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MicroRNA-155 mediates endogenous Angiotensin II Type 1 receptor regulation: implications for innovative Type 2 Diabetes Mellitus management

Papadopoulos *et al*. miR-155 and AT1 In T2DM

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Abstract

Type 2 diabetes mellitus (T2DM) is a lifelong condition and a threat to human health. Thorough understanding of its pathogenesis is acutely needed in order to devise innovative, preventative, and potentially curative pharmacological interventions. MicroRNAs (miRNA), are small, non-coding, one-stranded RNA molecules, that can target and silence around 60% of all human genes through translational repression. MiR-155 is an ancient, evolutionarily well-conserved miRNA, with distinct expression profiles and multifunctionality, and a target repertoire of over 241 genes involved in numerous physiological and pathological processes including hematopoietic lineage differentiation, immunity, inflammation, viral infections, cancer, cardiovascular conditions, and particularly diabetes mellitus (DM). MiR-155 levels are progressively reduced in aging, obesity, sarcopenia, and T2DM. Thus, the loss of coordinated repression of multiple miR-155 targets acting as negative regulators, such as *C/EBPβ*, *HDAC4*, and *SOCS1* impacts insulin signaling, deteriorating glucose homeostasis, and causing insulin resistance (IR). Moreover, deranged regulation of the Renin-Angiotensin

Aldosterone System (RAAS) through loss of Angiotensin II Type 1 receptor (AT1R) downregulation, and negated repression of *ETS-1*, results in unopposed detrimental Angiotensin (Ang) II effects, further promoting IR. Finally, loss of *BACH1* and *SOCS1* repression abolishes cytoprotective, anti-oxidant, anti-apoptotic, and anti-inflammatory cellular pathways, and promotes β-cell loss. In contrast to RAAS inhibitor treatments that further decrease already reduced miR-155 levels, strategies to increase an ailing miR-155 production in T2DM, e.g., the use of metformin, mineralocorticoid receptor blockers (spironolactone, eplerenone, finerenone), and verapamil, alone or in various combinations, represent current treatment options. In the future, direct tissue delivery of miRNA analogs is likely.

Core tip: MicroRNAs (miRNA) are small, non-coding, one-stranded RNA molecules that can target and silence over 60% of human genes thereby effectively regulating huge genetic networks. MiRNAs are abundantly found in every human cell and their production is tightly controlled. They play critical roles in regulating almost every cellular pathway, numerous human diseases, and have been linked to the development of diabetes mellitus (DM) and the regulation of blood pressure. In this minireview, we comment on crucial miR-155 effects in type 2 DM. Deeper mechanistical understanding of this miRNA's permeating action may lead to innovative therapeutic approaches in T2DM.

INTRODUCTION

Diabetes mellitus (DM), until recently considered a lifelong and irreversible condition, is a devastating burden to over half a billion people worldwide[1]. The overwhelming majority of diabetic patients-over 90%-suffer from **Type 2 DM (T2DM)** caused by an intricate interaction between lifestyle and genetics that through insulin resistance (IR) lead to metabolic syndrome, pre-diabetes, failure of insulin-secreting pancreatic β-cells and ultimately overt disease[2, 3]. Uncontrolled T2DM eventually progresses to a myriad of severe health complications (among which cardiovascular disease (CVD), chronic renal failure (CRF), and hypertension (HT)), and to an early death[1]. The syndemic of coronavirus disease 2019 (COVID-19) and T2DM has affirmed the latter's lethal effect[4]. Ominous future predictions estimate the number of DM-afflicted individuals to be over 800 million by 2045, up from the current 500 million[1]. Increased understanding of the T2DM pathogenesis is, therefore, acutely needed in order to devise innovative, preventative and potentially curative pharmacological interventions[5].

The pathophysiological role of the renin angiotensin aldosterone system (RAAS) and its major effector, Angiotensin II (Ang II) through the **Angiotensin II Type 1 receptor (AT1R),** in the development of IR in T2DM have long been recognized[6]. Furthermore, convincing evidence exists advocating the use of RAAS inhibition, ACE inhibitors (ACEi) or AT1 receptor antagonists/blockers (ARB), in patients with T2DM, not only for proteinuria and hypertension, but also as a means to improve IR and glucose homeostasis[6].

MicroRNAs (miRNAs or miRs) are small (21-25 nucleotides), non-coding RNAs, able to translationally repress and downregulate gene expression[7]. Present abundantly in all human cells, miRNAs are endogenously biosynthesized through a strictly regulated process that will ultimately result in a mature miRNA, with a 2-8 nucleotide long seed sequence in its 5'untranslated region (UTR), that will bind to a target messenger RNA (mRNA). If the miRNA seed sequence binds perfectly to the corresponding 3'UTR of a specific mRNA, the latter will be recruited to be degraded by an RNA silencing complex (RISC). If the binding is incomplete, mRNA translational machinery will be blocked,

thereby inhibiting protein translational efficiency, and repressing (silencing) gene expression[7]. As a specific miRNA can target multiple mRNA molecules, and equally, a single mRNA molecule can bind to multiple miRNAs, the host can modulate response feedback, through regulatory gene networks, in a concerted effort to control diverse aspects of cellular processes[7]. In this minireview, we present additional miRNA-modulated pathways that can modulate AT1R and Ang II effects that are of importance for the pathogenesis of IR, T2DM, and the development of cardiovascular and renal diabetic complications.

MiR-155 is of particular interest as it is intricately involved both in the pathogenesis of DM and the regulation of AT1R and Ang II effects[6, 8-12]. First identified in 1997, miR-155 is a highly conserved and ancient miRNA primarily expressed in the thymus and spleen. It exhibits unique expression profiles and multifunctionality but is minimally detected under normal physiological conditions[13]. With a target repertoire of over 241 genes, miR-155 plays critical roles in various physiological and pathological processes, such as hematopoietic cell line differentiation, inflammation, immunity (especially viral and parasitic infections), cancer, cardiovascular conditions, and notably, diabetes mellitus (DM) (Table 1)[5, 8, 12-20].

Table 1. Direct gene targets of microRNA-155 relevant to Type 2 Diabetes Mellitus (T2DM).

Gene symbol	Full gene name	Action

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AGTR1	Angiotensin II type 1 receptor gene	Repressed translation downregulates gene expression mediating endogenous AT1R antagonism.[9, 10, 21] Human *in-vitro* and *in-vivo* studies
ARG2	Arginase-2	Repressed translation prevents L-arginine depletion, supports dendritic cell maturation, and negates lung pathologies.[22, 23] Human and mouse *in-vitro* and *in-vivo* studies.
BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	Translational repression of *BACH1* leads to potent anti-inflammatory, cytoprotective, antioxidant programs through Heme Oxygenase-1. [12] Review of human *in-vitro* and *in-vivo* studies.
C/EBPβ	CCAAT/enhancer-binding protein β	Repression downregulates *Pyruvate Kinase 4* (*PDK4*) gene expression and negatively regulates Pyruvate kinase complex (PDC) activity, thereby improving glucose utilization. [16] Mouse *in-vitro* and human *in-vivo* studies.
ETS-1	E26 Transformation-specific Sequence-1	Translational repression averts Ang II effects involving gene regulation of vascular remodeling, angiogenesis, and inflammation.

		[9, 10, 24] Review of human *in-vitro* and *in-vivo* studies. Mouse *in-vitro* and *in-vivo* studies.
HDAC4	Histone Deacetylase 4	Its repression increases GLUT4 and enhances glucose uptake in insulin-sensitive tissues, i.e., skeletal muscle. [16] Mouse *in-vitro* and human *in-vivo* studies.
CACNA1C (*Cav1.2*)	L-type calcium channel subunit, LTCC	As a subunit of the L-type calcium channel, this pro-constrictive gene contributes to influx of calcium in vascular smooth muscle cells and reactive oxygen species (ROS) production, thereby mediating the important components of vascular aging: vasoconstriction and vascular oxidative stress.[21] Human *in-vitro* and *in-vivo* studies.
SOCS1	Suppressor of cytokine signaling 1	Repression prevents the degradation of IRS-1 (Insulin Receptor Substrate-1) protein that mediates the effect of insulin in muscle, liver, and adipose tissue. Supports the JAK2/Y343/STAT5 pathway through which the protective effects of EPO

		against ischemic injury are mediated.[16, 25]
		Human *in-vivo* study. Mouse *in-vitro* and *in-vivo* study.

In T2DM, miR-155 levels in plasma, peripheral blood cells, platelets, and urine are significantly and consistently decreased, with surprising congruence between different ethnicities[8]. Ranging from obesity to IR to diabetic complications in T2DM, miR-155 levels are progressively reduced[8, 14, 15, 17]. MiR-155's underlying molecular mechanism in enhancing insulin signaling, improving glucose homeostasis, and alleviating IR in T2DM, occurs partly through the coordinated repression of multiple negative regulators, such as *CCAAT/enhancer-binding protein β* (*C/EBPβ*), *Histone Deacetylase 4* (*HDAC4*), and *Suppressor of cytokine signaling 1 (SOCS1)* (Table 1)[16]. MiR-155-mediated *C/EBPβ* repression downregulates *Pyruvate Kinase 4* (*PDK4*) gene expression and negatively regulates Pyruvate kinase complex (PDC) activity, thereby improving glucose utilization[16]. *HDAC4* repression increases GLUT4 and enhances glucose uptake in insulin-sensitive tissues, i.e., skeletal muscle, while *SOCS1* repression prevents the degradation of IRS-1 (Insulin Receptor Substrate-1) protein that mediates the effect of insulin in muscle, liver, and adipose tissue[16].

Aging, obesity, sarcopenia, chronic RAAS activation, and IR, invariably predate the development of T2DM[26]. Shared miRNA signatures have been reported, highlighting the central role of miR-155 in the common pathogenesis of those conditions[8, 14, 26]. One particularly important observation is the activation of the classical RAAS axis arm that involves Ang II/AT1R signaling in aging skeletal muscle and white adipose tissue (WAT), both fundamentally involved in T2DM pathogenesis[26, 27]. In WAT, a chronically activated RAAS axis increases lipogenesis and reduces lipolysis, while in the aging skeletal musculature RAAS hyperactivity promotes protein degradation, and

sarcopenia, altogether ultimately leading to oxidative stress, inflammation, fat accumulation, muscle atrophy, and IR[26, 27]. In addition, RAAS's protective arm, involving Ang 1-7/AT2R/MasR signaling, is inhibited at the same time, further augmenting an unfavorable AT1R/AT2R imbalance[26, 27]. MiR-155, acting as a master regulator, is the key player in chronic RAAS/Ang II/AT1R activation, thereby, intricately associated with the development of IR[6]. Through its repression of the *AGTR1* (the gene that codes for the AT1R) miR-155 regulates the homeostasis of the AT1R receptor, its membrane presence, and thus the biological activity of Ang II (Table 1)[9, 10, 28-30]. Moreover, its regulation of the *E26 Transformation-specific Sequence-1 (ETS-1)* averts several detrimental vascular Ang II effects involving gene regulation of inflammation, proliferation, remodeling, fibrosis, and angiogenesis (Table 1)[9, 13, 24]. Furthermore, its repressive effects on *Arginase-2 (ARG2)* prevent the depletion of l-arginine, the obligate substrate of endothelial nitric oxide (NO) synthase (eNOS), improving substrate availability and further increasing NO-production and NO-bioavailability that further support NO-dependent cardio- and renoprotection in T2DM (Table 1)[13, 23]. From the sum of these actions, it is thus evident that the reported loss of miR-155 in T2DM has profound effects leading to persistent RAAS hyperactivity through chronic Ang II stimulation of the AT1R, thereby exerting its detrimental, pro-oxidant, pro-fibrotic, proliferative, and pro- inflammatory actions. Additional miR-155 effects through repressive actions on *BTB and CNC homology 1, basic leucine zipper transcription factor 1 (BACH1) and SOCS1*, synergistically enhance cytoprotective, anti-oxidant, anti-apoptotic, and anti-inflammatory cellular pathways and promote a protective cellular milieu, which is subsequently lost following miR-155 downregulation (Table 1)[12, 13]. Genetic variants that perturb miR-155's action (such as in carriers of AT1R + 1166C-allele) or that increase its synthesis (such as in trisomy 21 and the rs767649 polymorphism of miR-155) biochemically and molecularly demonstrate this central significance of miR in a plethora of DM-associated pathological conditions[11, 18, 31-33]. Moreover, clinical data in obese individuals demonstrate that miR-

155 levels correlate with improved insulin sensitivity post-bariatric surgery and are critical in mediating the effects of endurance exercise[34, 35].

While miR-155 is consistently reduced in serum and tissues in T2DM, it is reported to be upregulated in Type 1 (T1) DM, highlighting T1DM's autoimmune pathogenesis and miR's crucial and differential role in autoimmunity and innate and adaptive immunity[8, 36]. However, even if robustly elevated in newly diagnosed T1DM, miR-155 strikingly diminishes within 5 years of diagnosis[32].

AT1R substrate modulation (ACEi) and/or receptor inhibition (ARBs) may improve glucose homeostasis[6]. However, **strategies to increase an ailing miR-155 production in T2DM** could prove to be a more appropriate course of action. Metformin with ACEi/ARB improves HbA1c goals[6]. **Metformin** and the newer Glucagon Like Peptide 1 (**GLP-1**) analogs have been shown to repress *SOCS1* and *3* and increase IRS-1[37]. Metformin mediates miR-155 increases that repress *SOCS1* and reduce NF-κB (nuclear factor κB), thereby disrupting NF-κB-mediated high-fat induced inflammatory effects in T2DM[38, 39]. The clinical effects of GLP-1 analogs on miR-155 in humans are, to date, unknown, and additional research is needed, but miR-155 has been shown to promote GLP-1 production in the murine pancreas[40]. Moreover, in the resistance vessels of aging humans, elevated expression of mineralocorticoid receptor (MR) is accompanied by a decrease in miR-155 Levels and an upregulation of miR-155 targets such as the CACNA1C (Cav1.2) gene (a subunit of the L-type calcium channel (LTCC)), and the AGTR1 gene. These alterations in gene expression play a role in promoting vasoconstriction and oxidative stress in aging mice (Table 1)[21]. MR inhibition reverses and reinstates the significantly low basal serum miR-155 Levels in the aging blood vessels and blocks two interactive steps involving LTCC and *AGTR1* that underlie the pathogenesis of hypertension[13]. A correlation between improved blood pressure

response to therapy with MR antagonists and changes in miR-155 Levels in older individuals has been reported[21]. Moreover, the use of **MR-antagonists** (spironolactone, eplerenone, finerenone) has shown renal and cardiovascular benefits in T2DM[41-43]. LTCC blockade per se, through **verapamil** alone, or in combination with MR antagonists/metformin, will offer additional therapeutic options in T2DM[44]. Besides improved blood pressure regulation and cardio-renal protection, verapamil demonstrates additional benefits while avoiding many of the common adverse effects associated with ACEi/ARB[45]. Verapamil's mode of action is of particular interest in diabetes[46]. Apart from being present in cardiomyocytes, LTCCs are also present in pancreatic β-cells and participate in insulin homeostasis[47]. In the heart and the pancreas, effective pharmacological LTCC blockage can inhibit the expression of pro-apoptotic thioredoxin-interacting protein (TXNIP), a significant contributor to pancreatic β-cell dysfunction and a key gene regulated in response to hyperglycemia, thereby promoting the survival and proper functioning of β-cells and improving glucose homeostasis[46, 48]. Verapamil has, thus, the potential not only to enhance β-cell survival and function, but also improve and even prevent overt diabetes of both types[48, 49]. In a recent study, verapamil combined with metformin, significantly improved glycemic control in T2DM[49]. Finally, a drawback in the use of monotherapy as ACEi/ARBs (in conditions that already are associated with low miR-155 Levels) is that they significantly further decrease already reduced miR-155 Levels[50, 51]. RAAS inhibition could, thus, theoretically deprive T2DM patients of additional miR-155-engendered favorable immunological and cytoprotective effects and potentially explain ACEi's modest and ARBs' non-existent effects in preventing CVD or improving glycemic indices in DM and hypertension[13, 50-53].

CONCLUSION

The data presented above strongly support the role of miR-155 as a major player in the pathogenesis of T2DM and complications, by triggering insulin resistance and β-cell loss as well as through RAAS modulatory effects[5, 8]. Large multicenter trials are required to establish this role of miRNA as a reliable biomarker and potential therapeutic target in DM. Then, as increased mechanistic knowledge regarding miR-155 becomes available, novel miRNA-modulating approaches with miR-155 as a target are likely in T2DM. Even though these therapeutic modalities are still in their infancy and might yet be far from the clinic, research must address this knowledge gap in order to devise how to effectively deliver specific, synthetic miRNA mimics (T2DM, aging, obesity, sarcopenia) or inhibitors-antagomiRs (T1DM, cancer), to a specific tissue, in the diabetic patient, as miR-155 actions are tissue-sensitive[54]. In addition, a better understanding is needed on how several miRNAs work synergistically on the same mRNA targets and how miRNA networks function. As disease-specific miRNA expression pattern is ubiquitous in all related tissues, it can prove challenging in a complex disease like DM to accomplish precise delivery to certain tissues/organs and avoid adverse off-target effects in others[5].

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