GATA factors in pancreas development and disease

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Abstract
There is an urgent need for the development of novel therapeutics options for diabetic patients given the high prevalence of diabetes worldwide and that, currently, there is no cure for this disease. The transplantation of pancreatic islets that contain insulin-producing cells is a promising therapeutic alternative, particularly for type 1 diabetes. However, the shortage of organ donors constitutes a major limitation for this approach; thus, developing alternative sources of insulin-producing cells is of critical importance. In the last decade, our knowledge of the molecular mechanisms controlling embryonic pancreas development has significantly advanced. More importantly, this knowledge has provided the basis for the in vitro generation of insulin-producing cells from stem cells. Recent studies have revealed that GATA transcription factors are involved in various stages of pancreas formation and in the adult β cell function. Here, we review the fundamental role of GATA transcription factors in pancreas morphogenesis and their association with congenital diseases associated with pancreas.

KEYWORDS
β cell, cellular glucose metabolism, diabetes, pancreatic islet cells, pancreatic transcription factors

1 | INTRODUCTION TO PANCREAS DEVELOPMENT

The mammalian pancreas is a mixed exocrine and endocrine organ. The exocrine tissue is composed of clusters of acinar cells that produce and secrete digestive enzymes through a network of ductal cells. The endocrine pancreas is organized in clusters of hormone-producing cells called islets of Langerhans. The islets of Langerhans are scattered throughout the exocrine compartment and produce hormones that
regulate glucose homeostasis. The pancreatic islets contain five types of endocrine cells: glucagon-producing α cells, insulin-producing β cells, somatostatin-producing δ cells, pancreatic polypeptide-producing cells, and ghrelin-producing ε cells.

Due to the difficulty of obtaining human pancreatic samples at embryonic stages, our understanding of pancreas development derives mainly from mouse studies. However, morphogenesis events and key regulators of pancreas organogenesis appear to be greatly conserved between human and mouse, as recently reviewed.1 Pancreas formation can be separated into three major stages. In the mouse, the primary transition occurs between embryonic stage (E) 8.5 and E12.5 and includes the formation of the pancreatic rudiments from the endodermal primitive gut tube and specification of the different pancreatic cell types. During the secondary transition (E12.5–E15.5), the pancreatic epithelium undergoes extensive expansion and branching concomitant with the differentiation of acinar, ductal, and endocrine cells. The third phase of pancreas formation takes place after E15.5 with further expansion and maturation that continues postnatally (Figure 1).

In the mouse, the pancreas originates from a discrete region of the endodermal primitive gut tube forming a dorsal and a ventral evagination (bud) starting around E8.5.2,3 The formation of the pancreatic buds is dictated by signals from their adjacent tissues, such as the notochord, cardiac mesoderm, and aortic endothelium. Some of the extrinsic signals controlling pancreas specification have been identified and include, among others, fibroblast growth factor (FGF), bone morphogenetic protein, retinoic acid (RA), and Hedgehog.4 Interestingly, the dorsal pancreatic and ventral pancreatic

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**FIGURE 1** Overview of morphogenic events and key regulators of mouse pancreas development. Pancreas specification occurs at embryonic stage (E) 8.5 in discrete regions of the foregut endoderm that escape from Hedgehog (Hh) inhibitory signals. The early pancreatic epithelium is formed of multipotent pancreatic progenitor cells (MPCs) that proliferate and expand by the action of intrinsic transcription factors, including GATA4 and GATA6 (E9.5–E10.5). Between E12.5 and E15.5, the pancreatic epithelium undergoes a process of branching resulting in the formation of tip domain (proacinar) and trunk (ductal/endocrine) domains. In the mature pancreas, Gata4 expression is largely restricted to acinar cells. GATA6 is expressed in islets of Langerhans, acinar, and ductal cells. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License (https://smart.servier.com)
primordia are induced by markedly different signals. This is well illustrated by the negative role of sonic hedgehog (Shh) in regulating pancreas formation. In contrast to other endoderm-derived organs, the induction of the dorsal pancreas requires the inhibition of Shh, an event mediated by signals provided the notochord.\(^5\) Also, RA signaling is selectively required for the development of the dorsal pancreas.\(^6\) These extrinsic signals activate the expression of transcription factors specifically in the prepancreatic endoderm. Among these transcription factors, pancreatic and duodenal homeobox 1 (PDX1) and pancreas specific transcription factor 1a (PTF1A) are the earliest expressed in the pancreatic domain and are critical for the initiation of the pancreatic genetic program.\(^7\)-\(^11\) Once the dorsal and ventral pancreatic primordia are formed, the pancreatic epithelium undergoes a marked expansion from E9.5 until E12.5 when the two buds fuse. During this period, vascularization and innervation of the pancreas are also established.\(^12\)-\(^13\)

The early pancreatic epithelium is composed of multipotent pancreatic progenitor cells (MPCs) that have the potential to give rise to all types of pancreatic cells. The expansion of MPCs is mediated by extrinsic signals such as Wnt and FGF signaling. In addition, a substantial number of intrinsic transcription factors are involved in MPCs expansion and maintenance including PDX1, PTF1A, SOX9, FOXA1, FOXA2, GATA4, GATA6, HES1, HNF1B, and HNF6, among others (see for recent reviews\(^14\)-\(^17\)). There is an extensive cross-regulation among these transcription factors generating a gene regulatory network that maintains multipotency of MPCs. Recent studies have reported that the same gene regulatory network controls human MPCs.\(^18\)

Between E12.5 and E15.5, a period known as the secondary transition, a complex process of branching morphogenesis takes place in the pancreatic epithelium that results in the formation of tip and trunk domains that define different pancreatic cell fates.\(^19\) Thus, after E13.5, the tip domains exclusively generate acinar cells while the trunk domains give rise to endocrine and ductal cells. The segregation of pancreatic lineages is mediated by transcriptional cross-repression between PTF1A (that promotes tip identity) and NKX6.1/6.2 (that promotes trunk identity).\(^20\) During the secondary transition, the master regulator of endocrine differentiation, the transcription factor neurogenin 3 (Ngn3), gets activated in individual cells of the trunk. These Ngn3-expressing cells differentiate into the pancreatic endocrine lineage that is forming proto-islets.\(^21\) The cells in the trunk domain that do not express Ngn3 give rise to adult ductal cells. Acinar cell differentiation from tip cells is regulated in a coordinated manner by the transcription factors PTF1A, RBP-JL, and NR5A2.\(^22\)-\(^23\) By E15.5, there is substantial growth of the pancreas, mainly through acinar cell proliferation. Also, further differentiation of endocrine and exocrine pancreatic cells occurs, a process that continues into postnatal life (Figure 1).

## 2 | MUTATIONS IN GATA FACTORS AND PANCREAS ABNORMALITIES IN HUMANS

The association between mutations in GATA factors and a range of developmental anomalies in humans has been known for some time but it was only until recently that a link to pancreatic pathologies was reported. In 2011, Lango Allen et al. performed exome sequencing to identify the genetic mutation/s causing pancreatic agenesis, a rare congenital anomaly in which the pancreas is absent or extremely reduced.\(^24\) They found that heterozygous GATA6 mutations were the most common cause of this disease. The majority of GATA6 mutations in these individuals were de novo heterozygous mutations. Subsequent work confirmed this finding and revealed a more complex picture.\(^25\)-\(^34\) First, patients with GATA6 mutations often present defects in other organs including the heart and gut. Second, patients with GATA6-inactivating mutations show a broad spectrum of pancreatic abnormalities, from complete agenesis of the pancreas to moderate diabetes developed during adulthood with or without exocrine insufficiency (that might be suggestive of some degree of acinar function defects). Indeed, it has been reported that family members harboring the same GATA6 mutant allele can present with markedly different phenotypes.\(^32\)-\(^34\) Finally, mutations in GATA4 has also been more recently linked to neonatal and childhood-onset diabetes mellitus with or without exocrine insufficiency.\(^35\) Altogether, these genetic studies indicate a critical role for GATA4 and GATA6 in normal development of the human pancreas, a notion confirmed by studies on human embryonic stem cells (hESCs) (at least for GATA6, see in the following).

## 3 | ROLE OF GATA FACTORS IN MOUSE PANCREAS FORMATION

The subfamily of GATA factors Gata4/5/6 is expressed in the definitive endoderm that gives rise to pancreas as well as other organs of the gastrointestinal system. An exhaustive survey of GATA factors expression in the mouse pancreas by RNA in situ hybridization has shown that only Gata4 and Gata6 are expressed in the embryonic pancreas.\(^36\) Both factors are expressed in the presumptive pancreatic endoderm and continue to be expressed throughout the pancreatic embryonic development. Although earlier studies reported contradictory Gata expression data during pancreas formation,\(^36\)-\(^39\) recent immunohistochemistry studies have
clarified their expression patterns in the embryonic pancreas. Thus, *Gata4* expression becomes progressively restricted to the acinar compartment, while *Gata6* is expressed in both the endocrine and exocrine compartment in the adult pancreas. Mouse transgenic reporter assays have demonstrated *Gata4* and *Gata6* expression patterns in the pancreas that are similar to what it is observed in immunohistochemistry studies. However, a study has reported detectable (albeit low) levels of *Gata4* expression in mouse islets, as assessed by RT-PCR.

The study of GATA factors in pancreas formation has been hampered due to the early embryonic lethality of *Gata4* or *Gata6* null mice. Inactivation of *Gata4* in the germ line cause embryonic lethality around E8.5 due to defects in extraembryonic endoderm. Similarly, *Gata6* null mice embryonic development is arrested after gastrulation stages. Attempts to circumvent the early lethality of *Gata4* have been done by tetraploid complementation, in which *Gata4*−/− embryos have been provided with *Gata4*+/+ extraembryonic endoderm. These studies showed that ventral pancreatic induction was abrogated in the absence of GATA4. Consequently, *Gata6* null mice embryonic development is arrested after gastrulation stages. However, it is not clear from these studies whether GATA4 plays a cell-autonomous role in pancreas development. More recently, the use of conditional knockout approaches mediated by the Cre-loxP system has helped to define the multiple roles of GATA factors in pancreatic development.

GATA factors act as pioneer factors in initiating organ-specific gene expression during embryonic development. As discussed previously, both *Gata4* and *Gata6* are expressed in the foregut endoderm and have been reported to be involved in activating liver- and intestine-specific genes, respectively, in the primitive gut tube. However, conditional inactivation of *Gata4* and *Gata6* in the prepancreatic endoderm using the Foxa3-Cre driver did not block pancreas specification nor initial pancreas budding. Thus, GATA4 and GATA6 factors do not seem to be required for pancreas specification in mice. Interestingly, GATA5 transcription factor has been described to be involved in pancreas specification in zebrafish and *Xenopus*. However, *Gata5* does not seem to be expressed in the mouse embryonic pancreas. Thus, it is unlikely that GATA5 may be required for pancreas formation in mice. In agreement with this, no pancreatic abnormalities have been reported in patients with congenital heart disease associated with GATA5 mutations.

GATA4 and GATA6 are, however, essential regulators of the expansion and maintenance of MPCs during early mouse pancreas development. Interestingly, and contrary to what it is observed in humans with GATA6 mutations, GATA4 and GATA6 are functionally redundant in these early stages in mouse pancreas development. Conditional inactivation of either *Gata4* or *Gata6* in the pancreatic progenitors using the pancreas-specific *Pdx1-Cre* line does not cause major pancreatic defects. However, simultaneous inactivation of both genes causes pancreatic agenesis or severe pancreatic hypoplasia. The pancreatic bud in *Gata4*/*Gata6* double mutant embryos is formed but fails to expand, exhibiting defects in epithelial branching as well as MPCs proliferation. Furthermore, the segregation of endocrine and acinar lineages is also severely affected. As discussed previously, the activation and maintenance of the pancreatic developmental program in MPCs are achieved by a complex regulatory program composed by a range of transcription factors. Many of these transcription factors directly regulate each other expression. Thus, GATA factors regulate *Pdx1* expression during early pancreas formation. Therefore, some of the pancreatic defects observed in *Gata4*/*Gata6* mutant embryos might be due to decreased *Pdx1* expression. Interestingly, although GATA factors are not needed for pancreas specification in the foregut endoderm, they are necessary to maintain pancreatic identity in MPCs. The absence of both GATA4 and GATA6 results in the conversion of the dorsal and ventral pancreas to intestinal and gastric cell fates, respectively. This phenotype is caused, at least in part, from the aberrant activation of the Shh pathway in pancreatic progenitors (Figure 1). Thus, GATA4 and GATA6 contribute to maintaining pancreas identity through suppression of the Shh pathway. GATA4 and GATA6 seem to directly repress Shh expression in the pancreatic endoderm by binding the foregut endoderm enhancer MACS1.

GATA factors also act at later stages of pancreas formation. A recent study in *Gata6*-deficient mice has demonstrated an important role for GATA6 in β cell development and function. In this study, *Gata6* was inactivated during early pancreas development using the mouse *Pdx1-Cre* line. No pancreatic endocrine defects were observed in young *Gata6*-deficient mice. However, as they age (6 months old), they develop glucose intolerance, a phenotype indicative of defects in β cell function. Indeed, loss of GATA6 activity causes a vast array of abnormalities in adult β cells including impairment of insulin biosynthesis and secretion, defects in mitochondrial ultrastructure, and disorganization of the endoplasmic reticulum (Figure 2). These latter results are in agreement with an earlier study showing that GATA factors are required for endoplasmic reticulum integrity in mouse β cells. Studies performed in isolated human islets also point to an important role for GATA6 in the response to endoplasmic reticulum stress. Loss of GATA6 has a broad impact on β cell gene expression. Transcriptome analysis of GATA6-deficient islets shows decreased expression of genes critical for different aspects of β cell function including glucose sensing (*Slc2a2*, encoding for GLUT2 transporter), insulin biosynthesis (*Ins1*, *Ins2*, and *Pck1*), and insulin secretion (*Abcc8*, *Cacna1c*, and SNAP-
Importantly, the expression of key transcriptional regulators of adult β cell function such as Pdx1, MafA, and Nkx6.1 was also diminished. Similar to what is observed during pancreas embryonic development, GATA6 directly regulates the expression of Pdx1 in adult β cells. Thus, the effects of GATA6 loss on adult β cell function might be indirect. While these studies demonstrate an essential role for GATA6 in mouse β-cell function, it remains to be determined whether the defects in glucose homeostasis in Gata6-deficient mice are caused by Gata6 inactivation during embryonic stages or in adult β-cells. Further studies inactivating Gata6 at different time points during pancreas formation as well as in adult stages will help to solve this issue. In this regard, the use of a Cre line to efficiently delete Gata6 in adult β-cells would be highly valuable.

GATA6 also plays a role in acinar mouse cell differentiation. As discussed earlier, mice deficient for Gata6 are born and reach adulthood with no apparent pancreatic defects. However, genes important for acinar cell function including transcription factors and digestive enzymes are downregulated in Gata6-deficient pancreatic tissue. Importantly, GATA6 directly regulates the expression of key genes that code for acinar transcription factors such as Mist1 and Rbpj.

These results indicate that GATA6 is necessary for full acinar differentiation. Furthermore, aged Gata6 mutant mice display extensive loss of acinar cells, acinar-to-ductal metaplasia, fibrosis, and lipomatosis indicating that GATA6 is also required to maintain acinar cell identity in the adult pancreas (Figure 2). Interestingly, loss of GATA6 activity has also been linked to acinar cell dedifferentiation and tumorigenesis in a mouse model of Kras-driven pancreatic ductal adenocarcinoma (the most common type of pancreatic cancer) as well as in human pancreatic ductal adenocarcinoma. Indeed, GATA6 has been proposed to act as a tumor suppressor in the pancreas by maintaining acinar cell differentiation as well as regulating inflammatory and cancer-related pathways.

**FIGURE 2** Function of GATA transcription factors in adult mouse pancreas. Gata6 mutant pancreas exhibits acinar cell loss, acinar-to-ductal metaplasia, lipomatosis, and decreased expression of key transcription factors for acinar cell function (such as Mist1 and Rbpj). In β cells, loss of GATA6 activity results in decreased expression of genes critical β cell function including key transcriptional regulators (Pdx1, MafA, and Nkx6.1), glucose sensing (Slc2a2, encoding for GLUT2 transporter), insulin biosynthesis (Ins1 and Ins2), and insulin secretion (Abcc8, encoding for SUR1 and SNAP-25). Defects in mitochondrial (mito) ultrastructure and disorganization of the endoplasmic reticulum (ER) is also observed in Gata6-deficient β cells. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License (https://smart.servier.com)
patterns are similar to what it is observed in mouse development. GATA4 is first observed in the pancreatic-specified gut endoderm at Carnegie Stage (CS) 12 of human embryonic development (around E9 of mouse development) and continues to be expressed in the pancreatic progenitor cells. Around CS19 (E14.5–E15 in mouse), the expression becomes more localized to the tip domain and later is restricted to acinar cell lineage. GATA6 expression has been reported in the early pancreatic progenitor domain (CS16-CS18) but information at other embryonic stages is scarce. Transcriptomic data indicate that GATA6 is expressed in adult human pancreatic islet cells. The recent development of protocols for stepwise differentiation of hESCs and induced pluripotent stem cells (hPSCs) toward β-cells constitutes a relevant in vitro model for studying human pancreatic formation. The expression of GATA4 and GATA6 in hPSC differentiation cultures has been recently reported. Both GATA4 and GATA6 were found to be coexpressed at the definitive endoderm as well as early pancreatic progenitor stages, similar to what is observed in mice. As pancreatic progenitor cells differentiate into β-like cells, GATA4 and GATA6 expression decrease (particularly GATA6) with no substantial expression in mature insulin-secreting cells.

The findings that GATA4 and GATA6 play redundant roles in mouse pancreas development seem slightly at odds with the human genetic studies showing that GATA6 haploinsufficiency is associated with pancreatic agenesis. However, hPSCs differentiation studies have recently demonstrated that additional factors might be involved in the pancreatic defects associated with GATA6 heterozygosity in humans. In these studies, the effects of GATA6 on human pancreas differentiation were evaluated by inactivating GATA6 via CRISPR/Cas9 technology or generating induced pluripotent stem cells from a patient with pancreatic agenesis carrying a mutation in GATA6 and performing directed differentiation toward β cells in vitro. In contrast to what it is observed in patients with pancreatic agenesis, GATA6 inactivation only has a moderate effect on the formation of pancreatic progenitor cells during in vitro differentiation. Furthermore, dosage-sensitive requirements for GATA6 and GATA4 in the formation of both definitive endoderm and pancreatic progenitor cells were demonstrated, similar to the results described in double Gata4/Gata6 mutant mice. A very interesting finding is that the reducing levels of RA during pancreatic differentiation in GATA6 mutant hPSCs lines lead to more severe phenotypes. Remarkably, the reduction of RA also causes a decrease in GATA4 expression that could potentially contribute to the pancreatic defects caused by GATA6 inactivation. These findings might have important clinical implications, since they suggest that environmental signals may influence the pancreas phenotypes caused by GATA6 mutations. However, differences in individual genetic backgrounds among patients with GATA6 mutations might also account for the variation in clinical presentation. The effect of RA on in vitro pancreatic cell formation in GATA6 mutant lines also illustrates the importance of culture conditions in hPSCs differentiation studies. Slight variations exist in the current pancreatic β cell differentiation protocols that may potentially lead to different results. The use of standardized protocols and reagents would ensure that results can be properly compared among different studies.

The hPSCs in vitro differentiation approaches have also made it possible to directly assess the effect of GATA6 inactivation on human β cell development. All the studies reported the generation of β cells from both homozygous and heterozygous GATA6-deficient cell lines albeit with decreased efficiency. Furthermore, hPSC-derived β cells carrying mutations in GATA6 displayed decreased expression of genes important for β cell function including endocrine hormones and proteins involved in insulin secretion. One of the studies also reported defects in glucose-stimulated insulin secretion in GATA6-deficient β cells, although it was not observed in other studies. Collectively, these results suggest a potential role for GATA6 in human β cell formation, a finding that could help to explain why certain patients with heterozygous GATA6 mutations develop diabetes without exocrine insufficiency at adult stages.

5 CONCLUSIONS

Studies performed over the last few years have uncovered a critical role for GATA4 and GATA6 in pancreas development and adult function. Mutations in both the transcription factors have been associated with the congenital anomalies of the pancreas in human. The use of genetically modified mouse models and hPSCs has demonstrated that GATA4 and GATA6 play multiple roles at different stages of pancreas formation as well as in adult pancreas. However, the specific cellular and molecular mechanisms through which GATA factors regulate these processes remain to be elucidated. Thus, GATA-regulated direct targets during pancreas development are largely unknown. Similarly, loss of GATA6 activity has a profound impact on β cell gene expression, but whether a direct or indirect effect remains has to be established. Understanding the molecular link between GATA6 and β cell function is of special interest as it might shed light on the mechanisms underlying diabetes onset in patients with GATA6 mutations. These future studies may also help to uncover whether GATA6 plays a more general role in β cell dysfunction in diabetes mellitus. To this regard, it would be interesting to analyze the function of GATA6 in β cells under stress conditions associated with
diabetes such as obesity, hyperglycemia, and chronic low-grade inflammation. The analysis of GATA4 activity in β cells will also be necessary to determine whether GATA4 and GATA6 show some degrees of functional redundancy as they do during embryonic pancreas development. To this end, double inactivation of Gata4 and Gata6 at different time points and/or cell lineages during pancreas formation and postnatal life using specific mouse Cre lines would be very informative. The establishment of in vitro generated pancreatic β-like cells from hPSCs combined with the use of genomic editing tools constitutes a very powerful technology to analyze human pancreas development and function as demonstrated by the studies inactivating GATA6 in hPSCs. Similar studies evaluating the inactivation of GATA4 as well as the simultaneous inactivation of both GATA4 and GATA6 in hPSCs will clarify the precise roles of GATA factors in human pancreas development and adult function. Ideally, these approaches should be performed in three-dimensional in vitro organoids derived from hPSCs as they more closely resemble native organs.

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