Review Article

Emerging role of long noncoding RNAs and circular RNAs in pancreatic β cells

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Abstract: The advent of high-throughput genomic technologies revealed the expression of thousands of long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) in human. Both classes of RNAs have limited or no protein coding potential. Both lncRNAs and circRNAs regulates gene expression by interacting with target DNA, RNA or proteins. Accumulating studies have demonstrated that lncRNAs and circRNAs play important role in various human diseases, including diabetes. Pancreatic β cell function is at the core of development of diabetes. This review will highlight the present knowledge about the role of lncRNAs and circRNAs in pancreatic β cell function and their association with development of diabetes.

Keywords: Diabetes; insulin; lncRNAs; circRNAs; miRNA

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Introduction

Diabetes mellitus affecting more than 400 million people across the globe is one of the major health challenges today (1). Diabetes is a metabolic disorder characterized by dysfunctional production or sensing of insulin, leading to high glucose levels in the blood. Diabetes mellitus is categorized into Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), defined by the deficiency of insulin production by pancreatic β cells and by the loss of insulin receptor sensitivity on the target cells respectively (2,3). T1DM is an autoimmune disease leads to the destruction of pancreatic β cells accounts for less than 10% of the diabetic population while the rest 90% is T2DM. Glucose homeostasis in the body is regulated by the hormone insulin, which is secreted by pancreatic β cells (4-6). Increased extracellular glucose triggers a rapid upregulation of insulin secretion from the insulin storage granules and the secreted insulin is replenished by increased insulin biosynthesis through the activation of diverse gene regulatory mechanisms (5,7,8). In early stages of the T2DM, β cells increase their mass and insulin production to maintain glucose levels and overcome insulin resistance (9,10). However, long-term hyperglycemia leads to β cell failure by various mechanisms, including endoplasmic reticulum stress, mitochondrial dysfunction, and glucolipotoxicity (10). Most of the studies focused on elucidating the role of key protein-coding genes that are involved in insulin expression, secretion, and β cell apoptosis. However, the protein-coding sequences account for only 2% of the human genome (11,12). Interestingly, more than 85% of the human genome is likely to be transcribed and the majority of the transcripts are noncoding (nc) RNAs in nature (11-13). The ncRNAs include ribosomal (r)RNAs, transfer (t)RNAs, micro(mi) RNAs, piwi-interacting (pi)RNAs, small nuclear (sn)RNAs, long noncoding (lnc)RNAs and circular (circ)RNAs (14-17). In fact, we now know that these ncRNAs play a key role in regulating various steps of gene expression. While classical ncRNAs like rRNAs, tRNAs, snRNAs, and miRNAs are well characterized for their function in gene regulation, lncRNAs and circRNAs are poorly characterized (14-16,18-22). In this review, we will discuss the function of lncRNAs and circRNAs in insulin production.

LncRNAs are a heterogeneous class of >200 nucleotides

linear transcripts that lack the protein-coding ability (11,23). The Encyclopedia of DNA Elements (ENCODE) Project Consortium revealed that more than 28,000 distinct lncRNA are transcribed from the human genome (11). LncRNAs are known to regulate every step of gene expression, including transcription, pre-mRNA splicing, mRNA stability, and translation (19,24-26). LncRNAs may act as a decoy for transcription factors, as sponges for miRNAs and RNA-binding Proteins (RBPs), as host genes for miRNAs, as scaffolds for protein complexes, and as stabilizer/destabilizer of mRNAs to regulate gene expression (25). A few lncRNAs are reported to associate with polyribosomes and translate into peptides (27). Various key cellular events like cell proliferation, growth, senescence, differentiation, and secretion are known to be regulated by lncRNAs. LncRNAs have been functionally associated with several diseases including cancer, nervous disorder, cardiovascular disease, muscular dystrophy, and diabetes (28-31).

Another class of novel ncRNA called circRNA was initially discovered in 1980s in plant viroid (32). Later few circRNAs were found to be expressed in eukaryotic cells and thought to be generated from erroneous splicing (33-36). Recently, high-throughput RNA-sequencing and unbiased analysis of mammalian transcriptome identified thousands of endogenous covalently closed loop circRNAs (37-39). CircRNAs are abundant, ubiquitously expressed, conserved, and show tissue-specific expression pattern (37,39-41). CircRNAs are shown to be originated from the pre-mRNAs by a process called backsplicing where an upstream 5' splice site is ligated to a downstream 3' splice site by head-to-tail nonlinear splicing (37,42,43). Although little is known about its function, increasing pieces of evidence established that they may act as miRNA sponges, act as a decoy for RBPs, compete with linear splicing, and translated into proteins (21,44). Although a numerous number of circRNAs have been identified to date, few circRNAs were reported to be involved in various diseases including cancer, muscular dystrophy, and diabetes (45). The role of lncRNAs and circRNAs in diabetes is not yet well understood but there is increasing evidence about their involvement in the development of diabetes.

Characteristics and function of lncRNAs

The protein-coding mRNAs represent less than 5% of the whole transcriptome while the rest are ncRNAs (16). The ncRNAs are broadly divided into short ncRNAs which are less than 200 nucleotides (nt) and long ncRNA with a length of more than 200 nt. Development in the next-generation sequencing (NGS) approaches revealed the expression of thousands of lncRNAs with no apparent protein-coding role. Recent ENCODE project revealed that the number of lncRNAs expressed from the human genome is more than the number of protein-coding genes (11). LncRNAs are mainly transcribed by RNA Polymerase (Pol) II or III in humans (46). Epigenetic modifications like H3K4me3 and H3K36me3 are associated with lincRNA transcription by RNA Pol II while H3K4me1/2/3 or H3K4ac are associated with Pol III transcription (46-48). Although it's difficult to classify the lncRNAs, they are broadly classified into sense overlap, antisense overlap, bidirectional, intronic lncRNAs, and intergenic lncRNAs depending on their location relative to the nearest protein-coding gene (49). Many lncRNAs are low abundant and not well conserved during evolution, posing a challenge for their functional characterization (50). However, some of the lncRNAs are ultra-conserved in various organisms ranging from Xenopus and chicken to human (11,51). Although lncRNA sequences are not well conserved, the lncRNA promoters show higher conservation similar to the mRNA promoters suggesting their regulated expression (11). LncRNAs are differentially localized into distinct subcellular compartments like nucleus, cytoplasm, and mitochondria to execute different activities. The nuclear lncRNAs can regulate transcription and splicing while the cytoplasmic lncRNAs can regulate posttranscriptional events such as mRNA stability, translation, protein localization, and protein modification (25).

For a long time, lncRNAs were thought to be non-functional transcripts generated by transcriptional errors and termed as junk RNA. Recent development in transcriptomic moved the lncRNAs to the forefront of RNA research. Although the function of most of the identified lncRNAs is unknown, some of the lncRNAs are very well characterized. LncRNAs can regulate gene expression by interacting with diverse molecules and forming macromolecular complexes. As shown in *Figure 1*, lncRNAs can regulate transcription by recruiting chromatin modifiers to specific genes or by interacting with various transcription factors (24,52). Some lncRNAs can directly interact with the pre-mRNAs or splicing factors to regulate pre-mRNA splicing (53). Moreover, lncRNAs also recruit RNA modifiers like ADAR and regulate RNA modifications (54). LncRNAs can act as a decoy for proteins and inhibit the protein from interacting with its target mRNA or modify catalytic activity of the protein (19,25). Additionally, the presence of miRNA target sites on lncRNAs makes them a competitive endogenous

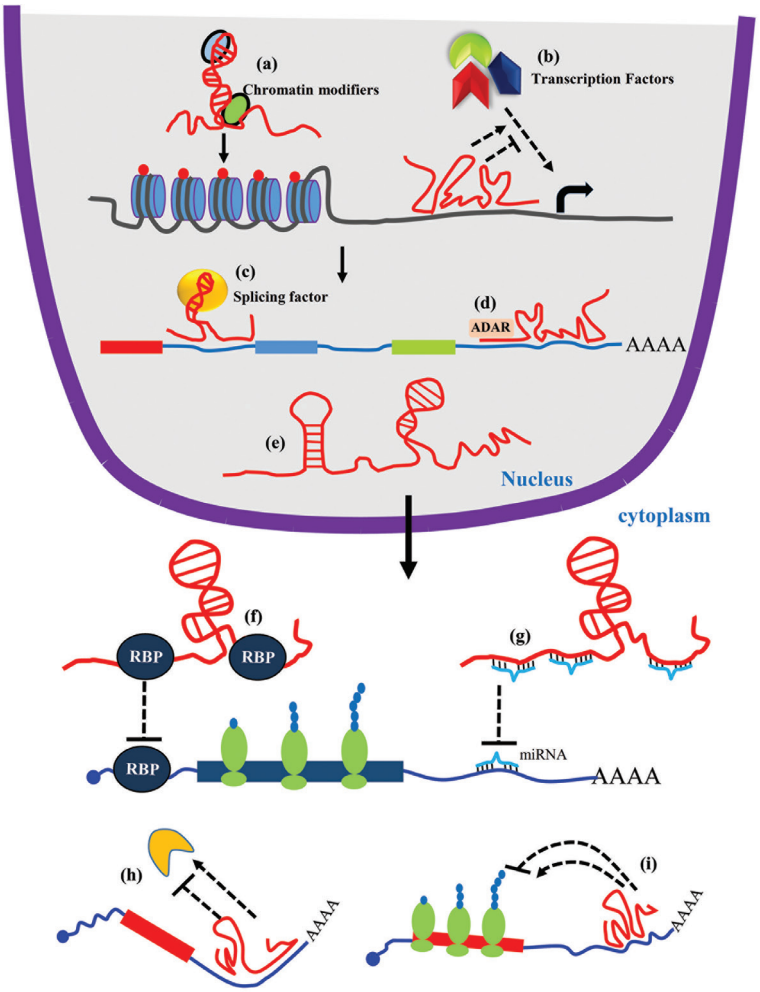

Figure 1 Mechanisms of lncRNA action. (a) LncRNAs can recruit chromatin modifiers to the target chromatin and alter the epigenetic histone marks; (b) LncRNAs regulate the transcription of the target gene by altering the availability of transcription factors; (c) LncRNAs can regulate splicing events by interacting with the pre-mRNAs or splicing factors; (d) LncRNAs control RNA modifications by recruiting RNA modifiers; (e) Several lncRNAs harbor miRNAs and act as a host gene for that miRNA; (f-g) LncRNAs can regulate mRNA translation and/stability by acting as a sponge for RBPs (f) and miRNAs (g); (h) LncRNAs control mRNA turnover by inhibiting or recruiting degradation machinery; (i) LncRNAs regulate mRNA translation by altering the polysome loading to mRNAs.

(ce)RNA for the miRNAs and inhibit miRNA activity by its sponging ability (19,25). Interestingly, direct interaction of lncRNAs with translating mRNAs can alter the stability and/or translation of the target mRNA (19).

LncRNAs regulate β cell function

A major portion of the genome is transcribed as ncRNAs and many of them are reported to regulate gene expression. LncRNAs are shown to regulate gene expression at the transcriptional and posttranscriptional level. Indeed, lncRNAs are reported to play critical role in various biological processes including cell proliferation, differentiation, senescence and aging (28,29,55,56). Although many studies suggest the important role of lncRNA in development and disease, few studies investigated the functional significance of lncRNAs in the context of pancreatic β cell function and diabetes. Many lncRNAs are reported to be expressed in cell-type specific manner in islet β-cells and are termed as islet lncRNAs. In this section, we describe and discuss the emerging role of lncRNAs in pancreatic β cells (*Table 1*).

Table 1 List of functional lncRNAs and circRNAs in **β** cells

LncRNA/circRNA	Aliases	Overlapping/closest protein-coding gene	Target	Function			Reference
				β cell proliferation	β cell apoptosis	Insulin biosynthesis and/or secretion	
MALAT1	HCN, LINC00047	–	miR-17, TXNIP	–	–	↓	(57)
GAS5	NCRNA0003, SNHG2	–	PDX1, MAFA	↑	–	↑	(58)
HI-LNC25	LINC01370, HILNC25	–	GLIS3	↑	–		(59)
HI-LNC78	Tunar	–	–	–	–	↑	(60)
PLUTO	HI-LNC71, PDX1AS1, PLUT	PDX1	PDX1	–	–	↑	(60)
βLINC1	HI-LNC15	–	Nkx2.2	↑	–	↑	(61)
βlinc2	–	–	___	–	↑	–	(62)
βlinc3	–	–	–	–	↓	–	(62)
TUG1	ENSG00000253352	–	caspase-3/9, PDX1, MAFA		↓	↑	(63)
MEG3	Gtl2, FP504, LINC00023, ENSG00000214548, PRO0518, PRO2160	–	PDX1, MAFA		↓	↑	(64)
LncRNA-p3134	ENST00000545923	–	PDX1, MAFA, GLUT2		↓	↑	(65)
LncRNA uc.322	–	SOX6	PDX1, FOXO1, SOX6	↑	–	↑	(66)
ciRS-7/CDR1as	hsa_circ_0001946	CDR1	miR-7, Myrip, Pax 6	↑	–	↑	(67,68)
circHIPK3	hsa_circ_0021592	HIPK3	miR-124, miR-338	↑	↓	↑	(68)
circAFF1	–	AFF1	–	–	↓	–	(68)

↑, increased; ↓, decreased.

MALAT1

Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is one of the best-characterized lncRNAs for its molecular function and diseases association. Expression levels of thioredoxin - interacting protein (TXNIP) and *MALAT1* are upregulated while mafA and miR - 17 are downregulated in MIN6 cells with cigarette smoke extract (CSE) (57). TXNIP controls the redox state of the cell by inhibiting thioredoxin and reported to inhibit insulin production in β cells (69). Interestingly, silencing of *MALAT1* upregulates the expression of miR-17 and miR-17 is known to promote insulin production. *MALAT1* silencing in β cells increases miR - 17 expression leading to suppression of TXNIP and increased insulin production (57). Intriguingly, diabetic patients who smoked shows a high level of *MALAT1* and low level of miR - 17 in the serum compared to non-smokers. In sum, CSE induces *MALAT1* expression that reduces miR-17 levels leading to upregulation of TXNIP which in turn inhibits insulin production in β - cells of smoking individuals.

GAS5

Growth arrest specific transcript 5 (*GAS5*) is a well-characterized lncRNA, known to generate many small nucleolar (sno)RNAs (70). As the name indicates *GAS5* is known to regulate cell proliferation and growth. The serum level of *GAS5* in diabetic patients is significantly lower compared to non-diabetic one (71). *Gas5* is abundantly expressed in normal pancreas while in db/db mice *Gas5* is significantly downregulated (58). In Min6 cells, silencing of *Gas5* promotes cell cycle arrest at G1 and decreases insulin biosynthesis and secretion. Additionally, *Gas5* knockdown in islets reduces the expression of insulin as well as transcription factors like Pdx1 and MafA underscore its role in insulin production and β cell function (58).

HI-LNC25

HI-LNC25 is a multiexonic lncRNA transcribed from the islet-specific active chromatin domain. It is specifically expressed in pancreatic β cells while the *MAFB*, the closest protein-coding gene localized in the same genomic loci is expressed in several tissues along with its abundant expression in β cells (59). Overexpression of *HI-LNC25* by lentivirus in human β cells promotes the expression of GLIS3 that encodes an islet-specific transcription factor. Although silencing of *HI-LNC25* does not affect glucose-induced insulin secretion, it downregulates GLIS3 mRNA levels (59). Interestingly, GLIS3 is involved in β cell growth and proliferation in response to insulin resistance. Thus, HI-LNC25 is a regulator of GLIS3 which regulates the development of type 2 diabetes.

HI-LNC78

HI-LNC78 is another islet lincRNA orthologous to mouse lncRNA *Tunar* is regulated by glucose (60). Silencing of *HI-LNC78* reduces insulin content and show decreased glucose-stimulated insulin secretion in human EndoC-bH3 cells. Silencing of *HI-LNC78* regulates the expression of various genes in β cells which is highly correlated with changes in gene expression by islet-specific transcription factors like HNF1A and MAFB suggests its role in β cell function (60).

PLUTO

PLUTO (*PDX1 locus upstream transcript*), also known as *HI-LNC71* is an abundant antisense transcript lncRNA located 3 kb upstream of PDX1 gene. PDX1 is a well-known transcription factor involved in pancreas development and β cell differentiation. In T2DM patients or patients with glucose intolerance, *PLUTO* and PDX1 are significantly downregulated (60). Interestingly, the decrease in *PLUTO* expression alters chromatin architecture reducing the association of PDX1 promoter with its enhancer resulting in reduced expression of PDX1 (60). These data highlight the role of lncRNAs in the regulation of β cell development and function.

βLINC1

β*LINC1* (β-*cell long intergenic noncoding RNA 1*), a 6.8 kb long lncRNA located nearly 20kb upstream of the transcription factor called NKX2.2 (61). In mammals, it is highly conserved and show islet specific expression pattern similar to other islet lncRNAs. The orthologous mouse β*linc1* has no protein coding ability. In mouse H3K27ac & H3K4me1/3 modifications are enriched around β*linc1* genomic region and the binding of transcription factors like Foxa2, NeuroD1, and Pdx1 to the putative β*linc1* promoter region is evident (61). The expression of β*linc1* is restricted to trunks and islets of the developing pancreas and it is mostly localized in the nucleus suggesting its role in transcriptional regulation. Knockdown of β*linc1*

reduces the expression of Nkx2.2 which is responsible for transcription of many genes involved in β cell maturation and function. Interestingly, β*linc1* knockout mice show abnormal insulin secretion in low glucose and displays elevated glucose intolerance (61). Silencing of β*linc1* in MIN6 cells upregulates the expression of somatostatin and suppresses the expression of Scg5 and Pax6 further indicates the involvement of β*linc1* in β cell function.

βlinc2 and βlinc3

Human lincRNA β*LINC3* expression is downregulated in T2DM patients compared to normal donors and its expression is negatively correlated with BMI of the patients (62). Two mouse orthologue lncRNAs β*linc2* and β*linc3* are reported to be differentially expressed in the islets of diet-induced obese mice (62). β*linc3* is localized mostly in the nucleus while β*linc2* is present in both nucleus and cytoplasm. Tissue specific analysis of β*linc2* and β*linc3* found them to be expressed mostly in pancreatic islets compared to other tissues. The expression level of β*linc2* is positively correlated with body weight, insulinemia, and glycemia while the level of β*linc3* expression is negatively correlated. Interestingly, upregulation of β*linc2* or downregulation of β*linc3* promotes apoptosis in MIN6 cells without affecting the insulin secretion suggests their role in development of T2DM (62).

TUG1

Taurine upregulated gene 1 (TUG1) is a multiexonic, spliced, polyadenylated lncRNA, highly conserved in mammals but absent in other vertebrates. It is highly expressed in pancreas compared to other organs and its expression is downregulated with glucose treatment (63). Low level of *TUG1* expression in NOD mice compared to BALB/c suggests its strong association with diabetes. *TUG1* knockdown induces β cell apoptosis evidenced by increased expression of apoptosis-related proteins such as caspase-3, caspase-9, GLUT2, Pdx1, MafA and decreased expression of antiapoptotic protein bcl-2 (63). Furthermore, silencing of *TUG1* reduces insulin biosynthesis and secretion suggesting a definitive role of *TUG1* in β cell function.

MEG3

Maternally expressed gene 3 (MEG3) is an imprinted lncRNA known to be involved in neurogenesis and retinal development. Furthermore, the low level of *Meg3* is associated with diseases like Huntington's disease and cancer (72,73). *Meg3* is also required for overall growth and survival of mice. Interestingly, *Meg3* is very abundantly and specifically expressed in pancreatic β cells (64). Its expression in β cells is also regulated by the level of glucose. Silencing of *Meg3* decreases mRNA and protein levels of Ins2, pdx1 and MafA underscore its role in insulin synthesis and secretion (64). Silencing of *Meg3* promotes apoptosis by increasing Bax and caspase3 expression and reducing Bcl 2 levels supports its role in the development of β cells. Together, these findings indicate that *Meg3* regulates insulin production from β cells.

LncRNA-p3134

LncRNA-p3134 (also known as ENST00000545923) is known to suppress the expression of HOMA-β, that is associated with beta cell function (65). *LncRNA-p3134* expression is upregulated with glucose treatment (65). Interestingly, overexpression of *lncRNA-p3134* by glucose in MIN6 cells induces the expression of transcription factors like Pdx-1 and MafA as well as upregulation of GLUT2 expression, a glucose transporter associated with glucose-stimulated insulin secretion. In db/db mice induction of *lncRNA-p3134* results in upregulation of TCF7L2 expression (65). Furthermore, *lncRNA-p3134* act as a protective lncRNA to prevent beta cell destruction and apoptosis.

LncRNA uc.322

LncRNA uc.322 is a 224 base pair lncRNA originates from exonic region of SOX6 (SRY related HMG box 6). *LncRNA uc.322* is highly conserved in mammals and found to be abundantly expressed in pancreatic tissue compared to other tissues (66). Overexpression of *lncRNA uc.322* upregulates the expression levels of PDX1 and FOXO1. Knockdown of *lncRNA uc.322* in MIN6 cells reduces insulin secretion and ATP concentration while its overexpression has opposite effect. SOX family proteins especially SOX 4 is associated with beta cell growth and development whereas SOX 6 promotes secretion of insulin (66). Expression level of *lncRNA uc.322* is positively correlated with SOX6 expression which suggest its role in beta cell development and insulin transcription.

Characteristics and functions of circRNAs

In the early 1980s, a novel class of covalently closed loop

circRNAs was initially discovered in plant viroid (32). For a long time, this kind of molecules was considered as splicing artifacts. Their existence and functional significance were neglected until the recent development of NGS technologies (37,39). Unlike linear RNAs, circRNAs are identified by the presence of the backsplice sequence at the 5' to 3' ligation junction (74). Global analysis of circRNA expression relies on high-throughput RNA-sequencing (RNA-seq) followed by identification of non-linear backsplice reads using bioinformatics tools (75). CircRNA enrichment methods like depletion of linear RNA by RNase R and/or poly(A) RNA and rRNA depletion were developed for specific identification of circRNAs by RNA-seq, as non-linear reads may be generated by various mechanisms including backsplicing, template switching of RT or trans splicing (40,74-78). High throughput RNA-seq discovered more than hundred thousand circRNAs in humans (39,77-79). As many of the circRNAs are low in abundance, high sequencing depths of 50 million reads or more are required for their identification and quantification (75,78,80,81). Although RNA-seq is the most preferred method for circRNA identification, methods like microarrays, RT-PCR, and northern blot analysis are also used for checking their expression (39,74,82,83).

As the majority of the identified circRNAs are generated from the exons of mRNAs, the canonical splicing machinery is believed to be involved in the biogenesis of circRNAs (42,43,84). CircRNAs are generated from the pre-mRNAs by a process called backsplicing where a downstream 5' splice donor reversely attacks an upstream 3' splice acceptor site of pre-mRNA forming a covalently closed circRNA lacking the 5' and 3' ends (42,43). For multiexon circRNAs, backsplicing is often coupled with canonical splicing to remove the intervening introns generating the mature exonic (E)circRNAs (37-40). Although backsplicing is not that efficient compared to linear splicing, the canonical splicing signals and the speed of transcription by RNA pol II have been shown to regulate the biogenesis of circRNAs (43). Various RBPs and inverted repeat sequences in the flanking intron are also involved in the regulation of circRNA biogenesis by backsplicing (42,85,86). Very often, the formation of EcircRNA leads to skipping of those exons from the mature mRNAs leading to expression of splice variants. Some EcircRNAs retains the intervening intron and termed as Exon-Intron (EI)circRNAs (87). Many intronic lariats have been reported to be resistant to degradation by the debranching enzyme leading to the generation of stable circular intronic (ci)RNAs (88). A recent study identified thousands of circRNAs generated from the intronic sequences with unknown mechanism of biogenesis and termed as intronic (I)cicRNAs (78).

As shown in *Figure 2*, circRNAs generated from the pre-mRNA by backsplicing and regulate gene expression by various mechanisms. The EcircRNAs are localized in the cytoplasm and regulate posttranscriptional gene regulation (21,38). Many circRNAs harbor miRNA response elements (MRE) can act as miRNA sponges (89). CircRNAs with miRNA binding sites can act as an ceRNA and inhibit the miRNA from binding to its target mRNAs leading upregulation of target gene expression (21,89). *CDR1as* is one of the highly conserved and abundant circRNA discovered to contain more than 60 MREs for miR-7 (38,39). Accumulating studies have established that the circRNA-miRNA-mRNA network plays a critical role in gene expression (89,90). Besides miRNA sponging, circRNAs can act as a decoy for the RBPs and modulate the expression of the RBP target gene. For instance, *circPABPN1* acts as a decoy for HUR and sequester HUR from binding to *PABPN1* mRNA leading to decrease in PABPN1 translation (82). Another circRNA *circMBL* act as a sponge for the splicing factor MBL and regulate MBL expression (91). Various web tools have been developed to explore the interaction of circRNAs with miRNAs and RBPs (92-94). The ciRNAs and EIcircRNAs are localized in the nucleus and associate with RNA Pol II to regulate transcription of target genes (87). Although circRNAs are categorized as ncRNAs, a few circRNAs are reported to translate into peptides (95-97). Recent studies have shown that circRNAs can act biomarkers for human diseases (98). Although the function of some circRNAs have been characterized, the function of majority of circRNAs remains to be explored.

CircRNAs regulate β cell function

CircRNAs are emerging as novel regulators of gene expression and shown to be involved with disease pathogenesis. There is increasing evidence that circRNAs play critical roles in various pathophysiological conditions. Although some circRNAs are very well characterized and considered as important biological molecules for gene regulation, very little is known about their role in β cell function and diabetes. In this section, we review the functional significance of circRNAs in pancreatic β cells.

Cdr1as

Cdr1as (also termed as *ciRS-7*) is generated from antisense

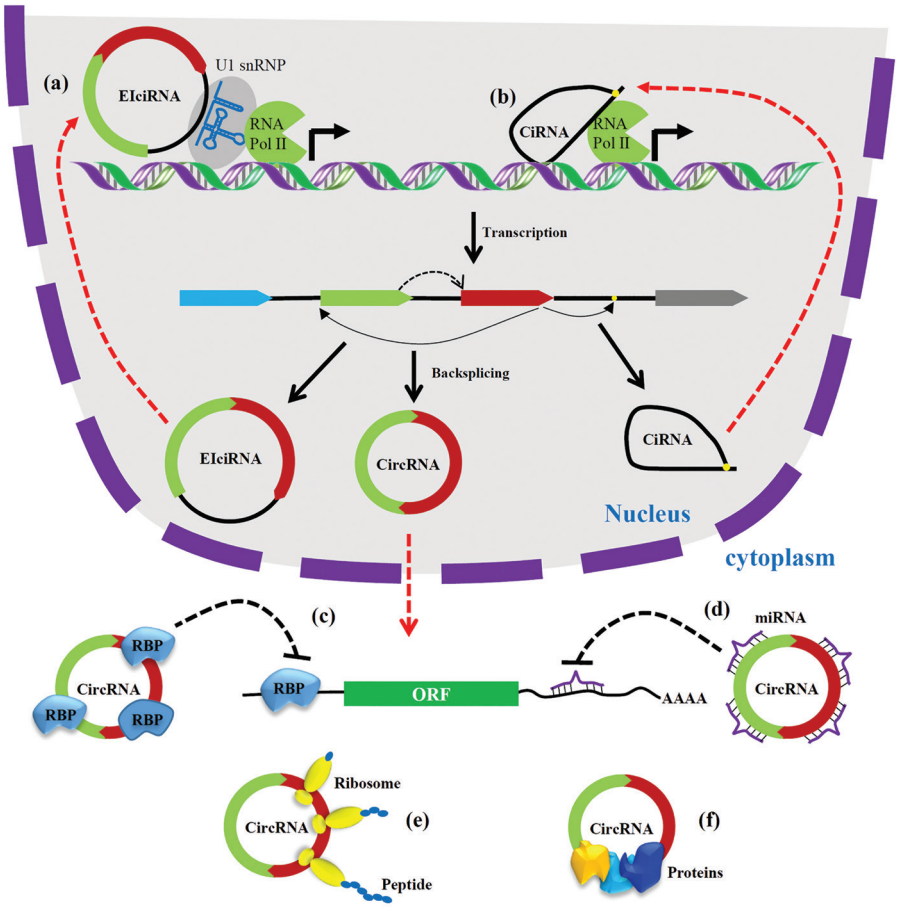

Figure 2 Schematic representation of circRNA biogenesis and functions. CircRNAs are generated from the exons of pre-mRNAs by backsplicing. Backsplicing coupled with splicing removes the intervening introns to generate exonic circRNAs while some exonic circRNAs with retained intron are generated by backsplicing and called EIcircRNAs. Some of the intron lariats generated during canonical splicing escape the debranching process and form stable ciRNAs. (a,b) The EIcircRNAs (a) and ciRNAs (b) are localized in the nucleus and promote the transcription of their parental genes; (c,d) the exonic circRNAs in the cytoplasm can act as a sponge for RBPs (c) and miRNAs (d) to regulate the translation and/or stability of the target mRNA; (e) Some circRNAs contains IRES and ORFs to translate into proteins; (f) CircRNAs can act as a scaffold for protein assembly in the cells regulating the function of the associated proteins.

transcript of cerebellum degeneration-related antigen 1 (CDR1). Cdr1as was one of the first functional circRNAs identified to act as a sponge for miRNA. Interestingly, *Cdr1as* contains more than 60 binding sites for miR-7 leading to inhibition of miR-7 activity (39). *Cdr1as* is upregulated in mouse islets with forskolin and PMA treatment. As miR-7 is known to decrease insulin secretion, upregulation of *Cdr1as* promotes insulin secretion by inhibiting miR-7 activity (99). In fact, the myosin VIIA and Rab interacting protein (Myrip) and paired box 6 (Pax 6) are the direct targets of miR-7 (67). Myrip is known to be involve in secretory granules transportation and release, while Pax 6 is a transcription factor which binds to the promoter of *ins1* and *ins2* genes and leads to enhanced insulin biosynthesis and secretion. Expression of miR-7 inhibits Myrip and Pax 6 expression in mouse islets and MIN6 cells. Interestingly, overexpression of *Cdr1as* upregulates expression of Myrip and Pax 6 resulting in increased insulin transcription as well as increased insulin secretion in islets and MIN6 cells (67). Interestingly, *Cdr1as* expression is downregulated in the islets of db/db and ob/ob mice (68). Silencing of *Cdr1as* decreases the prolactin-stimulated proliferation of rat β cells and MIN6B1 cells. Together, *Cdr1as* regulate insulin biosynthesis and secretion

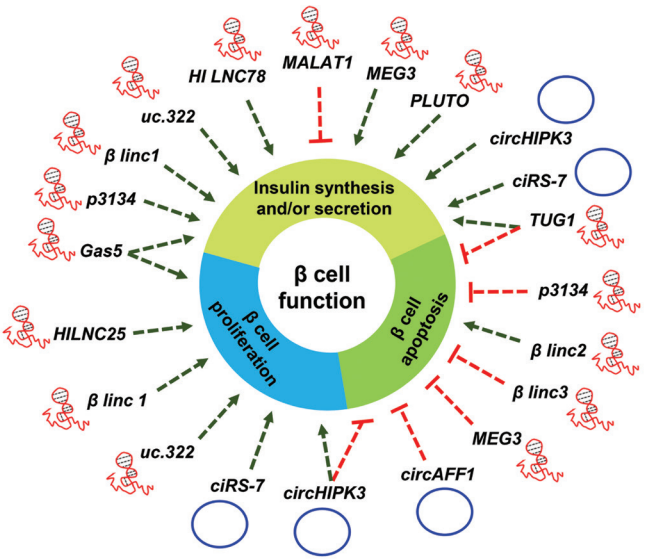

Figure 3 Schematic representation of the role of lncRNAs and circRNAs in β cell function.

by acting as a sponge for miR-7.

circHIPK3

CircHIPK3 is one of the most abundant circRNA expressed in a variety of cells including pancreatic β cells. *CircHIPK3* is generated from the exon2 of *HIPK3* mRNA by backsplicing. Reduction in *circHIPK3* levels promotes apoptosis and reduces proliferation of β cells (68). In fact, prolactin fails to induce proliferation of *circHIPK3* silenced MIN6B1 and β cells. Silencing of this circRNA reduces *insulin* mRNA levels as well as impairs glucose-stimulated insulin secretion. Modulation of *circHIPK3* level impairs the function of the promoter region of insulin in MIN6B1 cells. Additionally, knockdown of *circHIPK3* reduces insulin content in MIN6B1 cells suggesting that *circHIPK3* plays a critical role at the level of translation or post-translation. The effect of *circHIPK3* silencing on β cell function is also partly regulated through sponging miRNAs including miR-124-3p and miR-338-3p (68).

circAFF1

As the name indicates *circAFF1* is produced from the AFF1 gene. *CircAFF1* is one of the abundant circRNA expressed in pancreatic islets of human, mouse and rat as well as in insulin-secreting INS832/13 and MIN6B1 cell lines (68). *CircAFF1* silencing induces apoptotic phenotype in MIN6B1 cells and primary rat β cells similar to apoptosis after treatment with proinflammatory cytokines. In contrast, silencing *circAFF1* in MIN6B1 cells and of primary rat β-cells has no effect on cell proliferation and no effect on insulin biosynthesis or insulin secretion (68).

Concluding remarks and perspectives

In summary, with the development of various genomic technologies, a numerous number of lncRNAs and circRNAs are identified in various organisms. Many of the lncRNAs and circRNAs regulate gene expression by controlling gene transcription, pre-mRNA splicing, mRNA stability, and translation. Increasing pool of data indicates the crucial role of lncRNAs and circRNAs in development and diseases. Recent studies have focused on the use of lncRNAs and circRNAs as biomarkers for detection and prognosis of diseases. Increasing evidence showed that lncRNAs and circRNAs are involved in the pancreatic β cell physiology and development of diabetes. In this review, we discussed the emerging functional roles of lncRNAs and circRNAs associated with the development of diabetes focusing on pancreatic β cell function (*Figure 3*).

Without any doubt, lncRNAs and circRNAs are emerging as critical tools for diseases diagnosis and treatment. However, our current knowledge of lncRNAs and circRNAs in β cell function remains rather limited. Although functional role of various lncRNAs and circRNAs have been characterized, vast majority of lncRNAs and circRNAs remains to be explored in the context of diabetes. To gain insight into function of lncRNAs and circRNAs in development of diabetes, few major issues need to be addressed; (I) development of NGS with computational algorithms for identification and functional analysis is essential; (II) as lncRNAs and circRNAs regulate gene expression by interacting with DNA, RNA, and proteins, computational algorithms and integrated datasets needs to be developed to understand the complex crosstalk of regulatory molecules in gene regulation; (III) we need to develop suitable animal models to study the specific effect of lncRNA and circRNAs in diabetes. With these technological advancements, we expect to gain a deeper understanding of the role of lncRNAs and circRNAs in β cell function which may lead to development of effective therapies for diabetes.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/ncri.2018.11.02). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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