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Review

Lineage determinants in early endocrine development

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ABSTRACT

Pancreatic endocrine cells are produced from a dynamic epithelium in a process that, as in any developing organ, is driven by interacting programs of spatiotemporally regulated intercellular signals and autonomous gene regulatory networks. These algorithms work to push progenitors and their transitional intermediates through a series of railroad-station-like switching decisions to regulate flux along specific differentiation tracks. Extensive research on pancreas organogenesis over the last 20 years, greatly spurred by the potential to restore functional β-cell mass in diabetic patients by transplantation therapy, is advancing our knowledge of how endocrine lineage bias is established and allocation is promoted. The field is working towards the goal of generating a detailed blueprint of how heterogeneous cell populations interact and respond to each other, and other influences such as the extracellular matrix, to move into progressively refined and mature cell states. Here, we highlight how signaling codes and transcriptional networks might determine endocrine lineage within a complex and dynamic architecture, based largely on studies in the mouse. The process begins with the designation of multipotent progenitor cells (MPC) to pancreatic buds that subsequently move through a newly proposed period involving epithelial plexus formation-remodeling, and ends with formation of clustered endocrine islets connected to the vascular and peripheral nervous systems. Developing this knowledge base, and increasing the emphasis on direct comparisons between mouse and human, will yield a more complete and focused picture of pancreas development, and thereby inform β-cell-directed differentiation from human embryonic stem or induced pluripotent stem cells (hESC, iPSC). Additionally, a deeper understanding may provide surprising therapeutic angles by defining conditions that allow the controllable reprogramming of endodermal or pancreatic cell populations.

Contents

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1. Introduction

Since the successful (albeit temporary) removal of brittle type 1 diabetic patients from insulin-dependence by transplantation of cadaver-derived islets, there has been a great push to understand the methods that could be applied *in vitro* to obtain functioning β cells [1]. Added incentive comes because the demand for β cells (or islets, as discussed later) far outstrips any conceivable cadaver-based source. To date, *in vitro* protocols have attempted to entrain hESC along a still poorly defined sequence of events, initially biasing them towards definitive endoderm, then channeling them to primitive gut tube epithelium, posterior foregut, pancreatic-endoderm progenitors and endocrine precursors, *en route* to insulin (Ins)-expressing cells. However, mature endocrine cell formation is inefficient and incomplete; the number of β-like cells formed is low, and unlike their endogenous counterparts they are poorly glucose-responsive [1,2]. And yet, intermediate-stage pro-pancreatic cells derived from such protocols that are transplanted into mice can, after several months, apparently yield mature, functional β cells [3]. This *in vivo* maturation is a real 'black box' phenomenon: what happens post-implantation remains cryptic. It could be useful to determine the local or systemic signals and/or other factors that are driving the transplanted cells along a proper endocrine/β-cell differentiation program. The corollary is that all current *in vitro* differentiation protocols induce a slightly incorrect, off-track program, leading to pseudo-β-cells [4]. Which begs the question: What are the missing *in vivo* components that induce complete β-cell maturation *in vitro*? Is it a lack of 'community effect' involving planar cell polarity and apical-basal polarization present in the normal 3D epithelial structure, or is the *in vitro* differentiation process being forced along too quickly? Or, could there be just one or two missing critical factor(s), which might be identified or mimicked soon by appropriate screening methods?

hESC generally grow and differentiate in two-dimensional (2D) monolayer conditions. In contrast, the pipeline for endocrine development *in vivo* involves potentially complex 3D niches within the developing pancreatic epithelium, and these may be highly dynamic as the plexus intermediate moves towards an organized, tubular network [5]. It is possible, therefore, that the current applications of inducer cocktails, although designed to mimic the normal developmental process, fail to recapitulate the finely timed triggers and signaling thresholds that exist in real tissue. We have gained some broad knowledge of how regionalized transcription factor (TF) expression distinguishes the evolving epithelial compartments [6]. But, more information is needed regarding the timing, context, and expression thresholds of specific transcription factors (TFs), and how they interact with epigenetic control factors in individual genes, and in entire gene regulatory networks (GRN), to control endocrine-biased differentiation [7]. The development of additional prospective cell markers and a detailed appreciation of local tissue landmarks should aid in obtaining a fundamental, sequential map of cellular transitional phases. We therefore predict, over the next few years, an almost certain substantial overhaul of our understanding of endocrine ontogeny. The new realization that the pancreatic epithelium develops through a plexus intermediate, whose resolution (to an epithelial tree) and differentiation is temporally asynchronous across the organ [6,8], means that understanding lineage allocation could involve defining the epithelium using classical developmental biology terms such as equivalence groups (which could be relatively small, reiterated groups of cells, having a specific interaction structure), and how these groups are tiled through the plexus and resolving epithelial tree. This concept is important because cells expressing the endocrine commitment factor Neurogenin 3 (Neurog3 or Ngn3) are already delaminating from the epithelium in large numbers at the plexus stage (Fig. 1C). How regionally distributed cells or groups advance developmentally with respect to each other, and how they give birth to various numbers of each hormone-positive endocrine cell, could tell us how the pancreas grows to its final size with the appropriate endocrine cell ratio within the islets of Langerhans [9]. Most importantly, characterizing transient niches within the developing plexus, along with any facilitating (permissive) signals from the adjacent mesenchyme [10–12], could identify factors missing from hESC differentiation protocols. As we shall address below, small molecule or miRNA treatments, together with synthetic scaffolds and embryoid/spheroid cultures could effectively substitute for the signaling pathways operative *in vivo*.

One critical unknown is whether it is strictly necessary to reproduce all aspects of endocrine ontogeny operating *in vivo* to achieve large-scale differentiation of functional β-cells *in vitro*. Instead of triggering a multitude of steps each *via* manual intervention, it is possible that a systemic factor or small set of local signals initiate relatively autonomous ('autopilot') gene regulatory 'modules' that push cells relatively automatically along the endocrine pipeline (Fig. 2). Here, we highlight some of the most important instructive steps determined to date, and address the biggest current deficiencies. When appropriate, we discuss similarities and differences between mouse and other model organisms, and try to make direct comparisons to what is known on human pancreas development. At the end of the review, a perspective section describes ideas on how genetic and other studies, done with cellular resolution, would allow a reductionist deconstruction of lineage commitment and differentiation. Our goal is to convince the reader that fundamental investigations into the mechanisms regulating the spatiotemporal and cell-type specificity of endocrine development should continue as a high-energy avenue towards a deeper knowledge base for translational application involving cellular transplantation-reconstitution or reprogramming (reviewed by Nostro and Keller; this issue).

2. The primary (1°) transition: MPC proliferation and priming phase (E9.5–E12.5)

2.1. Multipotent progenitors of the pancreatic buds

Pancreas fate is specified from dorsal and ventral regions of 'pre-differentiated' epithelial cells arising within the posterior foregut endoderm – although, interestingly, the ventral pancreas emerges from common bile duct, while the dorsal bud is attached to duodenum (suggesting different specification strategies; as reviewed by Dr. J. Wells). The first signs of pancreas organogenesis are noticed at approximately embryonic day (E)9.5–E10 when epithelial cells adopt a columnar character, evaginate, and thicken into surrounding mesenchyme. These initial processes result in formation of two 'fist-like,' complex stratified epithelia surrounding a primary central lumen [5], and comprising a pool of non-polarized multipotent progenitor cells (MPC) committed to pancreas-fate and defined as 'proto-differentiated' [13]. Pulse-chase lineage-tracing experiments using *Pdx1*-CreER demonstrate that all subsequent progenitors and adult pancreas cells (acinar, duct, and islet endocrine) derive from these MPC [14]. Limited experiments with individual cell labeling suggest that each MPC is competent to produce descendants in all three compartments [15]. The term 'primary (1°) transition' (E9.5 to ~E12.5) encompasses, in addition to the aforementioned events, a proliferative epithelial expansion of 'primary' MPC, a 'first-wave' α-cell-biased window of endocrine cell formation, a re-establishment of apicobasal cell polarity and microlumen formation, and fusion of the dorsal and ventral pancreatic buds (Fig. 1A and B, 2) [16].

While distinct developmental programs drive formation of the dorsal and ventral pancreatic buds [17], the total number of primary