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REVIEW

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Primary Cilium, An Unsung Hero in Maintaining Functional β-cell Population

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A primary challenge in type 2 diabetes (T2D†) is the preservation of a functional population of β-cells, which play a central role in regulating blood glucose levels. Two congenital disorders, Bardet-Biedl syndrome (BBS) and Alström syndrome (ALMS), can serve as useful models to understand how β-cells are normally produced and regenerated. Both are characterized by obesity, loss of β-cells, and defects in primary cilia – the sensory center of cells. Primary cilia are cellular protrusions present in almost every vertebrate cell. This antenna-like organelle plays a crucial role in regulating several signaling pathways that direct proper development, proliferation, and homeostasis. Mutations in genes expressing ciliary proteins or proteins present at or near the base of the cilium lead to disorders, collectively called ciliopathies. BBS and Alström syndrome are such disorders. Though both BBS and Alström patients are obese, their childhood diabetes rates are vastly different, suggesting distinct pathogenesis underlying these two ciliopathies. Clinical studies suggest that BBS patients are protected against early onset diabetes by sustained or enhanced β-cell function. In contrast, Alström patients are more prone to develop diabetes. They have hyperinsulinemia, yet their β-cells fail to sense glucose and to regulate insulin secretion accordingly. These data suggest a potential role for primary cilia in maintaining a functional β-cell population and that defects in cilia or in ciliary proteins impair development and function of β-cells. Identifying the respective roles of primary cilia and ciliary proteins, such as BBS and ALMS1 may shed light on β-cell biology and uncover potentially novel targets for diabetes therapy.

INTRODUCTION

Most nucleated vertebrate cells generate one immotile primary cilium [1]. This organelle acts as a signaling center, regulating a growing list of signal transduction pathways controlling developmental programs, cell proliferation, cell fate determination, and metabolic homeostasis [1]. Molecules involved in signal transduction such as ligands, channels, receptors, transcription factors, and ciliary structural proteins are specifically trafficked to primary cilium in a tightly regulated fashion. Any mutation perturbing the structure or operation of primary cilia can cause havoc on development and organogenesis. It also disrupts the normal functioning of differentiated cells [2]. The disorders of primary cilia and motile cilia are collectively referred to as ciliopathies.

Defects in primary cilia are also implicated in metabolic disorders, such as obesity and type 2 diabetes (T2D) [3,4]. Obesity is linked to T2D due to their association with insulin resistance, a risk factor of T2D. However,

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†Abbreviations: T2D, Type 2 diabetes; BBS, Bardet-Biedl syndrome; ALMS, Alström syndrome; IFT, Intraflagellar transport; FGF, Fibroblast *growth factor*; Wnt, Wingless/Integrated; TGF-β, Transforming growth factor β; NGN3, Neurogenin3; RFX3, Regulatory Factor X3; ALMS1, Alström syndrome protein 1; iPS, induced pluripotent stem.

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about 7.5 percent to 21 percent of T2D patients are lean [5-8], suggesting that diabetes in lean people is independent of obesity and may have other causes, such as anomalies in β-cells that produce insulin.

Successful production of β-cells, either by *in vitro* culture or by stimulation of *in vivo* regeneration, is uncommon, partially due to limited knowledge of the factors required for specification of these cells. Naturally congenital disorders with impaired β-cells, can serve as powerful models to unravel β-cell biology. In particular, disorders in which the onset of diabetes is directly linked to β-cell production and maintenance could be invaluable. Two ciliopathies Alström Syndrome and Bardet-Biedl syndrome represent such disorders. While both are characterized by highly penetrant obesity, they display vastly different childhood diabetes rates (75 percent and 2 to 6 percent respectively) [9-14]. This discrepancy raises the possibility that the incidence of diabetes in these two ciliopathies is independent of obesity and rather due to distinctions in the maintenance of β-cell population or function. Though the exact mechanism of how primary cilia maintain a functional population of β-cells have yet to be elucidated, the scientific community have started to acknowledge its significance at different aspects of β-cells. This review will focus on the current knowledge on roles of primary cilia and ciliopathy proteins in the production and maintenance of functional β-cell population.

PRIMARY CILIUM AND CILIOPATHIES

Animals generate a variety of cilia and the synonymous flagella for a wide array of tasks. Defects in cilia underlie many congenital disorders in human, collectively referred to as ciliopathies. A primary cilium is immotile, supported by an axonemal structural scaffold consisting of nine microtubule doublets (Figure 1). The axoneme is enclosed by the ciliary membrane, that is physically connected to the plasma membrane. The protein composition in these two membranes differs substantially. Ciliary membrane proteins, such as receptors, channels and other molecules involved in the signaling cascade, are selectively transported into the ciliary compartment [15,16]. The axonemal microtubules are nucleated from the basal body, a modified centriole that contains nine triplet microtubules [17,18]. The ciliary pocket, an invagination of plasma membrane, is found at the base of each primary cilium [19]. Ciliary proteins assembled around the basal body are selectively transported into the cilium through the transition zone, which operates as a gate at the ciliary base [20-24]. The transition fibers that connect the distal basal body to the base of the ciliary membrane and the distinct Y-shaped structures between the membrane and axoneme form a diffusion barrier and, along with other proteins, they control the entry of ciliary proteins [25-30]. The movement between the ciliary base and the cilium is commonly called intraflagellar transport (IFT), a bi-directional trafficking mechanism carried out by IFT particles to transport cargoes into and out of the cilium. Anterograde motors, kinesins which are associated with IFT-B complexes are responsible for the tip-ward movement, whereas retrograde dynein motors associated with IFT-A complexes bring the cargo back to the cell body [31,32] (Figure 1).

Ciliopathies resulting from defective primary cilia are numerous, including Bardet-Biedl syndrome, Alström syndrome, polycystic kidney disease, nephronophthisis, Meckel-Gruber syndrome, Joubert syndrome, Senior-Loken syndrome, oral-facial-digital syndrome, Leber congenital amaurosis, and Early-onset severe retinal dystrophy, Jeune syndrome [33,34] (Table 1). Clinical overlap has been observed with mutations in the ciliopathy genes. The involvement of genetically distinct transcripts in multiple ciliopathies is a common feature as these proteins affect the same cellular structure: the primary cilium [33,34].

As primary cilium is present in nearly every nucleated human cell transducing a wide array of stimuli [33], any defect in primary cilium-related genes could result in a spectrum of phenotypes ranging from retinal degeneration, skeletal defects, kidney disease, cognitive disorders, to more specific defects in cellular fate determination during tissue development [34]. The latter is being implicated in regulating energy metabolism, body weight maintenance and glucose homeostasis [3,4]. The review will focus on the role of primary cilium in maintaining glucose homeostasis by regulating a functional pancreatic β-cell mass.

PRIMARY CILIUM IN PANCREAS AND β-CELLS

Pancreas, essential in maintaining glucose homeostasis, comprises exocrine and endocrine compartments. The endocrine pancreatic compartment, known as islets of Langerhans, contains distinct cell types producing different hormones: β-cells for insulin, α-cells for glucagon, δ-cells for somatostatin, ξ-cells for ghrelin, and pancreatic polypeptide cells for pancreatic polypeptides. Primary cilium is present in β, α, and δ-cells as well as in the ductal cells and centroacinar cells of exocrine pancreas [35]. As primary cilia regulate several signaling pathways, such as FGF, Sonic Hedgehog, Wnt, TGF-β, Notch, that are critical for proper pancreatic organogenesis and function, defects in this cellular organelle directly affect pancreas development [35].

Pancreatic β-cells play a central role in maintaining glucose homeostasis. Several studies identified multiple diabetes associated genes, that are involved in main-

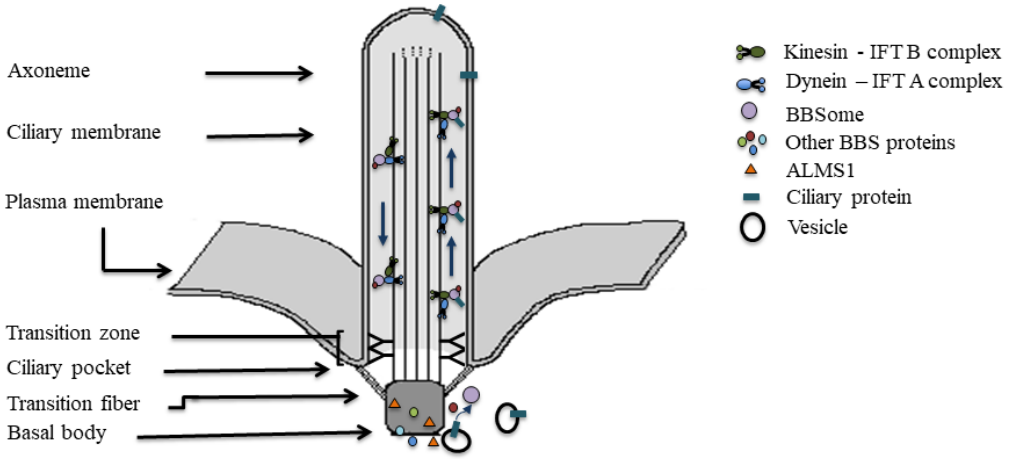

Figure 1. Primary cilium: Structural organization, protein trafficking by IFT and BBSome, and proposed functional localization of ALMS1 and BBS proteins.

taining a functional β-cell population [36-41]. Loss of these cells is a primary cause of progression of diabetes. A significant loss of β-cell differentiation along with increased Shh signaling was observed when primary cilia are specifically ablated in β-cells [42]. The following sections will provide an overview of current knowledge, describing the roles of primary cilia and ciliary proteins in β-cells.

HOW DO PRIMARY CILIA AFFECT INSULIN PRODUCING β-CELLS?

During pancreas development, progenitor cells expressing transcription factor NGN3 specify the fate of endocrine cells [43,44]. These NGN3 positive endocrine precursor cells and differentiated endocrine cells express another transcription factor RFX3, which is necessary for ciliogenesis [45,46]. *Rfx3*$^{-/-}$ mice possess fewer and shortened primary cilia, as well as fewer α, β, ξ-cells during perinatal stages [45,46]. Adult *Rfx3*$^{-/-}$ mice exhibit impaired glucose tolerance, consistent with their characteristic smaller islets, decreased insulin production and reduced glucose stimulated insulin secretion. Given its role in regulating critical developmental signaling, defects in cilia are probably causative to the observed abnormal β-cell development in embryonic (E15.5) *Rfx3*$^{-/-}$ mice [45].

Kinesin family member 3A (KIF3A) is another protein that is necessary for ciliogenesis as well as for intraflagellar transport. When suppressed, its deficiency leads to reduction in the number of primary cilia. This is also correlated with decreased proliferation of β-cells. This observation is consistent in cultured mouse insulinoma β-cells (Min6), dispersed primary mouse islet and human islet cells. [47]. Lack of primary cilia along with decreased proliferation, in *Kif3a* depleted β-cells, provide direct functional evidence for the involvement of cilia in β-cell proliferation. This phenomenon could be explained by the fact that the ciliary disassembly is important in proliferating cells. The assembly and disassembly of primary cilia and lifecycle of centrosomes are tightly linked to cell division [48-50]. The absence of primary cilia could prevent the β-cells from dividing, potentially by disrupting the communication between external signals that trigger β-cell proliferation and internal machineries that lead to cell division. Moreover, this gene is differentially expressed in non-diabetic and T2D human islets. A significantly higher expression is observed in non-diabetic human islets as well as in islets of obese non-diabetic mouse model when compared to the respective diabetic controls [47], further confirming its significance in maintaining functional β-cells.

The B6.V-Lepob/ob (B6-ob/ob) mouse develops severe obesity but is protected from diabetes, whereas the New Zealand Obese (NZO) mouse is obese and is a model for polygenic T2D. Interestingly, these obese mouse models are significantly different from each other in terms of number of primary cilia and expression of cilia-genes including *Kif3a* and genes involved in cell division in pancreatic islets. The diabetic NZO mouse islets are shown to have significantly less primary cilia and reduced expression of cilia-genes when compared to non-diabetic B6-ob/ob. The primary cilia in B6-ob/ob

Table 1. Ciliopathies, causative genes, functional localizations of the proteins in primary cilia and the respective phenotypes of the syndromes.

Ciliopathy	Genes	Ciliary localization	Phenotypes
Alström syndrome [58]	*ALMS1*	Basal body	Truncal Obesity, T2D, Insulin resistance, hepatic dysfunction, hyperlipidemia, hypothyroidism, hypogonadism, short stature, wide feet, retinal degeneration, hearing loss, mental retardation
Bardet-Biedl syndrome [71,93]	*BBS1-20*	Axoneme, Basal body	Obesity, T2D, hypertension, hyperlipidemia, polydactyly, male hypogonadism, retinal dystrophy, renal dysfunction, learning disabilities, cognitive impairment, fatty liver
Autosomal dominant polycystic kidney disease [94,95]	*PKD1*	Axoneme	Renal cyst formation, loss of renal function, abnormalities in cardiovascular, portal, pancreatic and gastrointestinal systems
Nephronophthisis [96]	*NPHP1-9*	Basal body Transition zone, Axoneme	Kidney cyst, tubulointerstitial nephropathy, retinal degeneration, liver fibrosis, cerebellar hypoplasia, *situs inversus*, and mental retardation
Meckel-Gruber syndrome [97]	*MKS1, TMEM216, TMEM67, TMEM231 TMEM138, TMEM237, CEP290, RPGRIP1L, CC2D2A, NPHP3, TCTN2, B9D1 B9D2, EVC2, C5orf42, SEC8*	Basal body, Transition zone, Ciliary membrane	Lethal, cystic renal disease, central nervous system malformation, occipital encephalocele, polydactyly, hepatic fibrosis, polydactyly, *situs inversus,* skeletal defects
Joubert syndrome [98]	*CEP290, CEP120, CEP41, INPP5E, ARL13B, CC2D2A, RPGRIP1L, TMEM216, TMEM67, TMEM237, TMEM231, TMEM138, NPHP1, AHI1, CXORF5, OFD1, TTC21B, KIF7, TCTN1, TCTN3 C5ORF42, ZNF423, CSPP1, ARMC9, FAM149B1*	Basal body, Transition zone	Cerebellum and midbrain abnormalities: molar tooth sign, hypotonia, psychomotor delay, irregular breathing pattern and oculomotor apraxia. developmental delay, truncal ataxia, speech apraxia, polydactyly, chorio-retinal colobomas, retinal degeneration, congenital hepatic fibrosis, fibrocystic kidney disease, cleft palate
Senior-Loken syndrome [99]	*SDCCAG8, NPHP4, NPHP5, NPHP6, WDR19, TRAF3IP1*	Basal body Transition zone, Axoneme	renal nephronophthisis, retinal degeneration, retinitis pigmentosa
Oral-facial-digital syndrome (OFD) [100]	*OFD1-15, two unclassified*	Basal body Transition zone, Axoneme	abnormalities of the face, oral cavity and digits, pancreatic, renal, hepatic, ovarian cysts, cognitive defects
Leber congenital amaurosis and Early-onset severe retinal dystrophy [101]	*GUCY2D, RPE65, SPATA7, AIPL1, LCA5, RPGRIP1, CRX, CRB1, NMNAT1, CEP290, IMPDH1, RD3, RDH12, LRAT, TULP1, KCNJ13, GDF6, OTX2, CABP4, CLUAP1, IQCB1, DTHD1, IFT140, ALMS1, PRPH2*	Basal body, Transition Zone, Axoneme	early infantile onset rod–cone dystrophies, retinal dystrophy
Jeune syndrome [102]	*TCTEX1D2, DYNC2H1, WDR34, WDR60, IFT80, IFT172, IFT144, IFT139, IFT140, CEP120, CSPP1*	Basal body, Axoneme	multiple skeleto-muscular abnormalities, narrow thorax, shortened ribs, variable limb shortening, brachydactyly, polydactyly, renal dysfunction, hepatic dysfunction, retinal dystrophy

islets are able to disassemble in response to high carbohydrate diet and in turn can trigger β-cell proliferation, an important phenomenon that was not evident in NZO mice islets. The lack of primary cilia also correlated with increased apoptosis due to glucose toxicity in NZO mice [47]. An inability to fine tune insulin secretion in response to different carbohydrate concentrations is also observed in NZO mice, but not in B6-ob/ob mice. The observed insulin hypersecretion, even in low glucose conditions, in NZO mice is possibly due to the lack of proper regulation in glucose stimulated insulin secretion as a consequence of reduced primary cilia.

Other ciliary proteins that have been implicated in β-cell development are ALMS1 and BBS proteins. Mutations in these genes lead to autosomal recessive disorders — Alström syndrome and Bardet-Biedl syndromes respectively. They are also categorized as obesity ciliopathies due to highly penetrant childhood obesity [10,12,51-58]. However, they differ in susceptibilities to childhood onset T2D. About 75 percent of Alström patients develop T2D as early as by their first decade, while only 2 to 3 percent of BBS patients develop T2D in childhood. These discrepancies could be explained partly by severe insulin resistance observed in Alström patients [58], a risk factor that is not as apparent in BBS patients [59].

Alström syndrome is caused by loss-of-function mutations in the *ALMS1* gene, which is enriched at the basal body of primary cilia [60] (Table 1). This syndrome is characterized by multi-organ dysfunctions, such as cardiomyopathy, retinal degeneration, fibrosis and renal dysfunction. In addition to that, Alström patients exhibit childhood obesity, severe insulin resistance, hyperinsulinemia, and early onset T2D [58]. *Alms1* is highly expressed in adult and fetal pancreatic islets [61,62] and loss of *Alms1* expression in mutant mice results in pancreatic hyperplasia, partial degranulation of β-cells and islet cysts [63,64]. Clinical reports suggest that the progression of diabetes in Alström patients is not due to further worsening of insulin resistance but as a result of failure in insulin secretion from β-cells [10]. In zebrafish, its absence during development leads to significant loss of β-cells [65], due to both decreased proliferation and increased apoptosis of β-cells specifically without any significant effect on progenitor cell population (Figure 2). These embryos are also unable to regenerate β-cells after specific depletion [65]. An increased β-cell apoptosis correlates with the decrease in the expression of genes involved in RNA and protein processing in *alms1*$^{-/-}$ zebrafish model [66].

An important property of β-cells is to proliferate during increased demand [67]. However, zebrafish β-cells lacking Alms1 do not expand in response to prolonged exposure to glucose [65,66] (Figure 2). Cultured *si-Alms1* β-cells (Min6) fail to alter *Glut2* expression [66], a glucose transporter that also acts as glucose sensor in β-cells of rodents, and subsequent insulin secretion in high glucose condition [68], suggesting a potential involvement of *Alms1* in glucose sensing. Consistent with hyperinsulinemia in patients bearing mutations in *ALMS1* [13,58], *alms1*$^{-/-}$ zebrafish also exhibit hyperinsulinemia in the normal diet, but no further increase in total insulin after exposure to glucose. This observation is further confirmed by the insulin hypersecretion by *Alms1*-depleted Min6 cells at basal glucose concentration [66]. In zebrafish, *alms1* regulates the expression of genes that are involved in cellular transport and secretion, including the expression of Ca^{2+} and K^{+} channels. A significantly increased expression of these genes in *alms1*$^{-/-}$ zebrafish explains the dysregulated insulin secretion in unstimulated conditions [66]. This ciliary protein probably regulates secretion by regulating the expression of genes involved in membrane potential regulation as well as in cAMP production and glucose sensing [66]. These data suggest a role for *ALMS1* gene in both β-cell proliferation and function.

In contrast, BBS patients and mouse models of BBS display elevated insulin sensitivity, but normal glucose management [59,69]. BBS is an autosomal recessive disorder caused by mutations in one of 21 genes [70,71], out of which, eight BBS proteins form a complex, called BBSome. The BBSome is an integral component of basal body and plays crucial role in trafficking membrane-associated cargoes to primary cilium [72,73]. Mutations in these genes result in abnormal structure and functions of primary cilia. Defects in BBS genes have differential effects on insulin secretion. In *Bbs4*$^{-/-}$ mice, the absence of BBS4 leads to generation of β-cells that are inefficient in first phase insulin release, possibly due to deficient signaling through insulin receptor [74], but, downregulation of *Bbs5*, *Bbs7*, and *Bbs9* lead to insulin hypersecretion in Min6 cells [69].

Interestingly, although β-cell proliferation increased, apoptotic β-cell numbers are also significantly higher both in basal and high glucose conditions in BBS models of zebrafish [65]. A decrease in endocrine progenitor cells along with differentiated α- and δ-cells also accompanied the increase in β-cell numbers [65] (Figure 2). These suggest that BBS patients possibly have higher β-cell mass to begin with, in the expense of other differentiated cell types and that the increased proliferation compensates for the increased apoptosis, at least for some time before patients start depleting their β-cell mass. This observation supports the low rate of diabetes susceptibility in BBS patients in the childhood [9,11,13,14].

These findings suggest that different ciliary proteins play different roles in the regulation of β-cells. ALMS1 is a basal body protein [60] (Figure 1) while the BBSome octamer (BBS 1, 2, 4, 5, 7, 8, 9, 18) functions as a cargo

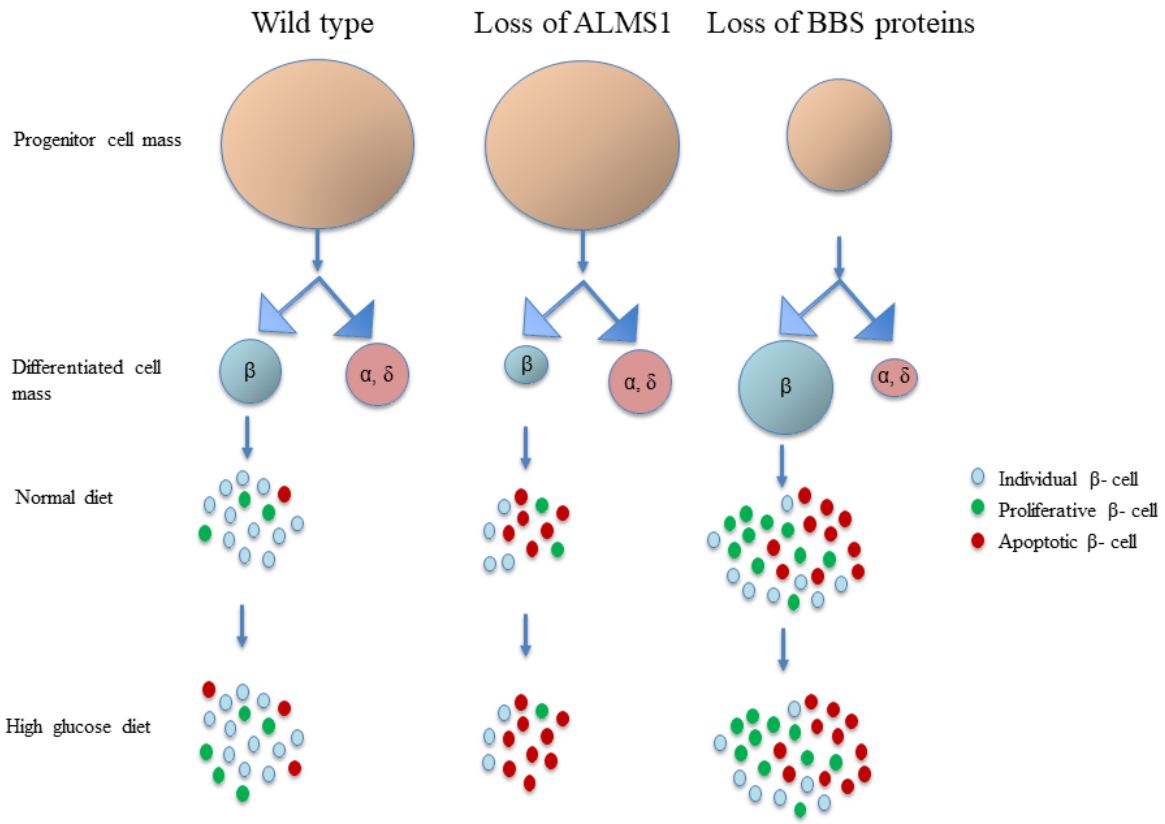

Figure 2. Alström and BBS genes regulate the pancreatic β-cell production. In wild-type pancreas, progenitor cells differentiate into endocrine cell types. β-cells proliferate in the presence of high glucose to meet the increased demand of insulin. Loss of ALMS1 does not have any effect on progenitor cell population but specifically decreases β-cell mass as a result of increased apoptosis and decreased proliferation. Elevated systemic glucose probably decreases β-cell mass due to further increase in the rate of apoptosis. ALMS1 depleted β-cells do not proliferate in high glucose condition. Loss of BBS genes leads to fewer pancreatic progenitors, that produce fewer endocrine α- and δ-cells. An increase in β-cell production is observed at the expense of other differentiated cell types. A compensatory increase in proliferation maintains the high number of β-cells, that are prone to apoptotic cell death in the absence of BBSs. The rate of proliferation and apoptosis remain unchanged and the elevated β-cell mass is maintained in high glucose environment in the absence on BBS proteins.

for anterograde and retrograde transport [75]. Other BBS proteins either form a chaperonin complex (BBS 6, 10, 12) to facilitate the BBSome formation [76,77] or function at the base of the cilium and in the basal body to recruit the BBSome to deliver cargo to ciliary compartment [78] (Figure 1). Different sub-ciliary locations of ALMS1 and BBS proteins as well as their specific functions in those locations allow the primary cilia to maintain a delicate balance in establishing a functional β-cell population.

β-cells are normally arranged as rosettes around capillaries in islets, where the primary cilia are generally found in the lateral surfaces of β-cells. Maintaining this organization of primary cilia, relative to blood vessels, is also an important factor in β-cells [79]. Defects in organization of primary cilia in β-cells, due to the absence of LKB1 protein, a tumor suppressor, impacts the size of β-cells and insulin secretion. *Lkb1*$^{-/-}$ mice have larger β-cells, where unlike wild type mice, primary cilia are not found in lateral surfaces of β-cells. Instead, they are positioned to the cell surface opposite to the blood vessels [79,80]. This change in cellular polarity potentially changes the cellular microenvironment and results in hyperactivation of mTOR pathway [81], leading to the generation of larger β-cells. *Lkb1* deficient mice have an elevated blood glucose level after birth. However, the blood glucose level returns to normal in older pups.

The adult mice tend to have faster clearance of glucose in glucose tolerance tests [82], a phenotype supported by insulin hypersecretion in those knockout mice [81]. Shifting the primary cilia away from the capillaries, where insulin secretion takes place, probably affects the intensity of how primary cilia sense and in turn regulate insulin secretion. Reduced sensing due to altered organization of primary cilia in β-cells could explain the observed insulin hypersecretion in *Lkb1*$^{-/-}$ mice.

As discussed in this review, maintaining the structural integrity and proper organization of primary cilia are important for maintaining a functional population of β-cells. Further investigation on the roles of primary cilia and associated proteins in the specification, proliferation, and functionality of β-cells, will identify the mechanisms contributing to their roles in modulation of signal transduction pathways during specific stages of β-cell specification.

CONCLUSION AND OUTLOOK

Recent studies have started to explore the role of primary cilia in β-cells. However, the direct association of insulin producing β-cells with ciliopathies are, so far, limited to only Bardet-Biedl and Alström syndromes, two ciliopathies that impart discrepant effects on β-cells. These disorders serve as useful models to identify novel factors involved in β-cell production and maintenance. Further investigations are needed to study previously unexplored roles of primary cilia, ciliary proteins and basal body proteins in the specification, maintenance, and regeneration of functional β-cells and to characterize the β-cell phenotype resulting from the loss of gene expression at different stages of development and to elucidate the mechanism by which disruption occurs. The opposing effects on β-cells from depletion of proteins localized to distinct structures central to the primary cilium, suggest the critical yet complicated roles of this organelle in β-cell biology. Therefore, these opposing effects of primary cilia offer a unique opportunity to elucidate the mechanism underlying β-cell production and survival.

Ciliopathy proteins are critical in the developmental program signaled by Sonic hedgehog (Shh), Wnt, and Notch [83-88]. These pathways are also key players at different stages of pancreatic development [89] and are targets in cellular reprogramming of induced pluripotent stem cells (iPS) into insulin producing β-cells [89-92]. Therefore, unraveling the roles of ciliary proteins including ALMS1, BBS proteins and primary cilium will shed critical insight on β-cell specification pathways *in vivo* and *in vitro*. These results will, in turn, identify novel potential targets, such as specific signaling pathways, specific molecules that regulate different aspects of β-cells through this evolutionary conserved organelle, for therapeutic intervention, and for the preservation and regeneration of β-cells.

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