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Therapeutic aspect of microRNA inhibition in various types of hypertension and hypertensive complications

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Abstract

Hypertension has attained an epidemic level and it has been predicted that by 2025, 25% of adult individuals worldwide will be hypertensive. Despite considerable advances in illustrating the molecular pathways involved in the pathophysiology of hypertension, the regulatory function still remains unknown and there are certain limitations in the effectiveness of diagnosis and treatment of various types of hypertension. On the other hand, non-coding RNAs called microRNAs which are short with 16-27 nucleotides in length can serve as diagnostic, prognostic and therapeutic targets for various diseases, including hypertension. Interestingly, anti-miRs, a miRNA inhibitor blocks the target miRNA molecules to suppress the disease progression. At present there are many studies concentrating on miRNA inhibition in the treatment of different types of hypertension, but still their molecular mechanisms and therapeutic applications are yet to be evaluated. In this review, we provide an in-depth examination of the current understanding regarding the role of miRNA inhibition as a therapeutic target in various types of hypertension and its complications in heart, brain, eyes and kidney.

Keywords: Hypertension; Hypertensive Complications; MicroRNAs; MicroRNA inhibition; Therapeutic targets

Abbreviations

EH- Essential Hypertension

PH- Pulmonary Hypertension

PAH- Pulmonary Arterial Hypertension

RNAi- RNA interference

miRNAs- microRNAs

AMOs- Anti-microRNA oligonucleotides

DGCR8- DiGeorge Syndrome Critical Region 8

Pri-miRNA- Primary microRNA

Pre-miRNA- Precursor microRNA

AGO2- Argonaute2

RISC- RNA-induced silencing complex

CVD- cardiovascular disease

cIMT- carotid intima- media thickness

HPECs- Human pulmonary endothelial cells

KLF15- Kruppel Like Factor 15

PAECs- Pulmonary arterial endothelial cells

AAV9- Adeno-associated virus 9

IUGR- Intrauterine growth retardation

PASMCs- Pulmonary Arterial Smooth Muscle cells

PAAT- Pulmonary Arterial Acceleration Time

KCNK3- Potassium Two Pore Domain Channel Subfamily K Members 3

SLC45A3- Solute Carrier Family 45 Member 3

CFs- Cardiac fibroblasts

CTGF- Connective tissue growth factor

PE- Preeclampsia

MMP2- Metalloproteinase-2

SOCS1- Suppressor of cytokine signaling 1

AVMSMCs- Arteriovenous malformations smooth muscle cells

XBP-1- X-Box Binding Protein 1

RVLM- Rostral ventrolateral medulla

SIH- Stress-induced Hypertension

UUO- Unilateral ureteric obstruction

FOXO3- Forkhead Box O3

CTCL- Cutaneous T-cell lymphoma

1. Introduction

Hypertension or high blood pressure, is a non-communicable disease characterized by perpetual raised pressure in the blood vessels. According to the World Health Organization (WHO), 1.13 billion people across the globe have hypertension and are facing serious medical condition (Liu *et al*., 2021). Hypertension has recently attained an epidemic proportion, and it is also predicted that by 2025, 25% of adult individuals worldwide will be hypertensive (Adler *et al*., 2015). Hypertension continues to be a major public health issue, with wide-ranging consequences for global burden of diseases like cardiovascular and final stage kidney diseases etc. (Dodoo *et al*., 2017). Hypertension can be classified as either primary or secondary depending on the cause. Primary or essential hypertension (EH) is the most common type of hypertension, accounting for almost 90% of all cases without any underlying medical condition, which can lead to secondary hypertension that influence complications in the heart, kidney or endocrine system (Batkai *et al*., 2012). Moreover, there are other types of hypertension, namely pulmonary hypertension (PH), pulmonary arterial hypertension (PAH), gestational hypertension, preeclampsia, nocturnal hypertension, portal hypertension and white coat hypertension (Carretero *et al*., 2000). Even though the occurrence of hypertension is unknown, both environmental and genetic factors may play a major role in the pathophysiologic mechanisms in modern societies (Sekar *et al*., 2017). Despite considerable advances in research, discovery of biomarkers and therapeutic molecules are still required to treat hypertension.

Interestingly, RNA interference (RNAi)- based therapeutics which include microRNAs (miRNAs) and short interfering RNAs (siRNAs) have the ability to downregulate proteins which are associated to disease progression. RNA-based formulations have become effective therapeutic alternatives for a wide range of diseases as a result of their extensive targeting capabilities and research in RNA modification and delivery systems (Zhu *et al*., 2022). In this study we mainly focused on the role of miRNA inhibitors in hypertension. MicroRNAs are short, noncoding RNA fragments that monitor or control protein expression by targeting the 3ˈ-untranslated region (3'-UTR) of mRNA post-transcriptionally (Batkai *et al*., 2012). On the other hand, anti-microRNA oligonucleotides (AMOs) and locked nucleic acids (LNAs) including anti-miRs are chemically modified single-stranded oligonucleotides designed to inhibit miRNA function by direct Watson-Crick binding to complementary targets (Lennox *et al*., 2013). Once bound by the anti-miR, endogenous miRNAs are no longer able to associate with target mRNA molecules, as a result, the mRNA is no longer repressed, leading to

increased expression of its protein product (Krutzfeldt et al., 2005; Preethi *et al*., 2021). Anti-miRs can act as a bio-stable compounds and improve cell permeation and distribution to treat a variety of diseases, including hypertension (Preethi *et al*., 2021). At present there are many studies concentrating on miRNA inhibition in the treatment of different type of hypertension, but still their molecular mechanisms and signaling pathways are yet to be evaluated. In this paper, we provided the current understanding regarding the role of miRNA inhibition as a therapeutic target in treating various types of hypertension namely EH, PH, PAH and Preeclampsia. We also reviewed miRNA inhibition in relation to hypertension complications mainly affecting heart, brain, eyes and kidney.

2. Overview of microRNAs

Non-coding RNAs called miRNAs are 16-27 nucleotides in length and play a crucial role in gene expression predominantly by post-transcriptional silencing of target genes (Selvaraj *et al*., 2020; Li *et al*., 2014). MicroRNA biogenesis is classified into two pathways namely, canonical and non-canonical pathways. In canonical pathway, miRNAs are transcribed as a long transcript called primary microRNAs (pri-miRNAs) in the nucleus, either through their own promoters or those of their host gene (Bartel *et al*., 2018). The transcription of pri-miRNA is carried out predominantly by RNA polymerase II, with evidence for RNA III polymerase in some cases (Di Pascale *et al*., 2018). Pri-miRNAs are refined into precursor microRNAs (pre-miRNAs) by microprocessor complex consisting of a Drosha (a ribonuclease III enzyme) and DiGeorge Syndrome Critical Region 8 (DGCR8) (an RNA binding protein). Pre-miRNA has a staggered cut with a 3′2 nucleotide overhang and 5′ phosphate (Lee *et al*., 2003). This pre-miRNA enters the cytoplasm via the interaction of exportin-5 and Ran-GTP. Further processing is carried out by RNA III endonuclease Dicer in the cytoplasm, removing the terminal loop and resulting in a mature miRNA duplex. This duplex is then loaded onto an argonaute2 (AGO2) protein to form the RNA-induced silencing complex (RISC) [Vishnoi *et al*., 2017]. Both ends of the miRNA are protected by AGO2 proteins once they enter RISC, conferring stability on the miRNA (O Brien *et al*., 2018).

Aside from canonical miRNA biogenesis pathways presented above, various other mechanisms can generate miRNAs. Deep sequencing of small RNAs from cells lacking DGCR8/Drosha or Dicer revealed that novel miRNAs can be produced without the use of a microprocessor or Dicer (Chong *et al*., 2010). Mirtron production was first described in non-canonical pathway, in which the Drosha-mediated processing step is circumvented in favor of pri-miRNA splicing,

which results in the production of pre-miRNA (Okamura *et al*., 2007; Flynt *et al*., 2010). Drosha, on the other hand, degrades endogenous short hairpin RNA transcript to produce dicer-independent miRNAs (Ha *et al*., 2014). These pre-miRNAs require AGO2 to mature within the cytoplasm because they are too short to be a dicer-substrate. As a result, AGO2 dependent 3p strand slicing and loading of the entire pre-miRNA is promoted, and their maturation is completed by trimming the 5p strand (O Brien *et al*., 2018; Ha *et al*., 2014). **Figure 1 represents the overview of microRNA biogenesis.**

3. MicroRNA inhibition and hypertension

3.1 MicroRNA inhibition in Essential Hypertension (EH)

Essential hypertension (EH) continues to be a key modifiable risk factor for cardiovascular disease (CVD) and has indeed been major public health concern due to its link to an enhanced danger of certain vascular disorders such as myocardial infarction and stroke etc. Evidences suggested that genetic factors play a role in the severity of EH (Carretero *et al*., 2000; Garfinkle, 2017). Interestingly, in response to cardiac stress, miR-21 was upregulated, and inhibiting it with an antagomiR was shown to prevent cardiac hypertrophy and fibrosis in rodents. Whereas, when miR-21 LNA-modified anti-miR was injected to mice, it failed to block the response of the heart. Therefore, it was suggested that in comparison to LNA-modified anti-miR, 2 O methyl modified anti-miR can play a major role in preventing cardiac hypertrophy and fibrosis in rodents (Patrick *et al*., 2010). A study by Krishnan *et al*, suggested that the expression levels of miR-510 was upregulated in the blood sample of hypertensive patients when compared to that of the normal patients. In addition, their methylation analyses have also confirmed miR-510 levels were high in hypertensive subjects. Thus, the use of anti-miR may help in the reduction of miR-510 levels and might be used as a therapeutic molecule for the treatment of hypertension (Krishnan *et al*., 2017). Ye *et al*, recognized 257 differently expressed miRNAs in EH of 4 Uyghur patients. Microarray results showed that miR-198 and miR-1183 were up-regulated and miR-30e-5p and miR-144-3p were down-regulated. The study stated that it's unknown how these four microRNAs have a role in EH. The large variations in their expression seen in this study could pave the way for more investigation. Thus, the use of miRNA inhibition to inhibit the upregulated miRNAs could provide experimental data for more research into the pathophysiology and use of anti-miR in treating EH (Ye *et al*., 2019).

Furthermore, a study by Torres-Paz *et al*, investigated the relationship between miR-33a expression (5p and 3p) and carotid intima- media thickness (cIMT) in monocytes and serum

samples from hypertensive patients. The study involved a total of 84 participants in which 42 subjects were with EH and 42 were normal subjects. It was observed that the miR-33a-3p expression was downregulated, while miR-33a-5p expression was significantly upregulated in the monocytes and also was associated with a greater risk of exhibiting cIMT in hypertensive patients when compared to that of control subjects. However, the miR-33a expression of both strands didn't show any significance in the serum sample of the participants. Thus, we suggest the use of anti-miR technology to miR-33a-5p may help in the reduction of cIMT in EH patients (Torres-Paz et al., 2018). Thus, anti-miRs can be used in treating EH and also many studies are required to address their role in pathophysiology of EH for further investigations.

3.2 MicroRNA inhibition in Pulmonary Hypertension (PH)

Pulmonary hypertension (PH) is a complex and multidimensional pulmonary vascular condition that is becoming more common worldwide. Currently, PH treatment predominantly targets three major vasodilator pathways namely endothelin, prostacyclin signaling and nitric oxide, but these pathways were not able to highlight the ambiguous molecular causes of PH (Chun *et al*., 2017). In 2018, Jiang *et al*., explained the impact of miR-190a-5p on chronic hypoxia-induced PH which was investigated in mice lung tissue and human pulmonary endothelial cells (HPECs). According to *in vitro* experiments on HPECs, it was observed that the levels of miR-190a-5p were significantly elevated by hypoxia and also noted that miR-190a-5p transfection mimicked HPECs suppression of Kruppel Like Factor 15 (KLF15) expression. In the case of in-vivo studies, anti-miR-190a-5p was administered intravenously which remarkably attenuated the right ventricular systolic pressure and escalated the expression levels of KLF15 in lung tissue of PH. KLF15 is involved in cell proliferation and migration, heart failure, the creation of aortic aneurysms, and the activation of proinflammatory processes in vascular smooth muscle and atherogenesis. KLF15 has also been shown to have a role in maintaining pulmonary endothelium homeostasis by modulating the expression of endothelial Arg2 and eNOS. Thus, anti-miR-190a-5p can be served as a therapeutic molecule in the treatment of PH (Jiang *et al*., 2018). Liu *et al*., explored whether the expression of miR-17-5p contributed to the proliferation of pulmonary arterial smooth muscle cells (PASMCs) caused by hypoxia in PH. It was observed that the miR-17-5p levels were upregulated in PASMCs which led to increase in the cell proliferation and migration. Whereas, after the administration of anti-miR-17-5p there was a reduction of cell proliferation and migration in PASMCs by targeting PTEN. Thus, this study proved that inhibition of miR-17-5p can be a novel therapeutic molecule for the management of hypoxia-induced PH (Liu *et al*, 2018).

In addition, Fu *et al* (2019) performed an experiment both *in vivo* and *in vitro* using male C57BL/6J mice and pulmonary arterial endothelial cells (PAECs) respectively. To induce PH, male C57BL/6J mice were injected with SU5416 once a week for three weeks while exposed to 10% oxygen. The effects of adeno-associated virus 9 (AAV9) delivery in the PH model's lungs, which was particularly intended to suppress miR-495 was first tested. Later, under hypoxic condition, the biological activity of miR-495 was investigated in cultured PAECs. The techniques like flow cytometer and CCK8 assay revealed that miR-495 inhibitor enhanced the cell viability in the G2/M+S phase, and wound healing studies revealed that PAECs transfected with miR-495 inhibitor had greater migratory potential than inhibitor-NC cells. These findings imply that delivering AAV9-TuD-miR-495 to PH mice not only improves hemodynamic and pulmonary vascular structural alterations, but also restored the pulmonary microcirculation integrity which could be a unique treatment approach for human PH (Fu *et al*., 2019). Therefore, advances in understanding the role and function of anti-miR therapies may provide a critical foundation for future research in PH.

3.3 MicroRNA inhibition in Pulmonary Arterial Hypertension (PAH)

In general, pulmonary arterial hypertension (PAH) is a rare condition distinguished by profound vascular re-modelling in the small peripheral arteries of the lung, resulting in an escalation in pulmonary vascular resistance over time. PAH is a fatal disease with a mortality rate of 5-10% and the current treatment strategies are not able to address the underlying cellular and molecular abnormalities (McGoon *et al*., 2013). A study by Gao *et al* in 2019 showed that increased levels of miR-410 in human pulmonary artery endothelial cells (HPAEC) suppressed basal and VEGF- induced proliferation, migration and apoptosis, whereas inhibition of miR-410 has the inverse effect. By targeting a modulator of pulmonary vascular remodelling, miR-410 may play a pivotal role in PAH pathogenic mechanism (Gao *et al*., 2019). Moreover, Lv *et al*, stated that the expression levels of miR-206 and potassium voltage-gated channel subfamily A member 5 (Kv 1.5) in primary cultured PASMCs and pulmonary artery smooth muscle from IUGR rats were assessed with or without the administration of miR-206 inhibitor. Inhibition of miR-206 increased the expression of Kv1.5-protein and KCNA5 both in *in vivo* and *in vitro* condition and whereas decreased right ventricular systolic pressure and cell proliferation were observed in PASMCs and IUGR rats after chronic hypoxia. These findings imply that the inhibition of miR-206 may be a therapeutic molecule for chronic hypoxia-PAH via Kv1.5 (Lv *et al*., 2019).

A study by Le Ribeuz *et al* (2020) found that miR-138-5p was overexpressed in PASMCs. The administration of anti-miR-138-5p via nebulization to rats with monocrotaline-induced PAH productively declined right ventricular systolic pressure and escalated pulmonary arterial acceleration time (PAAT). Moreover, the first channelopathy in PAH was discovered to be caused by mutations in the KCNK3 gene. Furthermore, the study discovered that PAH is associated with KCNK3 dysfunction in the pulmonary vasculature and high right ventricular levels. The study also stated that SLC45A3 was overexpressed in monocrotaline-anti-miR-Control-treated rats, however, it was normalised after anti-miR-138-5p therapy. In the lungs of the rat, miR-138-5p inhibition re-established KCNK3 mRNA expression and SLC45A3 protein expression in *in vivo* conditions (Le *et al*., 2020). Thus, the study confirmed that miR-138-5p inhibition has the propensity to turn down the spread of PAH.

Recently, a study by Liu *et al* (2021) aimed to establish whether right ventricular remodeling in PAH model rats might be prevented by inhibiting miR-1 expression. Rats were exposed to hypoxia to create PAH model rats, while cardiac fibroblasts (CFs) from PAH model rats were treated to hypoxia to establish an *in vitro* model. MiR-1 antagomiR transfection inhibited the progress of right ventricle fibrosis and also decreased the expression of mRNA levels in collagen I, collagen III, smooth muscle actin (α-SMA) and connective tissue growth factor (CTGF) in right ventricular tissue of PAH rats. Additionally, the upregulation of collagen I, collagen III, α-SMA and CTGF expression levels in hypoxia-treated CFs were reversed by transfecting with miR-1 antagomiR. These findings suggested that inhibiting miR-1 could reduce RV hypertrophy and fibrosis in the PAH rat model (Liu *et al*., 2021). Although, many of the above enumerated miRNA inhibition have shown miRNA expression in animal model and human derived cell line studies, their potential function remains unknown in the development and advancement in treating PAH.

3.4 MicroRNA inhibition in Preeclampsia (PE)

Preeclampsia (PE) is a pregnancy specific syndrome that causes severe clinical hypertension and proteinuria in the mother. PE affects 2% to 8% of pregnancies worldwide (Rana *et al*., 2019). Despite recent advancements in medication, the disease mechanism remains unknown. Till date there is no cure for treating PE and the only option available is the delivery of the foetus as premature baby or still birth. The approaches accessible to treat and prevent PE may be inefficient due to the lack of basic knowledge of the disorder's cause and pathophysiology. According to Liu *et al* (2019), in trophoblast and placental tissue, overexpression of miR-142-

3p inhibited the mRNA expression and activities of matrix metalloproteinase-2 (MMP2) and MMP9 which are involved in cell invasion and migration. TGF-β1 has been identified as a direct target of miR-142-3p. Eventually, the research found that inhibiting miR-142-3p increased cell invasion and migration by reactivating the TGF-β1/Smad3 signaling pathway. As a result, miR-142-3p may play a vital role in human placental development by inhibiting trophoblastic cell invasion and migration. Therefore, miR-142-3p may be used as a therapeutic target in the treatment of PE (Liu *et al*., 2019).

Moreover, in 2020, a study by Han *et al*, has used trophoblast and placental tissue to explore the miR-342-3p expression. Their study stated that the cell proliferation and invasion was promoted in the trophoblastic cells with the help of miR-342-3p inhibitor by directly targeting DNA binding 4 (ID-4). ID protein family members have been found to be important regulators of cell proliferation, migration, angiogenesis, and permeability of endometrial epithelial cell. Thus, the *in vitro* studies suggested that inhibiting miR-342-3p expression may reduce the incidence of PE (Han *et al*., 2020). Liu *et al*., reported that miR-491-5p expression was shown to be higher in the placental tissues of women with PE, and overexpression of miR-491-5p inhibited the invasion and migration of trophoblast cells, by targeting MMP-9. These findings suggested that miR-491-5p could be a promising therapeutic target for treating PE. However, the study has shown limitations that miR-491-5p expression levels were only examined in placental tissues taken from pregnant women undergoing caesarean sections, implying that its expression levels may not be ideal for prenatal screening of PE patients (Liu *et al*., 2020). In 2021, Wang *et al*, studies revealed that the transcriptional levels of miR-155 in the placenta were higher among patients with PE than in healthy pregnant women. Inhibiting miR-155 levels boosted sonic hedgehog (SHH) expression and enhanced morphology in primary trophoblasts from patients with PE. This finding revealed that miR-155 regulates trophoblast apoptosis in PE, suggesting that it could be used to forecast PE risk and as a therapeutic target for treating PE (Wang *et al*., 2021). Li *et al*., demonstrated that in PE placenta tissues, the expression of circ_0001438 and NLRP3 was increased. In HTR-8/Svneo cells, circ_0001438 knockdown increased cell proliferation, migration, and invasion but decreased apoptosis and inflammatory responses, which were reversed by inhibiting miR-942. Thus, Circ _0001438 sponged miR-942 to modulate NLRP3 expression, and via mediating the miR-942/NLRP3 axis, circ_0001438 exacerbated the dysfunctions of human villous trophoblasts (Li *et al*., 2021). Hence, these examinations revealed that miRNA-inhibition treatment strategies may help in studying the role and mechanism of PE and also large numbers of studies are needed to

prove their mechanism in the cells. **Table 1 represents the role of microRNA inhibition in EH, PH, PAH and preeclampsia.**

4. Hypertension complications

Hypertension complications are clinical consequences that occur as a result of persistently increased blood pressure. Some of the complications of hypertension that affect different organs like heart, brain, eyes, kidney are hypertensive cardiomyopathy, myocardial infarction, stroke, hypertensive encephalopathy, hypertensive retinopathy and hypertensive nephropathy which are all predisposing factors for hypertension (Biswas *et al*., 2003).

4.1 Hypertensive complication affecting heart

Hypertensive heart diseases are caused by anatomical and functional alterations like left ventricular hypertrophy, hypertensive cardiomyopathy and myocardial infarction etc, in the cardiovascular system (Piskorz *et al*., 2021). Despite the advancement of appropriate treatments to approach heart failure, the disease eventually progressed, resulting in repetitive diagnosis and, finally death. As hypertension acts as the primary complication leading to these secondary complications, early diagnosis and treatment of hypertension can reduce the chances of health deterioration. A study by Duan *et al*., elucidated the molecular and clinical importance of miR-214 dysregulation in heart failure. It was observed that miR-214 levels were upregulated in the serum sample of patients with chronic heart failure reduced endothelial cell proliferation and angiogenesis by targeting X-Box Binding Protein 1 (XBP-1). Thus, inhibition of miR-214 by targeting XBP-1 can play an essential role in cardiac angiogenesis (Duan et al., 2015). In 2019, Hu *et al*, demonstrated that inhibition of miR-155 reduced lipopolysaccharide-induced macrophage inflammation and nuclear factor-kB pathway activation while increasing suppressor of cytokine signaling 1 (SOCS1) expression in male mice model. Findings revealed that miR-155 inhibition reduced endoplasmic reticulum stress-induced cardiomyocyte apoptosis after myocardial infarction (Hu *et al*., 2019).

Interestingly, Heinkel *et al*, developed a porcine model of pressure-overload- induced heart failure and to see how inhibition of miR-132 affects the progression of heart failure in animals. The results stated that at day 56, treatment with antimiR-132 reduced cardiomyocyte cross-sectional area and enhanced overall heart performance. Thus, inhibition of miR-132 is a viable method for preventing the progression of heart failure in hypertrophic heart disease and could

be used as a treatment for non-ischemic heart failure (Hinkel *et al*., 2021). However, large number of studies are required to understand the role of miRNA inhibition in hypertensive cardiomyopathy.

4.2 Hypertensive complication affecting the brain

The brain is an initial target of hypertension-induced organ damage, stroke, subclinical cerebrovascular abnormalities, intracerebral hemorrhage and dementia are all possible outcomes (Kelly *et al*., 2020). Interestingly, Huang *et al*., (2017) performed an experiment using arteriovenous malformations smooth muscle cells (AVMSMCs) and it was noted that miR-137 and miR-195 levels were considerably lower in AVMSMCs. Furthermore, increasing the levels of these miRNAs in *in vivo* condition reduced AVMSMC migration, tube formation, survival as well as the establishment of vascular rings. On the other hand, inhibition of miR-137 and miR-195, had no effect on cell migration, tube formation or survival in AVMSMC cultures, implying that AVMSMCs have lower baseline levels of miR-137 and miR-195 than normal vascular smooth muscle cells (VSMCs). Thus, inhibition of miR-137 and miR-195 inhibit vasculogenesis in brain AVM (Huang *et al*., 2017).

In 2020, Yan *et al*., stated that in an *in vivo* middle cerebral artery occlusion (MCAO) mouse model of ischemic stroke, inhibiting miR-9-5p or miR-128-3p reduced MCAO-induced infraction volume and prevented apoptosis. Overall, the findings helped in understanding how miR-9-5p and miR-128-3p induced brain damage in ischemic stroke and can be used as a promising therapeutic target (Yan *et al*., 2020). Recently, Zhang *et al* (2022), evaluated the role of miR-335 and miR-674-3p in the rostral ventrolateral medulla (RVLM) in the stress-induced hypertension (SIH) rat model. The upregulation of miR-335 and miR-674-3p in RVLM resulted in the significant increase of the heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP). While, the intra-RVLM microinjection of anti-miR resulted in the reduction of heart rate, SBP, DBP and MAP in the SIH rats. Thus, the inhibition of miR-335 and miR-674-3p can be exploited as a potential treatment for RVLM and SIH (Zhang *et al*., 2022). More validated research is required for the better understanding of the pathological changes in miRNA inhibition that occur in the cells or tissue of the brain.

4.3 Hypertensive complication affecting eyes

Hypertensive retinopathy is characterised by retinal arteriolar intimal thickening, and hyperplasia of the intima-media end with sclerosis as a response to high blood pressure (Erden *et al*., 2012). Studies have proved that miRNAs are essential regulators of retinal endothelial cell proliferation and migration in patients with hypertension and hypertensive retinopathy (Heggermont *et al*., 2012). In 2017, Wang *et al*, induced acute ocular hypertension (AOH) in the left eye of adult albino rats and the opposite eye served as the control. The AOH versus control group miRNA microarray research showed 31 differently expressed miRNAs (miR-133b-3p, miR-336-5p, miR-22-3p, miR-532-3p, miR-190a-5p, miR-136-3p, miR-144-5p, miR-350, miR-3571, miR-3580-3p, miR-1912-3p, miR-628, miR-3084b-5p, miR-378b, miR-215, miR-3120, miR-3568, miR-17-5p, miR-291a-3p, miR-450a-5p, miR-672-5p, miR-210-3p, miR-493-3p, miR-93-5p, miR-206-3p, miR-1-3p, miR-539-5p, miR-383-5p, miR-592, miR-490-5p and miR-6324), and the regulation of 12 chosen microRNAs was validated by qRT-PCR. Among the 31 miRNAs, 12 miRNAs were upregulated. Thus, the use of miRNA inhibition in the upregulated miRNAs may be used as a therapeutic target for the treatment of AOH. However, the results showed that variations in the expression of miRNAs, whose target genes were linked to the modulation of microglia-mediated neuroinflammation or neural apoptosis, were seen in response to acute intra-ocular pressure (IOP) rise. Thus, microRNAs may open new opportunities in preventing retinal ganglion cell apoptosis and may serve as a target for future therapeutic regimens in AOH and retinal ischaemic conditions (Wang *et al*., 2017**).**

Yang *et al*., performed an experiment using 42 patients with hypertension, 42 healthy patients and 42 with hypertensive retinopathy. It was shown that hypertensive retinopathy patients showed lower expression of miR-637 when compared to hypertensive patients and luciferase assay revealed that STAT3 was a target gene for miR-637. Thus, according to the findings, miR-637 could be a non-invasive diagnostic for hypertensive retinopathy patients. The impact of miR-637 on STAT3 and use of anti-miR-637 may prevent retinal endothelial cells from proliferating and migrating, making it a potential target for hypertensive retinopathy treatment (Yang *et al*., 2021). Thus, these studies revealed that the use of anti-miR might help in solving out the problems in eye caused due to hypertension. However, further investigations on molecular mechanisms are required to prove that anti-miRs has a crucial role in treating hypertensive retinopathy.

4.4 Hypertensive complication affecting kidney

Hypertension is one of the major causes of chronic kidney disease because of the negative effects of high blood pressure on the renal vasculature. Hypertension has been observed to affect 85-95% of chronic kidney disease patients (Kalatizidis *et al*., 2018). The connection between high blood pressure and chronic kidney disease is cyclic. Gomez *et al*, demonstrated that miR-21 contributed to the pathogenesis of cellular ATP generation, reactive oxygen species (ROS) production, mitochondrial dysfunction and inflammatory signaling in chronic kidney disease. Whereas, miR-21 inhibition protected glomerular and interstitial cells from TGF-β induced fibrogenesis and inflammation and also enhanced mitochondrial function (Gomez *et al*., 2015). This finding showed that inhibition of miR-21 is a possible treatment approach for chronic kidney disorders.

In 2021, Bai *et al*., investigated the role of miR-27b-3p in the development of renal fibrosis in HK-2 cells and unilateral ureteric obstruction (UUO) mice model. It was observed that miR-27b-3p overexpression reduced UUO-induced renal fibrosis by STAT1, α-SMA, and collagen III expression in HK-2 cells. Thus, *in vivo* and *in vitro* studies suggested that inhibition of miR-27b-3p could reduce renal fibrosis via decreasing STAT1. As a result, miR-27b-3p could be a suitable therapeutic target for renal fibrosis treatment (Bai *et al*., 2021). A study by Liu *et al*., 2022 suggested that miR-122-5p promoted renal injury and fibrosis in spontaneously hypertensive rats by targeting Forkhead Box O3 (FOXO3). Whereas, the inhibition of miR-122-5p reduced renal fibrosis and injury including inflammation in spontaneously hypertensive rats which exhibited its importance in the treatment of hypertensive renal injury and fibrosis (Liu *et al*., 2022). However, further investigations on miRNA inhibition are required to provide their role in hypertensive nephropathy. **Table 2 represents the role of microRNA inhibition in hypertensive complications.**

5. Clinical shortcomings of miRNA inhibition

RNAi is a regulatory mechanism of most eukaryotic cells. Meanwhile, in a relatively short period of time RNAi therapeutics have advanced significantly (Kim *et al*., 2022). Interestingly, it was noted that miRNAs are the endogenous substrates for the RNAi machinery. In today's competitive world, finding a novel therapeutic molecule that can function as a new medicine is a challenging task. Some of the key considerations that are designed for RNAi therapeutic as new drug are it must address an unmet medical need; the drug must have the good pharmacokinetic feature and also the drug must be safer and/or more effective than the existing standard of care. MicroRNAs have the capacity to pleiotropically target hundreds or even

thousands of genes and some of which have unique functions for distinct organs or cell types which are some of the common features (Nie *et al*., 2021). Accordingly, this raised the possibility that one miRNA candidate may have the power to control entire biological pathways that are disturbed by pathogens in a patient.

Moreover, some of the microRNAs like miR-17, miR-29, miR-208 and miR-155 etc., are already in the preclinical and clinical trials in the treatment of various diseases like polycystic kidney disease, cardiac fibrosis, chronic heart failure and cutaneous T-cell lymphoma (CTCL) respectively. A study by Li *et al*., (clinical trial registration no.: NCT00420784) demonstrated that miR-296-5p and let-7e showed a novel link between human cytomegalovirus (HCMV) and EH in plasma samples of 24 hypertensive patients and 22 control subjects. It was observed that hypertensive group had higher seropositivity and quantitative titers of HCMV than the control group. These results might provide crucial information on the pathophysiology of EH [Li *et al*., 2011]. A clinical trial study by Zhou *et al*., reported that simvastatin inhibited miR-15a-5p to enhance Bcl-2 expression and Bak expression and protected myocardium from apoptotic damage after cardiac surgery [Zhou *et al*., 2018]. Patients are still being enrolled in a phase 1 trial (Clinical trial number: NCT03603431) for the drug MRG 110, an antisense oligonucleotide modified with locked nucleic acid (LNA) to inhibit miR-92 which is used for the treatment of heart failure. Recent reports have also stated that there are only about 20 miRNA therapeutics in clinical trials and none of them advanced to phase III trials [Diener et al., 2022]. However, most of the clinical trials are being studied in cancer when compared to hypertension. Nevertheless, research on miRNAs and/or miRNA inhibitions in preclinical and clinical trials for the treatment of hypertension are yet to be studied. Recent reports have stated that some of the clinical trials have failed due to the following reasons: Firstly, off-target biological effects are unquestionably a problem when it comes to miRNAs because of their pleiotropic character, but conventional therapies that focus on a single protein-coding gene have also been known to cause comparable nonspecific reactions. Secondly, efficacy for specific target sites varies greatly between the miRNAs. This can be overcome by following some of the criteria like target mRNA accessibility, position-specific determinants, and thermodynamic end stability (Bajan *et al*., 2020). Thirdly, at present a key challenge in the drug treatment is the drug resistance (Chakraborty *et al*., 2020). However, some of the other challenges that are faced in the miRNA therapeutics are rapid clearance and degradation in blood and bodily fluids, low penetration and the ability to activate the immune system.

Some of the unique considerations for pre-clinical studies to progress are one should perform an absorption, distribution, metabolism, and elimination (ADME) research for the drug discovery and development investigations for miRNA inhibition studies in hypertension. Through these investigations, chemists, biologists, doctors, toxicologist, researchers and the pharmaceutical industries can collaborate interdisciplinary. In addition, the delivery of anti-miR drugs should take into account their efficacy and specificity in reaching the target cell. This delivery system can be overcome by safeguarding anti-miR from early dissociation into the blood, bringing anti-miR close to the target cells, facilitating cellular uptake, not inducing any immunogenic response and finally containing components that are biocompatible and biodegradable (Chakraborty *et al*., 2020 and Dzau *et al*., 2019).

6. Future perspectives

MicroRNA inhibitors can be used to restore altered mRNA expression in various diseases, including hypertension. Due to microRNA's small size and evolutionary conservation among different species, inhibition of miRNAs has an encouraging attribute as new therapeutic strategies for various diseases along with hypertension. Disruption in the expression of miRNAs can cause cellular dysfunction and promote the development of pathological events associated with hypertension. This cellular dysfunction caused by few miRNAs can be treated with the help of anti-miRs. Several miRNA-based drugs are currently under investigation, and none have so far achieved a pharmaceutical breakthrough. Despite considerable improvements in the technologies used for the discovery and validation of novel anti-miRs, their clinical applicability remains a challenge as therapeutic targets and biomarkers. However, some of the hurdles that must be overcome if miRNAs are to be therapeutically useful for the treatment of hypertension and hypertensive complications are ensuring effective and safe management and delivery, avoiding undesirable off-target effects, developing strategies for evaluating systemic bioavailability of drugs in subcellular localization and also to prevent intracellular entrapment. In addition, the use of other miRNA therapeutics like LNAs, phosphorodiamidate morpholino oligonucleotides (PMOs), miRNA sponges, peptide nucleic acids (PNA) and also CRISPR/Cas9- based genome editing technique are useful in treating hypertension and hypertensive complications (Saiyed *et al*., 2022). The side effects and toxicity of modulating gene expression studies need to be carefully examined. It is also important to know the distinct role of molecular pathways in various cells that should be taken into account when designing

therapeutic mechanisms. More research in cell lines, humans and animal models will be required to determine the exact mechanism and potential therapeutic applications. Accumulating evidence in *in vivo* and *in vitro* preclinical studies proved that anti-miRs are efficient therapeutic targets in hypertension, hence further evidence or studies on anti-miRs in clinical trials can pave way for prognosis, diagnosis and treatment of various hypertension.

7. Conclusion

Hypertension continues to be a major public health issue, with wide-ranging consequences for global burden of diseases like heart failure, stroke, myocardial infarction, aortic dissection and final stage kidney diseases etc. Development of anti-miRs in treating hypertension may improve therapeutic outcomes for patients with this condition. In conclusion, this review highlighted the role of miRNA inhibition in various types of hypertension namely EH, PH, PAH and preeclampsia and hypertensive complications affecting the heart, brain, eyes and kidney. Substantial advancements in the technology used to find and validate novel miRNA inhibitors as useful therapeutic targets are yet to be established. More research on miRNA inhibitors in clinical trials could act as the diagnostic, and therapeutic targets for the treatment of miscellaneous hypertension.

Declarations

Author Contributions

K Auxzilia Preethi collected the related papers, wrote and drafted the manuscript. Sushmaa Chandralekha Selvakumar worked on the visualization and reference correction. Kehinde Ross edited the manuscript. Durairaj Sekar initiated the study, revised and finalized the manuscript. All authors read and approved the final manuscript.

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Figure

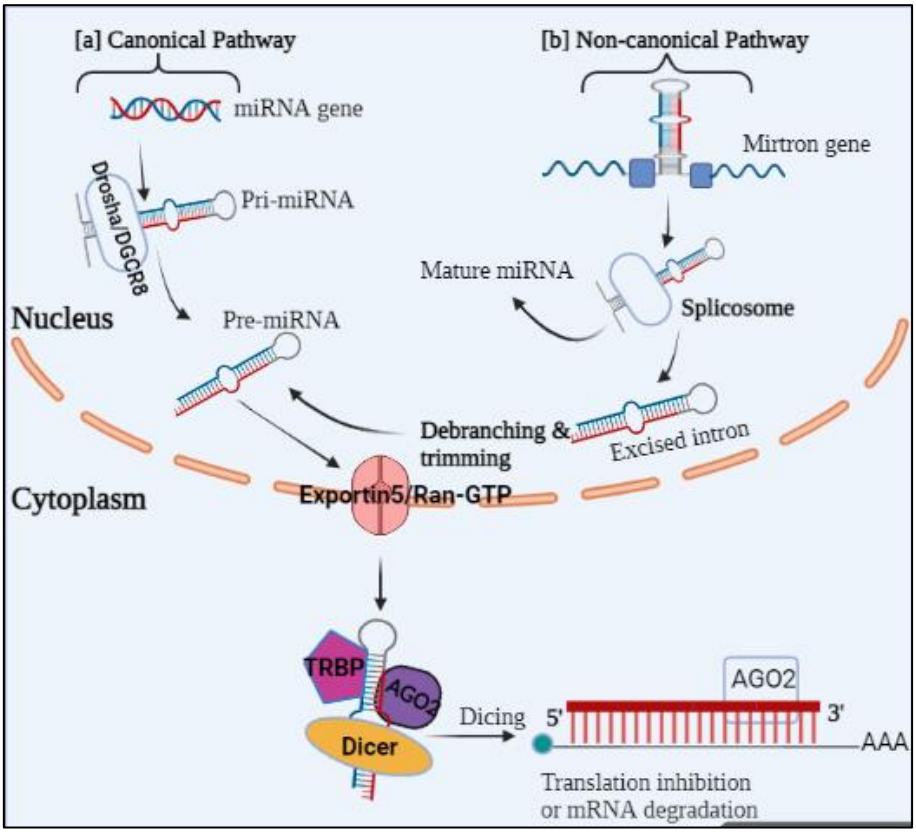

Figure 1 represents the overview of miRNAs biogenesis (a) Canonical Pathway: The first step in the biogenesis of the canonical pathway is the production of the primary miRNA (pri-miRNA) transcript carried out by RNA polymerase II. Drosha RNase III endonuclease cleaves the pri-miRNA into the precursor miRNA (pre-miRNA). This pre-miRNA enters the cytoplasm via the interaction of exportin-5/Ran-GTP and further carried out by Dicer. Finally, dicer generates a small RNA duplex, after which it is loaded onto an AGO protein to configure the RNA-induced silencing complex (RISC). **(b) Non-canonical Pathway:** In non-canonical pathways, mirtron production was first described, in which the Drosha-mediated processing step is skipped. Having followed splicing and the generation of mature mRNA, the excised intron is debranched and trimmed to produce pre-miRNA, which is then exported by Exportin5/Ran-GTP and finally processed to the canonical pathway for biogenesis of miRNA.

801 **Tables**

802 **Table 1 represents the role of miRNA inhibition in EH, PH, PAH and preeclampsia**

803

MiRNA inhibition	Species	Cell Type/Tissue	Target Gene	Function	Hypertension type	Reference
miR-21	mice	Cardiac tissue	TMEM49	Prevented cardiac hypertrophy and fibrosis	Essential hypertension	Patrick *et al*., (2010)
miR-33a-5p	Human	Monocytes and serum sample	SREBPs	Helped in reduction of carotid intima-media thickness	Essential Hypertension	Torres-Paz *et al*., (2018)
miR-190a-5p	Mice	Human pulmonary endothelial cells and lung tissue	KLF15	Helped in maintaining pulmonary endothelium homeostasis and reduced the right ventricular systolic pressure	Pulmonary Hypertension	Jiang *et al* (2018)
miR-495	Mice	Pulmonary arterial endothelial cells (PAECs)	VEZF1	Improved both vascular remodeling and angiogenesis	Pulmonary Hypertension	Fu *et al*., (2019)
miR-17-5p	Rat	Pulmonary arterial smooth muscle cells	PTEN	Reduced cell proliferation and migration	Pulmonary Hypertension	Liu *et al.*, (2018)
miR-410	Mice	Pulmonary Artery Endothelial Cells/ Lung Tissue	NAMPT	modulator of pulmonary vascular remodelling	Pulmonary arterial Hypertension	Gao *et al*., (2019)

miR-206	Rat	Pulmonary Artery Smooth Muscle Cell/ Lung tissue	KCNA5	Decline in right ventricular systolic pressure and cell proliferation	Pulmonary Arterial Hypertension	Lv *et al*., (2019)
miR-138-5p	Rat	Lung tissue	KCNK3, SLC45A3	Reduced right ventricular systolic pressure	Pulmonary Arterial Hypertension	Le Ribeuz *et al*., (2020)
miR-1	Rat	Rat cardiac fibroblasts	CTGF	Reduced right ventricular hypertrophy and fibrosis	Pulmonary arterial hypertension	Liu *et al* (2021)
miR-142-3p	Human	Trophoblast cell lines and Human 293T cells/ Placental Tissue	TGF-β1	Human placental development	Preeclampsia	Liu *et al*., (2019)
miR-342-3p	Human	Trophoblast cell lines HTR-8/SVneo and Placental tissue	ID-4	Promotes cell proliferation and invasion	Preeclampsia	Han *et al*., (2020)
miR-491-5p	Human	Trophoblast cell lines HTR-8/SVneo	MMP-9	Inhibits trophoblast cell migration and invasion	Preeclampsia	Liu *et al*., (2020)
MiR-155	Human	HTR8/Svneo cells	SHH/GLi1/ BCL2	Improved the phenotype in primary trophoblast	Preeclampsia	Wang *et al*., (2021)

804

805

806 **Table 2 represents the role of miRNA inhibition in hypertensive complications**

807

miRNA Inhibition	**Species**	**Cell Type/Tissue**	**Target Gene**	**Function**	**Hypertensive complication affecting**	**Reference**
miR-155	Mice	Bone marrow-derived macrophage cells	SOCS1	Reduced macrophage inflammation	Myocardial infraction (Heart)	Hu *et al*., (2019)
miR-132	Porcine	Endothelial cells	NRF2	Preventing the progression of heart failure	Cardiac Hypertrophy (Heart)	Hinkel *et al*., (2021)
miR-137 and miR-195	mice	AVM smooth muscle cells	FMNL2	Inhibit vasculogenesis	Brain	Huang et al., (2017)
miR-9-5p and miR-128-3p	Mice	Neuronal Cells	Caspase-3	Prevented apoptosis	Ischemic stroke (Brain)	Yan *et al*., (2020)
miR-637	Human	HUVECs	STAT3	Inhibited the proliferation and migration of retinal endothelial cells	Hypertensive retinopathy (Eyes)	Yang *et al*., (2021)
miR-21	mice	Proximal kidney tubule cells	PPARα	Protected glomerular and interstitial cells	Chronic kidney disease (Kidney)	Gomez *et al*., (2015)
miR-27b-3p	Mice	HK-2	STAT1	Inhibited the progression of renal fibrosis	Chronic Kidney disease	Bai *et al*., (2021)
miR-122-5p	Rat	Primary renal tubular interstitial fibroblasts	FOXO3	Reduced renal fibrosis and injury	Chronic Kidney disease	Liu *et al*., (2022)