Pancreatic ductal cell heterogeneity: insights into the potential for β-cell regeneration in diabetes

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Abstract

Diabetes mellitus is a significant and fast-growing health problem worldwide. Cost, donor shortages, and immune rejection limit current treatment strategies. While considerable progress has been made in creating β-cells *in vitro* with remarkable morphological and functional resemblance to those in primary pancreatic islets, exploring alternative sources for β-cell replacement is crucial. With adult pancreatic stem cells still not conclusively identified, researchers focus their attention on heterogeneity within pancreatic ductal epithelial cells, exploring these cells as a potential source of progenitor cells for pancreatic regeneration and β-cell formation. Recent studies using techniques such as fluorescence-activated cell sorting, immunostaining and single cell RNA-sequencing have identified ductal cell heterogeneity with several subpopulations of ductal cells with progenitor-like properties and their capacity for differentiation into insulin producing cells. Here, we have reviewed the most recent studies on pancreatic ductal cell subpopulations that offer insights into potential stem-cell populations to form β-cells in diabetes treatment.

Keywords: Diabetes, pancreatic ductal heterogeneity, stem/progenitor cells, stem cell differentiation, β-cell regeneration, pancreatic endocrine cell differentiation.

Introduction

Diabetes is a growing global health concern. According to the World Health Organization (WHO), 422 million adults worldwide have diabetes, and 1.5 million deaths occur annually directly from the disease [1]. Additionally, the prevalence rate is expected to increase by 46% as the International Diabetes Federation (IDF) projects that 1 in 8 (738 million) adults will have diabetes by 2045 [2]. In addition to the widespread prevalence, complications from long-term diabetes include an increased risk of cardiovascular disease, coronary artery disease, stroke, kidney failure, and eye and nerve damage [3, 4].

Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated blood glucose levels. Endocrine β-cells are located in the islet of Langerhans in the pancreas and are responsible for the production and release of insulin, which regulates blood glucose levels. The main types of diabetes are type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes [4]. T1D is an autoimmune disease in which the β-cells are destroyed by the immune system, causing a lack of insulin production, whereas T2D is associated with insulin resistance and its increased demand for insulin that the body can no longer match [3]. Thus, in both T1D and late-stage T2D, there is an insufficient mass of β-cells.

The current treatments for DM include non-invasive pharmacological interventions, such as insulin sensitizers, glucagon-like peptide-1 (GLP1) receptor agonists, and invasive approaches, such as pancreas and islet transplantation, and insulin injections, each of which has its limitations [5]. Insulin sensitizers, such as metformin and thiazolidinediones, improve insulin sensitivity but are associated with gastrointestinal discomfort, weight gain, and an increased risk of heart failure in some patients [5]. GLP-1 receptor agonists enhance glycemic control and promote weight loss [5, 6]; however, their use is restricted by gastrointestinal side effects, and

risk of gallbladder disease [6]. Insulin therapy, while essential for glucose regulation, is hindered by its high cost and the need for multiple daily injections, leading to poor compliance and inadequate glycemic control [5, 7–11]. Despite the life-saving capabilities of insulin therapy, it does not provide a cure for diabetes [5, 7]. Another treatment is pancreas or islet transplantation, which offers a more long-term solution as affected patients would receive functional islets restoring insulin independence through the presence of active β-cells. However, due to the significant shortage of donors and the need for life-long immunosuppressive treatment, this treatment is not a viable option for most patients [8–13]. Despite their shortcomings, current management for DM primarily relies on costly insulin injections or pancreatic islet cell transplantation, highlighting the need for alternative solutions [14]. One promising alternative treatment is the generation of β-cells derived from pluripotent stem cells [15–17].

Compelling evidence has demonstrated the *in vitro* differentiation of pluripotent stem cells, embryonic stem cells, and induced pluripotent stem cells (iPSC) into insulin-producing β-cells [15–18]. Presently, the generation of functional β-cells is in progress, and clinical trials are being conducted, with promising outcomes observed [19]. One promising clinical trial by Vertex Pharmaceuticals uses VX-880, an investigational allogeneic stem cell that is a fully differentiated, insulin-producing islet cell therapy for patients with T1D [20]. Results demonstrated that nearly all patients reduced or eliminated exogenous use of insulin following the treatment [20]. Although the use of stem cells is providing encouraging results, other alternative approaches need to be explored [21].

Another approach could involve inducing the generation of beta cells *in vivo* either through stimulating beta cell proliferation or differentiation of new β-cells (neogenesis) within the pancreas. Growing evidence suggests that β-cell neogenesis can occur in adult pancreases,

with cells within the ductal tree shown to differentiate into new β-cells under specific conditions [22, 23]. Given this potential, identifying and targeting stem cells in the pancreas would be an essential and valuable therapeutic approach for diabetes and pancreas regeneration to induce β-cells in the organ itself [24]. This would have an immense impact on patient therapy, especially for individuals with T2D, as they compose the majority of individuals with diabetes. However, the existence of stem/progenitor cells within the pancreas remains a subject of controversy. Pancreatic duct epithelial cells have long been suggested as a source of progenitors for pancreatic growth and regeneration [24–29]. However, lineage-tracing experiments using pancreatic duct-specific Cre expression have resulted in conflicting results [26, 30–33]. One possible explanation for these contradictory results is the heterogeneity among pancreatic ductal cells [27, 34, 35]. This paper aims to review primary studies exploring the heterogeneity of pancreatic ductal cells and their potential as progenitor cells in endocrine differentiation in diabetes, offering insights into new therapeutic avenues for β-cell regeneration. This paper examines over ten studies from the past eight years (2016-2024), emphasizing on findings from recent years. The selected papers were primary studies that focused on studying pancreatic ductal heterogeneity in diabetes using novel and high throughput approaches such as RNA sequencing (RNAseq), flow cytometry and organoid colony formation assays.

Non-ductal cells as potential pancreatic stem cells

The pancreas has two major components: the exocrine pancreas, consisting of acinar cells that produce digestive enzymes, as well as the ductal tree that serves as the plumbing to deliver the enzymes to the intestine, and the endocrine pancreas, consisting of clusters or micro-organs of five main endocrine cell types called the islets of Langerhans. Within the islets, various cell

types are present, including α-cells, β-cells, δ-cells, pancreatic polypeptide cells, and ε-cells that function by secreting glucagon, insulin, somatostatin (SST), pancreatic polypeptide hormones, and ghrelin, respectively, contributing to the regulation of metabolic processes [36–39].

A brief overview of research suggests that certain cells within the islets of Langerhans, particularly α-cells, may have progenitor-like properties and the potential to differentiate into new β-cells [40–43]. Wang and colleagues identified protein C receptor-positive (Procr+) cells located in pancreatic islets as endocrine progenitors capable of forming islet-like organoids and differentiating into functional β-cells [44]. Therefore, identifying progenitor-like cells among these populations could suggest the presence of cells that could be mobilized to replenish β-cell mass in response to injury or disease [45].

Additionally, exocrine function, driven by acinar cells, has recently been emphasized for its potential regenerative capabilities [36, 46]. Recent studies using fate maps have highlighted significant plasticity and regenerative potential of specific subpopulations of acinar progenitor-like cells, drawing attention to the locations of acinar cells expressing progenitor markers within the pancreas and their capacity for proliferation [46, 47]. This includes the identification of β-cell specific Moloney leukemia virus insertion site 1 (Bmi1) as a marker for a subpopulation of acinar cells that can self-renew and proliferate during tissue homeostasis post-injury, suggesting that regeneration is a feature that is not exclusive to undifferentiated stem cells [47, 48]. The transdifferentiation of acinar cells into β-cells remains a viable approach for β-cell replacement therapy [45]. Acinar cells can also undergo transdifferentiation into duct cells through a process called acinar ductal metaplasia, and depending on the microenvironment, they also have the potential to transdifferentiate into adipocytes and hepatocyte-like cells, highlighting their regenerative potential [45, 49, 50]. While there is considerable evidence for differentiation

from non-islet cell progenitors (neogenesis)[36], ductal cells have been suggested as the most probable source of pancreatic progenitor cell population [37].

Pancreatic ductal cells as a source of progenitors

Ductal cells (terminal or intercalated) form the epithelial lining of the pancreatic duct and deliver exocrine enzymes to the duodenum, and produce bicarbonate to neutralize stomach acidity [51]. During development, ductal cells express transcription factors required for pancreatic ductal formation and maturation [52]. The adult ductal cells share properties with primitive embryonic ducts and can maintain the ability to generate endocrine cells [51]. Among the populations of cells in the pancreas, ductal cells have been suggested as one of the most likely sources of progenitor/stem cells in the pancreas as described earlier [22, 26, 26, 34, 39, 53, 54]. This is supported by observations of endocrine cell formation in or near ducts, shared progenitors during development, and duct-specific lineage tracing experiments [53]. Recent single-cell transcriptomics and chromatin accessibility analyses refine models of pancreatic endocrine development and differentiation, reveal that *Neurogenin 3* (*Neurog3)* is expressed in high levels in a cluster of endocrine progenitors and show transcriptional heterogeneity among Neurog3^{pos} cells. These results suggest that *Neurog3* activation facilitates the transition of ductal cells into endocrine progenitors while challenging the necessity of bipotent progenitors in this process. This shows that transcription factors follow regulatory patterns in gene expression throughout the duct to progenitor differentiation process in endocrine lineages [52]. Additionally, the pancreatic duct glands (PDGs) function as a progenitor niche, serving as the primary site for epithelial regeneration and giving rise to differentiated progeny that migrate to populate and regenerate the pancreatic ductal epithelium [56]. Single-cell analyses, including those by Duvall

et al. [55], further delineate transcriptional trajectories and regulatory mechanisms underlying endocrine progenitor differentiation, enhancing our understanding of endocrine pancreas development in ductal epithelial cells. Complementing these findings, Rovira and colleagues isolated a subset of centroacinar and terminal duct (CA/TD) epithelial cells that express high levels of aldehyde dehydrogenase 1 (ALDH1) and were enriched in markers associated with pancreatic progenitor cell populations [57]. Furthermore, fluorescence activated cell-sorted (FACS) CA/TD cells formed self-renewing "pancreatospheres" in culture, leading to endocrine and exocrine differentiation of cells, as well as insulin secretion. These findings highlight the capacity for a subset of CA/TD cells to contain progenitor functions and be recruited for pancreatic regeneration [57].

Moreover, under conditions of metabolic stress or insulin resistance, ductal cells demonstrate notable plasticity, acquiring the ability to differentiate into insulin-secreting β-cells as a compensatory response to increased insulin demand [54]. In a liver-specific insulin receptor knockout (LIRKO) mouse model, Dirice et al. [54] observed β-cell mass expansion during pregnancy, with ductal cells contributing to this process by differentiating into functional β-cells. However, while these findings highlight the potential of ductal cell plasticity in β-cell expansion, most evidence remains limited to animal models, with limited human studies corroborating similar results [35, 54]. This emphasizes the need for further studies using human pancreatic tissue to determine and validate whether targeting heterogeneous ductal populations can effectively promote β-cell expansion in diabetic conditions in humans [54].

Pancreatic ductal cell heterogeneity

A notable body of research has emerged in recent years highlighting the heterogeneity among pancreatic ductal cells. As such, this section examines key findings from the most recent primary literature, highlighting distinct subpopulations, variation in gene expression patterns, differentiation potential, and functional plasticity within the ductal cell population [22, 27, 34, 35, 58–62]. For instance, Jin et al. [27] identified and characterized a subpopulation of ductal cells with progenitor-like properties in the adult murine pancreas, providing compelling evidence of the heterogeneity within pancreatic ductal cells. This subpopulation, marked by high surface expression of CD133 and low surface expression of CD71 (CD133highCD71low), exhibited significant tripotent colony-forming and differentiation potential. Through flow cytometry, they isolated CD133+ ductal cells and divided them into three major subpopulations: CD133+CD71-, CD133highCD71low, and CD133lowCD71low cells (Table 1). This differentiation potential was confirmed through immunofluorescence staining, which brought forth the presence of markers specific to each lineage within the colonies formed by these cells: KRT19 for ductal cells, amylase for acinar cells, and insulin for endocrine cells [27]. Additionally, self-renewal assays indicated that CD133highCD71low cells retained their colony-forming ability and differentiation potential over multiple passages, suggesting self-renewal properties [27]. This self-renewal capacity, coupled with the tripotent differentiation potential, underscores the critical role of these cells as a distinct progenitor-like subpopulation within the pancreatic ductal epithelium that could contribute to the regenerative potential of the pancreas. Moreover, quantitative RT-PCR and RNA-sequencing (RNA-seq) pathway analysis revealed that the CD133+CD71- and CD133highCD71low subpopulations expressed higher levels of ductal genes (KRKT19, KRT7,

and SOX9) and PDX1 and displayed tendencies for cell migration and immunological response, suggesting functional heterogeneity within the ductal cell population [27].

Similarly, in another study assessing gene expression profiles and colony formation abilities, Rezanejad et al. [34] identified subpopulations of murine ductal cells with different levels of HNF1β and SOX9, revealing dynamic expression patterns. Notably, $CD24^{high}$ subpopulations, marked by high HNF1β and SOX9 levels, showed greater organoid/sphere formation and proliferation. These cells also exhibited higher mRNA levels for HNF1β, SOX9, and HNF6 (Fig. 1). Organoids from these subpopulations were differentiated into β-like cells, suggesting that specific ductal cells can act as progenitor cells capable of endocrine differentiation [34].

Using carbonic anhydrase II (CAII) as a marker of differentiated ductal cells, Inada et al. demonstrated that after birth, $CAII^{+}$ expressing cells acted as progenitors and formed new islets in transgenic mice [26]. However, in adult human pancreas, Qadir et al. [29] reported that not all ductal cells express CAII, as both $CAII^{+}$ and $CAII^{-}$ cells were present among pancreatic ductal cells and that $PDX1^{+}/ALK3^{+}/CAII^{-}$ cells had progenitor-like properties and could differentiate into β-like cells. With FACS analysis, they identified $P2RY1^{+}/ALK3^{bright+}$ cells as the progenitor cell population and that transient BMP-7 treatment promoted cell growth and differentiation into pancreatic lineages [29]. Further studies of single-cell RNA-sequencing (scRNAseq) of sorted ALK3bright+ cells revealed a heterogeneous expression of $ALK3^{bright+}$ cells within the pancreatic ducts [58]. Specifically, one cluster of cells was characterized by high expression of PDX1, ID1-2 and -4, CTNND1, and CEACAM6, markers of a progenitor phenotype. Other clusters showed different markers indicating multiple lineages of ductal cells, including progenitors, mature ductal cells, and acinar cells. Furthermore, $P2RY1^{+}/ALK3^{bright+}$ sorted cells were

transplanted into hormone-negative cells and activity was induced with the treatment of a receptor agonist for ALK3, THR-123 [58]. The results showed that P2RY1^{+}/ALK3$^{bright+}$ cells differentiated into INS-secreting cells, indicative of functional β-cells. Additionally, based on the subpopulations of ductal cells, results revealed the presence of different axes of cells capable of differentiation, including a pro-ductal, pro-acinar, and ducto-endocrine axis. Thus, Qadir and colleagues provided evidence to highlight the heterogeneity of ductal cells within the pancreas, as well as suggesting that a subpopulation of ductal cells within the adult human pancreas possessed progenitor-like properties and was capable of differentiation to insulin-expressing β-cells [29, 58].

Further supporting the heterogeneity of pancreatic ductal cells, Hendley et al. conducted a scRNAseq analysis of murine ductal cells, identifying several diverse subpopulations of ductal cells (Table 1) [59]. This experiment involved using DBA+ lectin to sort and isolate all pancreatic ductal cell types. The scRNAseq analyses identified distinct clusters (subpopulations) of ductal cells, each varying in the expression of ductal cell markers. Employing a Uniform Manifold Approximation and Projection (UMAP) graph to reduce large datasets of gene expression counts to a two- or three-dimensional plot, the largest pancreatic ductal cell subpopulation exhibited an upregulation of genes related to pancreatic ductal cell function, such as secreted phosphoprotein 1 (Spp1), and contained progenitor capacity. However, other clusters expressed different genes or signaling pathways, such as those associated with cystic fibrosis transmembrane conductance regulator (CFTR) or calcium signaling for bicarbonate secretion, cilia biogenesis for intrapancreatic bile duct cells, and transcriptional regulation in pancreatobiliary ductal cells. The data from the comparison of specific clusters using cell type and lineage analysis demonstrated progenitor-like properties and indicated several different

stages of cell differentiation. This evidence is consistent with that observed by Qadir et al. [58] that progenitor-like cells within the ductal cell population exhibit no lineage restrictions [59].

To assess the capacity for ductal cells to redifferentiate into endocrine cells, SPP1 was knocked out in an immortalized human pancreatic duct cell line (HPDE6c7). Results indicated increased expression of pancreatic markers compared to the gRNA control, suggesting a cell phenotype with progenitor-like functions. Additionally, there was decreased expression of markers in mature ductal cells (HNF1β, SOX9, and KRT19) in the HPDE6c7 SPP1 KO, indicating that SPP1 is involved in the maturation and function of pancreatic ductal cells in humans. Furthermore, they demonstrated that HPDE6c7 SPP1 KO cells initiated the epithelial-to-mesenchymal transition (EMT) axis of ductal cells, as indicated by the reduction in ductal markers and increased EMT genes. These data suggest that the loss of SPP1 can lead to ductal cell dedifferentiation in humans [59]. Hendley and colleagues classified six subpopulations (Table 1) and identified a functional role of heterogeneity within pancreatic ductal cell populations and their progenitor capacity [59].

In another study, Quijano et al. [63] provided evidence that adult human pancreatic ducts contain progenitor-like cells (PCFUs) capable of self-renewing and demonstrating multi-lineage differentiation potential through *in vitro* assays. Using scRNA-seq, this study showed that functional heterogeneity is present within the sorted human pancreatic cell population (CD133+CD49f^{low}), revealing four distinct ductal cell subpopulations. Furthermore, results show the expression of PDX1, SOX9, and NKX6.1 progenitor markers in colonies and adult human ducts *in vivo*. These results highlight a subpopulation of ductal cells capable of expressing progenitor markers and potentially using human ductal tissue for pancreas regeneration [63].

Furthermore, Fernández et al. [60] utilized scRNA sequencing to assess the heterogeneity of the entire pancreatic ductal cell population in a SOX9:GFP transgenic mouse model. Initially, they determined that ductal cells have different abilities to form organoids. Organoids from medium-big ducts exhibited lower expression levels of Sox9 and Spp1 ductal markers, while Hnf1β expression remained relatively consistent across organoids from all duct sizes, highlighting the heterogeneity in the ductal cell population. Furthermore, medium and larger duct-derived organoids demonstrated the capacity for endocrine differentiation, as indicated by increased expression (mRNA and protein) of transcription factors *Neurog3* and *NeuroD1* [60]. Additionally, through scRNAseq and FACS isolation, fifteen ductal subpopulations were identified and found to be heterogeneous (Table 1)[60]. Notably, within the PDG, the Wnt-responsive population of ductal cells exhibited a high level of exocrine regeneration, and the Flrt-3 population demonstrated a high capacity for differentiation into insulin-producing cells. Their results indicate that heterogeneity exists within the pancreatic ductal cells and that specific ductal subpopulations with progenitor-like properties can lead to β-cell differentiation and regeneration.

Recently, zebrafish have been used by Mi et al. [62] as a model to provide insight into the mechanisms of β-cell regeneration. Evidence from Mi et al. indicates that a population of ductal cells expressing *krt4*, with tp1-driven Notch-signaling ductal cells, function as progenitor cells that generate endocrine cells postembryonic development [62]. Moreover, results demonstrate ductal heterogeneity in the pancreas of zebrafish, where krt4^{+} extrapancreatic, intermediate and luminal ducts serve as progenitor cells. This study expands on the lineage path of differentiation, highlighting that ductal cells expressing the dlb and hes6 regulator play a role in endocrine precursors and de-differentiation dynamics [62].

Building on the understanding of ductal cell heterogeneity and its implications for β-cell regeneration, recent research has further elucidated the mechanisms by which ductal cells contribute to β-cell neogenesis. Gribben et al. [64] employed advanced lineage tracing and single-cell RNA sequencing to reveal that distinct subpopulations of ductal cells, specifically those expressing Ngn3, can differentiate into new β-cells within the pancreas of adult mice. This neogenic process involves a transitional phase where Ngn3+ cells co-express somatostatin (SST+) and insulin (INS+), indicating a critical stage in their progression towards becoming functional β-cells [23, 64]. The ability of these cells to switch fates under specific conditions, such as metabolic stress or injury, highlights the functional heterogeneity of the pancreatic ductal cell population [23, 64]. Furthermore, these findings suggest that targeting specific heterogeneous ductal cell populations could significantly enhance β-cell mass and improve glucose homeostasis, particularly in diabetic contexts. This offers a promising avenue for developing novel regenerative therapies for diabetes. However, Magenheim et al. [65] expressed concern over findings from Gribben et al. [64], challenging the study's claim that ductal progenitors expressing Ngn3+ differentiate and lead to β-cell neogenesis in the adult pancreas. Instead, their evidence demonstrates that Neurog3 mRNA is expressed in a higher proportion in δ cells within pancreatic islets compared to β-cells. This provides contradictory evidence for whether β-cell originates from a ductal origin [65]. Therefore, Gribben and colleagues responded to Magenheim et al. [65], indicating that the use of a high-efficiency lineage-tracing approach in the Ngn3-creERT line reveals ductal populations that express Ngn3+ and diabetes mice show an increase in endocrine progenitors within the duct [64–66]. This evidence highlights that controversy is still present within lineage-tracing strategies.

Conclusion

The potential plasticity of pancreatic ductal cells in the adult pancreas continues to remain controversial among researchers in the field. In the developing embryonic pancreas, both endocrine and exocrine cells emerge from the pancreatic ducts, originating from PDX1/NGN3-expressing progenitor cells. This embryonic foundation has led scientists to speculate that the proximity of newly formed β cells to ductal structures indicates a possible interconversion process, wherein ductal cells give rise to functional β cells [53].

Altogether, there is consistent evidence indicating the existence of heterogeneity among ductal cells, and specific clusters of ductal cells demonstrate progenitor-like characteristics [27, 34, 58–60, 63]. There is a shared conclusion that cells expressing high levels of SOX9, HNF1β, PDX1, and Ngn3/Neurog3 among specific subpopulations have the capacity for endocrine differentiation into functional β cells, compared to low levels, highlighting the functionality of heterogeneity among ductal subpopulations. However, studies have examined different ductal cell markers to distinguish ductal cell subpopulations. Therefore, it remains unknown whether subpopulations with specific markers used in FACS lead to higher capacities for ductal to endocrine differentiation among progenitor subpopulations.

Investigations conducted in murine models have demonstrated that under conditions of stress, certain adult ductal cells can adopt a progenitor-like identity, exhibiting a remarkable degree of plasticity [28, 54] (Fig. 1). Such plasticity raises the question of whether ductal cells can indeed transdifferentiate into β-cells, a concept that has generated considerable debate within the scientific community. While some studies have provided evidence supporting this hypothesis, lineage tracing experiments have yielded conflicting results, complicating our understanding of

the origins of β-cells. These discrepancies are further compounded by concerns regarding the reliability of lineage tracing techniques themselves, which may introduce biases or inaccuracies in tracking cell fate over time [67]. However, we previously suggested that the heterogeneity within pancreatic ductal cells could explain this discrepancy [34].

Based on the studies reviewed in the current literature on pancreatic ductal heterogeneity, there is significant evidence of heterogeneity within pancreatic ductal cells and the existence of progenitor/stem cell subpopulations in different organisms, potentially contributing to β-cell regeneration. The identification of diverse pancreatic ductal cell subpopulations with progenitor-like properties underscores their potential to differentiate into insulin-producing β-cells, offering promising avenues for developing innovative treatments [68, 69]. Emerging evidence further suggests that these heterogeneous ductal cell populations may possess the capacity to enhance β-cell mass and improve glucose homeostasis, thereby supporting their therapeutic potential.

These findings emphasize the necessity of understanding the heterogeneity within ductal cell subpopulations as defined by distinct markers, gene expression profiles, and signaling pathways. Regenerative approaches emerging from this understanding may provide alternatives to traditional therapies that rely on external insulin administration or invasive transplantation methods, potentially addressing some of the current limitations and challenges in diabetes management [70]. By identifying a true stem cell population within the pancreas and enhancing the body's innate ability to regenerate pancreatic tissue, these therapies could pave the way for more sustainable and effective diabetes management strategies. However, it is important to consider that for T1D, the problem of autoimmune destruction of newly formed β-cells and invasive nature of β-cell transplants remains [70]. Additionally, most studies reveal a limited

number of newly differentiated β-cells in adults, raising concerns about the efficiency of these approaches in clinical settings [70].

This limitation highlights the need to identify more reliable sources of β-cell regeneration, particularly within pancreatic ductal cell populations. In this context, determining whether a true pancreatic stem cell population exists and understanding its role in β-cell regeneration could provide valuable insights for future studies. Further investigation into its characteristics may help refine *in vitro* differentiation protocols while also informing the development of drug or treatment strategies aimed at inducing β-cell regeneration *in vivo*. This could lead to more effective patient treatments, shifting diabetes management toward regenerative approaches rather than long-term insulin dependence.

Future research should intensify its focus on elucidating the mechanisms controlling and guiding pancreatic ductal cell differentiation and proliferation to fully harness their potential in regenerative medicine. One promising approach is the study of the transcriptome of purified human pancreatic ductal cells using techniques such as RNAseq. By analyzing gene expression profiles, researchers could identify specific signaling pathways responsible for the heterogeneity of these cells. A deeper understanding of these pathways and differential gene expression patterns may uncover potential therapeutic targets that can modulate ductal cell behaviour and promote β-cell regeneration, thus creating new opportunities for diabetes treatment. For example, research conducted by Al-Hassani et al. [68], isolated ductal cells from juvenile and adult human pancreatic tissue samples and inhibited EZH2, a histone methyltransferase gene to target ductal cells to form a β-cell identity. This study highlights the reprogramming or targeting of ductal cells at a genetic level where differentiated cells are glucose responsive and can lead to insulin secretion [68]. Therefore, therapies targeting ductal cell subpopulations in the pancreas are a vital

pathway for modulating pancreatic ductal cell differentiation and restoring β-cell mass and function in T1D patients.

Additionally, emerging evidence suggests that 3D culture models, such as organoids/spheres, may offer a more physiologically relevant environment for studying pancreatic ductal cells, as they preserve ductal characteristics better than traditional 2D culture methods [71–73]. Using 3D systems would allow for the expansion of knowledge regarding the regulatory and signaling mechanisms in the environment that lead to the differentiation of ductal cells, as well as differences that exist in the functionality of β-cells through activation of different ductal progenitor subpopulations. This shift towards the use of more advanced *in vitro* models could reveal previously hidden aspects of ductal cell biology, such as their plasticity, epithelial-mesenchymal transition, and polarity, which are crucial not only for understanding diabetes but also for their underexplored role in diseases like pancreatic cancer. Furthermore, human pancreatic slices can be used as a model to study the pathogenesis of diabetes and physiological conditions or processes at the organ level [54, 74, 75]. This novel technique would provide an understanding of the cellular mechanisms in endocrine tissue, organ environments, and single-cell heterogeneity [75], such as ductal cells. Therefore, this is a model that can be used to circumvent challenges faced when using mice models for lineage tracing, as previously mentioned. Exploring this potential further may provide insights into ductal cell behaviour and their regenerative capacity in health and disease. By leveraging these models, the development of non-invasive treatments that utilize the body's ability to regenerate β-cells could offer a novel approach to managing diabetes.

Abbreviations

DM: Diabetes mellitus
T1D: Type 1 diabetes
T2D: Type 2 diabetes
GLP1: Glucagon-like-peptide-1
iPSC: Induced pluripotent stem cells
Bmi1: B-cell specific Moloney leukemia virus insertion site 1
PDGs: Pancreatic duct glands
CA/TD: Centroacinar and terminal duct
ALDH1: Aldehyde dehydrogenase 1
FACS: Fluorescence-activated cell sorting
LIRKO: Liver-specific insulin receptor knockout
CD133: Prominin-1
CD71: Cluster of differentiation 71
RT-PCR: Reverse transcription polymerase chain reaction
RNA-seq: Ribonucleic acid sequencing
KRT19: Keratin 19
KRT7: Cytokeratin-7 or keratin-7
SOX9: SRY-box transcription factor 9
PDX1: Pancreatic and duodenal homeobox 1
HNF1β: Hepatocyte nuclear factor-1 beta
CD24: Cluster of differentiation 24
mRNA: messenger RNA
CAII: Carbonic anhydrase 2
ALK3: Activin receptor-like kinase 3
BMP-7: Bone morphogenic protein-7
ID1-2: Inhibitor of differentiation and DNA binding
ID1-4: Inhibitor of differentiation and DNA binding
CTNND1: Catenin delta-1
CEACAM6: Carcinoembryonic antigen-related cell adhesion molecule 6
scRNAseq: Single-cell RNA-sequencing
THR-123: Peptide agonist of ALK3
DBA: Dolichos biflorus agglutinin
UMAP: Uniform manifold approximation and projection
SPP1: Secreted phosphoprotein 1
CFTR: Cystic fibrosis transmembrane conductance regulator
gRNA: Guide RNA
HPDE6c7: Human pancreatic duct epithelial cell line
EMT: Epithelial to mesenchymal transition
GFP: Green fluorescent protein

Neurog3: Neurogenin 3
NeuroD1: Neurogenic differentiation 1
Ngn3: Neurogenin 3
SST: Somatostatin
INS: Insulin
PCFUs: Progenitor-like cells from the adult human pancreatic ducts

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Table 1 A summary of studies that identify and characterize pancreatic ductal cell subpopulations and their regenerative potential

Author, Year	**Number of Subpopulations (Species)**	**Subpopulations Identified**	**Biological Significance**
Jin et al. 2016 [27]	Three subpopulations (Mouse)	$CD133^{+}CD71^{-}$ and $CD133^{high}CD71^{low}$ and $CD133^{low}CD71^{low}$	Pancreatic ductal cell subpopulations were identified to have progenitor-like features and tripotent differentiation potential into ductal, acinar, and endocrine lineages. This highlights their role in pancreatic regeneration and their potential for developing regenerative therapies. [34]
Rezanejad et al. 2018 [34]	Two subpopulations (Mouse and human)	$HNF1\beta^{low}$ and $SOX9^{low}$ and $HNF1\beta^{high}$ and $SOX9^{high}$	They reported heterogeneity in the expression of HNF1β and SOX9 in pancreatic ductal cells. Organoids derived from $HNF1\beta^{high}SOX9^{high}$ cells can differentiate into islet cells, highlighting the potential for ductal cells as a source of progenitor cells.

Qadir et al. 2020 [58]	Seven subpopulations (human)	Stress/harboring progenitor-like cells, activated/migrating progenitor cells, small ducts, centroacinar, transitional to acinar (2), and immune cells.	A ductal subpopulation of $ALK3^{bright+}$ sorted cells ($PDX1^{+}/ALK3^{+}/CAII^{-}$) contain progenitor-like properties and could be activated pharmacologically as a therapy for diabetes to produce functional β-cells.[59]
Hendley et al. 2021 [59]	Six subpopulations (mouse)	Pancreatic duct cells (3), intrapancreatic bile duct cells, pancreatobiliary cells, and cycling duct cells.	Subpopulations reveal heterogeneous expression of ductal cell markers, highlighting variability in ductal cell lineage and function by activating ductal cell regulators, such as Spp1.
Fernández et al. 2024 [60]	Fifteen subpopulation (mouse)		Expansive analysis of ductal cell heterogeneity, revealing novel subpopulations. Results show diverse roles of ductal populations in pancreatic regeneration, differentiation, and disease pathogenesis.

Zook et al. 2024 [72]	Seven subpopulations (human)		Using a six soluble factor (6F) suspension culture, they reported that 6F allows for self-renewal and tri-lineage differentiation of ductal progenitor cells. Single-cell RNA-sequencing reveals that six subpopulations of 6F cells exhibited a ductal phenotype, as indicated by the expression of ductal markers, as well as multipotent progenitor markers (SOX9, PDX1, and NKX6.1). Results support the potential of ductal cells to act as progenitor cells capable of differentiating into insulin-producing β-cells, and the presence of non-progenitor cells, reflecting a heterogenous ductal cell population.

***Note**: The findings presented in this table are derived from studies conducted by the respective authors mentioned.*

Fig. 1

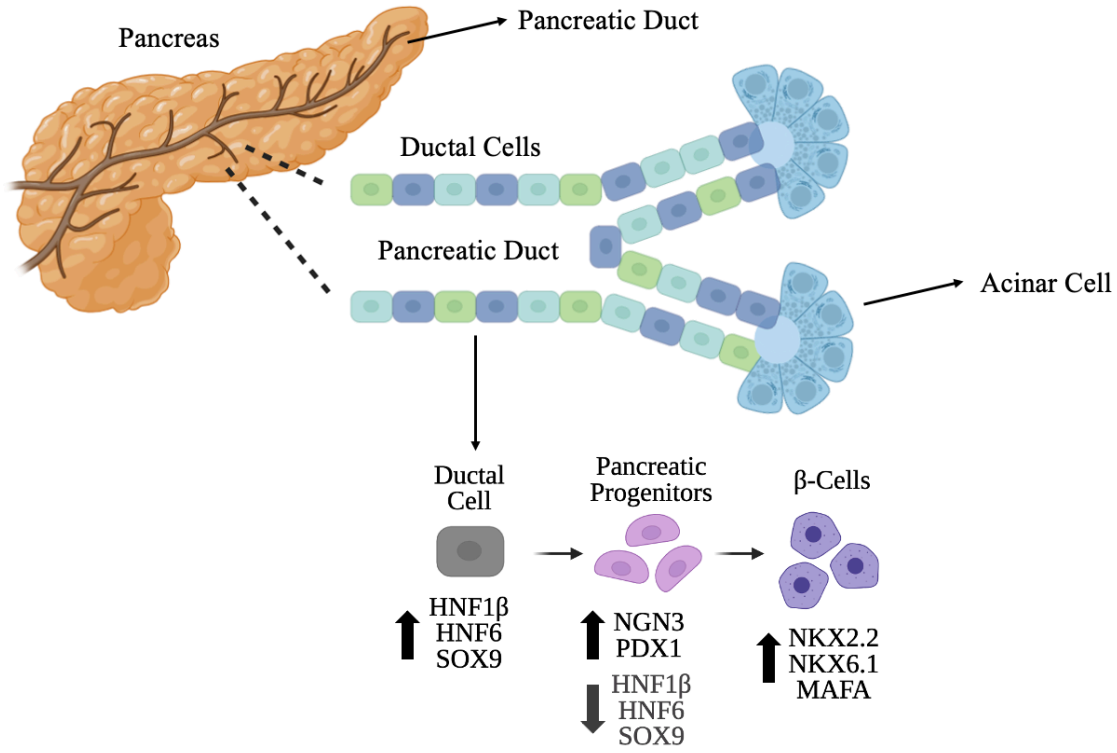
Pancreas
Pancreatic Duct
Ductal Cells
Pancreatic Duct
Acinar Cell
Ductal Cell
Pancreatic Progenitors
β-Cells
HNF1β
HNF6
SOX9
NGN3
PDX1
HNF1β
HNF6
SOX9
NKX2.2
NKX6.1
MAFA

Fig. 1 Schematic representation of pancreatic ductal cell subpopulations (green, teal, dark blue), and proposed mechanism for their transdifferentiation into beta cells. Ductal cells (grey) show high expression levels of ductal markers (HNF1β, HNF6, and SOX9). The ductal markers decrease as ductal cells dedifferentiate into pancreatic progenitor cells (pink), seen by an increase in NGN3 and PDX1 expression levels. Then, pancreatic progenitors differentiate into β-cells (purple), marked by high expression levels of NKX2.2, NKX6.1, and MAFA. *Figure created in BioRender and Powerpoint* [76]