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MicroRNA regulation of islet and enteroendocrine peptides: Physiology and therapeutic implications for type 2 diabetes

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ABSTRACT

The pathogenesis of type 2 diabetes (T2D) is associated with dysregulation of glucoregulatory hormones, including both islet and enteroendocrine peptides. Microribonucleic acids (miRNAs) are short noncoding RNA sequences which post transcriptionally inhibit protein synthesis by binding to complementary messenger RNA (mRNA). Essential for normal cell activities, including proliferation and apoptosis, dysregulation of these non-coding RNA molecules have been linked to several diseases, including diabetes, where alterations in miRNA expression within pancreatic islets have been observed. This may occur as a compensatory mechanism to maintain beta-cell mass/function (e.g., downregulation of miR-7), or conversely, lead to further beta-cell demise and disease progression (e.g., upregulation of miR-187). Thus, targeting miRNAs has potential for novel diagnostic and therapeutic applications in T2D. This is reinforced by the success seen to date with miRNA-based therapeutics for other conditions currently in clinical trials. In this review, differential expression of miRNAs in human islets associated with T2D will be discussed along with further consideration of their effects on the production and secretion of islet and incretin hormones. This analysis further unravels the therapeutic potential of miRNAs and offers insights into novel strategies for T2D management.

1. Introduction

Microribonucleic acids (miRNAs) are short RNA sequences ranging from 19 to 22 nucleotides in length that bind to complementary messenger RNA (mRNA) sequences and inhibit protein synthesis [41]. The first miRNA was identified in 1993, and since then, 4571 precursor and mature human miRNA sequences have been identified [56]. Mature miRNAs are hypothesised to control the expression of more than half of all mRNAs [13,52]. Individual mRNA strands can be targeted by multiple miRNAs, and conversely, multiple miRNAs can regulate a single mRNA [13,52].

miRNAs are transcribed by RNA polymerase and processed from long primary miRNAs into short mature sequences, as illustrated in Fig. 1 [71]. Through the canonical pathway, primary miRNAs are enzymatically cleaved by a ribonuclease III enzyme, Drosha, along with the RNA binding protein DiGeorge Syndrome critical region 8 (DGCR8) [71]. This results in the generation of a precursor miRNA, approximately 70 nucleotides in length [18]. Consequently, precursor miRNA exits the nucleus into the cytoplasm via exportin-5 where it is later cleaved by Dicer, another ribonuclease III enzyme, to liberate a small miRNA duplex [71]. After Dicer processing, the passenger strand is discarded whereas the guide strand is incorporated into the RNA induced silencing complex (RISC) through interaction with Argonaute proteins [71]. Subsequent inhibition of protein translation occurs through the interaction of the RISC complex with target mRNA through complementary binding of the seed sequence located at the 5' end of the guide strand to target mRNA sequences [52]. Most miRNAs bind to the 3' untranslated region (UTR) of target mRNAs, however, binding has been described at the 5' UTR and coding regions [52]. Supplementary binding between nucleotides at the 3' end of the guide strand enhances the stability of the complex [71]. The net result of this interaction is either the removal of the 3' poly-A tail with subsequent degradation, enzymatic cleavage, or halting of mRNA translation [52].

Within the pancreas, endocrine cells within the islets of Langerhans are responsible for regulating blood glucose and nutrient metabolism through the production and secretion of islet peptides [54]. Beta-cells are one of the most prevalent cell types within the islets and are responsible for the secretion of insulin, whilst alpha-cells secrete

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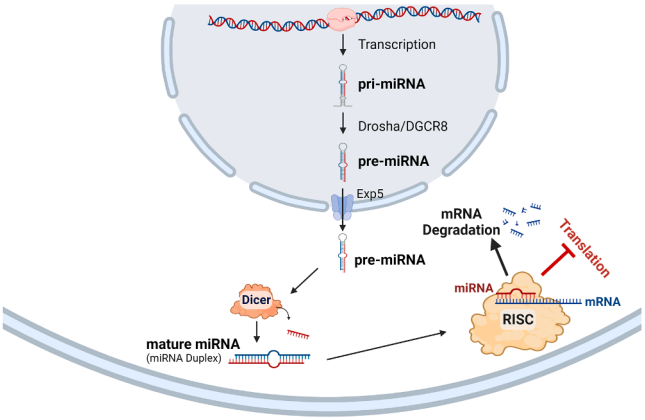

Fig. 1. Canonical pathway of miRNA production and their mechanism of action. The production of miRNA begins with transcription by RNA polymerase to generate pri-miRNA with ensuant generation of pre-miRNA through cleavage by Drosha/DGCR8. Pre-miRNA exits the nucleus through exportin-5 and is cleaved by Dicer to liberate a mature miRNA duplex. One of these strands, known as the passenger strand, is discarded whereas the guide strand is incorporated into the RISC and dictates mRNA targets based on complementarity between the sequences. Abbreviations: pri-miRNA, primary microribonucleic acid, DGCR8, DiGeorge Syndrome critical region 8, pre-miRNA, precursor miRNA, exp5, exportin-5, mRNA, messenger RNA, RISC, RNA induced silencing complex, ⊥, inhibited.

glucagon, delta-cells secrete somatostatin, gamma-cells secrete pancreatic polypeptide (PP), and epsilon-cells secrete ghrelin [10,47]. The former two peptides play a crucial role in the regulation of glucose homeostasis, although importantly other peptides produced by enteroendocrine cells of the gastrointestinal tract are also necessary for glucose control [54]. Central among these peptides are the incretin hormones: glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) [54,57]. Additionally, gut-derived peptides such as ghrelin, oxyntomodulin (Oxm), peptide YY (PYY), cholecystokinin (CCK), xenin, and gastrin may contribute to these regulatory processes [54].

In type 2 diabetes (T2D), changes in pancreatic islet miRNA expression have been reported when compared to nondiabetic (ND) islets [52,98]. This can manifest either as a compensatory mechanism (e.g. to augment beta-cell mass), or lead to subsequent dysfunction of beta-cells, thereby contributing to disease progression (e.g. apoptosis) [52]. As such, miRNAs present diagnostic and therapeutic potential, with diagnostic applications involving monitoring circulating miRNAs, and therapeutic avenues associated with altering miRNA expression [44]. Notably, miRNAs have been shown to regulate expression of both islet and incretin peptides [32,52]. This review will evaluate the evidence for altered miRNA expression in T2D and explore their actions pertaining to production and secretion of islet and enteroendocrine peptides. Subsequently, further analysis will explore the therapeutic potential of miRNAs in the treatment of T2D.

2. Differential expression of miRNAs observed in human islets obtained from donors with T2D

miRNAs play an important role in the maintenance of beta-cell identity through posttranscriptional modification of gene expression [32]. The importance of miRNAs in murine beta-cells was revealed in a study that selectively deleted the ribonuclease III enzyme, Dicer, leading to discernible effects towards beta-cell mass and function [50]. Dicer is required for the generation of mature miRNA sequences and thus ablation of this enzyme within the beta-cell highlighted the role of miRNAs by impeding their activity [58]. Dicer-deficient mice also displayed impaired glucose stimulated insulin secretion (GSIS) and were subsequently diagnosed with diabetes [50], suggesting a potential role of miRNAs underlying the pathophysiology of T2D and demonstrating the need for further research into the function of specific miRNAs in beta-cell physiology.

A comprehensive overview of differentially expressed miRNAs in human T2D islets is presented in Table 1. Studies were found through search strings on Google Scholar and PubMed using the following terms “miRNA AND T2D AND beta-cell AND human islets” and “miRNA expression AND beta-cell AND T2D.” Studies were also sourced from review papers in this field. Only those that investigated the expression of miRNAs in human T2D islets were included in this review (Table 1). These studies either investigated changes in miRNA expression overall or of specific miRNAs which were often investigated in human T2D islets after experiments in animal models [39,45,51,59,60,61,72,73,87,91]. However, it is notable that most of these studies employed small sample

Table 1
Differential expression of miRNAs in human islets from donors with T2D.

Study	Sample Size	Method of Analysis	Significantly Upregulated miRNAs	Significantly Downregulated miRNAs
[51]	n=7 (n=4 T2D, n=3 controls)	Small RNA sequencing	hsa-miR-187–3p hsa-miR-7–3–5p hsa-miR-216a-5p hsa-miR-589–5p hsa-miR-30a-5p	hsa-miR-369–3p hsa-miR-539–3p hsa-miR-487a-3p hsa-miR-495–3p hsa-miR-544a-5p hsa-miR-23c hsa-miR-656–3p hsa-miR-4716–3p hsa-miR-487b-3p hsa-miR-7–1–3p
[61]	n=20 (n=11 T2D, n=9 controls)	TaqMan Arrays	hsa-miR-187 hsa-miR-345*	
[60]	Not provided	qRT-PCR	hsa-miR-223	
[91]	n=12 (n=6 T2D, n=6 controls)	qRT-PCR		hsa-miR-344–5p
[39]	n=22 (n=9 T2D, n=13 controls)	qRT-PCR		hsa-miR-184–3p
[59]	(n=9–10)	qRT-PCR		hsa-miR-7a
[87]	(n=5 T2D, n=6 ND)	qRT-PCR	hsa-miR-124	
[45]	n=68 (n=34 T2D, n=34 ND)	qRT-PCR	hsa-miR-463–3p	
[73]	n=42 (n=20 NG, n=11 T2D, n=11 IGT)	qRT-PCR	miR-130a miR-130b miR-152	
[72]	n=34 (9 T2D, 25 ND)	qRT-PCR	miR-200c	

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction, NG, normoglycemic, IGT, impaired glucose tolerance, ND, non-diabetic.
*Did not replicate with confirmatory individual TaqMan miRNA quantitative polymerase chain reaction (qPCR) analysis of a different cohort of 20 islets (n=10 T2D, n=10 control).

sizes due to an inherent lack of tissue availability, and as such the results should be interpreted with some caution [28].

Interestingly, intriguing discrepancies exist among the various published studies (Table 1). For example, the sole commonality between the reports by Kameswaran et al. [51] and Locke et al. [61] was the upregulation of hsa-miR-187–3p. This incongruity may relate to modest sample size limitations, other confounding variables (e.g., obesity, differences in gut microbiota) or through variations in analytical methods used for miRNA quantification in the respective studies [34,51,61,89]. Animal studies may help overcome sample size limitations associated with human islet studies, but challenges remain in the applicability of derived observations to humans due to genetic variations of miRNAs and their respective targets between species [109,40]. It should also be noted that these studies have investigated miRNA expression levels in islets, not composite beta-cells alone, and therefore the potential impact of cell heterogeneity should be noted [23]. Furthermore, beta-cell specific miRNA expression analysis could provide further insight into beta-cell physiology and subsequent dysfunction seen in T2D. In addition, further studies are required to determine whether the observed changes in miRNA expression are associative or causal for T2D and from this the potential application of miRNA-based treatment for T2D could be evaluated.

3. miRNA regulation of islet peptides and association with T2D

miRNAs have recently emerged as potential key regulators of islet peptide production and secretion [26,31,40,76]. Importantly, these non-coding RNA molecules have been demonstrated to act through modulation of mRNA translation for islet peptides and other key cellular components involved in peptide secretion (e.g., homeodomain interacting protein kinase 3 (HIPK3), alpha-synuclein (SNCA)) [31,76]. Changes in miRNA expression observed in T2D have been shown to impact islet peptides in both animal and human studies [19,21,32,40,58,76]. Perhaps not surprisingly, to date, research into miRNAs and their association with islet peptides has predominantly focused on insulin [32], whilst the effect of miRNAs on the production and secretion of glucagon for example remains relatively poorly understood [32]. Further, the impact of miRNAs relating to the secretion of somatostatin, PP, or ghrelin by delta-, gamma-, and epsilon-cells, respectively, remains elusive. Remarkably, miRNAs were shown to be instrumental in cellular differentiation of islets cells upon deletion of Dicer within the pancreas of mice [63]. Consequently, Dicer ablation caused a significant decrease in islet cell mass and affected all cell types other than epsilon-cells [63]. Studies investigating the impact of miRNAs on islet peptide production and secretion were searched on Google Scholar and PubMed using the terms “miRNA AND islet peptide,” “miRNA AND islet hormones,” “miRNA AND insulin action” “miRNA AND glucagon production and secretion” and “miRNA AND glucagon.” Studies were also retrieved from other review papers in this field. Only studies that focused on T2D were included in this review.

3.1. miRNA regulation of insulin production and secretion

The importance of miRNAs in the regulation of insulin transcription and secretion was evidenced by the deletion of Dicer in murine beta-cells, with a consequential decrease in GSIS and a decrease in insulin expression at both mRNA and protein levels [50,66]. Moreover, elevated levels of miRNA involved in the regulation of insulin secretion were identified in the rodent Goto–Kakizaki (GK) model of T2D, compared to controls [33]. Thus, alluding to the significance of miRNAs in beta-cell function and insulin secretion [52]. The role of specific miRNAs on insulin production in beta-cells has undergone extensive investigation, revealing a wide range of miRNAs that have been shown to regulate both the synthesis and secretion of this key regulatory hormone (Fig. 2). Certain miRNAs, such as miR-30d, have demonstrated a stimulatory role in insulin synthesis and/or secretion [108,52,92]. Conversely, other

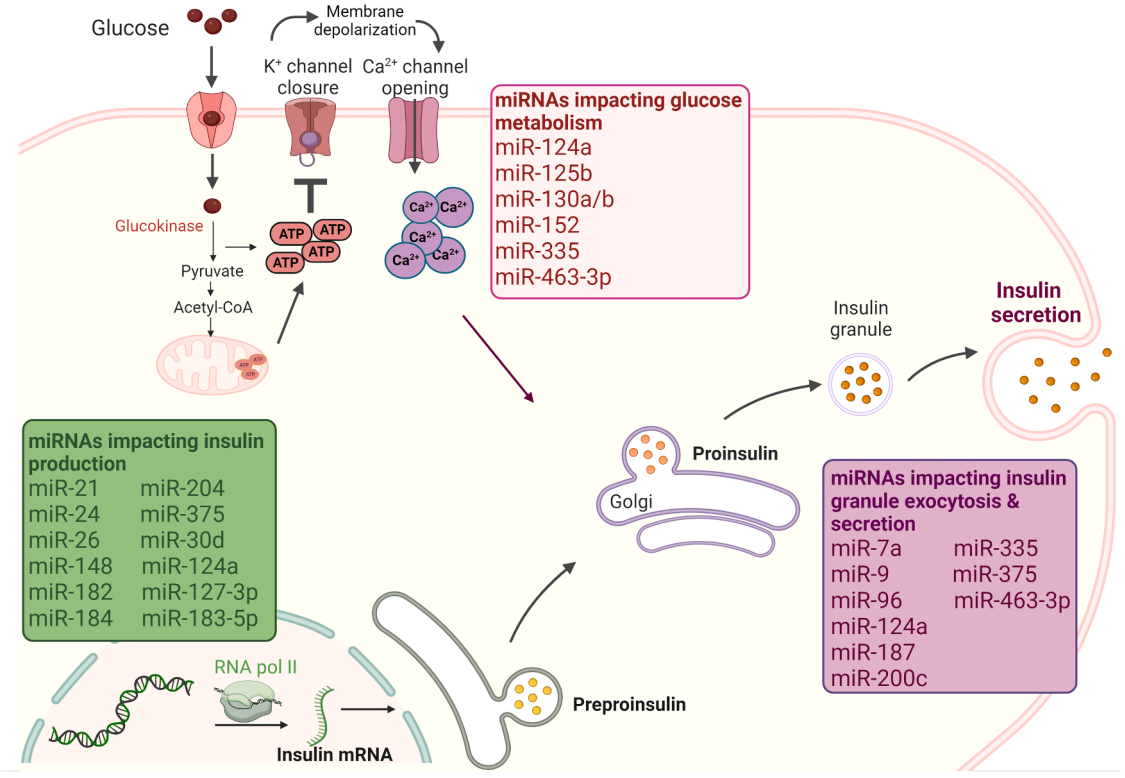

Fig. 2. miRNAs evidenced to regulate beta-cell glucose metabolism and the production and secretion of insulin in animal models, cell lines, or human islets. Abbreviations: ATP, adenosine triphosphate, acetyl CoA, acetyl coenzyme A, RNA pol II, ribonucleic acid polymerase II, mRNA, messenger RNA, ADP, adenosine diphosphate. Further consideration of microRNAs involved n beta cell function is included in other reviews [32,52,58,76].

Table 2
miRNAs involved in the control of insulin production and secretion with targets and results from experimental models and human islets.

Study	miRNA	Target(s)	Results from *In Vitro* Model(s)	Results from *In Vivo* Models	Results from Human Studies
[61]	miR-187	*Hipk3*	**Rat islets:** OE = ↓ GSIS **INS1 cells:** OE = ↓ GSIS		Upregulated in T2D Normoglycemic islets: negative correlation with GSIS
[59]	miR-7a	*Snca*	**Mouse islets:** OE = ↓ exocytosis, KD = ↑ GSIS or KSIS.	**Beta-cell miR-7a KO mouse model** = ↑ GSIS.	Downregulated in T2D KD = ↑ glucose or KCl induced exocytosis of insulin granules
[87]	miR-124a	*Foxa2* *Mtpn*	**MIN6 (pseudo-islets):** OE = ↓ GSIS		Upregulated in T2D
[45]	miR-463–3p	*Abcg4*	**MIN6 cells:** OE = ↓ GSIS, KD = ↑ GSIS		Upregulated in T2D Negative correlation with GSIS
[73]	miR-130a miR-130b miR-152	*Pdha1*	**INS1 832/13 cells:** OE of miR-130a/miR-152 = ↓ GSIS and ↓ insulin quantity. OE of all 3 individually = ↑ proinsulin:insulin and ↓ ATP (+↑ glucose). KD miR-130a/b = ↑ GSIS and KD of all 3 individually = ↑ insulin content.	**GK rat islets**: all upregulated	Upregulated in T2D and IGT
[85]	miR-335	*Snap25* *Stxbp1* *Syt11*	**INS1 832/13 cells:** OE = ↓ GSIS and ↓ exocytosis (↓ protein expression SNAP25, SYT11 and STXBP1), KD = ↑ exocytosis, ↓ insulin quantity **EndoC-BetaH1 cells:** OE = ↓ GSIS		Negative correlation with GSIS in islets of those with IGT
[72]	miR-200c	*ETV5*	**EndoC-betaH1 cells:** OE = ↓GSIS		Upregulated in T2D KD = ↑ GSIS
[111]	miR-24	*Neurod1* *Hfn1-alpha*	**MIN6 cells:** ↑ induced by palmitate, ↑ miR-24 = ↓ GSIS and ↓ proliferation, KD miRNA targets = ↓ GSIS, KSIS and ↓ proliferation. **HFD mouse islets:** KD = ↑ GSIS		↑ induced by palmitate
[46]	miR-21	*Tgfb2* *Smad2*	**INS1 cells:** ↑ miR-21 = ↓ GSIS, ↑ proinsulin: insulin and ↓ secretory granule staining **Tg(beta miR-21) mouse islets beta-cell:** ↑miR-21 = ↓ beta-cell identity and glucose intolerance.		↑ miR-21 = ↓ *SMAD2, PDX1, TGFB2* and ↑ *ALDH1A3* = dedifferentiated *
[88]	miR-212 miR-132		**INS1–832/3 cells:** GLP-1 = upregulates both. OE miR-132 = ↑ GSIS +/- GLP-1. **INS1–832/13 cells:** OE of either = significant improvement of GSIS in response to GLP-1 and ↑ glucose = similar response as INS1 832/3 cells.	**GLP-1 treated Sprague-Dawley rat islets:** Both upregulated **Ex-4 treated C57BL/6 N mice:** Both upregulated in islets (dose dependent)	GLP-1 = ↑ miR-212 and miR-132
[17]	miR-125b	*M6pr* *Mtfp1*	**MIN6 cells:** KD = ↑ GSIS and insulin quantity, ↑ miR-125b = ↓ GSIS, mitochondrial elongation and ↑ insulin quantity. **EndoC-Beta-H1 cells:** KO = ↑GSIS, shortened mitochondria	**MIR125B-Tg (↑ miR-125b) mouse:** hyperglycaemic, glucose intolerant, ↓ GSIS and KSIS, ↓ insulin and granules near membrane.	↑ glucose = ↑ miR-125b through inhibition of AMPK Positive correlation with BMI
[103]	miR-204	*Mafa*	**INS1 cells:** OE = ↓ insulin mRNA, quantity, and secretion		↑ miR-204 = ↓ insulin mRNA and insulin expression
[49]	miR-204	GLP-1R	**INS1 cells:** OE = ↓ GLP-1R mRNA and protein, KD = ↑ GLP-1R **Mouse islets:** OE miR-204 = ↓ GLP-1R	**miR-204 KO mice:** ↑ GLP-1R, significant ↓ blood glucose GTT + Ex-4, ↑ GSIS + Ex-4, ↑ cAMP + Ex-4. Protected against STZ induced diabetes. **Beta-cell specific TXNIP KO mice:** ↓ miR-204 = ↑ GLP-1R, ↓ blood glucose GTT + Ex-4	OE miR-204 = ↓ GLP-1R
[64]	miR-204		**EndoC cells:** ↓ or ↑ of miR-204 = no significant impact on C-peptide secretion, *INS, MAFA, TXNIP* and *TRPM3* mRNA expression		↑ or ↓ miR-204 = no significant impact on *MAFA, INS, TXNIP, TRMP3* mRNA expression
[22]	miR-375		**Rat islets:** OE = ↓ GSIS, ↓O_2 consumption and ↓glucose induced intracellular Ca^{2+} ↓ miR-375 = ↑ GSIS and O_2 consumption		OE = ↓ GSIS
[102]	miR-183–5p miR-375–3p miR-216b-5p miR-183–3p miR-7–5p miR-217–5p miR-7–2–3p miR-429–3p		**PANC1 cells:** OE no effect on (pro-)insulin mRNA, ↓ miR-183–5p, miR-375–3p, miR-7–2–3p, miR-7–5p, miR-183–3p, or miR-217–5p = ↓ (pro-)insulin mRNA		Top 8 (pro-)insulin miRNAs of 241 ↑ in insulin positive vs negative tissues = predict presence/absence of (pro-)insulin mRNA, with high specificity, and sensitivity. KD = ↓ (pro-)insulin mRNA levels **
[65]	miR-7	Beta-arrestin 1	**INS1 cells:** ↑ induced by GLP-1, OE = ↓ GLP-1 stimulated GSIS, cAMP production and ERK and		

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Table 2 (*continued*)

Study	miRNA	Target(s)	Results from *In Vitro* Model(s)	Results from *In Vivo* Models	Results from Human Studies
			CREB activation, KD = ↑ GLP-1 stimulated GSIS @ ↑ glucose and ↑ GLP-1 stimulated cAMP production		
[66]	miR-26 miR-148 miR-182 miR-24 miR-200/141 miR-7b		**MIN6 cells:** KD miR-148, −200/141, −26 and −182 = positive regulator of insulin transcription, KD miR-7 = ↑ insulin expression. **Mouse islets:** KD miR-26, −182, −148 and −24 = ↓ insulin mRNA		
[7]	miR-375 miR-122 miR-184 miR-127–3p				Positive correlation with insulin mRNA expression. Negative correlation between miR-184 and miR-127–3p and insulin secretion. No correlation = islets of glucose intolerant donors.
[83]	miR-375		**MIN6 cells:** OE =↓ GSIS, KSIS and tolbutamide stimulated insulin secretion, ↓ exocytosis. KD = ↑ GSIS		
[24]	miR-375	*Pdk1*	**INS-1E cells:** OE = ↓ glucose induced DNA synthesis, ↓ mass, proliferation, and viability. KD = ↑ *Pdk1* and insulin mRNA expression +/- glucose. ↑ glucose = ↓ pre-miR-375	**GK rat islets:** ↓ miR-375	
[82]	miR-375	*Mtpn*		**miR-375 KO mice:** hyperglycaemia, ↓ beta-cell mass and ↑ alpha-cell mass, ↑ glucagon secretion, ↓ GSIS, ↓ exocytosis in beta-cells and not alpha-cells, **miR-375 and leptin deficient mice:** ↓ beta-cell mass with failure to respond to IR, premature death, no effect on alpha-cells, ↓ blood insulin, 40% ↓ weight	
[69]	miR-375		**Dedifferentiated BCD cells:** OE = expression of beta-cell derived genes and ↑ insulin mRNA		
[92]	miR-30d		**MIN6 cells:** ↑ glucose = ↑ miR-30d, OE = ↑ glucose regulated insulin transcription, KD = ↓ insulin mRNA, OE or KD = no effect on insulin secretion **Mouse islets:** ↑ glucose = ↑ miR-30d		
[108]	miR-30d	*Map4k4*	**MIN6 cells:** OE = ↑ luciferase activity induced by rat insulin reporter, ↑ MafA expression. In the presence of TNF-alpha: OE = ↑ insulin protein expression, slight ↑ insulin secretion and retention of MafA activity and ↓ MAP4K4 protein. KD = further decrease in insulin protein and MafA activity in the presence of TNF-alpha. **Mouse islets:** OE = ↑ insulin and MafA expression	**db/db mice:** downregulated	
[16]	miR-152	*Pi3k-alpha*	**INS1 cells:** OE = ↑ GSIS and proliferation, KD = ↓ GSIS and proliferation **MIN6 cells:** OE = ↑ GSIS and proliferation, KD = ↓ GSIS and proliferation **Mouse islets:** ↑ glucose = ↑ miR-152 expression, OE = ↑ GSIS, KD = ↓ GSIS		**Human plasma glucose:** downregulated in T2D, negative correlation between T2D and blood glucose.
[80]	miR-9	*Oc2*	**INS1E cells:** OE = ↓ GSIS, KSIS, ↑ Granuphilin protein and mRNA levels		
[5]	miR-124a	*Foxa2*	**MIN6 cells:** OE = ↓ FOXA2 protein PDX1 mRNA and protein, ↓ *Ins* and *Kir6.2* and *Sur1*, ↑ intracellular Ca^{2+} with defective ↑ in response to glucose, no effect = GSIS, KD = ↑ FOXA2 mRNA and protein, ↑ PDX1 mRNA and protein, ↑ *Ins* and *Kir6.2* and *Sur1*, no effect = GSIS, **INS1 832/13 cells:** ↑ intracellular Ca^{2+} with defective ↑ in response to glucose		
[62]	miR-124a miR-96	*Snap25* *Rab3A* Synapsin-1A *Rab27A* *Noc2* Granuphilin	**MIN6B1 cells:** OE miR-124a = ↑ hormone secretion @ ↓ glucose, ↓ hormone secretion in response to secretagogues, ↑ SNAP25, Rab3A and synapsin-1A (↑ @ mRNA level also), ↓ Rab27A and Noc2 (Rab27A = validated target). OE miR-96 = ↓ hormone secretion in response to secretagogues, ↑ granuphilin (↑ @ mRNA level), ↓ Noc2		
[67]	miR-483	*Socs3*	**MIN6 cells:** OE = ↑ insulin secretion and transcription (latter @ ↑ glucose), ↓ SOCS3. KD = no effect = insulin secretion and ↓ insulin transcription (@ ↑ glucose), ↑ SOCS3, ↑ cytokine	**db/db mice:** ↑ miR-483, ↓ *Socs3*	

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Table 2 (*continued*)

Study	miRNA	Target(s)	Results from *In Vitro* Model(s)	Results from *In Vivo* Models	Results from Human Studies
			induced programmed cell death. **Mouse islets:** OE = ↑ insulin secretion and transcription, ↓ SOCS3.		

Abbreviations: KD, knockdown, KO, knockout, ATP, adenosine triphosphate, GK, Goto–Kakizaki, HFD, high-fat diet fed, +/-, presence or absence, ↑, increase, ↓, decrease, OE, overexpression, Ex-4, exendin-4, STZ, streptozotocin, BMI, body mass index, DNA, deoxyribonucleic acid, AMPK, adenosine monophosphate activated protein kinase, IGT, impaired glucose tolerance, cAMP, cyclic adenosine monophosphate, ERK, extracellular signal regulated kinase, CREB, cAMP-response element binding protein, IR, insulin resistance, KSIS, potassium stimulated insulin secretion, Hipk3, homeodomain interacting protein kinase 3, Foxa2, forkhead box A2, Abcg4, ATP binding cassette subfamily G member 4, Pdha1, pyruvate dehydrogenase E1 subunit alpha 1, Snap25, synaptosomal associated protein of 25 kDa, Stxbp1, syntaxin binding protein 1, Syt11, synaptotagmin 11, ETV5, ETS variant transcription factor 5, Neurod1, neuronal differentiation 1, Hfn1-alpha, hepatocyte nuclear factor-1 alpha, TGFB2, transforming growth factor beta 2, Smad2, suppressor of mothers against decapentaplegic family member 2, M6pr, mannose-6-phosphate receptor cation dependent, Mtfp1, mitochondrial fission process 1, GLP-1R, GLP-1 receptor, Pdk1, 3′-phosphoinositide–dependent protein kinase-1, Pi3k-alpha, phosphatidylinositol 3-kinase catalytic subunit alpha, Oc2, onecut2, Pdx1, pancreatic and duodenal homeobox 1, Ins, insulin, Aldh1A3, aldehyde dehydrogenase 1 family member A3, Trpm3, transient receptor potential cation channel subfamily M member 3, Sur1, sulfonylurea receptor 1, Socs3, suppressor of cytokine signalling 3.
*One donor = no change in the expression of *SMAD2, PDX1, TGFB2* and *ALDH1A3* after successful transduction with miR-21 lentivirus – data was included in the analysis [46].
**KD of miR-429–3p and miR-216b-5p had no effect on (pro-)insulin mRNA
Further consideration of microRNAs involved in beta cell function is included in other reviews [32,52,58,76].

miRNAs, including miR-187, miR-130a, and miR-152, are upregulated in T2D islets and may contribute to beta-cell dysfunction through their inhibitory effects on insulin [51,52,61,73]. Table 2 provides a summary of miRNAs associated with the regulation of insulin synthesis and secretion within beta-cells, and encompasses findings gathered using both experimental models and human islets

3.1.1. *miRNA regulation of insulin production*

The first key phase of insulin synthesis is transcription of the insulin gene into mRNA, a process known to be regulated by glucose, and more recently, by miRNAs controlling the expression of transcriptional inhibitors and activators of the insulin gene [101,76,81]. Several specific miRNAs have been implicated in controlling insulin synthesis with either stimulatory or inhibitory effects, as summarised in Table 2.

miR-375 is highly expressed in beta-cells and has been shown to regulate insulin synthesis, however, conflicting results have emerged regarding whether this miRNA upregulates or downregulates insulin transcription [24,32,7,69]. Both Bolmeson et al. [7] and Nathan et al. [69] observed that miR-375 increased insulin transcript levels in human islets and in dedifferentiated human derived beta-cells respectively. Specifically, a positive correlation was shown between insulin mRNA and miR-375 expression in human islets [7]. Interestingly, similar analysis on human islets from glucose intolerant donors yielded no correlation between miR-375 and insulin mRNA expression in which the authors hypothesised may reflect perturbations in miRNA activity arising due to T2D [7]. Moreover, insulin mRNA expression was found to increase 3.7-fold with miR-375 overexpression in dedifferentiated human derived beta-cells [69]. Putative targets for miR-375 were predicted by Bolmeson et al. however none of these targets were validated or further analysed [7]. In comparison, the indirect inhibition of glycogen synthase kinase-3-alpha (*GSK3A)* by miR-375 was postulated to contribute to the increase in insulin mRNA expression since corresponding protein expression decreased 1.6-fold compared to controls [69].

In contrast to the above studies, miR-375 was also shown to decrease glucose-induced insulin transcription in INS1E cells [24]. This was evidenced to occur through the direct post transcriptional inhibition of 3′-phosphoinositide–dependent protein kinase-1 (*Pdk1*) mRNA, consequently negatively impacting the phosphatidylinositol 3-kinase (PI3K) pathway [24]. This pathway has previously been evidenced to contribute to glucose stimulated insulin gene transcription [84]. Furthermore, miR-375 expression was shown to be glucose-dependent in INS1E cells and rodent islets.

Nathan et al. also found overexpression of miR-375 in human islets resulted in a 30% reduction in *PDK1* mRNA expression [69]. Thus, suggesting conservation of this interaction between rodents and humans. The authors postulated that overexpression of miR-375 contributed to redifferentiation of human islets by inhibiting the PDK1-AKT pathway [25,69]. However, as mentioned above, Nathan et al. demonstrated increased insulin transcript levels in human dedifferentiated beta-cells [69]. Variation between the studies could have arisen due to differences in primary cells compared to cell lines or variations in dosage [32]. Furthermore, these disparities highlight the complexity of miRNA interactions, the diverse range of miRNA targets and their ability to shift in response to varying cell conditions. Future studies aimed at clarifying the effect of miR-375 on insulin transcription in human beta-cells would be of benefit to clarify the role of this beta-cell abundant miRNA both in health and potentially T2D.

In addition to investigations on miR-375, miR-30d, miR-200, miR-26, miR-182, miR-24 and miR-148 were shown to upregulate insulin transcription in MIN6 cells and primary mouse islets [108,66,92]. Interestingly, increased expression of miR-30d, miR-24 and miR-26 has been demonstrated in response to hyperglycaemia [92], thus, highlighting a mechanism by which insulin transcription could be influenced by blood glucose through alterations in miRNA expression. In addition, mitogen-activated protein 4 kinase 4 (*Map4k4*), an insulin transcription factor, was validated as a target of miR-30d in MIN6 cells [108]. *Map4k4* expression is induced by tissue necrosis factor (TNF)-alpha and indirectly inhibits insulin transcription through the direct inhibition of *Mafa* [105,8,93]. Interestingly, the inhibition of *Map4k4* by miR-30d presents a novel approach to overcome the discernible effects of TNF-alpha on insulin synthesis and would benefit confirmatory analysis with human beta-cells [108].

In contrast, the stimulatory effects of miR-24/miR-148 and miR-182 towards insulin transcription in mice were postulated to occur by inhibiting two transcriptional suppressors, namely SRY-box transcription factor 6 (*Sox6*) and basic helix-loop-helix family member e22 (*Bhlhe22*), respectively [66]. These transcriptional suppressors were shown to increase at the mRNA level in response to the inhibition of respective miRNA in mouse islets, perhaps implying a potential interaction between them. Further target validation analysis revealed that miR-200, miR-24, miR-148 and miR-26 were shown to bind to the 3' UTR of *Sox6* and *Bhlhe22*, thus, revealing a potential mechanism by which these miRNAs promote insulin transcription [66]. Collectively, these studies emphasise the significance of miRNAs in regulating insulin transcription by modulating the expression of molecules that either indirectly or directly inhibit transcription.

Insulin content and processing were also shown to be under the control of miRNAs (Table 2). Insulin content was shown to be negatively regulated by miR-130a, miR-152 and miR-204 in rodents and the same was demonstrated for miR-125b in mice and EndoC-Beta-H1 cells [103,17,73]. Additional investigations demonstrated that miRNAs also

influence insulin processing. For example, overexpression of miR-130a, miR-130b, miR-152, and miR-21 were associated with increased proinsulin to insulin ratio in mice/rodent beta-cells [46,73]. Notably, miR-130a/b and miR-152 were also linked to disruptions in glucokinase (GCK) and pyruvate dehydrogenase E1 alpha (PDHA1), with interactions affecting adenosine triphosphate (ATP) levels and impairing insulin production/processing in INS1 832/13 cells [73]. Target validation analysis demonstrated miR-152 to target the 3' UTR of *Pdha1* mRNA in INS1 832/13 cells [73]. PDHA1 forms part of the pyruvate dehydrogenase complex associated with glucose metabolism via the conversion of pyruvate to acetyl-Co-A and thus, validation of this interaction indicates a potential mechanism by which miR-152 regulates insulin in rodents [78].

In comparison, miR-21 was associated with an increased proinsulin to insulin ratio within INS1 cells through inhibition of two validated targets, namely transforming growth factor-beta 2 (*Tgfb2*) and Smad family member 2 (*Smad2*) [46]. In this manner, miR-21 induced dedifferentiation of beta-cells, resulting in a reduction of prohormone convertases, and consequently, an increased proinsulin to insulin ratio [46]. Furthermore, in humans, increased expression of miR-21 also initiated dedifferentiation of islets and a significant decrease in *SMAD2* and *TGFB2* mRNA. However, for one donor transfection of miR-21 lentivirus resulted in no significant change in either of the above mRNA, likely related to genetic heterogeneity [46]. These studies, along with those mentioned previously, contribute additional evidence to the pivotal role of miRNAs in regulating insulin production, spanning from transcription to maturation of the insulin hormone.

3.1.2. Regulation of insulin secretion by miRNAs

Comparable to the regulation of insulin production by miRNAs, these non-coding RNA molecules also play an important role in controlling insulin secretion in response to glucose and other stimulants (e.g., incretins) [37,52]. Interestingly, miR-375 was the first miRNA identified to have a direct impact on insulin secretion which was postulated to occur through the translational inhibition of myotrophin (*Mtpn*), a molecule involved in exocytosis [83]. This was further evidenced in MIN6 cells, where decreased insulin exocytosis was demonstrated upon overexpression of miR-375 [83]. However, the regulation of *Mtpn* by miR-375 has been disputed in other studies where mRNA levels have not changed with increased miR-375 expression [22,69]. Nonetheless, in the original study performed in 2004, *Mtpn* mRNA levels were not affected by miR-375 overexpression, but corresponding protein expression was found to be significantly reduced [83]. Thus, suggesting a possible translational inhibition of the respective protein by miR-375-RISC binding to corresponding mRNA without subsequent mRNA degradation. Other studies critiquing this anomaly have failed to investigate corresponding protein levels after overexpression of miR-375 and therefore validation of this postulated interaction could be of potential interest for future studies [22,69]. Poy et al. also signified the importance of this miRNA in insulin secretion through miR-375 ablation in leptin-deficient mice, with resultant impairments towards beta-cell mass and plasma insulin in response to insulin resistance (IR) [82]. Thus, aligning with conflicting results observed with the impact of miR-375 on insulin transcription, it remains unclear whether this miRNA has an overall negative or positive impact on insulin secretion.

miR-7 is another highly conserved beta-cell abundant miRNA which, unlike miR-375, is differentially expressed in T2D, with most studies demonstrating a shift towards downregulation in human islets as seen in Table 1 [53,59,9]. Reduced miR-7 expression in T2D may occur as a compensatory mechanism to overcome its inhibitory effect on insulin secretion [59]. miR-7a was shown to decrease insulin secretion in mice by post transcriptionally inhibiting the expression of molecules involved in fusion of insulin granules to the membrane, and those involved in soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) protein interactions [59]. For instance, one validated target of miR-7a in mice is alpha-synuclein (*Snca*), which interacts with SNARE-protein vesicle associated membrane protein 2 (VAMP2) and promotes SNARE complex assembly [59]. miR-7a interrupts this process by binding to *Snca* with resultant decreases in mRNA and protein expression [59]. Consequently, decreasing insulin secretion by limiting the amount of SNCA available to interact with VAMP2 and assist SNARE complex formation [59]. Furthermore, knockout of miR-7a in mice and human islets resulted in improved GSIS [59].

The aforementioned study strongly indicates that miR-7 impairs insulin secretion, a proposition supported by another study confirming enhanced GLP-1-stimulated insulin secretion and cyclic adenosine monophosphate (cAMP) production in miR-7 knockdown INS-1 cells [65]. This effect was proven to occur through the direct translational inhibition of beta-arrestin 1 by miR-7 where absence of this inhibition mediated improved responses to GLP-1. Beta-arrestin 1 is involved in mediating downstream signalling in G-protein coupled receptor (GPCR) pathways and interestingly desensitisation of these receptors has been linked to beta-arrestin 1 [38]. However, it must be noted that the authors only performed investigations on INS-1 cells and thus, confirmatory analysis on human islets would be of benefit. Collectively, miR-7 has been shown to negatively affect insulin secretion by post transcriptionally modifying the expression of molecules involved in exocytosis of insulin granules or receptor desensitisation [59,65].

In contrast to miR-7, insulin secretion was shown to be positively regulated by miR-212 and miR-132 (Table 2), and unlike the above studies corresponding mRNA targets were not identified [88]. Instead, it was demonstrated that GLP-1 induced the expression of both miR-212 and miR-132 in INS-1 832/3 cells via protein kinase A (PKA) activation [88]. PKA activation was demonstrated to be essential as PKA inhibition negated the above alteration in miRNA expression induced by GLP-1. This shift in miRNA expression in response to GLP-1 was also demonstrated in human islets treated with GLP-1 [88]. In addition, miR-212 and miR-132 expression was increased following GLP-1 treatment *ex vivo* in rodent and mice islets and *in vivo* in mice [88]. Contrastingly, INS-1 832/13 cells were initially shown to exhibit no increase in miR-212 and miR-132 expression upon treatment with GLP-1. Furthermore, defects in the G-protein alpha-subunit stimulatory pathway was also exhibited when the GLP-1 analog exendin-4 (Ex-4) exerted no effects towards cAMP levels in INS-1 832/13 cells [88]. Thus, this further highlights the need for effective cAMP and PKA activation to augment miR-212 and miR-132 expression in response to GLP-1.

Overexpression of miR-212 and miR-132 in these cells was shown to restore GLP-1 stimulated insulin secretion to similar levels of INS1 832/3 cells [88]. Furthermore, overexpression of miR-132 in INS1 832/3 cells significantly improved GSIS with and without GLP-1 treatment. However, knockdown of miR-132 had no effect on GSIS in the presence or absence of GLP-1 [88]. Considering a single mRNA can be targeted by several miRNAs and one miRNA can have several mRNA targets, it is likely that inhibiting miR-132 had minimal impact as other miRNAs upregulated in response to GLP-1 (e.g., miR-212) could have induced compensatory mechanisms [13,52,88].

Previous studies have also demonstrated increased miR-212 and miR-132 expression in the islets of GK rats in response to hyperglycaemia [33]. Importantly, this could contribute to beta-cell dysfunction or beta-cell compensation in T2D, but further investigation is required to elucidate this. Similarly, expression of miR-132 was upregulated 8.2-fold in the pancreas of prediabetic mice and demonstrated positive effects on GSIS in rodent islets and increased proliferation in MIN6B1 cells [70]. Thus, the study concluded that increased miR-132 may act as a compensatory mechanism to improve beta-cell function and mass in response IR [70]. From the above studies, it would appear that the action of miR-212 and miR-132 positively impacts insulin secretion and cAMP production in response to GLP-1. Future studies to identify mRNA targets and how posttranscriptional inhibition by miR-212 and miR-132 benefits the actions of GLP-1 would certainly be beneficial, especially in human islets. Furthermore, clarification of the expression of both miR-212 and miR-132 in beta-cells from

donors with and without T2D would help to clarify the contribution of these miRNAs in the pathogenesis of T2D.

Other miRNAs shown to negatively impact insulin secretion include miR-187, miR-124a, miR-463–3p, miR-130a, miR-152, miR-335, miR-200c, miR-24, miR-21, miR-125b, miR-204, miR-9 and miR-96 [103,111,17,45,46,49,61,62,72,73,80,85,87]. Furthermore, expression analysis on human T2D islets revealed upregulation of miR-187, miR-124a, miR-463–3p, miR-130a, miR-152 and miR-200c [45,61,73,85,87]. Thus, the above miRNAs could be involved in the pathogenesis of T2D and contribute to beta-cell dysfunction due to their upregulation in T2D islets and negative effect on insulin secretion. In addition, a negative correlation was observed between GSIS and miR-187, miR-463–3p and miR-335 further supporting this hypothesis [45,61,85]. The results of these studies and associated targets of the above miRNAs are summarised in Table 2.

3.2. Control of Glucagon Production and Secretion by miRNAs

Glucagon has been implicated as a potential player in the pathogenesis of T2D, with dysregulation of both insulin and glucagon seen as key modulators in the pathophysiology of T2D [96]. Glucagon plays an important role in the regulation of blood glucose and responds to hypoglycaemia by stimulating glucose synthesis in hepatocytes [97]. Recently, research surrounding glucagon has intensified, particularly due to interest in its application in the development of new therapies for T2D, with glucagon/GIP/GLP-1 receptor dual and triple agonists currently undergoing clinical trials [15,20,4,79]. Therefore, unravelling the role of miRNAs in the complex mechanisms underpinning regulation of glucagon production and secretion could offer additional insights and reveal novel therapeutic strategies [32]. To date, relatively few studies have investigated the control of glucagon synthesis and release from alpha-cells by specific miRNAs (Table 3, Fig. 3).

One study of particular interest demonstrated miR-320a directly targeted the 3'UTR of glucagon mRNA consequently inhibiting translation [48]. Specifically, overexpression of miR-320a resulted in decreased glucagon expression at both the mRNA and protein level in alpha-TC1–6 cells [48]. Increased miR-320a was also shown to decrease glucagon secretion in both alpha-TC1–6 cells and ND human islets. Moreover, miR-320a expression was shown to be glucose-dependent, with hyperglycaemia potentiating reduced miR-320a expression and increased glucagon expression in both alpha-TC1–6 cells and human islets [48]. This was further reflected in human T2D islets where decreased expression of miR-320a and increased glucagon expression were demonstrated [48]. This highlights a potential mechanism that could contribute to the development of hyperglucagonemia observed in T2D. Notably, overexpression of miR-320a in alpha-TC1–6 cells attenuated glucagon production in a high glucose environment [48] which reveals the potential of miR-320a mimics as a potential novel therapeutic avenue for T2D.

Similarly, prolonged hyperglycaemia was shown to reduce miR-124–3p levels in murine islets, accompanied with increased expression of its validated mRNA targets ionotropic glutamate receptor 2/3 (*iGluR2/3*) [106]. In addition, miR-124–3p was shown to affect glucagon secretion in murine islets in response to glutamate, a positive regulator of glucagon secretion acting through iGluR2/3 [106,11]. Consequently, overexpression of miR-124–3p decreased glutamate-induced glucagon secretion with reduced protein expression of iGluR2/3 also confirmed [106]. Furthermore, increased iGluR2/3 expression was observed in response to fasting or hypoglycaemia in mouse islets [106]. Therefore, the above study highlights the importance of miR-124–3p in the regulation of glucagon secretion with prolonged hyperglycaemia inhibiting its action and potentially contributing to glucagon dysfunction.

Another miRNA, namely miR-483, was shown to be differentially expressed in beta- and alpha-cells with beta-cells presenting higher miR-483 expression [67]. Overexpression of miR-483 in alpha-TC1–6 cells and mouse islets resulted in decreased transcription and secretion of glucagon in a low glucose environment [67]. In contrast, overexpression of this miRNA was shown to have a beneficial effect on beta-cells [67]. In comparison, Poy et al. found that miR-375 ablation in mice exhibited positive effects towards alpha-cell mass and secretion, compared to the detrimental effects observed in beta-cells [82]. However, it is unknown if this effect on alpha-cells was a direct or indirect consequence of miR-375 deletion.

Highly expressed miRNAs in beta-cells have been demonstrated to control the expression of alpha-cell specific transcription factors in alpha-TC6 cells [55]. Specifically, overexpression of miR-200c was shown to decrease c-Maf and Zfpm2 at mRNA and protein levels with consequent reductions in glucagon expression also evidenced. In addition, overexpression of miR-125b or miR-182 decreased c-Maf expression at both mRNA and protein levels, with similar effects towards glucagon expression [55].

These various studies described above have revealed that miRNAs play a regulatory role on glucagon production and secretion in alpha-cells, with their dysregulation potentially contributing to the pathogenesis of T2D. Further investigations in this domain would be

Table 3
miRNAs involved in the regulation of glucagon production and secretion with targets and results from experimental models and human islets.

Study	miRNA	Target(s)	Results from *In Vitro* Model(s)	Results from *In Vivo* Model(s)	Results from Human Islets
[48]	miR-320a	Glucagon	**Mouse alpha-TC1–6 cells:** OE = ↓ glucagon mRNA and protein expression, ↓ glucagon secretion, inhibited glucose induced change in glucagon expression and secretion, ↑ IRS2. KD = ↑ glucagon expression and secretion. ↑ glucose = ↓ miR-320a and ↑ glucagon expression		Downregulated in T2D with ↑ glucagon expression. ND islets: OE = ↓ glucagon secretion. ↑ glucose = ↓ miR-320a and ↑ glucagon expression
[67]	miR-483	*Socs3*	**Alpha-TC1–6 cells:** OE = ↓ glucagon secretion and transcription (latter @ ↓ glucose). KD = ↑ glucagon secretion and transcription. **Mouse islets:** OE = ↓ glucagon secretion and transcription.		
[55]	miR-200c, miR-125b, miR-182	*c-Maf*	**Alpha-TC6 cells:** OE of all 3 individually = ↓ C-Maf expression (validated miR-200c target) (mRNA and protein level), ↓ glucagon. OE miR-200c = ↓ Zfpm2 (mRNA and protein level)		
[106]	miR-124–3p	*iGluR2, iGluR3*	**Mouse islets:** OE = ↓ iGluR2/3 protein, ↓ glutamate or kainate induced glucagon secretion, KD = slight ↑ iGluR2/3 ↑ glucose = ↓ miR-124–3p and ↑ iGluR2/3		
[82]	miR-375			**MiR-375 KO mice:** hyperglycaemia, ↑ alpha-cell mass, ↑ glucagon secretion	

Abbreviations: OE, overexpression, ↑, increase, ↓, decrease, ND, nondiabetic, Socs3, suppressor of cytokine signalling 3, Zfpm2, zinc finger protein, multitype 2, iGluR2/3, ionotropic glutamate receptor 2/3, IRS2, insulin receptor substrate 2, KO, knockout.

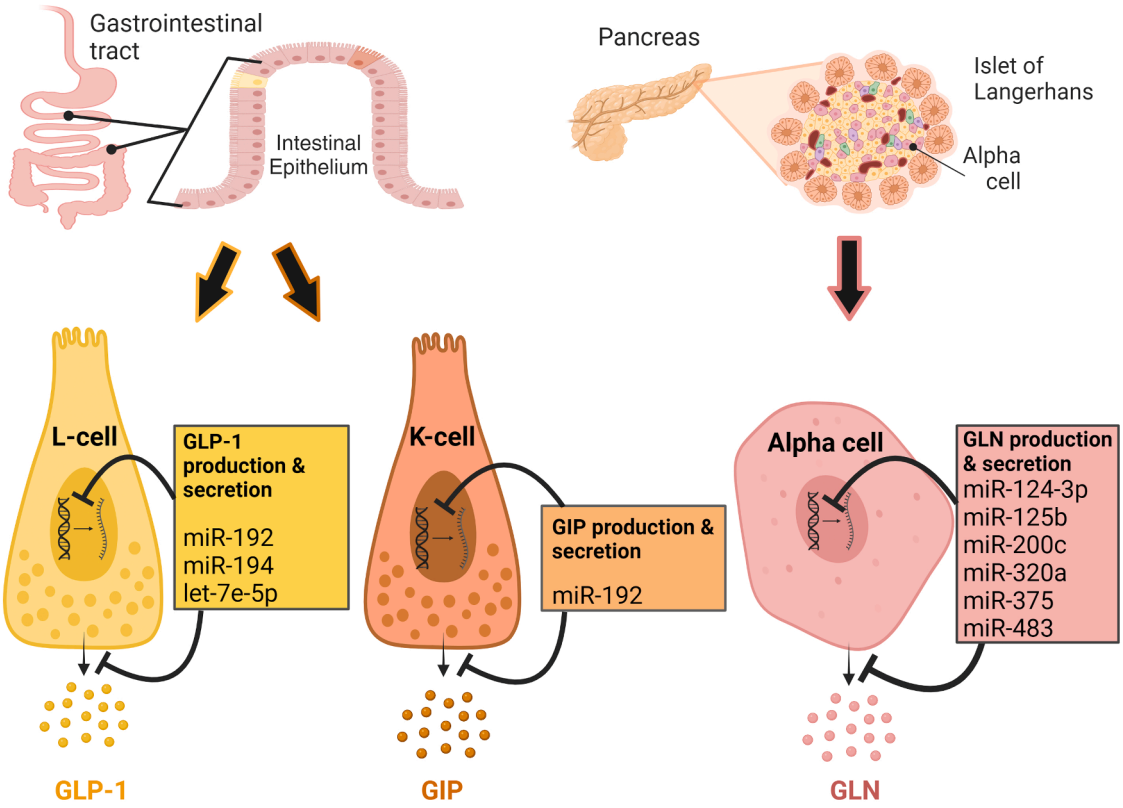

Fig. 3. Summary of miRNAs shown to regulate GLP-1, GIP and glucagon production and secretion in animal models, cell lines, or human islets. Abbreviations: GLP-1, glucagon like peptide 1, GIP, glucose dependent insulinotropic polypeptide, GLN, glucagon.

Table 4
miRNAs involved in the regulation of incretin production and secretion with targets and results from animal models, cell lines and human islets.

Study	miRNA	Focus on GIP/GLP-1	Target(s)	Results from *In Vitro* Models	Results from *In Vivo* Models	Results from Human Islets
[100]	miR-194	GLP-1	*TCF7L2, Foxa1*	**STC1 cells:** OE = ↓ GLP-1, glucagon mRNA, *Pcsk1* (mRNA and protein) and ↓ beta-catenin protein expression, ↓ IL-6 induced GLP-1 secretion	**HFD induced obese mice:** ↓ GLP-1 (serum and ileum) and ↑ miR-194 (ileum), negative correlation = serum GLP-1 and ileum miR-194 expression. KD = ↑ GLP-1	
[42]	let-7e-5p	GLP-1		**GLUTag cells:** OE = ↓ GLP-1 content, KD = ↑ GLP-1 content		
[110]	miR-155–5p	GLP-1 (Alpha-cell produced)	*Mafb*	**MIN6 cells:** OE = ↑ GLP-1 content, KD = ↓ GLP-1 content **Enteroendocrine L-cell line:** OE= no effect on GLP-1	**Mir155$^{(-/-)}$ Ldlr$^{(-/-)}$ mice:** ↓ internal islet GLP-1 content and ↓ serum GLP-1. OE = ↑ GLP-1 secretion **Ldlr$^{(-/-)}$ mice:** LPS = ↑ miR-155–5p and serum GLP-1	**Human islets:** OE = ↑ GLP-1 content and secretion in alpha-cells
[77]	miR-192	GLP-1	GLP-1	**NCI-H716 cells:** OE = ↓ GLP-1 (mRNA and protein expression), and ↓ extracellular GLP-1, KD = ↑ GLP-1 (mRNA and protein expression) and ↑ extracellular GLP-1		
[107]	miR-6796–3p miR-6763–5p miR-4750–3p miR-197–3p	GLP-1 GIP		**STC1 cells:** Exosomes with a differential expression of these miRNAs = ↓ GLP-1 and GIP secretion, ↓ GLP-1 and GIP content, ↑ *Gip* and glucagon mRNA. Individual OE = ↑ *Gip* and glucagon mRNA		

Abbreviations: OE, overexpression, KD, knockdown, HFD, high fat diet, *TCF7L2*, transcription factor 7 like 2, (-/-), knockout, LDLR, low density lipoprotein receptor, STC, secretion tumour cell line, *Pcsk1*, prohormone convertase 1/3

beneficial to comprehend the specific roles of miRNAs towards the regulation of glucagon and its potential contribution to the pathogenesis of T2D.

4. Regulation of incretin peptides by miRNAs

Similar to glucagon, miRNA regulation of glucoregulatory gut derived hormones remains poorly understood. To date, a limited number of studies have investigated the effect of specific miRNAs on incretin production and secretion (Table 4, Fig. 3). Studies discussed in this review were selected using search strings "miRNA AND incretins," "miRNA AND incretin secretion," "miRNA AND GIP AND K-cells," "miRNA AND GLP-1 AND L-cells," "miRNA AND enteroendocrine K-cells" and "miRNA AND enteroendocrine L-cells" on Google Scholar and

PubMed. Studies were also obtained from other review papers in this field. Due to the absence of studies on other gut hormones involved in the maintenance of glucose homeostasis (e.g., Oxm, PYY and CCK), this review concentrates on the regulatory role of specific miRNAs towards incretin production and secretion. Incretins are of particular interest in diabetes given their therapeutic potential and ability to potentiate insulin secretion, stimulate beta-cell expansion and regulate insulin production [3]. Given that incretins are responsible for approximately 60% of postprandial insulin secretion, these hormones have garnered significant interest in T2D research [3].

Key studies that have investigated the control of incretin synthesis and secretion by specific miRNAs are noted in Table 4, with validated mRNA targets and results from experiments performed on animal models, cell lines, and human islets. Interestingly, most of the studies investigating the impact of miRNAs on incretin production and secretion have also observed that the expression of prohormone convertase 1/3 (*Pcsk1*) was either positively or negatively affected [100,107,110]. Prohormone convertase 1/3 (PC1/3) is an enzyme encoded by *Pcsk1,* responsible for the cleavage and subsequent generation of mature GIP and GLP-1 from immature proGIP and proglucagon, respectively [90]. Notably, cleavage of proGIP to GIP in enteroendocrine K cells is a function of PC1/3 only, whereas the generation of mature GLP-1 is not solely reliant on this process [90,95]. Zhu et al. demonstrated miR-155–5p had a positive effect on GLP-1 content and secretion in human alpha-cells [110]. This was revealed after lipopolysaccharide (LPS) and hyperlipidaemia increased miR-155–5p expression in beta-cells, resulting in the translational inhibition of *Mafb,* which alleviated the suppression of interleukin 6 (IL-6) [110]. IL-6 secreted by beta-cells was evidenced to stimulate the production of PC1/3 in alpha-cells thus increasing GLP-1 production [110,30,29]. By increasing the production of GLP-1, beta-cell response and adaption to stress and IR was also improved.

In comparison, miR-6796–3p, miR-6763–5p, miR-4750–3p and miR-197–3p were shown to negatively impact GLP-1 and GIP production and secretion in STC1 cells by inhibiting PC1/3 expression [107]. This defect was highlighted when both *Gip* and glucagon mRNA levels were found to be increased by exosomes containing the mentioned miRNAs, with corresponding protein levels decreased [107]. Thus, the authors hypothesised that these miRNAs impaired hormone maturation and confirmed said theory by demonstrating decreased *Pcsk1* and PC1/3 [107].

Wang et al. also demonstrated the negative effect of miR-194 on the production of GLP-1 in L cells [100]. The authors validated two targets of miR-194 linked to GLP-1, namely transcription factor 7 like 2 (*TCF7L2*) and *Foxa1,* which impacted glucagon and PC1/3 mRNA and protein expression [100]. As the name suggests, *TCF7L2* is a transcription factor involved in the regulation of genes within the beta catenin/Wnt signalling pathway [104]. Glucagon expression is regulated by this pathway, and it is likely that translational inhibition of *TCF7L2* by miR-194 impeded glucagon transcription, thus decreasing GLP-1 expression [100,68].

Another contributor to decreased GLP-1 expression is miR-194-induced translational suppression of *Foxa1,* a transcription factor shown to bind to the promoter of *Pcsk1* [100]. Thus, it is likely miR-194 induced suppression of *Foxa1* impaired *Pcsk1* transcription, and reduced PC1/3 levels, thereby limiting conversion of proglucagon to GLP-1 [100]. This complex process illustrates the complexity of miRNA networks in the regulation of gene expression, and together these studies highlight the importance and complexity of miRNA networks in the regulation of incretin synthesis and secretion.

5. Therapeutic potential of miRNAs in T2D

The number of people diagnosed with diabetes, particularly T2D, continues to soar with estimations predicting 783 million cases by 2045 [4,74]. T2D is associated with a vast array of macrovascular and microvascular complications (e.g. coronary heart disease, nephropathy, and retinopathy) [12]. Novel treatment strategies are thus warranted to enhance patient wellbeing and prevent/limit complications, particularly due to limitations of current therapies, including cost and availability of leading biopharmaceuticals [14,4,94].

The administration of molecules that block or mimic the action of specific miRNA implicated in T2D represents an innovative therapeutic approach [44]. The term miRNA mimic is used to describe synthetic oligonucleotides which have the same sequence as target miRNA and thus increase the concentration and action of selected miRNA within cells [44]. In comparison, miRNA inhibitors, known as antagomirs, are antisense oligonucleotides that have complementary sequences to miRNA of interest, and act by binding to target miRNA and inhibiting miRNA and mRNA interactions [44]. Chemical modifications of these molecules (e.g., locked nucleic acids) have improved their efficacy *in-vivo* by improving circulating stability and reducing potential activation of the innate immune system [28,44]. However, obstacles remain with miRNA-based therapeutics, including tissue specific delivery, unwanted effects mediated by in-direct interactions, and appropriate methods for transport to target sites [52].

Clinical trials are currently underway with success for miRNA therapeutics where, for example, the drug MRG-110, a miR-92a inhibitor, has completed Phase 1 trials and was shown to successfully decrease circulating miR-92a and liberate mRNA targets [2,43]. In comparison, ABX464, a miRNA mimic, increases miR-124 expression and is currently undergoing Phase 3 clinical trials for ulcerative colitis [99]. These trials highlight the potential and applicability of miRNA-based therapeutics which could be adopted for treatment of T2D.

Exploitation of miRNAs in T2D therapy has been substantiated by studies identifying changes in the expression of miRNAs in human T2D islets (Table 1) [39,45,51,59,60,61,72,73,87,91]. Furthermore, investigations using animal models, cell lines, and human islets have demonstrated the effect of specific miRNAs on the production and secretion of hormones imperative for glucose homeostasis (Tables 2, 3, and 4). This is reinforced by studies revealing the beneficial actions of miRNA manipulation in animal models, highlighting the potential these small noncoding RNA molecules present for treating and potentially overcoming T2D [27]. For instance, complete deletion of the miR-200 family in mice was shown to protect mice from STZ-induced diabetes [6].

Therapeutically, miRNA mimics could be employed to increase expression of downregulated miRNAs in T2D (Table 1) and exert regenerative effects towards beta-cells (Tables 2, 3, and 4) or enhance insulin sensitivity to curtail IR [1,52]. Similarly, miRNA inhibitors could be utilised to inhibit miRNAs upregulated in T2D that contribute to beta-cell dysfunction and IR [1,52]. In this way miRNA mimics/inhibitors could replace existing therapies or improve their effectiveness. Interestingly, the exploitation of miRNAs has been proposed as a mechanism to overcome drug resistance for other diseases [86]. Alternatively, miRNAs could be utilised as biomarkers to stratify treatment strategies and facilitate disease prognosis monitoring [35,36,75]. For example, Flowers et al., identified specific miRNAs that could predict patient response to thiazolidinediones [35]. Further research is clearly warranted to elucidate the role of miRNAs in the development, progression, and treatment of T2D.

6. Concluding remarks

miRNAs are small noncoding RNA molecules that regulate gene expression by binding to complementary mRNA sequences with ensuant translational inhibition. In humans, differential miRNA expression has been seen in human islets from T2D donors compared to controls. Modulating the expression of specific miRNAs in experimental models and human islets has revealed both positive and negative effects on the production and secretion of insulin, glucagon, or incretin hormones. These studies would support the concept that alterations in miRNA

expression could function as either a compensatory mechanism, as seen in the downregulation of miR-7a, or their dysregulation could actively contribute to the development and/or progression of T2D, as exemplified by miR-187. The utilisation of miRNA mimics and antagomirs as a potential therapy has been highlighted by the success of recent clinical trials for other conditions. Collectively, the early observations presented in this brief review provide considerable impetus for further in-depth research into the regulatory actions of miRNAs and their association with T2D and other metabolic disorders with the view to providing further pathological insights and exciting new therapeutic avenues.

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E.R. Carr: Writing – original draft, Investigation, Formal analysis. **P. B. Higgins**: Writing – review & editing, Conceptualization. **N.H. McClenaghan**: Writing – review & editing, Conceptualization. **P.R. Flatt**: Writing – review & editing. **A.G. McCloskey**: Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of Competing Interest

The authors report no declarations of interest.

Data availability

No data was used for the research described in the article.

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