School of Advanced Studies of the Romanian Academy

PHD THESIS ABSTRACT

with the title

Identification of new epigenetic biomarkers for the improved prediction of atherosclerosis progression

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Contents

SUMMARY

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In the first part of the PhD thesis I briefly described theoretical aspects related to the cardiovascular system under both physiological and pathological conditions, especially related to the initiation of atherosclerotic plaque formation and progression. Aspects of the metabolism of circulating lipoproteins, especially HDL particles, and aspects of the biology of non-coding RNA molecules are also mentioned. Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide, despite technological and medical progress. Underlying these diseases is atherosclerosis, a chronic inflammatory multifactorial pathology affecting the medium and large arteries in various vascular beds (Schaftenaar et al. 2016). Atherosclerosis involves the proliferation of the extracellular matrix of resident cells in the arterial wall, accumulation of lipids and calcium there, adhesion of circulating monocytes and platelets to the endothelium, a process that triggers a cascade of molecular and structural changes that ultimately lead to obstruction of blood flow of the affected vessel (Libby et al. 2011).

Currently, there are a number of parameters used in medical practice that provide information on the risk that patients have for developing cardiovascular disease. These parameters include elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C) or low levels of high-density lipoprotein cholesterol (HDL-C), but these are not entirely sufficient to indicate patients at risk. For this reason there is still an urgent need to identify new sensitive, specific and accurate parameters to complement those listed above. In this context, a number of epigenetic molecules, such as microRNAs, stand out (Vogiatzi et al. 2018) or as long non-coding RNAs (Holdt et al. 2010), which are promising in terms of both diagnosis and early prognosis of disease progression in cardiovascular patients.

Epigenetics studies the totality of changes that occur in genetic material, changes that are not inherited but acquired, as a result of the impact of external factors on the genetic material. One line of research in epigenetics concerns non-coding RNA molecules (ncRNAs), which, through the functions they perform in the cell, have an indirect effect on gene expression and thus on DNA.

It is well known that only 2% of the human genome encodes proteins, while the rest has until recently been considered redundant genetic material of no biological importance, the so-called "junk DNA". The last two decades of genomics and transcriptomics research have disproved this idea and demonstrated that regions of DNA that do not encode proteins, which are transcribed to non-coding RNA transcripts, play a crucial role in maintaining cellular homeostasis.

The term 'non-coding RNA' (ncRNA) refers to all types of RNA molecules other than coding RNA, namely messenger RNA (mRNA).

NcRNAs are divided according to size into two main categories:

- small non-coding RNAs (<200 nucleotides) -microRNA (miRNA), small interfering RNA (siRNA) or piwi interfering RNA (piRNA);
- large non-coding RNAs (>200 nucleotides) - long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) or enhancer-type RNAs (eRNAs) (Zhang et al. 2019).

MiRNA molecules are highly conserved non-coding RNA molecules of approximately 20 nucleotides in size, thus falling into the category of small regulatory non-coding RNAs. They are involved in the regulation of gene expression by binding and blocking mRNA molecules.

Although this category of molecules was initially observed in the study of cancer, they have subsequently been shown to be involved in many other pathologies, such as diabetes, autoimmune diseases or cardiovascular diseases. Due to the possibility of being easily measured in circulation or tissue samples, as well as their high stability, miRNAs have rapidly become candidates to serve as biomarkers for various pathologies. Regarding CVD, there are various miRNAs that have been proposed as markers, such as miR-133 for heart failure or miR-21 for cardiac fibrosis and atherosclerosis (Barwari et al. 2016). Also, in a study conducted in our lab, my colleagues demonstrated that miR-486 and miR-92a have the potential to discriminate between patients with vulnerable versus stable coronary artery disease (CAD) (Niculescu et al. 2015).

A second category of non-coding RNA that appears to have prognostic or diagnostic potential for various pathologies, and which has received particular attention in recent years, are long non-coding RNAs (lncRNAs). These are over 200 nucleotides in length and are involved in a multitude of biological processes, such as the cell cycle, cell differentiation, as well as in various pathologies (Bridges et al. 2021). Studies show that lncRNAs have lower expression levels than mRNA or miRNA, but also have higher tissue specificity, which recommends them as markers of various pathologies. Another difference is the low degree of conservation of lncRNAs between species, although this is not a general rule (Johnsson et al. 2014).

In order to achieve the objectives proposed in this PhD thesis, I used molecular biology, biochemistry and cell culture techniques, which have been described in the "Experimental protocols and analytical methods" sections of each study. In the experiments conducted during the PhD program, I developed methods to quantify long non-coding RNA as well as extracellular circulating DNA (cfDNA) in patient plasma by Real-Time PCR.

The second part of the PhD thesis contains original contributions to the field of research and is structured in two main chapters, comprising a total of 9 studies (see Table of Contents).

The main results of the first **4 pre-clinical** *in vitro* and *in vivo* studies (**first chapter**) are as follows:

(i) Hyperlipidemia causes dysfunctional HDL production and thus decreases cholesterol efflux in the small intestine of the hyperlipidemic hamster. Ginger extract has the ability to reverse and improve these processes, contributing to lipid metabolism homeostasis. The results presented in this study were published in the paper entitled: **Hyperlipidemia Determines Dysfunctional HDL Production and Impedes Cholesterol Efflux in the Small Intestine: Alleviation by Ginger Extract**, authors: **Teodora Barbălată**, Mariana Deleanu, Mihaela Georgiana Cărnuță, Loredan Ștefan Niculescu, Mina Răileanu, Anca Volumnia Sima, Camelia Sorina Stancu, published in *Molecular Nutrition and Food Research* **2019**, 63(19) :e1900029. DOI: 10.1002/mnfr.201900029. **Journal impact factor (2019): 5,309 (Q1)**; impact factor in 2022: 5,2 (Q1).

(ii) Administration of probiotics or cessation of fatty diet in hyperlipidemic hamsters results in decreased serum and liver levels of miR-223-3p, miR-122a-5p, miR-92a-3p and miR-486-5p. The results of this study were published in the paper entitled: **Probiotics administration or the high-fat diet arrest modulates microRNAs levels in hyperlipidemic hamsters,** authors: Loredan S. Niculescu, Mădălina D. Dulceanu, Camelia S. Stancu, Mihaela G. Cărnuță, **Teodora Barbălată**, Anca V. Sima, in *Journal of Functional Foods* **2019**, 56, 295-302, DOI 10.1016/j.jff.2019.03.036. **Journal impact factor (2019): 3,701 (Q1)**, and in 2022: 5,6 (Q1).

(iii) Hyperlipidemia induced by fatty diet administration results in increased levels of miR-146a-5p, miR-21-5p, miR-223-3p in myocardium, liver, and small intestine of hyperlipidemic hamsters. The results obtained in this study were published in the paper entitled: **Regulation of microRNAs in high-fat diet induced hyperlipidemic hamsters,** authors: **Teodora Barbălată**, Lu Zhang, Mădălina D. Dulceanu, Camelia S. Stancu, Yvan Devaux, Anca V. Sima, Loredan S. Niculescu, in *Scientific Reports* **2020**, 10: 20549, DOI 10.1038/s41598-020-77539-4. **Journal impact factor (2020): 4,38 (Q1),** and in 2022: 4,6 (Q2).

(iv) Transcriptional activation of apolipoprotein A-I and paraoxonase 1 using gene editing by CRISPR/dCas9 technology in cultured human enterocytes improves dysfunctional HDL quality. Also, conditioned medium collected from transfected enterocytes has a beneficial effect on dysfunctional endothelial cells. The results presented in this study were published in the paper entitled **CRISPR/dCas9 Transcriptional Activation of Endogenous**

Apolipoprotein AI and Paraoxonase 1 in Enterocytes Alleviates Endothelial Cell Dysfunction, authors: Laura Toma, Teodora Barbălată, Gabriela M. Sanda, Loredan S. Niculescu, Anca V. Sima, Camelia S. Stancu, in *Biomolecules* **2021**, 11, 1769. DOI: 10.3390/biom11121769. **Journal impact factor (2021): 6,064 (Q2)**; and in 2022: 5,5 (Q1).

The main results of the 5 studies included in the **second chapter comprise studies on human material obtained from patients with cardiovascular disease**:

(i) HDL dysfunction is characterized by elevated levels of clusterin and myeloperoxidase as well as low levels of paraoxonase 1 in patients with peripheral artery disease (PAD), and these changes are aggravated by the presence of type 2 diabetes. Data from this study were published in the paper entitled **Clusterin, paraoxonase 1, and myeloperoxidase alterations induce high-density lipoproteins dysfunction and contribute to peripheral artery disease; aggravation by type 2 diabetes mellitus**, authors: Gabriela M. Sanda, Laura Toma, **Teodora Barbălată**, Oriana E. Moraru, Loredan S. Niculescu, Anca V. Sima, Camelia S. Stancu in *BioFactors* **2021**: 1-15, DOI 10.1002/biof.1800. **Journal impact factor (2021): 6,438 (Q1)**, and in 2022: 6,0 (Q1).

(ii) Plasma levels of miR-142-3p are elevated in PAD patients as well as in atheroma plaques isolated from them, which recommends this miRNA as a possible biomarker for the adverse postoperative outcome of patients. The results obtained in this chapter were published in the article entitled **Increased miR-142 Levels in Plasma and Atherosclerotic Plaques from Peripheral Artery Disease Patients with Post-Surgery Cardiovascular Events,** authors: **Teodora Barbălată,** Oriana E. Moraru, Camelia S. Stancu, Yvan Devaux, Maya Simionescu, Anca V. Sima, Loredan S. Niculescu, in *International Journal of Molecular Sciences* **2020**, 21: 9600, DOI: 10.3390/ijms21249600. **Journal impact factor (2020): 5,92 (Q1),**.

(iii) Increased levels of plasma miR-223-3p and atheroma plaques from patients with carotid artery stenosis (CAS) are correlated with patients' advanced age and proteins involved in HDL metabolism. The results obtained in this chapter were published in the article entitled **MiR-223-3p levels in the plasma and atherosclerotic plaques are increased in aged patients with carotid artery stenosis; association with HDL-related proteins,** authors: **Teodora Barbălată**, Oriana E. Moraru, Camelia S. Stancu, Anca V. Sima, Loredan S.

Niculescu, in *Molecular Biology Reports* **2021**, DOI: 10.1007/s11033-021-06636-y. **Journal impact factor (2021): 2,742 (Q4)**

(iv) Elevated plasma levels of mitochondrial DNA and miR-142-3p are an indicator of the adverse outcome of patients after ST-segment elevation myocardial infarction (STEMI). The results obtained in this chapter have been published in 2 articles: the first article is entitled **Mitochondrial DNA Together with miR-142-3p in Plasma Can Predict Unfavorable Outcomes in Patients after Acute Myocardial Infarction,** authors: **Teodora Barbălată**, Alina I. Scărlătescu, Gabriela M. Sanda, Laura Toma, Camelia S. Stancu, Maria Dorobanţu, Miruna M. Micheu, Anca V. Sima, Loredan S. Niculescu, in *International Journal of Molecular Sciences* **2022***, 23: 9947*, DOI: 10.3390/ijms23179947. **Journal impact factor (2022): 5,6 (Q1)**; the second article partly includes results from this chapter of the thesis and is entitled **miR-146a-5p, miR-223-3p and miR-142-3p as Potential Predictors of Major Adverse Cardiac Events in Young Patients with Acute ST Elevation Myocardial Infarction—Added Value over Left Ventricular Myocardial Work Indices**, authors: Alina Ioana Scărlătescu, **Teodora Barbălată**, Anca Volumnia Sima, Camelia Stancu, Loredan Ştefan Niculescu, Miruna Mihaela Micheu, in *Diagnostics (Basel)*, **2022** 12(8):1946, DOI: 10.3390/diagnostics12081946. **Journal impact factor (2022): 3,6 (Q2).**

(v) The unfavorable outcome of patients with acute coronary syndrome correlates with increased circulating levels of LIPCAR and MALAT1 lncRNAs, which recommends these two lncRNAs as possible biomarkers for patient outcomes. The results obtained in this chapter were published in an article entitled **Elevated Levels of Circulating lncRNAs LIPCAR and MALAT1 Predict an Unfavorable Outcome in Acute Coronary Syndrome Patients**, authors: **Teodora Barbălată**, Loredan S. Niculescu, Camelia S. Stancu, Florence Pinet, Anca V. Sima, in *International Journal of Molecular Sciences* 2023, 24(15):12076, DOI: 10.3390/ijms241512076. **Journal impact factor in 2022:** 5,6 (Q1).

Among the most relevant results of the studies carried out for this thesis are the following:

he study that examined the association between increased plasma miR-142 levels and atherosclerotic plaques in patients with peripheral artery disease (PAD) and their post-operative outcome found that compared to PAD patients without subsequent cardiovascular events (no CVE), PAD patients with CVE showed increased levels of miR-142-3p, miR-223-3p and miR-155-5p in atherosclerotic plaques, results that correlated positively with levels of primary miRNA transcripts (pri-miRNA) (Figure 1). Also, the

presence of pri-miRNA in PAD atheroma plaques, correlated with the presence of mRNA for Drosha and Dicer at this level, indicates possible local synthesis of the miRNA of interest in plaques. Statistical analysis (Table 1) of the results obtained in this study suggests that circulating miR-142-3p has the potential to be an independent predictor for the occurrence of post-operative CVD in PAD patients.

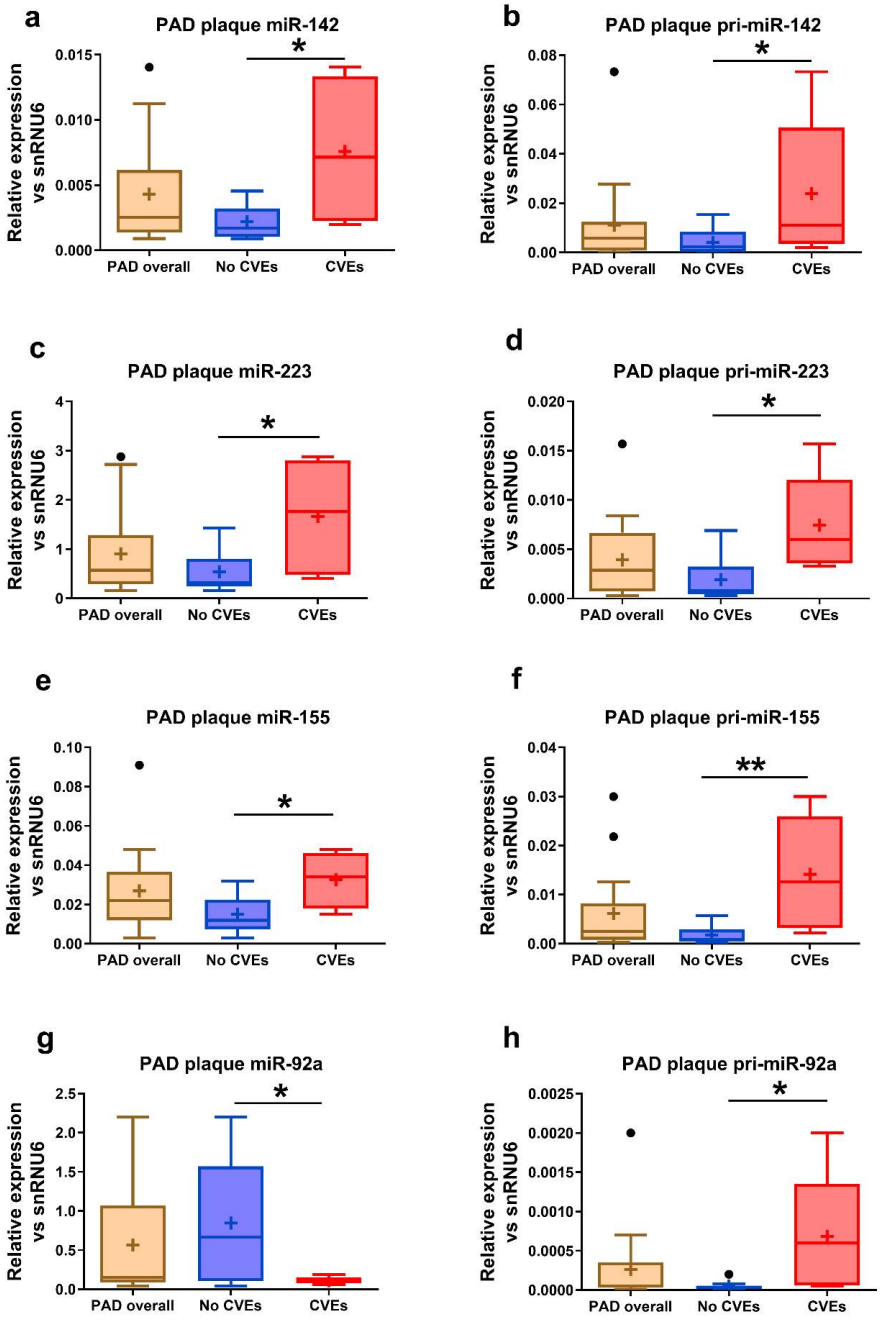

Figure 1. Levels of miRNAs and pri-miRNAs in the atherosclerotic plaques of peripheral artery disease (PAD) patients. Levels of miR-142 (a), miR-223 (c), miR-155 (e), miR-92a (g), pri-

miR-142 (b), pri-miR-223 (d), pri-miR-155 (f) and pri-miR-92a (h) in the atherosclerotic plaques of PAD patients with cardiovascular events (CVEs) compared to those with no CVEs. Data are illustrated as boxplots with Tukey whiskers, median line and mean cross (+). *p<0.05, **p<0.01 vs no CVEs.

Table 1. Receiver operator characteristic (ROC) analysis for predictive potentials of individual plasma miRNAs values and a combination of the four plasma miRNAs' values from peripheral artery disease (PAD) patients for follow-up cardiovascular events (CVEs).

Area Under the Curve					
Test Result Variable(s)*	**Area**	**Std. Error [a]**	**Asymptotic Sig.[b]**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Plasma miR-142	0.861	0.068	0.007	0.727	0.995
Plasma miR-223	0.451	0.124	0.717	0.207	0.695
Plasma miR-155	0.632	0.152	0.325	0.335	0.929
Plasma miR-92a	0.396	0.099	0.437	0.202	0.589
All plasma miRNAs	0.924	0.050	0.002	0.825	1.000

* Adjusted for age and gender. a. Under the nonparametric assumption. b. Null hypothesis: true area = 0.5

The main results of the study in which we examined the potential of novel markers such as extracellular ncRNA (miRNA) or extracellular DNA (cfDNA) and circulating mitochondrial DNA (mtDNA) to predict major cardiovascular events (MACE) in STEMI patients are: STEMI patients show the following changes at hospital discharge (T_1) compared to admission (T_0): (i) increased levels of all 6 miRNAs analysed (hsa-miR-223-3p, hsa-miR-142-3p, hsa-miR-155-5p, hsa-miR-486-5p, hsa-miR-125a-5p and hsa-miR-146a-5p); (ii) increased plasma levels of cfDNA and mtDNA. At the same time, compared to STEMI patients without MACE, those with MACE showed: (i) increased levels of all 6 miRNAs analysed (hsa-miR-223-3p, hsa-miR-142-3p, hsa-miR-155-5p, hsa-miR-486-5p, hsa-miR-125a-5p and hsa-miR-146a-5p) (Figure 2); (ii) increased levels of cfDNA and mtDNA (Figure 3). Following BLR and ROC statistical analysis, we reported for the first time that plasma mtDNA levels together with miR-142-3p levels can accurately predict the adverse outcome of STEMI patients (Table 2).

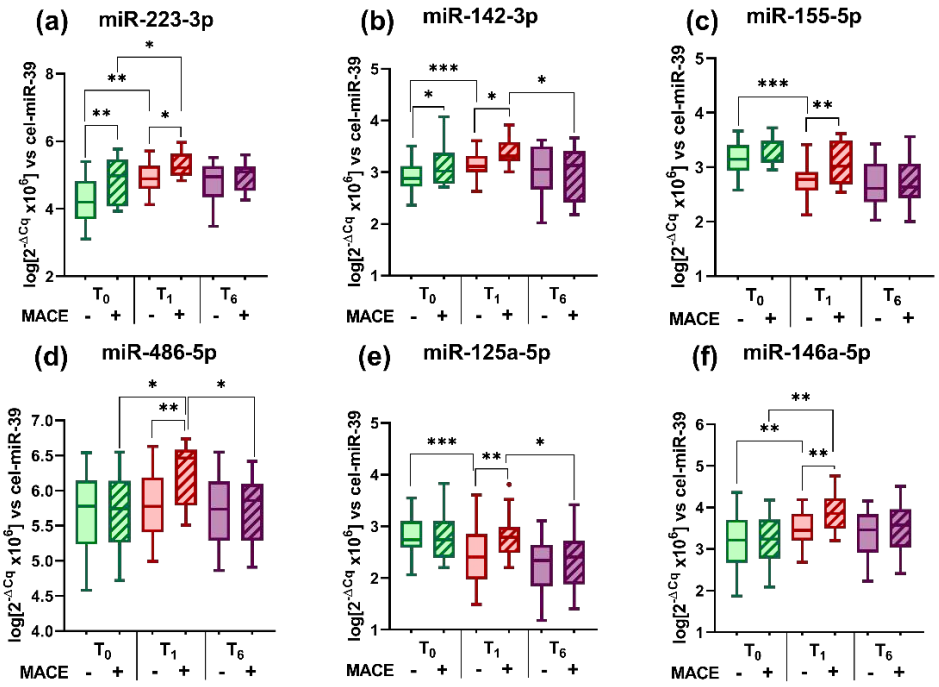

Figure 2. Variation of miRNAs levels in the plasma of ST-segment elevation myocardial infarction (STEMI) patients at T_0, T_1 and T_6 time points grouped by the occurrence of subsequent major adverse cardiovascular events (MACE) at 6-month follow-up: miR-223-3p (a), miR-142-3p (b), miR-155-5p (c), miR-486-5p (d), miR-125a-5p (e) and miR-146a-5p (f). Data are illustrated as boxplots with Tukey whiskers and median line. *p<0.05, **p<0.01, ***p<0.001.

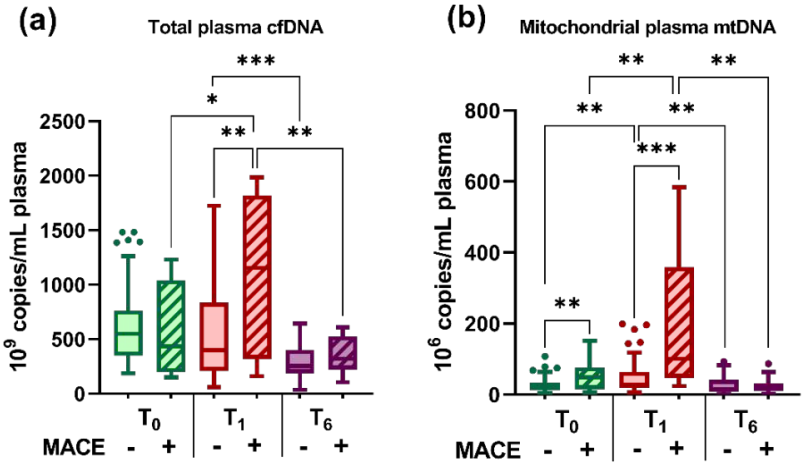

Figure 3. Variation of plasma levels of total cell-free (cfDNA) (a) and mitochondrial cfDNA (mtDNA) (b) in ST segment elevation myocardial infarction (STEMI) patients at T0, T1 and T6 time points grouped by the occurrence of subsequent major adverse cardiovascular events (MACE) at 6-month fol-low-up. Data are illustrated as boxplots with Tukey whiskers and median line. *p<0.05, **p<0.01, ***p<0.001.

Table 3. Receiver operator characteristic (ROC) analysis for predictive potentials of univariate and mul-tivariate models for follow-up major adverse cardiovascular events (MACE), using plasma miRNAs, total cell-free DNA (cfDNA) and cell-free mitochondrial DNA (mtDNA) values measured at hospital discharge (T_1) in ST-elevation myocardial infarction (STEMI) patients.

Area Under the Curve

Test Result Variable(s)*	Area	Standard Error [a]	p-value [b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Univariate models					
miR-223-3p	0.788	0.071	5.18×10^{-3}	0.648	0.927
miR-142-3p	0.832	0.069	1.27×10^{-3}	0.696	0.968
miR-155-5p	0.742	0.094	0.019	0.558	0.927
miR-486-5p	0.718	0.096	0.034	0.530	0.906
miR-125a-5p	0.782	0.074	6.21×10^{-3}	0.636	0.928
miR-146a-5p	0.785	0.083	5.67×10^{-3}	0.622	0.948
cfDNA	0.722	0.102	0.029	0.523	0.921
mtDNA	0.832	0.069	1.09×10^{-3}	0.698	0.966
Multivariate model 1 (all 6 miRNAs)	0.796	0.081	4.20×10^{-3}	0.638	0.954
Multivariate model 2 (all 6 miRNAs, cfDNA and mtDNA)	0.975	0.021	4.45×10^{-6}	0.934	1.000
Multivariate model 3 (mtDNA and miR-142-3p)	0.833	0.069	1.17×10^{-3}	0.697	0.968
Multivariate model 4 (mtDNA, miR-142-3p and miR-223-3p)	0.833	0.070	1.17×10^{-3}	0.696	0.970
Multivariate model 5 (mtDNA, miR-142-3p, miR-223-3p and miR-146a-5p)	0.903	0.046	8.37×10^{-5}	0.813	0.993

* All adjusted for age and gender (male). a. Under the nonparametric assumption. b. Null hypothesis: true area = 0.5

Following the study that invesigated the potential of LIPCAR and MALAT1 lncRNAs and two relevant miRNAs to discriminate patients with acute coronary syndrome (ACS) at risk, we concluded that in patients with stable angina (SA) and unstable angina (UA): (i) LIPCAR, MALAT1, hsa-miR-155-5p and hsa-miR-142-3p levels are significantly elevated in the plasma of vulnerable CAD patients

(UA - patients) compared to stable ones (SA); (ii) LIPCAR, MALAT1 and miR-142-3p levels are significantly increased in hyperglycemic UA patients (UA-HG) compared to normoglycemic (NG), while miR-142-3p also showed increased values in SA-HG vs SA-NG patients (Figure 4); (iii) the minimal multivariate ROC model that can discriminate patients with vulnerable coronary artery disease consists of LIPCAR and MALAT, with a slight improvement following the addition of miR-155-5p (Table 3).

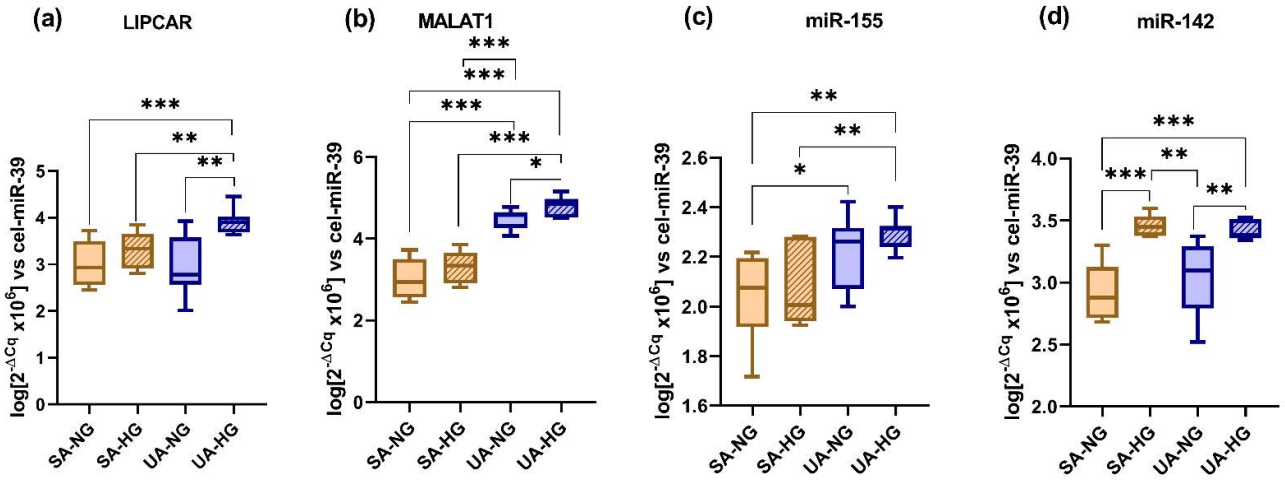

Figure 4. Levels of ncRNAs in the plasma of hyperglycemic (HG) SA and UA compared to normoglyce-mic (NG) patients: LIPCAR (a), MALAT1 (b), miR-155-5p (c), miR-142-3p (d). Data are illustrated as boxplots with Tukey whiskers and median line. *p<0.05; **p<0.01; ***p<0.001.

Table 3. Receiver operator characteristic (ROC) analysis for the discriminating potentials of univariate and multivariate models for diagnosis of acute coronary syndrome - unstable angina (UA) versus stable angina (SA) in patients, using plasma lncRNAs LIPCAR, lncRNA MALAT1, miR-155-5p and miR-142-3p values.

Area Under the Curve					
Test Result Variable(s)*	**Area**	**Standard Error [a]**	**p-value [b]**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Univariate models					
LIPCAR	0.737	0.094	0.022	0.554	0.921
MALAT1	0.773	0.080	6.27×10^{-3}	0.617	0.930
miR-155-5p	0.812	0.087	3.55×10^{-3}	0.643	0.944
miR-142-3p	0.596	0.104	0.355	0.393	0.799
Multivariate models					
Multivariate model 1 (LIPCAR and MALAT1)	0.870	0.065	7.34×10^{-4}	0.742	0.999

Multivariate model 2 (LIPCAR, MALAT1 and miR-155-5p)	0.938	0.047	4.08×10^{-4}	0.846	1.000
Multivariate model 3 (LIPCAR, MALAT1 and miR-142-3p)	0.836	0.081	5.92×10^{-3}	0.677	0.994
* All adjusted for age and gender (male as risk). A. Under the nonparametric assumption. B. Null hypothesis: true area = 0.5.					

In conclusion, the studies that I conducted during my PhD program with the common goal of identifying new epigenetic biomarkers that contribute to increase the prognostic accuracy of cardiovascular disease led to the identification of microRNA and lncRNA molecules that are useful in this regard. Thus, miR-142-3p, LIPCAR and MALAT1 have been noted.

The results obtained in these studies encourage the investigation of the molecular mechanisms in which these non-coding RNA molecules are involved and modulate, and their validation as biomarkers of various cardiovascular diseases in larger cohorts of patients. These insights opened by these results may support their introduction into current medical practice and the achievement of the main goal of modern medicine, namely personalised medicine.

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EFFECTIVENESS AND DISSEMINATION OF RESEARCH

Papers published in ISI journals - 6 as main author

1. **Teodora Barbălată**, Mariana Deleanu, Mihaela Georgiana Cărnuță, Loredan Ștefan Niculescu, Mina Răileanu, Anca Volumnia Sima, Camelia Sorina Stancu. Hyperlipidemia Determines Dysfunctional HDL Production and Impedes Cholesterol Efflux in the Small Intestine: Alleviation by Ginger Extract, *Molecular Nutrition and Food Research* 63(19) :e1900029, **2019**. **DOI**: 10.1002/mnfr.201900029. **Journal impact factor (2019): 5,309 (Q1)**; Journal impact factor in 2022: 5,2 (Q1).
2. **Teodora Barbălată**, Lu Zhang, Mădălina D. Dulceanu, Camelia S. Stancu, Yvan Devaux, Anca V. Sima, Loredan S. Niculescu. Regulation of microRNAs in high-fat diet induced hyperlipidemic hamsters. *Scientific Reports* 10: 20549, **2019. DOI:** 10.1038/s41598-020-77539-4. **Journal impact factor (2020): 4,38 (Q1),** and in 2022: 4,6 (Q2).
3. **Teodora Barbălată,** Oriana E. Moraru, Camelia S. Stancu, Yvan Devaux, Maya Simionescu, Anca V. Sima, Loredan S. Niculescu. miR-142 Levels in Plasma and Atherosclerotic Plaques from Peripheral Artery Disease Patients with Post-Surgery Cardiovascular Events. *International Journal of Molecular Sciences* 21: 9600, **2020**. **DOI**: 10.3390/ijms21249600. **Journal impact factor (2020): 5,92 (Q1),** and in 2022: 5,6 (Q1).
4. **Teodora Barbălată**, Oriana E. Moraru, Camelia S. Stancu, Anca V. Sima, Loredan S. Niculescu. MiR-223-3p levels in the plasma and atherosclerotic plaques are increased in aged patients with carotid artery stenosis; association with HDL-related proteins. *Molecular Biology Reports* 49(7):6779-6788, **2021**, **DOI**: 10.1007/s11033-021-06636-y. **Journal impact factor (2021): 2,742 (Q4),** and in 2022: 2,8 (Q3).
5. **Teodora Barbălată**, Alina I. Scărlătescu, Gabriela M. Sanda, Laura Toma, Camelia S. Stancu, Maria Dorobanțu, Miruna M. Micheu, Anca V. Sima, Loredan S. Niculescu. Mitochondrial DNA Together with miR-142-3p in Plasma Can Predict Unfavorable Outcomes in Patients after Acute Myocardial Infarction. *International Journal of Molecular Sciences 23: 9947*, **2022. DOI**: 10.3390/ijms23179947. **Journal impact factor (2022): 5,6 (Q1).**
6. **Teodora Barbălată**, Loredan S. Niculescu, Camelia S. Stancu, Florence Pinet, Anca V. Sima. Elevated Levels of Circulating lncRNAs LIPCAR and MALAT1 Predict an Unfavorable Outcome in Acute Coronary Syndrome Patients. *International Journal of Molecular Sciences* 24(15):12076, **2023. DOI**: 10.3390/ijms241512076. **Journal impact factor in 2022: 5,6 (Q1).**

Papers published in ISI journals - 6 co-authored papers

1. Loredan S. Niculescu, Mădălina D. Dulceanu, Camelia S. Stancu, Mihaela G. Cărnuță, **Teodora Barbălată**, Anca V. Sima. Probiotics administration or the high-fat diet arrest modulates microRNAs levels in hyperlipidemic hamsters. *Journal of Functional Foods* 56, 295-302, **2019. DOI:** 10.1016/j.jff.2019.03.036. **Journal impact factor (2019): 3,701 (Q1)**, and in 2022: 5,6 (Q1).

2. Gabriela M. Sanda, Laura Toma, **Teodora Barbălată**, Oriana E. Moraru, Loredan S. Niculescu, Anca V. Sima, Camelia S. Stancu. Clusterin, paraoxonase 1, and myeloperoxidase alterations induce high-density lipoproteins dysfunction and contribute to peripheral artery disease; aggravation by type 2 diabetes mellitus. *BioFactors* 48(2): 454-468, **2021. DOI**: 10.1002/biof.1800. **Journal impact factor (2021): 6,438 (Q1)**, and in 2022: 6,0 (Q1).
3. Laura Toma, **Teodora Barbălată**, Gabriela M. Sanda, Loredan S. Niculescu, Anca V. Sima, Camelia S. Stancu. CRISPR/dCas9 Transcriptional Activation of Endogenous Apolipoprotein AI and Paraoxonase 1 in Enterocytes Alleviates Endothelial Cell Dysfunction. *Biomolecules* 11, 1769, **2021**. **DOI**: 10.3390/biom11121769. **Journal impact factor (2021): 6,064 (Q2)**; and in 2022: 5,5 (Q1).
4. Alina Ioana Scărlătescu, **Teodora Barbălată**, Anca Volumnia Sima, Camelia Stancu, Loredan Ştefan Niculescu, Miruna Mihaela Micheu. miR-146a-5p, miR-223-3p and miR-142-3p as Potential Predictors of Major Adverse Cardiac Events in Young Patients with Acute ST Elevation Myocardial Infarction—Added Value over Left Ventricular Myocardial Work Indices D*iagnostics (Basel)* 12(8):1946, **2022. DOI**: 10.3390/diagnostics12081946. **Journal impact factor (2022): 3,6 (Q2).**
5. Mariana Deleanu., Laura Toma, Gabriela M. Sanda, **Teodora Barbalata**, Loredan S. Niculescu, Anca V. Sima, Călin Deleanu, Liviu Săcărescu, Alexandru Suciu, Georgeta Alexandru, Iuliana Crişan, Mariana Popescu, Camelia S. Stancu. Formulation of Phytosomes with Extracts of Ginger Rhizomes and Rosehips with Improved Bioavailability, Antioxidant and Anti-Inflammatory Effects In Vivo**.** *Pharmaceutics* 15 (4): 1066, **2023**, **DOI**: 10.3390/pharmaceutics15041066. **Journal impact factor (2023): 5,4 (Q1).**
6. Korina Karagianni, Alessia Bibi, Alisia Madé, Shubhra Acharya, Mikko Parkkonen, **Teodora Barbalata**, Prashant K. Srivastava, David de Gonzalo-Calvo, Constanza Emanueli, Fabio Martelli, Yvan Devaux, Dimitra Dafou, A. Yaël Nossent, and on behalf of EU-CardioRNA COST Action CA17129. Recommendations for detection, validation, and evaluation of RNA editing events in cardiovascular and neurological/neurodegenerative diseases. *Molecular Therapy Nucleic Acids* 35 (1) **2023. DOI:** 10.1016/j.omtn.2023.102085. **Journal impact factor (2023): 8,8 (Q1).**

Awards

1. **"Best Poster Award"** for the poster: **Barbalata T**, Oriana E. Moraru, Loredan S. Niculescu, Camelia S. Stancu, Maya Simionescu, Anca V. Sima. A panel of circulating miRNAs could predict age-related post-endarterectomy cardiovascular events in carotid artery stenosis patients. Poster presented during "The 4th MC and WG Meeting of COST Action CA17129 "Catalysing transcriptomics research in cardiovascular disease". 12-14 February 2020, Maastricht, The Netherlands.
2. **Award for the best poster** for: **Barbalata T**., Niculescu L.S., Stancu C.S., Pinet F., Sima A.V. LIPCAR and MALAT1, two long non-coding RNAs, are increased in the plasma acute coronary

syndrome patients and predict major adverse cardiovascular events. Conference of the Romanian Society of Cell Biology (SRBC), 16-17 November 2023, Bucharest, Romania.

Oral communications at international scientific conferences: 5

1. **Barbălată Teodora**. Oral presentation at the International Symposium Acad. N. Cajal, 17-19 October **2019**, Bucharest, Romania. Hyperlipidemia induces the production of dysfunctional HDL in the small intestine and liver of hamsters, and increases hepatic lipid-related miRNAs.
2. **Barbălată Teodora**. Oral presentation at the CardioRNA Live! Online Conference of COST Action CA17129 "Catalysing transcriptomics research in cardiovascular disease" (CardioRNA), 7-10 September **2020**. A panel of miRNAs and inflammatory markers are associated with hyperglycemia in peripheral artery diseases patients.
3. **Barbălată Teodora**. Oral presentation at the Conference of the Romanian Society of Cellular Biology (SRBC), 4-6 November **2021**, Bucharest, Romania. MicroRNAs profiling in the heart and liver of high-fat diet induced hyperlipidemic hamster.
4. **Barbălată Teodora**. Oral presentation at the 8th MC and WG Meeting of COST Action CA17129 "Catalysing transcriptomics research in cardiovascular disease" (CardioRNA), Pavia. Italy, 25-27 May **2022**. Analysis of long non-coding RNAs (LIPCAR) distribution in human blood compartments
5. **Barbălată Teodora.** Oral presentation at the „Institute of Cellular Biology and Pathology Nicolae Simionescu" Annual Scientific Symposium. Bucharest, Romania, 8-9 December **2022**. Increased plasma levels of miR-142-3p and mitochondrial DNA predict unfavorable outcomes in patients after acute myocardial infarction

Registered patent

Patent application no. a 2022 00502, with the filing date at the State Office for Inventions and Trademarks (OSIM) 18/08/2022 and title "Phytosomes with biologically active compounds from rhizomes of ginger and rose hip fruits with increased bioavailability and the process for obtaining them", authors: Deleanu M., Toma L., Sanda G.M., Niculescu L.S., **Barbalata T.**, Suciu A., Alexandru G., Crisan I., Popescu M., Stancu C.S.

COLLABORATIONS IN RESEARCH PROJECTS

1. **PNRR-III-C9-2022-I8 -** "Enhancement of endogenous antioxidant and cholesterol efflux potential by gene editing in fatty liver disease; preclinical studies (THERAGENLIV)", project director Dr. Shlomo Sasson;
2. **PN-III-P4-PCE-2021-0831 –** "Stimulation of endogenous antioxidant enzymes by gene editing with CRISPR/dCas9, an innovative approach for complementary therapy in metabolic diseases (OXIGENEDIT)", project director Dr. Camelia Sorina Stancu;

3. **PN-III-P2-2.1-PED-2021-1929 –** ” Experimental demonstration of an innovative biotechnology using CRISPR/dCas9 transcriptional activation to improve HDL function as a therapy in cardiovascular diseases (CARDIOCRISPR)", project director Dr. Camelia Sorina Stancu;
4. **PN-III-P2-2.1-PED-2019-1897** – "Validation of a protocol to measure a panel of epigenetic markers (microRNAs) in plasma for the prognosis of disease evolution and personalization of therapy of patients after acute myocardial infarction (EPITERAMI)", project director Dr. Loredan Ștefan Niculescu
5. **PN-III-P2-2.1-PED-2019-3552 –** "Development of innovative nanophytosomes designed for non-invasive and bioavailability-enhanced delivery of biologically active compounds with antioxidant and immunostimulatory properties (NANOGINROSA)", project director Dr. Camelia Sorina Stancu
6. **PN-III-P1.1-TE-2019-2044 –** "Peptides derived from apolipoprotein A-II with therapeutic potential in atherosclerosis (A2A)", project director Dr. Violeta Truşcă (collaborator in the project implementation team from 2022-2023)
7. **POC-A.1-A.1.1.4-E-2015 – ”**Improving institutional competitiveness in type 1 diabetes by developing an innovative concept of mesenchymal stromal cell immunotherapy" project director Dr. Nadir Askenasy (collaborator in the project implementation team in 2019-2020)