Reprogramming Cells to Make Insulin

Wendy M. McKimpson[1] and Domenico Accili[1]

[1]*Department of Medicine (Endocrinology), Columbia University, New York, New York 10032*

ORCiD numbers: 0000-0002-2262-5998 (W. M. McKimpson).

Type 1 diabetes is a disease characterized by the destruction of insulin-secreting β-cells in the pancreas. Individuals are treated for this disease with lifelong insulin replacement. However, one attractive treatment possibility is to reprogram an individual's endogenous cells to acquire the ability to secrete insulin, essentially replacing destroyed β-cells. Herein, we review the literature on the topic of reprogramming endodermal cells to produce insulin.

The American Diabetes Association estimates that ~1.25 million children and adults in the United States have type 1 diabetes (T1D) [1]. Furthermore, the rates of newly diagnosed individuals with this disease continue to climb, not only in the United States but also worldwide, especially in some Scandinavian countries and Australia [2–4].

There is irreversible destruction of pancreatic β-cells in T1D [3, 5]. As insulin-secreting β-cells are essential to maintain glucose homeostasis, patients with T1D are dependent on exogenously administered insulin to treat their disease [6]. This treatment is not only cumbersome for patients, but it also lacks the sensitivity achieved when glucose homeostasis is controlled by an individual's own β-cells [7–9]. An attractive therapeutic alternative to treat this disease is the replenishment of a patient's supply of β-cells [10].

One way to accomplish this is with the use of treatments that provide a new source of β-cells from either cadaveric islets [11–13] or differentiation of pluripotent stem cells [14–16]. Regarding the former, results from a recent phase 3 clinical trial suggest that it may be possible to control glucose levels in individuals with T1D, complicated by severe hypoglycemia, using this method [13]. However, this therapy is still constrained by the limited supply of cadaveric islets [17]. Whereas differentiation of β-cells from human pluripotent stem cells is possible, it has historically been limited by the ability to generate cells with a sensitive and robust insulin response. However, there have been encouraging advances with this in recent years [18–21]. The use of embryonic stem cells for treatment also requires host immunosuppression. It may be possible to circumvent this need by either the encapsulation of cells [22, 23] or the use of pluripotent stems cells derived directly from T1D patients [24]. Whereas the above-mentioned approaches have merit and show great promise, further discussion of these topics is beyond the scope of this review. The reader is directed to recent reviews for a comprehensive summary of these fields [25–27].

Recently, it has become evident that it is possible to convert an individual's own endogenous endocrine cells, located within the gut, to secrete insulin. As the "β-like" cells generated by this targeted strategy would effectively become a surrogate endocrine pancreas for individuals with T1D, this approach is becoming increasingly attractive as a diabetes

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Abbreviations: GI, gastrointestinal; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; NOD, nonobese diabetic; PMN, Pdx1; MafA, and Ngn3; STZ, streptozotocin; T1D, type 1 diabetes; T2D, type 2 diabetes.

treatment. Herein, we summarize knowledge about the reprogramming of cells to secrete insulin, as well as previous successes to generate gut-derived, insulin-producing cells. We will also address current hurdles in the progression of this field and discuss necessary considerations for the translation of this idea into a successful therapeutic.

1. Location, Location, Location!

Eguchi and Kodama [28] defined transdifferentiation as an irreversible switch of one type of differentiated cell to another. This definition requires that both the starting and ending material, in this case, the beginning cell type and the end-product—an insulin-secreting β-cell—be clearly defined and characterized. For the sake of this review, we will use the word transdifferentiation to refer to the conversion of cells from one distinct lineage commitment into one that correctly synthesizes insulin mRNA, processes these transcripts into functioning protein, and properly regulates the secretion of insulin in response to nutrients and other stimuli. This reprogramming process requires, among other things, a substantial reprogramming of gene-expression programs. Accordingly, transdifferentiation of cells into β-like cells requires activation of genes normally restricted to β-cells, such as those involved in insulin synthesis, maturation, and secretion, and suppression of genes that are normally absent in β-cells.

Previous research has demonstrated the complexity of defining the "best" starting material (*i.e.*, finding cells most susceptible to transdifferentiation) for such a reprogramming event. The developing embryo forms three different germ layers that give rise to all cells in an organism: endoderm, ectoderm, and mesoderm. All epithelial cells that line various organs in the body, including the gastrointestinal (GI) and respiratory tracts, as well as the pancreas, are derived from the endoderm. When one thinks about shifting the developmental program of a cell, it is logical to presume that cells more closely related to pancreatic β-cells would be more susceptible to the process of conversion. Thus, it is not surprising that cell types found experimentally susceptible to conversion are all derived from the endoderm (Fig. 1).
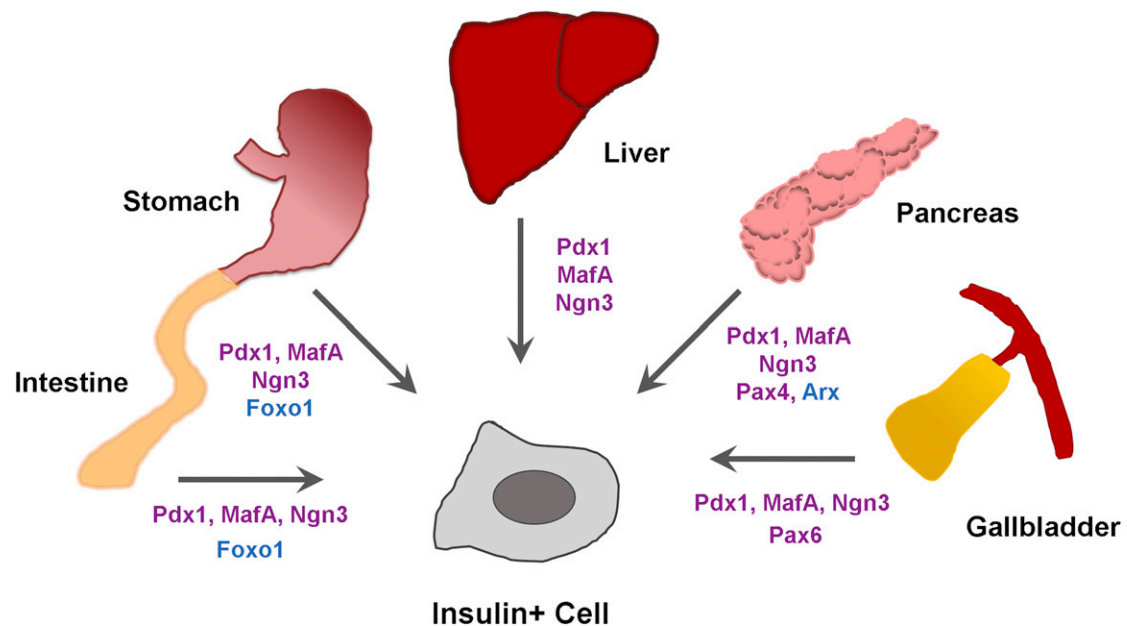

Figure 1. Schematic depicting tissues that can be reprogrammed to produce insulin. It is possible to transdifferentiate cells derived from intestine, stomach, liver, pancreas, and gallbladder into cells that make insulin. In the intestine and stomach, this is accomplished by overexpression of Pdx1, MafA, and Ngn3 (PMN) or ablation of Foxo1. Cells from liver, pancreas, and gallbladder are also transdifferentiated by PMN. Furthermore, Pax4 overexpression or Arx loss triggers this conversion in pancreatic cells, whereas the increase of Pax6 aids in the transdifferentiation of gallbladder cells. Gain-of-function factors are depicted in purple, whereas those requiring loss-of-function are shown in blue.

A. The Gallbladder: A Convenient Source of Cells

From a surgical perspective, the gallbladder is an attractive organ to target, as it is easily accessible by surgery. Whether a cholecystectomy is a reasonable tradeoff to restore insulin production is not for us to say, but theoretically, this represents a source of cells for autotransplantation.

Gallbladder-derived, insulin-producing cells were generated in mice by overexpression of transcription factors Pdx1, MafA, and Ngn3 (PMN; these three transcription factors will, from now on, be collectively referred to as PMN factors) [29]. Whereas this result validated the gallbladder as a potential source for transdifferentiated insulin-producing cells, cells thus generated made 1000-fold less insulin than pancreatic β-cells and did not exhibit glucose-dependent insulin release. Although cellular reprogramming was enhanced by the addition of retinoic acid and inhibition of Notch signaling, shedding light as to pathways important in this conversion process, the level of conversion achieved appears less than with other approaches [29].

In a second iteration, the same research group found that coexpression of another transcription factor (Pax6) with the PMN factors slightly increased efficiency of insulin conversion in mice [30]. Yet, even under these improved conditions, only 25% of cells were converted. Furthermore, insulin+ cells derived from gallbladder were heterogeneous in that they sometimes coexpressed other pancreatic hormones, such as somatostatin and glucagon, in addition to insulin. Interestingly, human-derived gallbladder cells have also been converted to insulin-expressing cells [31], although the potential application of such a technique to a treatment of diabetes is still in the distance. It is also important to keep in mind that once a gallbladder is removed from an individual, the source of cells for conversion is depleted.

B. Transdifferentiation of Liver Cells

An advantage of the use of the liver for generating insulin-producing cells is that cells from this organ have the capability for regeneration. Furthermore, both the pancreas and liver are derived from tissue that budded off of the foregut endoderm, suggesting that these two organs are developmentally related [32]. Similar to the gallbladder, liver cells have also been shown to be capable of differentiating into insulin-secreting cells, in this case, by overexpression of Ngn3 and Pdx1 [33–35]. Conversion of human liver cells is also possible [36].

Whereas many of the early studies simply overexpressed the transcription factor Pdx1 [36, 37], protocols that overexpressed Ngn3 in addition to Pdx1 were, not surprisingly, more effective in achieving cell conversion [38]. Furthermore, viral overexpression of combined PMN in livers of nonobese diabetic (NOD) mice triggered the production of functional insulin+ cells marked by Sox9 expression [39], and whereas insulin+ cells generated by this method have a short lifespan in CD-1 mice, coexpression of a peroxisome proliferator-activated receptor agonist seemed to promote their survival [40]. However, in this study, cells immunoreactive for insulin maintained some of their hepatocyte features. This suggests that transdifferentiation was incomplete. Accordingly, this is an area that needs more studies.

C. The Use of a Close Relative: The Pancreas

Transdifferentiation of cells in the gallbladder or liver requires either a complex protocol (remove tissue from an individual, transdifferentiate cells to produce insulin, and then implant insulin-secreting cells back into the individual) or overexpression of factors using viral constructs. It remains to be seen, as in all of these approaches, whether converted cells will avoid immune destruction. Moreover, the potential for cell transformation and aberrant growth has to be balanced against the relative safety of current insulin-based treatments. A second approach is to convert cells directly in an individual through activation or inhibition of the required factors. Whereas still experimental, these methods have met with some success in the pancreas and gut.

The pancreas can be divided into two major tissue types: the exocrine and endocrine pancreas. The majority of pancreatic mass is exocrine, including acinar and ductal cells. Acinar cells produce digestive enzymes, whereas ductal cells line the pancreatic structures that deliver enzymes to the intestine. Endocrine cells account for ~1% of total pancreatic mass. Similar to endocrine cells in other organs, pancreatic endocrine cells are characterized by the major hormone that they secrete. Thus, β-cells secrete insulin, α-cells secrete glucagon, δ-cells secrete somatostatin, PP-cells secrete pancreatic polypeptide, and ε-cells secrete ghrelin. Together, these cells cluster in "micro-organ" structures to form the islets of Langerhans.

Interestingly, many of the cell types mentioned above are capable of transdifferentiating into cells that make and produce insulin, although the exact methods vary. For example, there have been several studies demonstrating that pancreatic acinar cells [41, 42], ductal cells [43, 44], endocrine α-cells [45, 46], and δ-cells [47] can be converted into insulin-producing cells. However, one caveat is the extent by which these cells are, in fact, reprogrammed and recapitulate the function of native β-cells. In fact, this has not been completely characterized for many of the above-mentioned circumstances. For further in-depth discussion of the conversion of these pancreatic cells into insulin-secreting β-cells, the reader is directed to a recent comprehensive review [10].

D. The Unlimited Potential of the Gut

One of the limiting factors in using the above-mentioned cell types to generate insulin-producing cells is the low conversion rates observed. The average length of the adult intestine is 6 to 7 meters [48]. The intestinal epithelium is composed of a single layer of cells that forms finger-like projections, called "villi," which stretch into the gut lumen. When taking these protrusions into account, the actual surface area of the human gut is 32 m^2 [49]. Given this vast size, it is reasonable to presume that even if only a small portion of these cells was able to convert into insulin-secreting cells, it would still be a sufficient source of cells for the treatment of diabetes.

The field of generating insulin+ cells from enteroendocrine cells began in the early 2000s. Early proof-of-principle studies demonstrated by direct overexpression of the insulin transgene that it was possible to elicit insulin secretion from intestinal cell types. For example, transgenic mice harboring genetically engineered insulin-expressing K-cells, which normally secrete gastric inhibitory peptide (GIP), restored glucose tolerance in mice lacking pancreatic β-cells as a result of administration of the β-cell toxin, streptozotocin (STZ) [50]. Direct overexpression of insulin in L-cells, which secrete glucagon-like peptide 1 (GLP-1), also triggers the generation of cells that properly process and secrete insulin, demonstrating that it is possible to reprogram enteroendocrine cells to mimic β-cells [51].

Some of the transcription factors able to covert insulin+ cells in the gallbladder, liver, and exocrine pancreas were also shown to be important in gut-derived insulin+ cells. In fact, overexpression of each of the PMN factors alone in various intestinal cell types is able to generate cells positive for insulin in this organ. For example, overexpression of Pdx1 in IEC-6 cells, an intestinal crypt cell line, resulted in transdifferentiation into β-like cells [52, 53]. In addition, overexpression of the functional β-cell marker MafA in rat intestinal cells also triggered these cells to produce insulin [54]. Finally, *in vitro* experiments that overexpressed Ngn3 in a K-cell line (derived from the intestinal STC-1 cell line) that stably expressed Nkx6.1 increased insulin gene expression [55]. Although these studies resulted in cells that were immunoreactive for insulin, the cells generated did not acquire markers of mature β-cells—such markers were never assessed—and/or these cells did not show glucose-stimulated insulin secretion.

Many of the above-mentioned studies were performed in intestinal K- and L-cells. These cells secrete GIP and GLP-1, respectively, in response to feeding. Given that insulin is regulated in a similar manner, the ability of these cells to sense nutrients likely gives them an advantage in acquiring the ability to secrete insulin. However, as K- and L-cells are already fully differentiated, it is likely that insulin+ cells derived from these lineages would have a shorter lifespan than cells derived from a progenitor population.

Ngn3+ are endocrine progenitor cells. Whereas both pancreas and GI tract have Ngn3+ cells, they differ in that pancreatic Ngn3+ cells are mainly formed during embryonic development [56], whereas Ngn3+ cells are a continuous source of endocrine cells in the adult gut [57]. In this location, Ngn3 controls the differentiation of progenitor cells into the endocrine cell lineage [58, 59]. Interestingly, Ngn3+ cells in the GI tract have the potential to differentiate into both endocrine and nonendocrine cells [57].

Reprogramming GI Ngn3+ cells to produce insulin has been validated by independent research groups. The ability of Ngn3+ cells or their terminally differentiated daughter cells to secrete insulin was demonstrated by the observation that deletion of Foxo1 in Ngn3+ cells triggers the formation of functional β-like cells that produce insulin in the intestine. Importantly, these cells were capable of alleviating diabetes in mice [60]. Foxo1 is critical to β-cell function [61–63]. Although the function of intestinal Foxo1 remains to be determined, it is interesting to note that the majority (>80%) of Foxo1-expressing cells in the human gut colocalize with serotonin, a marker of enterochromaffin cells [64].

Overexpression of the PMN factors in multiple tissues of a mouse also resulted in insulin+ in the gut, further indicating the propensity of this organ for transdifferentiation [65, 66]. In this model, insulin+ cells were seen twice as often in the stomach than in the intestine, suggesting that stomach cells are more susceptible to conversion [66]. Importantly, these gastric insulin+ cells were also monohormonal and maintained glucose-stimulated insulin secretion, demonstrating that they were indeed differentiated β-like cells. Interestingly, we have recently identified a population of Foxo1+ cells in the stomach that also have the potential to convert into insulin+ cells upon Foxo1 ablation [67]. Although evidence suggests that stomach insulin-producing cells are longer lived than intestinal ones [66], a direct comparison has not yet been performed.

2. Finding the Right Factor

A. Major Players in Gut Reprogramming: PMN and Foxo1

The PMN combination of factors for the generation of β-like cells arose from an initial screen of nine factors [42]. Recent research has suggested that the combination of PMN (Pdx1, MafA, and Ngn3) is important in determining which cells are reprogrammed to secrete insulin. In studies of pancreatic acinar cells, adenoviral overexpression of PMN resulted in conversion to β-cells, whereas δ-like cells arose when Ngn3 was overexpressed alone and α-like cells when Ngn3 and MafA were coexpressed [41]. Consistent with this, removal of Pdx1 in β-cells triggers a phenotypic switch to α-like cells [68]. This suggests that Pdx1 is required, although not sufficient, for β-cell identity. Furthermore, the low levels of Pdx1, MafA, and Nkx6.1 in enteroendocrine cells, compared with insulinoma MIN6 cells, could be a reason why PMN overexpression is so efficient at generating insulin+ cells in the gut [69]. The determination of the best combination of factors will be important to create *bona fide* reprogrammed β-cells.

It is likely that Foxo1 ablation and overexpression of PMN factors target similar molecular pathways when converting cells to become β-like. First, all of the above-mentioned players are important in β-cell development. Prior evidence indicates that Foxo1 lies in the pathway of Ngn3 [70]. Moreover, Foxo1 protects against the failure of β-cells with diabetes by upregulating MafA [71], and Foxo1 has been linked to regulation of β-cell growth through Pdx1 [72]. Thus, the identification of shared effectors of PMN and Foxo1 can provide new mechanisms of β-cell conversion.

B. Other Participants in Reprogramming

Signaling cues that happen during the development of β-cells may also shed light on factors important to the transdifferentiation process. Interestingly, there is a large overlap in the developmental programs that control pancreas and gut development [73]. Given that Notch and Wnt signaling pathways are important in formation of these organs, it is not surprising that components of these pathways have been implicated in reprogramming β-cells. For

example, suppression of delta-notch signaling in combination with Ngn3 expression synergistically transdifferentiated pancreatic ductal cells into β-like cells [74]. In the gut, Foxo1 ablation represses the Notch signaling component of Hes1 [60], although this was not recapitulated in human organoids [64]. Furthermore, active Wnt signaling is necessary for liver cells to convert into insulin-producing cells [75].

Other developmental factors that have been shown to transdifferentiate β-cells include transcription factors Pax4 and Arx. Specifically in the pancreas, overexpression of Pax4 in α- and δ-cells convert them into cells that are β-like in nature [76, 77]. Likewise, loss of Arx in α-cells also triggers their formation into β-like cells [78–80]. It has also been suggested that methylation may be important in the conversion of insulin-producing cells. In cells induced by PMN, promoter demethylation may be the mechanism by which cells acquire the ability to secrete insulin [65]. In support of this, methylation of the insulin promoter is also a key difference in β-cell and enteroendocrine cell lines [69]. Interestingly, pancreatic α-cells can also be converted into β-cells through loss of the DNA methyltransferase Dnmt1 [80].

3. Developing Therapeutics

There is evidence to support that the gut can be a source of insulin+ cells. This idea gained traction from a study demonstrating that PMN factors overexpressed ectopically in various tissues, including exocrine pancreas, gallbladder, spleen, intestine, and skin (but not liver), gave rise to insulin+ cells in the intestine [65]. Interestingly, overexpression of PMN in Ngn3+ cells results in conversion of these cells to insulin+ at a rate of ∼50% [65]. One potential advantage of the use of the GI tract as a site of transdifferentiation is its "immune privilege" [81]. For example, gut lymphoid tissue makes gut-specific immune cells that do not circulate systemically.

In individuals with T1D, the majority of their native insulin-secreting cells has been destroyed by the immune system. Interestingly, a population of β-cells resistant to destruction by immune cells in NOD mice and human β-cells has recently been detected [82]. It is likely that gut-derived, insulin-secreting cells may be able to evade an immune response. There is some evidence to support this, as gut-insulin+ cells created by transgenic overexpression of preproinsulin (an unprocessed form of insulin) in intestinal K-cells escape immune detection in NOD mice [83]. Furthermore, the insulin generated from these programmed K-cells alleviated diabetes in these mice without disruption of intestinal function. As the majority of other *in vivo* studies reprogramming insulin+ cells in the intestinal tract have used STZ injections as a mouse model of diabetes, research in autoimmune models will be needed to validate that newly formed β-like cells can withstand immune destruction.

An outstanding question in developing therapies is how best to target cells in the GI tract. Several possibilities come to mind. First, it may be possible to transplant individuals with insulin-secreting cells derived on GI scaffolds. Although this method has had some success in mice [66], it is still experimental with regard to use in humans. Another approach is to drive cell conversion with small molecules. This approach does not lend itself to PMN, as a result of the involvement of multiple proteins, but is attractive with regard to inhibition of a single factor, as would be the case for the blocking of Foxo1. Importantly, it is possible to inhibit Foxo1 activity with molecular compounds [84, 85].

Regardless of the approach, one of the current hurdles in the translation of the transdifferentiation of cells into a viable therapy is the low conversion rate of insulin-secreting cells. For example, when Foxo1 is ablated in Ngn3+ cells of the intestine, only ∼4% of endocrine cells becomes positive for insulin [60]. Whereas this rate is slightly increased with PMN, it is still less than one out of every five Ngn3+ cells that are successfully converted [66]. Future studies that elucidate a clear pathway(s) by which cells transverse from gut to β-like will be necessary to design strategies that more specifically and effectively target cells for transdifferentiation.

4. How Do Reprogrammed Cells Impact the Local Environment?

Although the reprogramming of cells to produce insulin is an attractive possibility for diabetes therapy, it is important to consider possible off-target effects of such a treatment. The gut is the largest endocrine system in an individual [86]. Accordingly, the enteroendocrine cells located in the GI tract compose a diverse array of highly specialized cells that produce >30 different hormones [87]. Whereas many of the hormones secreted from these cells control GI motility and secretion, some also have important roles in the regulation of insulin secretion and control of glucose homeostasis.

Named by its ability to decrease gastric acid secretion [88], GIP is secreted from K-cells in the intestine to stimulate the secretion of insulin from β-cells [89]. Secreted by enteroendocrine L-cells, the incretin GLP-1 also stimulates the secretion of insulin upon glucose stimulation [90–92]. GLP-1 also acts to prevent gastric emptying and decrease food intake [93]. Together, GLP-1 and GIP account for the additional insulin secreted in an individual in response to oral vs intravenous stimulation by glucose, otherwise known as the incretin effect [94, 95]. Interestingly, both of the above-mentioned hormones also prevent the death of pancreatic β-cells and stimulate the proliferation of these cells [96–100].

There are also hormones produced by the gut that govern proper glucose homeostasis. Examples of such hormones include cholecystokinin, gastrin, and ghrelin [101]. Cholecystokinin is produced by I-cells, mostly located in the proximal gut, where its release suppresses appetite and aids in the digestion of fat and protein by stimulating the release of digestion enzymes from the pancreas and bile from the gallbladder [102]. A second hormone, gastrin, is secreted by enteroendocrine G-cells located in the stomach and proximal small intestine and stimulates parietal cells to release gastric acid. Both of these hormones can also stimulate glucagon release from islets and positively regulate β-cell proliferation [103–105]. Interestingly, ghrelin also has effects on pancreatic β-cells through protection [106] and prevention of insulin secretion [107], in addition to its effects on peripheral glucose use [108].

These cells are important regulators of metabolism, but it is less clear how they are affected by diabetes. Interestingly, there have been some changes in the number and distribution of enteroendocrine cell populations with obesity and diabetes. For example, there are differences in the abundance of K- and L-cells in human gut biopsies from healthy individuals and those with type 2 diabetes (T2D) [109]. L-Cells are also functionally altered in mice fed a high-fat diet [110]. Cells located in the stomach may also be altered with diabetes. For example, there is an increased prevalence of autoantibodies directed toward gastric acid-secreting parietal cells in patients with T1D [111, 112]. In fact STZ, a commonly used pancreatic β-cell toxin, also leads to decreased function of parietal cells [113]. However, the exact changes and the consequences of diabetes on cells in the GI tract remain to be elucidated.

There is also modulation of enteroendocrine cell populations with improvements in glucose homeostasis. For example, individuals that have undergone Roux-en-Y gastric bypass surgery have altered populations of enteroendocrine cells (including K-, L-, and I-cells) after surgery [114]. Many studies also demonstrate that bariatric surgery has effects on the improvement of insulin sensitivity beyond loss of adipose tissue [115, 116].

If there are changes to enteroendocrine cells in diabetes, this is more likely to occur in T2D rather than in T1D and thus, should not interfere with development of gut-based cell-conversion treatments. Increased numbers of intestinal Ngn3+ and chromogranin A+ cells have been seen in mice harboring Foxo1 deletion [60]. The expression of these markers is also increased in human intestinal organoids when Foxo1 is suppressed [64]. The above changes are not surprising, given that Foxo1 is involved in controlling the differentiation of many different cell types, including myoblasts [117], osteoblasts [118], neurons [119], and adipocytes [120]. These possibilities should be addressed by means of further basic research.

5. Search Strategies

For this article, we completed an extensive literature search of papers that conducted research on reprogramming cells to produce insulin. To do this, we used PubMed to search for

articles with the following key terms: cell reprogramming, transdifferentiation, β-cell, GI tract, intestine, stomach, liver, gallbladder, pancreas, enteroendocrine cells, pluripotent stem cells, insulin, diabetes treatment, T1D and T2D. Selection of the papers presented in this article was determined by scientific relevance and research methodology.

Acknowledgments

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