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Proper activation of MafA is required for optimal differentiation and maturation of pancreatic β -cells

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Abstract

A key therapeutic approach for the treatment of Type 1 diabetes (T1D) is transplantation of functional islet β -cells. Despite recent advances in generating stem cell-derived glucose-responsive insulin⁺ cells, their further maturation to fully functional adult β -cells still remains a daunting task. Conquering this hurdle will require a better understanding of the mechanisms driving maturation of embryonic insulin⁺ cells into adult β -cells, and the implementation of that knowledge to improve current differentiation protocols. Here, we will review our current understanding of β -cell maturation, and discuss the contribution of key β -cell transcription factor MafA, to this process. The fundamental importance of MafA in regulating adult β -cell maturation and function indicates that enhancing MafA expression may improve the generation of definitive β -cells for transplantation. Additionally, we suggest that the temporal control of MafA induction at a specific stage of β -cell differentiation will be the next critical challenge for achieving optimum maturation of β -cells.

Keywords

MafA; insulin transcription factor; maturation; differentiation; β -cells; stem cell differentiation

Introduction

The number of people affected with Diabetes has reached pandemic levels. According to the International Diabetes Federation report (sixth edition) an estimated of 382 million people around the world had diabetes in 2013, and by 2035 the disease is projected to affect 592 million people. Diabetes results from a reduction in the functional mass of pancreatic β -cells. In type 2 diabetes, there is reduction in both β -cell function as well as β -cell mass, collectively resulting in insufficient insulin secretion to control blood glucose levels. In type 1 diabetes, autoimmunity destroys most of the β -cells, forcing these individuals to take

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insulin injections to control their blood glucose levels. These observations suggest that restoring functional β -cell mass will be a beneficial therapy for both Type 1 and Type 2 diabetes. However, the absolute reduction in β -cell mass in individuals with Type 1 diabetes makes developing new approaches to regenerate and replace β -cells an urgent need for treatment of the disease.

There are many challenges in developing a “cure” for Type 1 diabetes, including controlling the autoimmune attack on β -cells. However, it is extremely likely that the next generation of therapies for type 1 diabetes will involve replenishing the lost β -cells with functionally mature β -cells, and potentially fully automated closed looped artificial pancreas. In attempt to achieve perfect glucose control, significant efforts, both in academia and industry, are underway to develop strategies aimed at regenerating mature β -cells *in vivo* and *ex vivo*. However, current limitations in preventing or reversing autoimmunity suggests that transplanting functional cells in immune-barrier devices is potentially the best approach for restoring physiologically regulated insulin release to control hyperglycemia in individuals with Type 1 diabetes.

Significant progress has been made over the last decade to generate a reliable source of β -cells for cell-based therapies. Most of this progress was achieved from directed differentiation approaches driving the differentiation of embryonic stem cells (ESc) or induced pluripotent cells (iPSc) into insulin-expressing cells. Several initial attempts lead to cells that acquired some insulin expression but lacked the other characteristics of “true” β -cells (1–4). Initial *in vitro*-directed differentiation protocols, based on the knowledge gained from the study of pancreatic development, generated multi-hormone expressing endocrine cells (5, 6). Transplanting *in vitro*-derived pancreatic progenitors in to mice lead to their maturation into glucose-responsive β -cells (7–9). These studies raised the concern that *in vitro* maturation of β -cell might be difficult, if not impossible. However, two recent studies (10, 11) showed significant progress toward achieving *in vitro* maturation of β -cells. These studies raised the possibility of successfully generating mature β -cells from *in vitro* differentiation of ES/iPS cells for cell-based therapies. We suggest that similar to the role played by an improved understanding of pancreatic development and differentiation of β -cells in the success of stem cells differentiation into insulin-producing cells, a better understanding of β -cell maturation process will be necessary to achieve *in vitro* differentiation of stem cells into “true” mature β -cells. In this review, we will discuss the current understanding of β -cell maturation process, the role of transcription factor MafA in this process, and the need for precise, temporal control of MafA induction in order to obtain mature β -cells.

Role of MafA in maturation and function of pancreatic β -cells

Our cloning of insulin gene transcription factor RIPE3b1, as MafA (12) initiated the systematic examination of the function of Maf factors in the endocrine pancreas. The first member of this family vMaf oncogene was isolated from a spontaneous musculoaponeurotic fibrosarcoma (Maf) in chicken and was shown to be the transforming gene of avian retrovirus AS4219. Maf proteins are subdivided into two classes, “large” [236–370 amino acids (AA); cMaf, MafB, NRL and MafA/L-Maf] and “small” (149–162 AA; MafF, MafG

and MafK) (13–16). All “large Maf” proteins have N-terminal serine/proline/threonine rich acidic activation domains that are absent in “small Maf” proteins (13, 17). Large Maf factors are important regulators of cellular differentiation: cMaf has been implicated in lymphopoiesis and lens development (18–20); loss of MafB function causes segmentation abnormalities in caudal hindbrain, defective inner ear development, abnormal differentiation of kidney podocytes, hematopoietic cells, and pancreatic α - and β -cells (21–25); and quail/chicken MafA/L- Maf is crucial for lens development in the avian system (15, 16, 26). Mammalian MafA is a glucose-responsive basic leucine zipper (bZIP) transcription that binds to the insulin Maf responsive element (MARE) and activates insulin gene expression (12, 27–29). Identification of mammalian MafA as a β -cell specific and glucose-responsive factor led to further characterization of its role in β -cell function (12, 27, 29). Studies conducted over the past decade by several groups strongly support a critical role of this transcription factor in regulating β -cell maturation.

A complete understanding of how embryonic insulin⁺ cell mature and become functional glucose-responsive insulin-secreting cells will likely be required for a full appreciation of the molecular basis of normal β -cell function and how perturbations in these mechanisms leads to the development of β -cell dysfunction during diabetes. Adult pancreatic β -cells are characterized by an exquisite physiological response to extracellular concentrations of glucose. In the adult islet, glucose-stimulated insulin secretion (GSIS) is highly sensitive to levels of glucose and intracellular concentration of Ca^{2+} . When extracellular glucose concentrations are above a basal threshold, glucose is internalized in β -cells through the glucose transporters GLUT2 and metabolized via glucokinase (GK) to increase intracellular ATP/ADP ratio. The closure of K^+ (ATP) channels in response to increased ATP/ADP ratio results in depolarization of the membrane and opening of Ca^{2+} channels. The increase of intracellular Ca^{2+} is the key step that activates insulin granules exocytosis (30–32).

In contrast to β -cells from adult animals, fetal and neonatal β -cells are immature and take several weeks before they respond to glucose like adult β -cells (33). The impaired glucose responsiveness of the neonatal β -cells is likely due to the relative reduction in the key metabolic and insulin secretory machinery in these cells when compared to adult β -cells (34, 35). Navarro-Tableros and colleagues (34) explored the factors that could partially explain the differences in insulin release in neonatal and adult β -cells. They observed that GLUT2 was predominantly expressed in the cytoplasm of neonatal β -cells rather than in the plasma membrane as in the adult β -cells. Interestingly, insulin mRNA, Ca^{2+} current density, and mRNA expression of α_1 subunit Ca^{2+} channel were lower in neonatal β -cells than in adult β -cells. Additionally, while Ca^{2+} channel subunits are mostly expressed in the plasma membrane of adult β -cells, many of these subunits were present in both the cytoplasm and plasma membranes of neonatal β -cells. Interestingly, the neonatal (neogenic) β -cells expressed genes like *MMP2*, *CK-19* and *SPD*, which are normally restricted to pancreatic ductal cells but not adult β -cells (36). These observations suggest that gene expression patterns seen in neonatal β -cells may reflect their recent differentiation from progenitors, and that the time required to turn on and off the appropriate set of genes may dictate the duration of immaturity.

Consistent with the recent differentiation of neonatal β -cells from progenitors, the expression of key β -cell transcription factors is also low in these cells in the first days following birth (37). At postnatal day 2 (P2), expression of key transcription factors such as PDX-1, NeuroD1, NKx6.1 and MafA in the islets from neonatal rat pups is lower than in adults. During the neonatal period, the pattern of MafA expression closely followed that of insulin, and by P21 the level of both genes is still lower compared to adults. In contrast, by P21 expression of several other transcription factors had already achieved their adult levels. Interestingly, although by P21 rat islets have acquired glucose stimulated insulin secretion (GSIS), maximal GSIS is only achieved by 3 months (33), coinciding with the time when MafA and insulin reach their adult levels (37). These observations suggest a dominant role of MafA over other transcription factors in regulating β -cell maturation. Other important metabolic genes such as Glut2, Glucokinase, Glp1r and Pcsk1 were found to be at very low levels at P2, and gradually increased with age (37). Similarly, genes including malate dehydrogenase, glycerol-3-phosphate dehydrogenase, glutamate oxaloacetate transaminase, pyruvate carboxylase and carnitine palmitoyl transferase 2 show reduced expression in the neonatal P2 than P28 β -cells (35), accounting for reduced GSIS by neonatal islets. The reduction in glucose-stimulated insulin release by neonatal β -cells also accompanies a comparable reduction in the glucose-stimulated increase in intracellular Ca^{2+} (38). The lack of glucose responsiveness of the fetal and neonatal islets has also been linked to differences in their architecture. In neonatal islets, gap junction proteins including N-CAM (neural cell adhesion molecule), α - and β -catenins, ZO-1, and F-actin are expressed at lower levels (39). Taken together, these studies show a correlation between increasing MafA expression and enhanced β -cell maturation, as judged by several key parameters including, GSIS, expression of mature β -cell markers and islet architecture.

In addition to its role in β -cell maturation, strong evidence supports a role of MafA as a key regulator of β -cell function. The global MafA Knockout (KO) mice demonstrated a critical requirement of MafA in regulating glucose stimulated insulin secretion *in vivo* (40). Although MafA KO mice displayed a normal islet phenotype at birth, abnormal islet architecture developed with age, and was accompanied by the impaired insulin secretion in response to glucose, arginine and KCl (40). A pancreas-specific deletion of MafA confirmed the importance of this factor in post-natal/adult β -cell function and glucose metabolism (41, 42). By 3 weeks of age, mRNA content of genes important in β -cell function including Insulin (1 and -2), Slc2a2 (known as Glut2), G6pc2, and Slc30a8 (Zinc transporter) are reduced. Interestingly, the first phase of insulin secretion was highly impaired, and insulin content was reduced by 40%, in islets from transgenic mice with pancreas-specific deletion of MafA (42).

MafA KO studies demonstrated a correlation between MafA expression and β -cell function. Several studies tested this correlation by directly examining the effect of enhancing MafA expression on β -cell activity. Wang and colleagues showed that overexpression of MafA in INS-1 cells enhanced GSIS and a number of genes important for glucose metabolism, proinsulin processing, and GLP-1R signaling (43). The expression of Glucokinase, the glucose transporter GLUT2, PDX-1, NKx6.1, GLP-1R, PCSK1 and pyruvate carboxylase (PC) was elevated upon overexpressing MafA. Consistently, overexpression of dominant

negative (DN)-MafA inhibited GSIS and expression of the same metabolic genes that were induced upon the overexpression of wild type MafA. The importance of MafA in β -cell function is further highlighted by the fact that a similar study overexpressing Pdx1, another critical β -cell-enriched transcriptional regulator, did not enhance GSIS (44). Interestingly, overexpression of PDX-1 increased insulin content by 37%, and the overexpressing DN PDX-1 impaired proinsulin processing, GLP-1R expression and cAMP content (44). These observations suggest that PDX-1, like MafA, regulates important indicators of β -cell function, but increasing PDX-1 expression alone (for duration comparable to that of MafA expression) was not sufficient to enhance glucose stimulated insulin secretion.

In addition to β -cell lines, MafA overexpression in islets also improved their function. Overexpression of MafA by 50% in isolated P2 islets, a model of β -cell dysfunction and immaturity due in part to low expression of MafA (10%), resulted in comparable fold-stimulation in GSIS to that observed in adult isolated islets (37). Furthermore, infection of P2 islet cells with MafA overexpressing adenovirus (Ad-MafA) significantly enhanced the expression of several critical genes including Glucokinase, GLP-1R, Neurod1 and Nkx6-1. The enhancement in GSIS in Ad-MafA infected neonatal islets resulted from an increase in the proportion of β -cells that secreted insulin as well as the level of insulin secreted by the individual β -cells. In contrast, overexpression of PDX-1 in neonatal islets for the same duration was unable to stimulate insulin secretion in response to glucose, further emphasizing a dominant role of MafA in regulating GSIS and β -cell function (37).

Consistent with the role of MafA in regulating β -cell function, reduction in MafA levels is also associated with β -cell dysfunction and diabetes in several animal models including: 90% pancreatectomized rats (45), db/db mice (45), Pdx1 heterozygous mice (46), PERK knockout mice (47, 48), ectopic expression of HNF6 (49), Smad7 expression in Pdx1-expressing cells and GDF11-deficient mice (50). More importantly, MafA expression is also decreased in islets from humans with type 2 diabetes (51, 52). These observations suggest that elevating MafA levels in β -cells in diabetic models may contribute to the restoration of β -cell function and reversal of diabetes. More recently, it was demonstrated that transgenic expression of MafA in pancreatic β -cells of diabetic db/db mice successfully reduced hyperglycemia in these animals (53). Increased expression of insulin and Slc2a2 and genes like Gsta1 and Gckr, implicated in decreasing β -cell stress, was also observed in the transgenic db/db animals along with elevations in plasma insulin levels and β -cell mass (53). Notably, the increase in β -cell mass in this study was attributed to decreased apoptosis rather than increased proliferation.

Together, these studies suggest that finding ways to induce MafA expression in immature β -cells, stem cell-derived insulin-producing cells, or dysfunctional β -cells, could lead to their conversion into mature β -cell populations and the amelioration of diabetes.

Strategies to enhance MafA expression and its consequences

Accompanying reviews in this issue highlight the current limitation in generating functionally mature β -cells from stem or progenitor cells, and recent progress that has been made to achieve this objective. We suggest that identification of regulators that induce

MafA expression will be extremely important to enhance functional maturation- and glucose-stimulated insulin secretion- of immature stem cells derived insulin-expressing cells. Indeed, numerous studies have identified different upstream regulators of MafA expression (54–58), and hence it is likely that implementing this knowledge to activate MafA expression may be beneficial to enhance β -cell maturation.

To identify potential extracellular modulators of MafA expression, we hypothesized that physiological regulators that affect the growth and development of neonates, like thyroid hormone, may also mediate the observed induction in MafA expression that is seen during this stage of development. We found that incubation of isolated neonatal islets with triiodothyronine T3 enhanced their maturation (59). Induction of dominant negative MafA in these islets blocked all effects of T3 thereby implicating the role of MafA in directly mediating the activity of T3 on the maturation of islets. Chromatin immunoprecipitation showed binding of thyroid receptors to MafA promoter thereby confirming that T3 directly regulates the expression of MafA (59). It is possible that the ability of T3 to enhance MafA expression, along with the major role of MafA in β -cell maturation, were key considerations in the use of T3 in the recent in vitro stem cell differentiation protocols that successfully generated “mature” insulin⁺ cells (10, 11). In the study by Rezania and colleagues, addition of T3 in combination with other factors during the last stages of differentiation resulted in a higher proportion of cells co-expressing PDX1, NKX6.1, NEUROD1 and insulin, without co-expression of glucagon or somatostatin (Stage 6) (10). Importantly, the expression of MafA was highly induced during stage 7 by the addition of a newly identified small molecule inhibitor of the tyrosine kinase receptor AXL. Further understanding of the mechanisms involved in enhancing β -cell maturation by this inhibitor may provide additional approaches to either enhance MafA expression or suggest a MafA-independent pathway of β -cell maturation. These potentially “mature” β -cells showed glucose-stimulated insulin release and reversed diabetes more rapidly than what was observed upon transplanting pancreatic progenitors in diabetic mice. T3 was also introduced in the final stages of another successful differentiation protocol published recently (11). The results from these studies further highlight the importance of strategies to enhance MafA expression in the terminal maturation steps of β -cells, and suggest that improved understanding of MafA induction and the role of this factor in β -cell maturation will have a significant impact on generating fully functional β -cells from stem cells.

To increase MafA levels in a cell we can also consider strategies to prevent its degradation. Over the past decade, we have gained significant understanding on the regulation of MafA protein stability. Several protein kinases including, glycogen synthase kinase 3 (GSK3), p38 MAPK, and ERK1/2 have been implicated in regulating MafA phosphorylation and degradation (17, 45, 60–65). Of particular interest is p38 MAPK, which is induced during oxidative stress as well as conditions of altered glucose metabolism. We have shown that under oxidative stress, MafA protein is predominantly degraded via the p38 MAPK pathway, as opposed to the GSK3 pathway (65). We suggest that including inhibitors of p38 MAPK, GSK3 and other regulators of MafA stability during the terminal stages of stem cell differentiation will prevent degradation of MafA, thereby enhancing its expression, and driving maturation of these cells closer to those of adult β -cells.

We propose that the impact of enhanced understanding of β -cell maturation pathways on generating “true” β -cells will be similar to the impact that the knowledge of pancreatic development had on the directed differentiation strategies for generating insulin⁺ cells from stem cells. Finding ways to enhance MafA expression in immature β -cells will be crucial for triggering the final maturation steps, however it is likely that enhancing MafA expression alone will not be sufficient. We suggest that a more precise understanding of when and in which cell type MafA is first expressed during β -cell differentiation, i.e., temporal regulation could be key to recapitulating the endogenous β -cell maturation process in the in vitro differentiation approaches focused on generating mature β -cells.

Consequences of altering timing and levels of MafA expression

During embryonic development in mice, MafB is expressed before MafA. The terminal differentiation of pancreatic β -cells proceeds with their transition from Ins⁺ MafB⁺ MafA⁻ state, to cells expressing higher levels of PDX1, to Ins⁺ MafB⁺ MafA⁺ cells, to finally an Ins⁺ MafB⁻ MafA⁺ cell. In adult rodent islets, MafA expression is restricted to β -cells, whereas expression of c-Maf is seen in both alpha and β -cells, while MafB is expressed only in alpha cells (66). After birth, MafA expression is low and it increases gradually with age (37). The increasing levels of MafA in neonatal β -cells, is accompanied by the gradual loss of MafB expression in these cells after birth (41, 66). These results suggest that the induction of MafA expression at a precise time and in a specific differentiation state of insulin⁺ cell might be critical for the normal β -cell maturation process. To test this hypothesis, we used doxycycline inducible MafA transgenic mice to mis-express MafA much earlier than normal during pancreatic development (67, 68). Expression of MafA transgene in PDX1⁺ pancreatic progenitors until embryonic day 12.5 (E12.5) reduced pancreatic mass, proliferation of pancreatic progenitors, and blocked the specification of endocrine cells (68). Additionally, MafA expression in the pancreatic progenitors enhanced the expression of cell cycle inhibitors p27 and p57, potentially explaining the phenotype of these MafA transgenic mice (68). To overcome such detrimental effects of MafA mis-expression at a very early stage of pancreatic development, we examined the consequence of forced MafA expression in endocrine progenitors, and embryonic insulin⁺ cells, using this doxycycline inducible system (67). Induction of MafA in Ngn3⁺ endocrine progenitors inhibited their differentiation into hormone⁺ cells without affecting the actual formation of endocrine progenitors during embryonic development (67). Interestingly, we observed that this MafA-dependent inhibition of endocrine differentiation was reversible. Stopping doxycycline administration, after the stage when endocrine progenitors stop differentiating into alpha cells (E14.5) (69), permitted these hormone-negative progeny of MafA-expressing endocrine progenitors to stop MafA expression and differentiate into all endocrine cell types including alpha cells. Hence, we suggested that this MafA-induced block in endocrine differentiation occurred after the progenitors committed to a specific endocrine fate, but before the induction of hormone expression. Interestingly, mis-expression of MafA in embryonic insulin⁺ cells did not show any detrimental effect on the differentiation of those cells (67). These results suggest that premature induction of MafA expression in pancreatic progenitors, endocrine progenitors, or at any stage before the induction of hormone expression, could be detrimental to the generation of insulin⁺ cells.

Hence, we suggest that the correct timing of MafA induction would be critical for any approach designed to enhance differentiation and maturation of β -cells.

Detrimental consequences of altering the timing of Maf factor induction are not unique to β -cells. Similar consequences of altering the timing of large Maf expression have also been observed in the lens differentiation program. Unlike pancreatic development where MafB expression precedes MafA (66), during lens differentiation MafA is expressed before cMaf, which are both followed by MafB expression (70). In chicken/quail, ectopic expression MafA can direct differentiation of lens fibers from ectodermal cells (15, 16, 26). Induction of MafA in ectodermal cells initiated expression of MafB and c-Maf, while the similar induction of c-Maf enhanced expression of only MafB. In contrast, induction of MafB in ectodermal cells resulted in reduced expression of MafA and c-Maf and altered the lens differentiation program (70). These studies suggested that the precise timing of Maf factor expression is critical for the progression of normal cell differentiation. We propose that improper timing and magnitude of Maf factor expression will have even a greater detrimental effect on the differentiation of β -cells since Maf factors affect both the differentiation and maturation of these cells.

The results from *in vivo* studies on pancreatic development and β -cell maturation suggest a need for precise temporal control of key transcription factor expression. However, several groups have successfully used simultaneous expression of MafA, in combination with PDX-1 and Ngn3 or NeuroD1, to transdifferentiate cells from liver, pancreas, and intestine, amongst others, to generate insulin⁺ cells (71–75). Importantly, some of these studies also showed a modest ability of these insulin⁺ cells to reverse hyperglycemia in diabetic mice, suggesting that these cells did achieve a degree of β -cell maturation. Explanation for this difference in the temporal requirements for the expression of transcription factors during embryonic development and maturation of β -cells, versus transdifferentiation of other adult cell types into insulin⁺ cells is unclear. It is possible that the co-expression of these factors results in differentiation via an alternate mechanism or that these cells are using the normal developmental pathway but not attaining the same level of maturity as adult β -cells.

Interestingly, several studies also suggest the correct combination and order of expression of different transcription factors may still be critical for the optimum differentiation of insulin-expressing cells from other cell types. In one study of acinar transdifferentiation, generation of these cells to insulin⁺ cells showed a preference for MafA over MafB (75). Additionally, a recent study by Nagasaki and colleagues showed that transduction of MafA in combination with PDX1 and Ngn3 in the liver of mouse-insulin promoter luciferase transgenic mice resulted in significant activation of the insulin gene reporter. However, this activity was mitigated when MafB was incorporated in place of MafA (76). It is also worth noting that not all efforts to enhance generation of insulin⁺ cell by forced MafA expression have been successful. Simultaneous mis-expression of MafA, PDX1, and Ngn3 using a polycistronic adenoviral construct showed detrimental effect on reprogramming of pancreatic ductal cells (77). On the other hand, sequential expression of PDX1 before MafA or Ngn3 in these cells enhanced the efficacy of reprogramming insulin-producing cells. These observations support the notion that proper temporal induction of various transcription factors, especially MafA,

would be critical for developing a robust method for generating glucose-responsive insulin positive cells.

It is important to emphasize that this hypothesis implicating the importance of appropriately timed transcription factor MafA expression for the generation of mature β -cells, is primarily based on rodent models of β -cell development and differentiation. Rodent and human islets and β -cells have several important differences. For instance, unlike adult mouse β -cells which only express MafA, human β -cells also contain MafB, which raises the possibility that MafB and MafA may perform similar function in adult human β -cells (52, 78). Hence, it is unclear if the differentiation and maturation of human ESCs and iPSCs derive insulin⁺ cells will be equally sensitive to mistimed induction of MafA. However, the on-going struggle to develop a robust *in vitro* differentiation protocol to generate mature human β -cells with sufficient MafA levels reinforces the idea that appropriate timing and magnitude of MafA expression may prove fundamental to achieving efficient differentiation of human stem cells into mature β -cells. Furthermore, the success in transferring the knowledge from mouse systems to human ESCs and iPSCs differentiation protocols indicates that the regulation of rodent β -cell maturation pathways may also be conserved during maturation of insulin⁺ cells derived from human stem cells. Hence, we suggest that a better understanding of the timing and degree of MafA expression during normal β -cell maturation may prove critical in optimizing protocols for differentiation of β -cells from pluripotent progenitors, and that novel inducers of MafA expression could be important tools toward the efficient generation of definitive, fully functional β -cells for transplantation.

Summary

The need for a reliable source of glucose-responsive β -cells for the treatment of type 1 diabetes is obvious. *In vitro* differentiation of embryonic stem cells and/or iPS cells represents the most likely approach to deliver this source of fully functional β -cells. While significant progress has been made towards differentiating stem cells into insulin⁺ cells over the past decade, the struggle remains in finding ways to transform these insulin⁺ cells into mature adult β -cells. We suggest that part of the difficulty results from our limited understanding of β -cell maturation process. Increasing evidence suggest that transcription factor MafA plays a critical role in regulating β -cell maturation and function (Table 1), and hence appropriately increasing its expression could enhance maturation of β -cells and help overcome β -cell dysfunction. Hence, approaches aimed at augmenting MafA expression during the terminal stages of stem cell differentiation protocols, by modulating expression of its upstream regulators, using physiological inducer of MafA like T3, or preventing degradation of endogenous MafA by inhibitors of protein kinases, may result in further maturation of these insulin⁺ cells. Importantly, animal studies that altered the timing of MafA expression during development demonstrated detrimental consequences on endocrine differentiation. Consequently, in addition to simply activating MafA expression during the differentiation process, determining the precise timing of MafA induction will be critical for the development of a robust differentiation protocol. We suggest that improved understanding of β -cell maturation process will be critical to successful *in vitro* differentiation of stem cells into mature β -cells. However, significant work remains towards determining whether the role MafA plays in supporting β -cell maturation and function in

rodents translates to human systems, and whether its manipulation will ultimately lead to differentiation of human stem cells into functional β -cells to treat Type 1 Diabetes.

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Research agenda

- Detailed understanding of the mechanisms that drive maturation of embryonic insulin⁺ cells into adult pancreatic beta cells will benefit generation of mature β -cells from stem cells.
- Determining the amplitude and timing of MafA expression during β -cell differentiation and maturation will be critical to improve the β -cell maturation efficiency of current differentiation protocols.
- The discovery of additional small- and large- molecule activators of MafA expression and function is needed for large-scale production of mature functional β -cells for cell-based therapy of diabetes.

Table 1

Effects of normal and altered MafA expression on β -cell function and glucose stimulated insulin secretion (GSIS).

MafA expression	Phenotype/Comments	References
<i>Developmental MafA expression</i>		
<u>Embryo</u>		
E12.5/13.5	Induction of MafA expression in a few ins ⁺ cells	41, 66
E15.5-until birth	Presence of Ins ⁺ MafA ⁺ MafB ⁺ , Ins ⁺ MafA ⁻ MafB ⁺ , and a few Ins ⁺ MafA ⁺ MafB ⁻ cells	66
<u>Neonate</u>		
P0–P21	Gradual increase in MafA expression & loss of MafB expression in Ins ⁺ cells	37
P0–P21	Gradual increase in GSIS, and expression of insulin, Glut2, Glp1r, glucokinase, Pcsk1 is enhanced	37
<u>Adult</u>		
~3months	Further increase in GSIS, as well as in expression of insulin and MafA	37
<i>Reduction/Loss of MafA expression</i>		
Dominant negative (DN)-MafA in INS1 cells	Inhibits GSIS and expression of key metabolic genes	43
Animal models of β -cell dysfunction	Reduction in MafA expression correlates with the development of β -cell dysfunction	45–50
Islets from T2D humans	Reduced MafA expression	51, 52
Global & pancreas-specific MafA KO	Reduced β -cell function, 1 st phase insulin secretion, and key regulators of glucose metabolism	40–42
<i>Enhance/Restore MafA expression</i>		
Overexpression of MafA in INS1 cells	Enhanced GSIS and genes regulating insulin synthesis & secretion	43
Adenoviral-mediated MafA expression in neonatal islets	Improved GSIS, increases both in number of cells secreting insulin and the amount of insulin secreted	37
T3 treatment of neonatal islets	Improved GSIS, increases both in number of cells secreting insulin and the amount of insulin secreted	59
T3 treatment of immature insulin ⁺ cells derived from hESC	Improved GSIS	10,11
Enhancing MafA expression in db/db islets	Reduced hyperglycemia, increased plasma insulin levels, β -cell mass, & expression of key β -cell genes	53
<i>MafA Misexpression</i>		
Misexpression in pancreatic progenitors	Inhibits progenitor proliferation, pancreatic size, and β -cell area (until E17.5)	68
Misexpression in endocrine progenitor	Number of endocrine cells unchanged, but they do not induce hormone expression. Differentiation is blocked after progenitors commit to a specific endocrine fate	67