GATA believe it: new essential regulators of pancreas development

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Understanding the transcriptional mechanisms that underlie pancreas formation is central to the efforts to develop novel regenerative therapies for type 1 diabetes. Recently, mutations in the transcription factor *GATA6* were unexpectedly shown to be the most common cause of human pancreas agenesis. In this issue of the *JCI*, Carrasco et al. and Xuan et al. investigate the role of *Gata6* and its paralogue *Gata4* in mouse embryonic pancreas and show that GATA factors are essential regulators of the proliferation, morphogenesis, and differentiation of multipotent pancreatic progenitors.

Type 1 diabetes results from autoimmune destruction of insulin-producing β cells. The major goal of current research efforts to reverse this disease is focused on learning how to generate new β cells to replace those that have been destroyed while at the same time blocking the autoimmune process. This challenge has fueled intense research to uncover the extracellular signals and transcriptional regulators that promote the generation of β cells during embryonic development (1–3). Proof-of-concept experiments have shown that this type of knowledge can indeed be exploited to derive insulin-producing cells from multipotent cells in vitro (4) or through transdifferentiation of somatic cells (5).

One of the critical stages of the developmental process that leads to β cell differentiation is the formation and orderly expansion of pancreatic multipotent progenitors. The latter originate from the foregut endoderm at around E8.5 in mice (6), and prior to 26 days post conception (dpc) in humans (ref. 7 and Figure 1). Nearly two decades ago, mouse genetic knockout experiments first proved that *Pdx1* is essential for the expansion of the early pancreatic bud (8, 9). Since then, numerous transcription factors have been shown to be essential at this initial developmental stage, including Pdx1, Ptf1a, Mnx1, Sox9, and Hnf1b (3). In mice, only homozygous null mutations of these factors (either germline, chimeric, or conditional) have revealed a pancreatic phenotype. For some transcription factors, such as Foxa1/2, Onecut1, or Nkx6.1, critical regulatory roles have been uncovered by the complete inactivation of more than one gene, reflecting a degree of functional redundancy (10–12). Although most of these discoveries have relied on mouse genetics, mutations in *PDX1*, *PTF1A*, and *HNF1B* have also been identified in human patients with monogenic diabetes and pancreatic aplasia or hypoplasia, consistent with the notion that pancreatic developmental programs are fundamentally conserved in humans and mice (3).

Human genetics uncovers a key pancreatic regulator

Genome sequencing technologies have recently provided a major unexpected discovery in this field. Allen et al. examined 27 human patients who had neonatal diabetes due to pancreas agenesis and found that only one had a homozygous mutation in a known pancreatic regulator. An exome sequencing strategy revealed that 15 of the remaining patients had heterozygous loss of function mutations in the gene encoding the zinc finger transcription factor *GATA6* (13). Patients also had developmental cardiac defects, in some cases mild, consistent with earlier reports of *GATA6* mutations causing cardiac outflow defects. Several patients with mutations had endoderm developmental abnormalities, including thyroid, pituitary, gut, and biliary tract defects. In short, most humans with pancreatic agenesis have mutations in a gene that had so far been largely left off the radar screen in the pancreas development field. Intriguingly, another case report described a mutation in the paralogue *GATA4* in a single patient with pancreatic agenesis and a cardiac malformation (14). The human genetics findings therefore establish that GATA6 (and less conclusively GATA4) is an essential regulator of pancreas development, although they do not shed light on the underlying molecular mechanism, nor do they define the precise cell types or developmental stages in which the essential role takes place.

Modeling GATA function in mice

In this issue of the *JCI*, Carrasco et al. and Xuan et al. (15, 16) report two mouse genetic studies that define a key regulatory role of *Gata6* and *Gata4* in pancreas development. In both reports, the two Gata genes were inactivated in embryonic multipotent pancreatic progenitors using Cre/LoxP technology. Conditional mutations were required because germline homozygote null mutations cause early embryonic lethality, whereas heterozygote mice have no known pancreatic defect (17, 18). Unexpectedly, the inactivation of either *Gata4* or *Gata6* in pancreatic progenitors did not cause severe pancreatic defects. However, the simultaneous deletion of both factors caused a major reduction in the pancreatic size related to a severe block in the proliferation of multipotent pancreatic progenitors. Furthermore, there was abnormal branching morphogenesis and a severe defect in differentiation, including failure to separate into peripheral acinar-committed progenitors and central endocrine-committed progenitors. Xuan et al. performed an additional experiment in which they deleted *Gata* genes in the endoderm prior to the appearance of the pancreas and again found that the pancreatic bud was formed, yet failed to expand (16). This means that, at least with the genetic tools that were used in these studies, there is no indication that GATA factors in foregut endoderm cells have a cell-autonomous role in pancreas specification. Instead,

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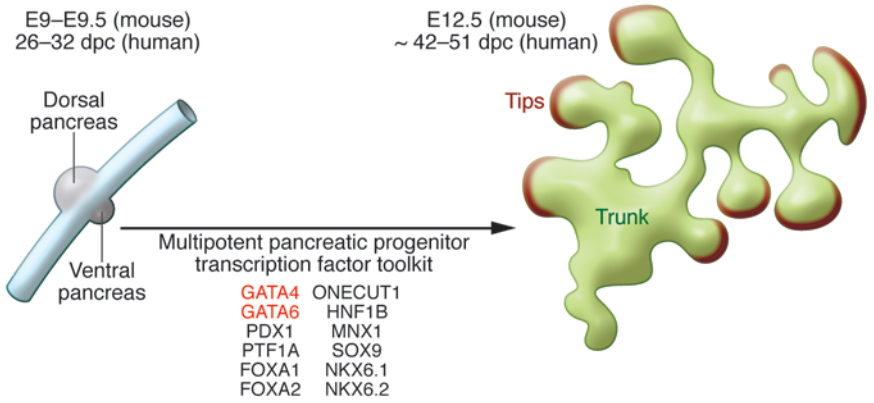

Figure 1

Representation of early pancreatic development. At approximately E9 in the mouse, pancreatic progenitors form dorsal and ventral buds off of the developing gut tube. Just a few days later, these multipotent progenitors give rise to a branched structure in which the tips are committed to an acinar cell fate, and the trunk contains duct-endocrine progenitors. The box lists transcription factors required for this remarkable transition.

the data show that Gata4 and Gata6 play essential functions in embryonic pancreatic progenitors.

Although the most severe phenotypes result from loss of GATA function in pancreatic embryonic multipotent progenitors, the studies also suggest that they may have important functions beyond this stage. For example, Carrasco et al. show that mice with pancreatic deletions of both *Gata4* alleles and one *Gata6* allele have severe loss of acinar cells (15). Another recent report has now shown that mice in which only *Gata6* is completely inactivated in pancreatic progenitors are histologically normal at birth, but eventually develop a severe loss of acinar cells (19). Thus, GATA factors appear to exert distinct essential functions throughout different steps of pancreatic cell formation.

Why does GATA6 haploinsufficiency cause pancreatic agenesis in humans, whereas in mice, three or four GATA alleles need to be deleted to elicit a severe pancreatic phenotype? It is important to note that haploinsufficiency of transcription factor genes is not uncommon in human developmental disorders. In pancreatic disease, heterozygous loss-of-function mutations in *HNF1B*, *HNF4A*, and *HNF1A* genes cause diabetes in humans but not in mice (2). The exact reason for this mouse-specific tolerance to haploinsufficiency of transcription factor genes has not been clarified. One candidate explanation is that some developmental stages in human last weeks longer than in mice. This could increase the likelihood that there are deleterious consequences from fluctuations in transcriptional levels that are not sufficiently buffered by the presence of the other healthy allele. In the case of GATA6 and GATA4, four alleles are in play. It is possible that the gene expression patterns of these two genes exhibit greater overlap at critical steps of early pancreas development in mouse versus human embryos, thus rendering mice more resilient to genetic perturbations of a single GATA gene.

GATA factors regulate Pdx1

How do GATA factors exert their essential functions in pancreatic progenitors? GATA4 and GATA6 transcription factors have been shown to initiate developmental programming steps important for heart, gut, and liver development (17, 18). The current studies now show that GATA factors are important for the activation of *Pdx1*, which encodes another essential pancreatic developmental transcription factor (8, 9). Carrasco et al. created a transgenic mouse containing the upstream sequence of *Pdx1* linked to a reporter and showed that deleting GATA-binding sites prevented the activation of the reporter in early embryonic multipotent pancreatic progenitors (15). This is consistent with the observation that Pdx1 expression is reduced in pancreatic progenitors of mutant mice, although it is slightly at odds with the experiments from Xuan et al., suggesting that GATA factors are not required in the endoderm to initiate the expression of *Pdx1* and the inception of pancreatic buds (16). It is possible, however, that enhancers of the *Pdx1* gene that were not included in the transgenic construct and do not require GATA factors are sufficient to initiate *Pdx1* expression in foregut endoderm.

An organ-building tool kit

Gata6 and *Gata4* are broadly expressed in endodermal and mesodermal lineages (20), in contrast with *Pdx1* and *Ptf1a*, which are expressed in the pancreatic anlage and only few other embryonic cell types. Early studies showing that *Pdx1* and *Ptf1a* have a selective expression pattern coupled with the fact that their knockout mice develop pancreas agenesis suggested that these two factors might work as master regulators of pancreas organogenesis (8, 21). It has now become clear, however, that pancreatic multipotent progenitors require a larger transcription factor tool kit, which the current work suggests includes GATA4 and GATA6 in addition to PDX1, PTF1A, MNX1, ONECUT1, NKX6.1/NKX6.2, FOXA1/2, SOX9, and HNF1B (Figure 1). The inactivation of each one of these factors, either alone or in combination, disrupts the progenitor program and thereby blocks pancreas development. Due to the combinatorial nature of transcription factor function, it is the unique coalescence of factors that drives the pancreatic progenitor program, and therefore it does not matter whether a particular transcription factor is more or less specific to the pancreatic lineage. Based on the genetic findings, we can surmise that all of these factors are required to bind to regulatory elements that control the transcription of genes that are important for all of the things that pancreatic multipotent progenitors need to do, namely to proliferate, undergo morphogenetic changes, and differentiate. This likely also involves conferring responsiveness to the extracellular signals that promote developmental processes. In short, an interesting combination of mouse and human genetics has provided an inventory of the key transcription factors for pancreas organogenesis. This represents an important step in untangling the mechanisms of pancreas organogenesis, which can now be pursued with novel technologies to manipulate whole animal and in vitro human models and to analyze them with genome-scale tools. Knowledge of the components of this developmental tool kit and the instructions followed are invaluable in allowing us to recapitulate the process for cell replacement therapies. The studies from Carrasco et al. and Xuan et al. provide a landmark advance in this direction (15, 16).

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Defending the cornea with antibacterial fragments of keratin

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In addition to its role in refraction, the cornea forms a barrier between the eye and environmental and infectious insults. Corneal infections are surprisingly rare, suggesting that multiple aspects of the immune system are at play in mediating protection. In this issue of the *JCI*, Tam et al. describe the unexpected role of a structural protein, cytokeratin 6A, in this process.

The usual healthy appearance of the cornea and conjunctivae of the human eye should puzzle you. Why is it that this surface looks so healthy, most of the time? How is it that despite the almost certain diversity of microbes that come in contact with it, we so rarely see infection, or its associated sign, inflammation, evidence of the body's mechanisms that are called forth to fight off microorganisms? Lysozyme in tears can defend the eye, but organisms that inhabit the upper airway, such as *Staphylococcus aureus* are resistant to this enzyme (1), and other antimicrobial systems must be at work, because in the various dry-eye syndromes, the reduced tear production is not associated with frequent bacterial infections. More perplexing is the fact that a corneal transplant will not necessarily develop infections at the incision or around the suture tracks, and antibiotics are not necessarily required postoperatively (2). Thus, even the wounded cornea seems to handle microbes in some mysterious — and remarkably effective — fashion.

Layers of protection

The cornea is a wonderful, close-up example of a site protected almost completely by the chemical and physical defenses of our innate immune system. Tears contain high concentrations (about 1 to 2 mg/ml) of each of three antimicrobial proteins: lysozyme, lactoferrin, and lipocalin (3). The presence of lactoferrin, which chelates iron, and lipocalin, which captures the iron-transporting siderophores used by many bacteria, tells us that many microbes would discover the microenvironment of the corneal epithelium to be unfavorably iron-depleted. The corneal epithelium secretes several types of mucin that adhere to the corneal surface (4) and form a barrier that both provides a physical shield from invaders and creates an "unstirred" micron-thick fluid layer between itself and the corneal epithelial cell. Antimicrobial peptides are secreted into this barrier and can accumulate without diffusion (or dilution) into to the tear fluids. Furthermore, we have known for some time that the epithelium expresses several well-characterized antimicrobial peptides, both constitutively and induced following injury (5). These include several of the β-defensins and LL-37 (cathelicidin), which are believed

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