Koronarna arterijska bolest (KAB) predstavlja vodeći uzrok smrtnosti širom sveta. Osnovni patološki proces koji karakteriše KAB je ateroskleroza, koju odlikuju različiti patofiziološki mehanizmi kao što su progresivna inflamacija, poremećen metabolizam lipida, oksidativni stres, itd. Aterosklerotski plak sužava lumen koronarnih arterija, što dovodi do nastanka ishemijskih promena na srčanom mišiću, posledično dovodeći do kliničkih komplikacija ove bolesti, kao što je akutni infarkt miokarda. Trenutno ne postoje biomarkeri koji bi mogli da predvide sudbinu aterosklerotskog plaka, kao ni razvoj akutnih koronarnih događaja ili razvoj velikih uznapredovalih kardiovaskularnih događaja. Mnogobrojne vrste nekodirajućih RNK molekula utiču na osnovne ćelijske funkcije i kao takve igraju ulogu u nastanku i progresiji KAB. Do sada su od svih vrsta nekodirajućih RNK-a najviše istražene mikro RNK-a (miRNK) i dugolančane nekodirajuće RNK-a (dnkRNK). Uzimajući u obzir da nekodirajuće RNK-a dospevaju u ekstracelularnu tečnost iz različitih ćelija, trenutno se veliki napori ulažu u ispitivanje mogućnosti upotrebe cirkulišuih nekodirajućih RNK-a kao potencijalnih biomarkera u dijagnostici i prognostici KAB. U ovom naučno-istraživačkom članku sažećemo trenutna saznanja o molekularnim mehanizmima preko kojih miRNK i dnkRNK doprinose razvoju KAB, kao i o potencijalnoj primeni ovih molekula kao budućih biomarkera u dijagnozi i prognozi ove bolesti.

Ključne reči: koronarna arterijska bolest, nekodirajuće RNK, miRNK, lncRNK, biomarkeri