MicroRNAs and Hypertension

Nikolina Kolobarić 1,2, Ines Drenjančević 1,2*

1 Department of Physiology and Immunology, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Croatia
2 Scientific Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek, Croatia

*Corresponding author: Ines Drenjančević, ines.drenjancevic@mefos.hr

Abstract

MicroRNAs (miRNAs) are non-coding, highly conserved RNAs found in all biological fluids, that are emerging as master regulators of gene expression, consequently impacting a variety of biological processes in both healthy and diseased environments. There are still certain limitations regarding analysis of circulating miRNAs, specifically concerning standardisation and accuracy of obtained data. However, there is an indisputable therapeutic and diagnostic potential, confirmed by recent research. Hypertension, as one of the leading causes of death in modern world, has been in the focus of scientific society for several decades now. So, it is of outmost importance to investigate and pinpoint appropriate miRNAs for early indication and diagnosis of hypertension in general population. More in vivo and clinical research is necessary in animal and human models in order to exploit the full potential of this novel technology.

(Kolobarić N, Drenjančević I. MicroRNAs and Hypertension. SEEMEDJ 2022; 6(1); 53-67)
**Introduction**

MicroRNAs (miRNAs) represent a class of short length (20-24 nucleotides), highly conserved, non-coding RNAs that have a role in regulation of gene expression through inhibition of transcription, translation or degradation of target genes, depending on their origin (1,2). MiRNAs can be categorized as tissue-derived miRNAs (t-miRNAs) and freely circulating miRNAs (c-miRNAs), with t-miRNAs being mainly associated with hypertension and cardiac function (more evidence needed), while the latter are described as a potential specific biomarkers for early disease detection, with their abnormal expression being largely associated with diseases in humans (1,3).

C-miRNAs or extracellular miRNAs can be found in all biological fluids, mainly carried in vesicles exosomes, such as serum, plasma, saliva, urine, while serving a hormone-like purpose in processes of signalisation, mediation and regulation of a variety of biological and cellular activities, physiological responses and pathological conditions (3–5). Even though the sampling is minimally invasive and there is a potentially high reproducibility of results, analysis of miRNAs still represents a challenge due to low concentrations of target compounds, lack of standardized methodology and eventual accuracy obtained regarding their in vivo role and therapeutic properties (1,6).

---

**Figure 1.** Schematic presentation of therapeutic and diagnostic potential of microRNAs in atherosclerosis, blood pressure regulation and hypertension, endothelial function, inflammation and lipid profile.

miR – microRNA; BP – blood pressure.
These highly stable regulators are primarily found in human serum and saliva (a total of 1881 miRNAs identified in human genome) and they can reduce gene expression post transcription by binding to the 3’ untranslated region (UTR) of a messenger RNA (mRNA) leading to repression of translation and/or degradation of target (2,7). Lately, efforts are being made regarding investigation of therapeutic use of miRNAs in a wide variety of diseases, including cardiovascular disease, immune disorders, rheumatoid arthritis and cancer (8). The fact that mature miRNAs are highly conserved and short, makes them perfect for therapeutic manipulations towards modulation of cellular pathways and networks (9). There are two possible proposals for such actions: (1) therapeutic restoration of miRNA activity/functionality; and (2) inhibition of miRNA function by so-called antimiRs – antisense oligonucleotides (4,9,10). In order to draw firm conclusions, it is necessary to take into account that there are great variations in miRNA expression levels present, depending on their origin (tissue or cell type) and pathological state, which requires further extensive in vivo experiments in appropriate animal models. Schematic representation of miRNA breakdown regarding therapeutic/diagnostic potential is presented in Figure 1.

**Emerging role of non-coding RNA in endothelial function and blood pressure regulation**

Hypertension is, alongside diabetes and cancer, the most common chronic disease and leading cause of death in modern society (11). Yet, in a large number of affected individuals, the main cause of elevated blood pressure (BP) cannot be determined with certainty. Some of the risk factors associated with hypertension include genetic predisposition, advanced age, low physical activity, obesity and overall poor dietary habits characterized by high sodium chloride (NaCl) and low potassium intake (12,13). High-salt (HS) diet normally has a suppressive effect on renin-angiotensin system (RAS) – a physiological system that regulates BP – and leads to reduced renin, angiotensin II (Ang II) and aldosterone plasma levels (14,15), inciting endothelial dysfunction (16–18). However, in hypertensive and diabetic patients, RAS activation is not suppressed following salt loading, thus salt loading tends to aggravate the cardiovascular risks in those particular individuals (14,19,20).

So far, animal studies have shown that Ang II, HS diet and exercise change miRNA levels in hypertension (6). Several studies listed miR-22 (21), miR-181a (22) and miR-25 (23) as potential therapeutics in stress response, hypertension and vascular remodelling. Furthermore, it is necessary to address environmental factors in future research and find out what influence they could potentially have on miRNAs expression, especially in case of hypertension and related complications (6). Table 1 represents a cross-section of research studies regarding non-coding RNA role in endothelial function and BP regulation.

**Non-coding RNAs in hypertension treatment**

Currently, there is a lack of evidence supporting a significant role of non-coding RNAs in BP regulation and hypertension but it has been frequently mentioned that miRNA activity is deregulated in the state of illnesses.

MiR-21 is among the first identified non-coding RNAs (24) and has been suggested by several studies as a biomarker for early atherosclerosis and hypertension (25–27). In a study by Kara et al. (2021), significantly increased levels of miR-21 and aldosterone were found in patients with resistant hypertension compared to newly diagnosed hypertensive patients and healthy controls (28). Further, higher levels of miR-21 were reported in hypertensive, stroke and atherosclerotic patients compared with healthy controls, while it also negatively correlated with plasmatic levels of eNOS in hypertensive patients (29–31). Renal miR-214-3p has also been mentioned as a contributor to hypertension by directly targeting eNOS in rats and potentially humans (32,33).
### Table 1. Cross-section of research studies regarding non-coding RNAs role in endothelial function and BP regulation

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Study model</th>
<th>Targets</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>miR-21</strong></td>
<td>Human model – hypertensive, ischemic stroke and atherosclerotic patients</td>
<td>mt-Cytb, eNOS</td>
<td>↑ Mir-21 and aldosterone levels in RHT group Positive correlation with aldosterone, age, office SBP, 24-h ABPM all-day SBP.</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>Rat model – SHR rats</td>
<td></td>
<td>↑ CRP level, plasma mir-21 expression level and CIMT in HT group</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>Mice model - C57BL/6J, TAC mice</td>
<td></td>
<td>↓ NOx and eNOS levels in HT group</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ miR-21 in stroke and atherosclerotic patients</td>
<td>(31)</td>
</tr>
<tr>
<td><strong>miR-214-3p</strong></td>
<td>Human model - HT and hypertensive nephrosclerosis patients</td>
<td>eNOS</td>
<td>Upregulated in kidneys of HT patients and HS-fed SS rats</td>
<td>(33)</td>
</tr>
<tr>
<td></td>
<td>Rat model – Dahl SS rat, SS.13GNN(L26) rat</td>
<td></td>
<td>↓ hypertension and albuminuria in SS model following inhibition of miR-214-2p</td>
<td></td>
</tr>
<tr>
<td><strong>miR-22</strong></td>
<td>Rat model – SHR rats</td>
<td>Chga, TGFβR I</td>
<td>↓ BP following inhibition ↓ cardiac hypertrophy and ↑ fibrogenesis of cardiac fibroblasts in knockout mice</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Mice model – cardiac-specific knockout</td>
<td></td>
<td>↑ cardiac contractility and function following inhibition</td>
<td>(36)</td>
</tr>
<tr>
<td><strong>miR-181a</strong></td>
<td>Mice model – BPH/2J mice, miR-181a knockout</td>
<td>RAS</td>
<td>↑ BP and salt-sensitivity in knockout mice ↓ BP and renal renin mRNA following treatment with miR-181a mimic</td>
<td>(40)</td>
</tr>
<tr>
<td>miR</td>
<td>Model &amp; Conditions</td>
<td>Effect</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>-----------</td>
<td></td>
</tr>
</tbody>
</table>
| miR-19a| Human model – AMI and CAD patients, Rat model – Dahl SS rat, Mice model - ApoE^{-/-} mice, c57BL/6 mice | ADRB1: ↑ proliferation and arteriogenesis  
HBP-1: ↓ apoptosis in endothelial cells  
↓ atherosclerotic plaques and lipids load in mice fed with high-fat diet following administration of antagonist | (1), (83), (47), (84) |
| miR-192-5p| Human model - hypertensive and hypertensive nephrosclerosis patients, Rat model – Dahl SS rat, SS.13^{B/H}(L26) rat, Mice model – mir-192 knockout | Atp1b1: ↑ MAP following HS diet in antimiR-treated L26 model  
↑ MAP, SBP, DBP following HS diet in knockout mice  
↓ miR-192-5p in hypertension animal model and following HS diet | (32) |
| miR-25 | Human model – diabetic patients, Rat model – SD rat, Mice model – WT mice, C57BL/6 mice, db/db mice | CDC42: ↓ glomerular fibrosis and BP following inhibition in db/db mice  
↑ RAS, hypertension, renal dysfunction following inhibition in normal mice | (49) |
| miR-451 | Human model – PAH and HCM patients, Rat model – Wistar rat, Mice model - miR-451 knockout | TSC1: ↓ development of PAH in hypoxia-exposed rats following inhibition  
↑ development of HCM following down-regulation | (51), (52) |
| miR-29a | Human model – hypertensive patients, Rat model – SHR rat | PTEN/AKT/mTOR signalling pathway: Negatively correlated with the glomerular filtration rate, but positively with CRP, TGF-β1, and UACR  
↓ hypertrophy and associated indices following inhibition | (57), (59) |
<table>
<thead>
<tr>
<th>Mice model – TAC mice</th>
<th>(85)</th>
</tr>
</thead>
</table>
| SHR rats – spontaneously hypertensive rats; TAC mice – transverse aortic constriction mice; mt-Cytb - mitochondrially encoded cytochrome B; eNOS - endothelial nitric oxide synthase; HS – high-salt; RHT – resistant hypertension; SBP – systolic blood pressure; ABPM - ambulatory blood pressure monitoring; CRP – C-reactive protein; CIMT - carotid intima-media thickness test; HT – hypertension; NOx – nitric oxide; SS rat – salt-sensitive rat; SS.13BN26(L26) rat – salt-insensitive rat; BP – blood pressure; Chga – chromogranin A; TGFβR I - transforming growth factor βR I; BPH/zJ mice – hypertensive mice; RAS - renin-angiotensin system; AMI - acute myocardial infarction; CAD - coronary artery disease; ApoE−/− mice – model of atherosclerosis; ADRB1 - adrenoceptor beta 1; HBP-1 - HMG-box transcription factor 1; ATP1B1 - ATPase Na+/K+ transporting subunit beta 1; MAP – mean arterial pressure; DBP – diastolic blood pressure; SD rat – Sprague Dawley rat; WT mice – wild type mice; C57BL/6 mice – basic background mouse strain; db/db mice – type II diabetes model; CDC42 - cell division cycle 42; PAH – pulmonary arterial hypertension; HCM – hypertrophic cardiomyopathy; TSC1 - TSC complex subunit 1; mPAP - mean pulmonary artery pressure; BNP - brain natriuretic peptide; ADMA - asymmetric dimethylarginine.
The above-mentioned miR-22 targets chromogranin A (Chga) mRNA— a protein expressed peripherally and in the central nervous system (CNS). Chga influences BP, vasodilatation, insulin sensitivity, and inflammation (34). Animal studies report overexpression of Chga and polymorphism of untranslated binding region (UTR) for miRNAs in Spontaneously Hypertensive Rat (SHR) animal model (13), which consequently increases the binding of miR-22 (34,35). Inhibition of miR-22 caused a decrease in BP of SHRs, which potentially makes it a therapeutic agent in terms of navigating hypertension treatment (7,34). Furthermore, results show that miR-22 is an essential regulator of cardiac function and remodelling, since its genetic ablation suppresses induced cardiac hypertrophy and enhances fibrogenesis of cardiac fibroblasts in murine model (21,36,37). Wahlquist et al. (2014) suggested miR-25 inhibition as a treatment strategy for heart failure, since it improves cardiac contractility and function. It is a repressor of cardiac function and has been upregulated in heart failure events in both murine and human model (7,23).

MiR-181a is another miRNA that has a potential influence on BP. It is the most abundant miRNA in lymphoid tissue and it regulates T cell function (38). It showcases somewhat anti-inflammatory effect and contributes to adaptive immunity (39). In knock-out mice, deletion of miR-181a led to salt sensitivity and increased BP while it was downregulated in hypertensive murine and human model (40,41). In mice model of hypertension (Schlager BHP/2J mouse), treatment with miR-181a mimics resulted in decreased BP and renal renin mRNA (6). Recently, we published several papers concerning effects of high-salt intake on inflammation and endothelial function and found altered leukocyte activation status followed by advancement of vascular low-grade inflammation in both animal and human model (16,42), and also impaired microvascular reactivity in healthy individuals (43).

Langlo et al. (2021) exposed Dahl salt-sensitive (Dahl/SS) rats to low-salt (LS) and HS diet, with first resulting in mild to moderate hypertension over time, while the latter resulted in severe hypertension following severely increased systolic blood pressure (SBP) (1). Out of 145 studied c-miRNAs assessed in that study, 68 of them were associated significantly with hypertensive complications and can potentially serve as biomarkers for diagnostic purposes. Among others, miR-19a-5p was suggested as a biomarker in cases of hypertensive encephalopathy and endothelial dysfunction (ED), since it was recognized as the main regulator of platelet activation, coagulation and inflammation (44). Enhanced expression of c-miR-19a was reported in cases of pulmonary arterial hypertension (45), acute myocardial infarction (46), coronary artery disease and atherosclerotic patients (47).

Baker et al. (2019) reported antihypertensive effects of renal miR-192-5p in animal hypertension models with an emphasis on the role of Atp1b1 target genes (32). They noted decreased levels of miR-192-5p in L26 (SS.13BN26; mild hypertension) and Dahl/SS rats following HS loading, with more pronounced effect of HS diet in the latter model. Furthermore, when treated with antimiR-192-5p, L26 rats had increased mean arterial BP (MAP) following HS diet. Similar effect was detected in miR-192 knockout mice where MAP, systolic (SBP) and diastolic BP (DBP) increased significantly compared to wild type (WT) mice as a response to a HS diet. These results suggest that deletion or a decrease in miR-192-5p levels leads to exaggerated renal damage and hypertension, suggesting protective role of miR-192-5p against hypertension development.

In another study, high salt loading caused renal and cardiac dysfunction in uninephrectomized Sprague Dawley rats (SD) to a larger extent compared to normal rats fed a HS diet. This was accompanied by an increase in levels of miR-25, miR-451, miR-155 and a decrease in levels of miR-99 of the heart, with opposite effect on same circulatory miRNAs (48). According to Liu et al. (2017), miR-25 levels are lower in blood and tissue samples from diabetic patients/animals and cell cultures exposed to glucose when compared to controls (49). They investigated the effect of knock-down/inhibition of miR-25 on
BP, among other parameters, since hypertension is associated closely to diabetic nephropathy. In WT mice, such venture resulted in RAS activation and hypertension contributing to renal dysfunction. MiRNA-25 has also been described as an oncogenic miRNA and an important regulator in acute myocardial infarction, left ventricular hypertrophy and heart failure (50). Expression levels of miR-451 have been cited in literature as a diagnostic reference in pathogenesis of pulmonary hypertension (51–53), while circulating miR-155 has been positively correlated with BP (both SBP and DBP) and inflammatory markers, with significantly higher expression levels in hypertensive patients compared to healthy controls (54). As suggested by Sun et al. (2012), miR-155 acts as a key regulator of cardiovascular functions, since, when overexpressed, it targets endothelial nitric oxide synthase (eNOS) expression, decreases it and impairs endothelium-dependent vasorelaxation (55).

Another potential therapeutic target and diagnosis biomarker (e.g. early stages of hypertensive nephropathy) is miR-29, depicted as necessary for both normal endothelial function and its restoration in animals and humans (56–58). For example, hypertensive patients with left ventricular hypertrophy had significantly higher levels of miR-29a compared to patients with hypertension, while anti-miR inhibition in transverse aortic constriction (TAC) mice model resulted in suppressed hypertrophy and associated indices (59). Alongside miR-29, miR-30a and miR-133 were also assessed for diagnostic accuracy in case of white-coat hypertension, where their expression levels were associated with BP-related parameters and BP monitoring (60).

Potential of non-coding RNAs in endothelial dysfunction treatment

In the last decade, there has been a lot of research dealing with therapeutic inhibition of miR-33 in animal models. There are two isoforms of miR-33 present in humans, miR-33a and miR-33b, embedded within SREBF1 and SREBF2 genes (SREBP family of transcription factors), while there is only one isoform present in rodents and non-human primates – miR-33a (9,61,62). These serve a purpose in progression of cardiometabolic diseases such as atherosclerosis and obesity and are crucial factors in lipid metabolism regulation, since they are responsible for maintenance of cholesterol, fatty acid and triglyceride homeostasis (62,63).

Previous studies reported improved lipid profile (increased circulatory HDL-cholesterol levels), mitigated inflammation and decreased formation of atherosclerotic lesions following inhibition or genetic ablation of miR-33 in mice (63–65). Similar effects were also detected in case of inhibition by antimiR when administered subcutaneously in Ldlr−/− mice (deficient for LDL receptor) which resulted in reduced plaque size and atherosclerosis regression as well as improved HDL-cholesterol functionality (66,67). On another note, in non-human primates, both normal and metabolic disease model, treatment with miR-33 targeting antimiRs resulted in increased HDL-cholesterol levels, while in normal males’ pharmacological inhibition also resulted in decreased VLDL triglycerides (68,69). These results suggest potential therapeutic utility of miR-33s in treatment of atherosclerosis, dyslipidaemia and related metabolic disorders.

Nuclear factor kappa B (NF-κB), a fairly general transcription factor, regulates a variety of biological processes, particularly in stress response and progression of inflammation, both playing large roles in vascular damage and onset of cardiovascular diseases (70,71). Downregulation or decreased expression of miR-150 occurs in acute coronary syndrome and correlates with its onset (72). It targets pentraxin-3 (PTX3) and negatively regulates it through inhibition of NF-κB signalling pathway, furthermore attenuating vascular remodelling and restoring endothelial cell function. Mir-150 has also been associated with therapeutic potential for thrombosis treatment, since its upregulation has an effect on endothelial progenitor cell differentiation and increases angiogenic potential (73). MiR-155, a master
MicroRNA identification and screening

Choosing the right miRNA for diagnostic/therapeutic purposes and identification of key targets responsible for specific phenotype is somewhat of a challenge of its own (77). Understanding of phenotypes evoked by certain miRNAs requires usage of computational tools (databases of validated miRNAs, prediction algorithms) as well as an experimental approach (gene expression analysis and proteomics), in order to perform functional cell-based screenings in both health and disease conditions (77,78). Several reviews and research articles listed functional genomics for appropriate validation of critical proteins in biological networks for the purpose of creating prognostic risk models using miRNA data (77–79).

Eulalio et al. (2015) (77) provided a review of functional cell-based screening technologies for miRNA function, aimed at biological processes and illness-related events, including proliferation, signaling, cell maintenance and differentiation. For characterization of mechanisms of action, it is of crucial importance to identify potential key targets for different miRNAs. Such information can be detained from computational, prediction algorithms based on complementarity between miRNAs and target sequences (80–82).

Several prediction tools for miRNA targets have been developed in the last two decades (miRanda, miRanda-miRVR, TargetScan, DIANA-microT-CDS, MirTarget2, ma22-GUI, TargetMiner, SVMicroO, PITA, RNAhybrid), although it should be clarified that despite the predictive power each approach has, there are also limitations and weaknesses calling for future efforts and research towards the upgrade of available tools (79,81).

Conclusions

Circulating miRNAs could have a potential for early diagnosis of end-organ injury in hypertension and hypertensive emergency. Pathway prediction tools elucidate possible mechanisms in hypertensive emergency that may be the subject for further investigations. Further mechanistic studies are needed (e.g. with miRNA-214-3p and miRNA-29, which have recently been shown to be involved in the development of hypertension). However, currently there are several limitations in using miRNAs in clinical diagnosis and therapy, such as large number of miRNAs as potential biomarkers that require high-throughput technology (e.g. functional screenings) for investigation, followed by mechanistic studies in animals and humans (the latter much more difficult).

Acknowledgement. None.

Disclosure

Funding. No specific funding was received for this study.

Competing interests. None to declare.


Southeastern European Medical Journal. 2022; 6(1)


1 Author contribution. Acquisition of data – N.K., I.D.; Administrative, technical, or logistic support – I.D.; Analysis and interpretation of the data - N.K., I.D.; Conception and design - N.K., I.D.; Critical revision of the article for important intellectual content - N.K., I.D.; Drafting of the article - N.K., I.D.; Final approval of the article - I.D.