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Chapter

MicroRNAs in Aldosterone Production and Action

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Abstract

The secretion of aldosterone by the adrenal cortex is a tightly regulated process. Loss of this control can result in severe hypertension and end-organ damage, so detailed understanding of the various mechanisms by which the body regulates aldosterone biosynthesis is key. The emergence of microRNAs (miRNAs) as negative regulators of numerous physiological processes has naturally led to their study in the context of aldosterone production. We summarise several studies that have demonstrated a significant role for microRNAs in aldosterone biosynthesis and action, thereby presenting a possible therapeutic role in the treatment of common forms of hypertension such as primary aldosteronism. Furthermore, the presence of microRNAs in the circulation offers the prospect of accessible and informative biomarkers that may simplify the currently protracted and technically difficult diagnosis of such conditions.

Keywords: aldosterone, microRNA, hypertension, adrenal cortex

1. Introduction

High blood pressure, or hypertension, is a major risk factor for coronary disease, heart failure and stroke. Hypertension is a contributing factor in over 7 million deaths per year, which provides strong motivation to understand the systems regulating normal blood pressure and how such control can be lost. Our own studies have focused on the role of the hormone aldosterone, a key determinant of blood pressure, and the various factors regulating its secretion from the adrenal gland.

Aldosterone is synthesised in the adrenal cortex and acts on specific mineralocorticoid receptors (MR), principally in epithelial tissue, to regulate fluid balance, electrolyte homeostasis and blood pressure. Excess secretion of aldosterone, as in primary aldosteronism (PA), leads to severe hypertension with markedly increased risk of myocardial infarction, stroke and left ventricular hypertrophy [1]. Originally believed to be a rare condition (principally due to practical difficulties in accurate diagnosis), the reported frequency of PA in all hypertensives has risen steadily over the years and is now generally regarded to lie somewhere between 10 and 20%; PA is therefore the single most common form of secondary hypertension [2]. Independent of its effects on blood pressure, excess aldosterone also has detrimental effects on various target organs including the renal and cardiovascular systems [3]. Such negative effects are not necessarily confined to PA; even when present in minimal excess, aldosterone associates with higher blood pressure and

substantial cardiovascular morbidity [4]. Although major advances have been made in understanding aldosterone and its regulation in the 60 years since its discovery, many aspects remain incompletely understood. New factors capable of regulating aldosterone secretion are still emerging, and evidence generated by ourselves and others indicate that we must add microRNA (miRNA) to this list.

In this article we summarise the major findings to date regarding miRNA and its effects on aldosterone secretion and action. We also anticipate the future direction and outcomes of such studies—including the possible role for miRNA in the accurate diagnosis of PA and other subtypes of hypertension—and related therapeutic strategies that could be employed to modify hormone production and action in such patients to yield major health benefits.

2. Aldosterone biosynthesis

Aldosterone biosynthesis is confined to the adrenocortical zona glomerulosa (ZG) and in normal circumstances is principally controlled by the renin-angiotensin system (RAS) and potassium status. Synthesis consists of a series of enzymatic reactions commencing with the conversion of cholesterol by the side-chain cleavage enzyme, CYP11A1. The terminal reactions in aldosterone biosynthesis are catalysed by the enzyme aldosterone synthase, the product of the *CYP11B2* gene, which is expressed only in the ZG. Its expression is principally controlled by angiotensin II (AngII) and potassium through transcription factor binding of its 5′ regulatory region [5]. The key second messenger in this process is calcium; influx of Ca^{2+} through channels in the ZG cell membrane raises aldosterone production by various means that include stimulation of *CYP11B2* transcription and increased availability of the cofactor NADH. Extensive study of the *CYP11B2* gene has shown it to be highly polymorphic, with multiple genetic sequence variations present across its introns and in the untranslated regions (UTRs) lying to the 5′ and 3′ ends of the locus. Certain of these 5′ and intronic variants associate with altered gene activity and also with raised plasma aldosterone, increased urinary excretion of aldosterone metabolites and high blood pressure, demonstrating that relatively subtle changes in expression of this gene can have significant cardiovascular effects. The discovery of microRNAs and their role in post-transcriptional repression of specific genes, principally through sites located in the 3' UTR, has now focused interest on that region of *CYP11B2*.

3. MicroRNA

miRNAs are a class of endogenous, small (~20–25 nucleotides), single-stranded non-coding RNA molecules which act to post-transcriptionally regulate expression of specific target mRNAs. They are often regarded as having only subtle 'fine-tuning' roles in gene expression but are nevertheless capable of significant effects including roles in human disease, including numerous cancers [6, 7].

Synthesis of miRNA is a multistep process (see **Figure 1**), beginning with the transcription in the nucleus from miRNA genes located mainly in intergenic or intronic chromosomal regions of chromosomes, although some are also present in exons [8, 9]. This produces primary transcripts (pri-miRNA), which are processed by Drosha endonuclease into pre-miRNAs ~70 nucleotides in size. Due to self-complementary nucleotide binding, these pre-miRNAs have distinctive 'hairpin loop' structures and are transported by Exportin-5 from the nucleus to the cytoplasm, where they are processed further by Dicer to form a miRNA duplex.

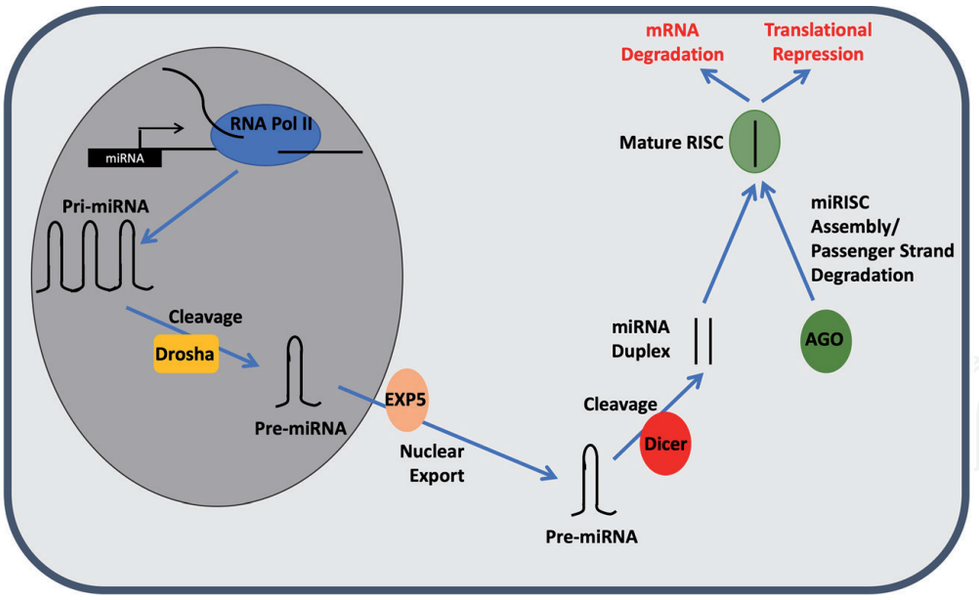

Figure 1.
Overview of miRNA biogenesis and post-transcriptional repression mechanisms. MicroRNA genes are transcribed in the nucleus as primary transcripts (pri-miRNA) before being processed into ~70 nucleotide pre-miRNAs by Drosha endonuclease. The pre-miRNA is transported from the nucleus into the cytoplasm by Exportin-5 where it is processed further by dicer. The mature miRNA (red) is then loaded into Argonaute 1–4 and assembled into the miRNA-induced silencing complex (miRISC), which is subsequently guided to the 3'UTR of the target mRNA. mRNA translation is inhibited by miRISC through one or more repressive mechanisms, including mRNA cleavage, degradation and translational repression.

The strand of the miRNA duplex with lower thermodynamic stability (usually the 3′ arm) is termed the passenger strand; this is removed, resulting in the formation of the mature miRNA [10]. It was initially thought that the passenger strand had no biological function and was automatically targeted for degradation, but recent studies show that passenger strands can have a functional role in mRNA regulation, prompting their study in current miRNA research [11, 12]. The mature miRNA then recruits a ribonucleoprotein complex called the miRNA-induced silencing complex (miRISC). At the core of the mammalian miRISC is one of the four Argonaute proteins (AGO1–4) and a 182 kDa protein, GW182. While the miRNA sequence determines which mRNAs are targeted for repression, it is the miRISC proteins that actually mediate the silencing [13]. The miRISC post-transcriptionally represses gene expression by initiating decay of target mRNAs and/or inhibiting their translation. It achieves this by recognising and binding to specific sequences on the target mRNA, usually in its 3'UTR, that is complementary to the miRNA seed site (located at nucleotides 2–8 of the miRNA, at its 5′ end). If the mRNA is sufficiently complementary to the miRNA, it will be cleaved by the slicer AGO and these cleaved mRNA fragments targeted for degradation [14, 15]. If binding is imperfect, AGO is unable to cleave the mRNA. However, complementary binding beyond the seed sequence can also initiate silencing; in this case the GW182 protein recruits deadenylation factors which destabilise the mRNA through the removal of its polyadenylated tail, again targeting it for degradation. Although the majority of miRNA-controlled gene silencing is achieved by mRNA cleavage or destabilisation, translation can also be repressed. This mechanism is less well understood but is thought to involve miRNA interaction with factors essential to the initiation of translation, such as cytoplasmic poly(A)-binding protein (PABPC) and cap-binding complex eIF4F [16].

While miRNA-mediated regulation is typically mild in nature, individual miRNAs can have significant and diverse biological effect due to their ability to target numerous different mRNA species within the same cell [17] and even several

components within a single pathway [18]. Indeed, it is believed that the majority of protein-coding genes are regulated in some way by miRNAs given that >60% of human protein-coding genes contain a minimum of one conserved miRNA-binding site [19].

The naming of miRNAs follows a specific set of rules. Each miRNA name identifies first its source species (e.g. 'hsa' for human and 'mmu' for mouse) and is numbered according to its order of submission to the miRNA database [20], with mature sequences labelled 'miR' and precursor hairpins 'mir' [21]. Identical sequences found in different species are assigned the same numbers, while identical sequences found within the same species but arising from different genomic locations are given numerical suffixes (e.g. hsa-miR-1-1, hsa-miR-1-2). miRNAs of similar sequence are grouped into a miRNA 'family' and are allocated an additional lowercase letter to aid identification (e.g. hsa-miR-320a, hsa-miR-320b, hsa-miR-320c). Finally, given that mature miRNAs derive from a 'hairpin' precursor, the current nomenclature assigns either a -5p or -3p suffix, depending upon whether the miRNA was generated from the 5′ or 3′ arm of that hairpin (e.g. hsa-miR-34c-5p and hsa-miR-34c-3p).

4. Extracellular microRNAs

In addition to acting within the cell where they are transcribed, miRNAs can be released from those cells and have been detected in various bodily fluids, including the bloodstream. This has raised interest in the potential utility of circulating miRNAs as disease biomarkers [22, 23]. The majority of miRNAs within the circulation are associated with AGO2 in nuclease-resistant complexes. miRNAs also circulate within exosomes, which are small membrane vesicles that form within multivesicular bodies and are secreted upon fusion with the plasma membrane. Exosomes contain specific miRNAs rather than the complete spectrum of miRNAs of a cell, indicating as yet unknown mechanisms for their recognition, packaging and secretion. miRNAs may also be incorporated into high-density lipoprotein and low-density lipoprotein particles although this process is again not fully understood. Secreted miRNAs can, in principle, be transferred from one tissue to another through the circulation, but it is unclear whether a miRNA species taken up by a cell in this way can achieve sufficient levels to inhibit its target transcripts significantly. This mechanism of action raises the intriguing possibility that extracellular miRNAs participate in long-range signalling between tissues, in a manner analogous to endocrine systems. In this regard, miRNAs have been reported to act as agonists of Toll-like receptors and to trigger downstream pathway activation in target cells [24]. Distinctive expression patterns of extracellular miRNAs have also been associated with a variety of cardiovascular disorders, including atherosclerosis, myocardial infarction, heart failure, hypertension and type 2 diabetes [22]. However, whether these miRNAs participate in the disease process or simply serve as markers of disease progression has not been established. Greater patient cohorts will be needed to reach firm conclusions regarding the diagnostic and prognostic power of extracellular miRNAs.

5. MicroRNAs and corticosteroid production

Various studies have demonstrated the importance of microRNAs to adrenal development and maintenance in animal models [25, 26], but their effects on the human adrenal gland are less well defined. As far as secretion of corticosteroids

such as aldosterone is concerned, miRNAs could have direct influence through the post-transcriptional repression of corticosteroidogenic or other related genes, which has been investigated. In 2008, Romero et al. identified miR-21 as a key modulator of aldosterone production. Overexpression of miR-21 in vitro significantly increased aldosterone production and cell proliferation in the H295R human adrenocortical carcinoma cell line [27]. These findings supported a role for miR-21 in both corticosteroid production and oncogenesis but possible target genes of miR-21 or a regulatory mechanism by which it increases aldosterone production and cell proliferation were not described. However, subsequent studies have demonstrated that miRNAs target numerous stages of the aldosterone biosynthesis pathway.

We have carried out comprehensive analysis of miRNA effects on aldosterone and cortisol production, as well as identifying and confirming target genes. We used a siRNA approach to knock down expression of Dicer, the protein essential to miRNA maturation, in H295R cells and studied its effects on cellular levels of steroidogenic mRNAs. Interestingly, only those encoding cytochrome P450 enzymes in the pathway (*CYP11A1*, *CYP21A1*, *CYP17A1*, *CYP11B1*, *CYP11B2*) were significantly increased in the absence of miRNA [28, 29]. Steroid production was also correspondingly changed, with levels of the end products aldosterone and cortisol—as well as many intermediate compounds in their biosynthesis—all increased relative to control cells (DOC, corticosterone, 18-OH corticosterone).

We then used a combination of bioinformatic prediction and experimental in vitro experimentation in H295R cells to confirm miRNA-24 as a direct regulator of *CYP11B1* and *CYP11B2* expression via sites in the mRNA 3'UTRs. We also observed changes in aldosterone and cortisol production rates that correlated with miRNA-altered levels of *CYP11B1* (11β-hydroxylase) and *CYP11B2* (aldosterone synthase). These experimental results were consistent with canonical miRNA action, whereby reduced levels of the mature miRNA result in less target mRNA degradation and therefore more abundant steroid product due to higher gene expression. These data therefore demonstrated a significant regulatory role for miRNAs in human steroidogenesis. Furthermore, other studies show miR-24 to be upregulated following MR activation in the kidney, leading to the proposal that it might form part of a feedback loop to the adrenal gland, repressing *CYP11B2* expression when aldosterone levels are high [30].

Subsequent studies have expanded the array of miRNAs known to regulate directly the expression of late enzymes in this pathway: Nusrin and colleagues showed that miR-10b also negatively regulates both *CYP11B1* and *CYP11B2* expressions in H295R cells, subsequently modulating aldosterone and cortisol production [31]. We found that miRNAs -125a-5p and -125b-5p modulate *CYP11B2* (but not *CYP11B1*) in H295R cells [29], as did Maharjan et al. with miR-766 [32], although either study determined the effect of this on aldosterone production or whether cortisol remains unaffected. Looking to steroidogenic enzymes earlier in the pathway, we demonstrated a direct regulatory effect of miR-320a-3p on *CYP11A1* and *CYP17A1* in H295R cells [29]. Furthermore, Hu and colleagues reported that miR-132 regulates steroidogenesis by inhibiting StAR protein expression, thereby inhibiting basal progestin production and stimulating 20α-OHP production in Y1 mouse adrenocortical cells [33]. They also demonstrated a secondary method of miR-132 regulation in Y1 cells, through which miRNA overexpression reduces methyl-DNA-binding (MECP2) protein and alters levels of 3β-HSD and 20α-HSD; this stimulates the conversion of progesterone to its inactive metabolite, 20α-OHP {Hu:2017iz}. The same group also showed negative regulation of HDL cholesteryl ester uptake and HDL-stimulated progesterone production by miR-125a and miR-455, acting via scavenger receptor class B type I (SR-B1), although this work

was primarily carried out in Leydig testicular cells {Hu:2012by}. It is therefore clear that miRNAs—including those mentioned above and likely many more, as yet undiscovered—regulate steroidogenesis within the adrenal cortex, at many different points, although the full impact of these individual regulatory miRNAs acting concurrently across the entirety of the corticosteroid pathway is yet to be assessed.

Studies have also expanded to examine non-steroidogenic genes with regulatory influence. Decreased expression of TWIK-related acid-sensitive K^+ (TASK-2) channels is associated with increased *CYP11B2* and StAR expression and with raised aldosterone levels; miR-34 and miR-23 reduce TASK-2 expression by direct binding of its mRNA 3'UTR [34]. Regulation of RAS genes by the miRNA has also been shown, with miR-181a and miR-663 both binding the 3'-UTR of the renin transcript [35]. The same study showed both of these miRNAs to be downregulated in the renal cortex of hypertensive subjects relative to normotensives, providing a possible mechanistic factor. Separately, miR-483 has been shown to repress four RAS components, including angiotensinogen [18]. Furthermore, angiotensin II (AngII) causes downregulation of miR-483 itself in vascular smooth muscle cells, implying that RAS activation could derepress itself through reduction of miR-483.

In addition to understanding which miRNAs target which elements of corticosteroid production and regulation, if miRNA-mediated control is to be fully understood, then we must improve our understanding of how production of the individual miRNAs is itself regulated. It is intuitively obvious that levels of these miRNAs should fluctuate in response to physiological demands and there are plentiful instances of this from various studies. Of the miRNAs already mentioned here, it is known that miR-21 expression is increased in H295R cells following angiotensin II stimulation [27], that miR-10b levels increase in response to hypoxia in H295R cells [31] and that miR-212 and miR-132 are more abundant in adrenal cells in vitro and the adrenal gland in vivo in response, respectively, to cAMP and ACTH stimulation [33].

6. Adrenal microRNAs

Numerous studies have profiled circulating or adrenal tissue miRNA expression in patients with adrenal carcinoma and/or aldosterone-producing adenoma (APA), confirming that miRNA expression is altered by these conditions relative to healthy controls [28, 29, 34, 36–51].

Notably, of the 11 miRNAs that have been shown to regulate corticosteroid biosynthesis in adrenal tissue (above), 8 are dysregulated in patients with benign adenoma or adrenal carcinoma: miRNAs -10b, −24 and -125a are downregulated in APA tissue [28, 29]; miR-125b is downregulated in carcinoma vs. benign adrenal tumour tissue [43]; miR-21, miR-320a-3p and miR-34 are significantly increased in adenoma tissue [28, 29, 41] and miR-21 is further increased in carcinoma tissue [41]; serum levels of miR-34a are raised in patients with adrenocortical carcinoma relative to patients with benign adrenocortical neoplasm [42]. TASK-2 expression is reduced in APA tissue relative to healthy adrenal tissue and negatively correlates with miR-23 and miR-34 levels [34]. Given that APA increases aldosterone secretion, it is perhaps unsurprising that miRNAs known to modulate corticosteroid biosynthesis, such as miR-24, show altered expression. Interestingly, one of the two genomic locations from which miR-24 is transcribed is a cluster on chromosome 9, where miR-24 is produced alongside miR-23b and miR-27b; our studies show all three to be downregulated in APA, which is consistent with this clustering and implies that many and diverse biological effects could result from the change in regulation to all three microRNAs [28]. Overall, existing studies of miRNA changes

in adrenal tumours add weight to the hypothesis that miRNA targeting of transcription is a common feature of such conditions and is likely to be of relevance to adrenal pathology generally.

Expression of several other miRNAs has also been found to be altered in multiple independent studies of adrenal disease. Most notably miR-483 is increased in tumour [36, 38, 41, 43, 47] and circulating (i.e. plasma or extracellular vesicle) [38, 42, 44, 48] samples from patients with adrenocortical carcinoma when compared to samples from patients with adrenocortical adenomas or healthy controls. miR-210 [37, 41, 48, 49] and miR-184 [48, 49] levels are also increased in tumour and plasma samples from patients with adrenocortical carcinoma in comparison to patients with adrenocortical adenoma or healthy controls and in some cases are increased in adrenocortical adenoma vs. healthy controls [41]. Other miRNAs are downregulated in tumour and serum samples of patients with adrenocortical carcinoma or adrenocortical adenoma relative to patients with adrenocortical adenoma or to healthy controls, respectively: these include miR-195 [38, 41, 43, 47] and miR-335 [38, 47]. However, little work has been done to assess the biological impact of these miRNAs on the regulation of corticosteroid biosynthesis in the context of these diseases, and this remains an obvious priority area of future study. Clearly defined miRNA profiles that are specific to certain tumour types clearly have the potential to facilitate and expedite the differential diagnosis of adrenocortical tumours and enhance our understanding of disease pathogenesis (reviewed by Singh et al.) [52].

7. MicroRNA and the mineralocorticoid receptor

As the nuclear receptor to which aldosterone binds in all aldosterone-responsive tissues, the mineralocorticoid receptor is clearly a key factor in mediating the hormone's effects and is itself subject to miRNA regulation. In silico analysis predicts the *NR3C2* gene, which encodes the MR and contains between 23 and 411 distinct miRNA-binding sites in its 3'UTR, depending on the prediction algorithm utilised. Of these predicted miRNAs, five were selected for further experimental validation; two—miRNA-124 and miRNA-135a—were subsequently confirmed to bind the *NR3C2* 3'UTR, as evidenced by a reduction in luciferase activity following *NR3C2* 3′UTR luciferase reporter and miRNA expression vector cotransfection. Neither miRNA decreased *NR3C2* mRNA level, suggesting miRNA-124 and miRNA-135a are involved solely in translational inhibition of MR [53]. Interestingly, miRNA-124 has also been identified as a regulator of the glucocorticoid receptor (GR), associated with cortisol action [54]. Given its brain-specific expression [55], miRNA-124 cannot directly influence aldosterone biosynthesis but may have a role in the regulation of its production and action through centrally mediated systems. It is currently the subject of much investigation in light of its apparently beneficial effects post-stroke [56–59]. A role in neuronal differentiation has been proposed, and it is possible that the beneficial effects of miRNA-124 are achieved through downregulation of MR expression or activation, mirroring the beneficial effects of MR antagonists.

In addition to demonstrating that MR is regulated by miRNA, other studies identify MR as a mediator of miRNA expression. For example, aldosterone treatment of aortal or vascular smooth muscle cells (SMCs) causes downregulation of miRNA-29b, but this effect can be prevented through MR blockade with the antagonist eplerenone [60]. Interestingly, this MR-regulated change does not occur in mouse endothelial cells, which demonstrates the cell specificity of this MR effect in the vasculature. As with miRNA-124, the benefits of miRNA-29b delivery to the brain post-stroke is currently being investigated [61, 62], although other reports suggest it may actually promote neuronal cell death [63].

Another key factor in MR action is the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). As cortisol is capable of binding MR and circulates at levels far higher than aldosterone, 11β-HSD2 effectively confers aldosterone selectivity on tissues where it is expressed (such as the renal tubule), by converting cortisol to inactive cortisone; this leaves aldosterone free to bind MR without significant competition. 11β-HSD2 activity is therefore important, and its loss can result in salt-sensitive hypertension. Although direct inhibition of 11β-HSD2 expression by miRNAs targeting the 3'UTR of its mRNA has been demonstrated in rats, the existence and importance of such regulation in human aldosterone-selective tissues is yet to be confirmed [64].

Despite the various inconsistencies and gaps in our current knowledge, such ongoing studies of miRNA targeting, action and expression are likely to provide valuable insights into aldosterone action in the future.

8. MicroRNAs as biomarkers and therapeutic targets in endocrine pathologies

Excessive aldosterone production and the consequent activation of MR are now generally accepted to be important and common factors in the pathogenesis of hypertension and a number of related comorbidities. Given that specific changes in miRNA expression and regulation are associated with certain disease states and that miRNAs can be released into extracellular fluids, the potential exists to use circulating microRNAs as biomarkers for conditions that are otherwise difficult to diagnose. This includes various endocrine pathologies, including PA, where the difficulty of accurately identifying and distinguishing aldosterone-producing adenoma and bilateral adrenal hyperplasia (BAH) is acknowledged to have restricted diagnosis and effective treatment. Given that miRNAs are known to regulate corticosteroid biosynthesis and that adrenal miRNA expression is altered in cases of adrenal pathology, it is reasonable to hypothesise that changes in the array of circulating miRNAs might result from diseases affecting corticosteroid regulation or other forms of adrenal function. A current ongoing initiative in this regard—which arose in part from the COST ADMIRE network—is the ENS@T-HT study. This is an EU-funded Horizon 2020 research and innovation project designed to define specific 'omics' for various forms of endocrine hypertension, including PA, Cushing's syndrome and phaeochromocytoma. Our particular focus as part of this project has been the profiling of circulating miRNAs in patient plasma, with the aim of identifying signature miRNAs of diagnostic value. Initial miRNA profiling has now been completed in archived samples, and analysis is under way to develop a signature for testing in a new study population. This study is part of a wave of current diagnostic initiatives aiming to improve diagnosis and better target patient treatment through a stratified medicine approach. MicroRNA is likely to be a focus of many such projects which share the implicit assumption that if miRNA profile is altered by disease, then manipulation of miRNA might also form part of an effective treatment. The longer-term aspiration of such studies— including ENS@T-HT—is therefore the progression from diagnostic applications to therapeutics.

The therapeutic potential of miRNAs is derived from the ability to inhibit miRNA function with antimiRs. These are small oligonucleotides that can be delivered subcutaneously or intravenously and inhibit the interaction of miRNAs with their targets by binding the miRNA seed site with high affinity [65]. Pharmacokinetic and pharmacodynamic studies of antimiR action suggest they are taken up from the circulation by endocytosis and accumulate within endosomes or multivesicular bodies, but much remains unknown about the precise mechanisms

of action and cellular handling of antimiRs. In contrast to classical drugs, the action of antimiRs appears to be delayed, often taking several days to exert an effect. This reflects the time required to rebalance the proteome of a target cell as a consequence of the relatively modest changes in numerous miRNA targets. Conversely, the actions of antimiRs are long-lived, owing to their high stability and accumulation within intracellular depots from which they are slowly released. They show efficacy at doses acceptable for therapeutic development, and further chemical modifications may enhance their uptake, stability and/or action.

An additional challenge with respect to the development of miRNA-based drugs is the inability to correlate target engagement with mechanism and therapeutic efficacy. Because of their many targets and the summation of relatively small repressive effects that contribute to the therapeutic actions of miRNAs, it is difficult or impossible to directly ascribe the activity of an antimiR to a specific target. An individual miRNA may have a beneficial activity in one tissue and an adverse activity in another. Therefore local delivery systems are likely to be useful in reducing off-target effects. While the sustained activity of antimiRs allows for effective treatment, the long-term consequences of antimiR accumulation in different tissues and the inability to rapidly reverse their activity or eliminate the presence of a toxic antimiR raise obvious concerns. AntimiRs accumulate predominantly in the liver and kidney, necessitating substantially higher doses to achieve efficacy in other tissues. This poses challenges with respect to achieving sufficient intracellular concentrations that evoke a therapeutic effect without causing liver and renal toxicity.

Of course, miRNAs may also play beneficial rather than pathogenic roles so strategies for elevating their levels are also required, including the administration of miRNA mimics. These are double-stranded synthetic oligonucleotides that are processed into single-stranded miRNAs when introduced into cells. However, the delivery of miRNA mimics still requires significant optimisation [66]. Lipid formulations for enhancing uptake may help in this regard, while adenoviral delivery methods may assist targeting to the tissue of choice. As with antimiRs, though, it is crucial to avoid repression of nontarget mRNAs or toxic accumulation of mimics.

Finally, a further factor needs to be considered regarding miRNA and its role in the personalisation or stratification of diagnosis and therapy: genetic polymorphisms. Although single-nucleotide polymorphisms that occur in protein-encoding or upstream regulator regions of genes are commonly accepted to contribute—sometimes dramatically—to disease phenotype, it is increasingly recognised that polymorphisms in miRNA genes themselves or in those transcribed but untranslated regions of the genes that they target might contribute to interindividual phenotypic variability and possibly predispose to disease [67]. This may become a major factor in the future 'personalisation' of medicine and effective targeting of therapeutic agents.

9. Conclusion

MiRNAs are providing us with fresh insights into aldosterone regulation, action and pathology while offering the prospect of new diagnostic and therapeutic approaches. It is apparent that miRNAs are important regulators of adrenal function and have the ability to regulate the expression of multiple enzymes within the corticosteroidogenic pathway, modifying the steroid profile as a result. Consistent changes in miRNA expression in APA or adrenocortical carcinoma tissue relative to healthy controls imply a role in the pathogenesis of these diseases and/or their resulting dysregulation. While the effect of each individual miRNA may be small, as numerous miRNAs can target the steroidogenic pathway in the adrenal cortex

and are altered in disease, the sum of multiple small individual effects could result in significant changes to corticosteroid synthesis within the adrenal cortex. The specificity with which miRNAs target their effect is potentially mirrored by the specificity with which dysregulated miRNAs might themselves be therapeutically targeted. The ability to do so raises the tantalising possibility of a new generation of therapeutic 'magic bullets'. However, much remains to be learned about the precise mechanisms by which an individual miRNA affects different physiological pathways within single and different tissues and cell types. This may add significantly to the complexity and consequences of manipulating miRNA for therapeutic ends. A deeper understanding as well as a 'systems biology' approach is required to fully explain miRNA activity under conditions of homeostasis and disease. Despite these challenges and uncertainties, it seems likely that some of the numerous miRNAs currently implicated in cardiovascular disease will eventually emerge as viable biomarkers and possibly drug targets, although the timescale and the reach of such miRNA-based approaches cannot yet be predicted.

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