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Stem Cells to Insulin Secreting Cells: Two Steps Forward and Now a Time to Pause?

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Two groups recently reported the in vitro differentiation of human embryonic stem cells into insulin-secreting cells, achieving an elusive goal for regenerative medicine. Herein we provide a perspective regarding these developments, compare phenotypes of the insulin-containing cells to human β cells, and discuss implications for type 1 diabetes research and clinical care.

Type 1 diabetes (T1D) results from an autoimmune destruction of insulin-producing pancreatic β cells (Atkinson et al., 2014). Patients require multiple daily injections of insulin that, while life-saving, are associated with significant alterations in lifestyle, the potential for hypoglycemia and hyperglycemia, and an increased risk of life-threatening complications. Although islet transplantation could represent a definitive therapy for T1D, multiple issues have precluded its widespread use including the need for chronic immunosuppression, inadequate supply of cadaveric organ donors, and limited duration of graft function (e.g., 2–5 years). As a result, extensive research has been directed at improving technologies for regulating metabolism (such as insulin pumps and continuous glucose monitors) and developing β cell replacement therapies, including xenogeneic islet cells, regeneration of β cells in vivo, and surrogate insulin-producing cells generated from stem cells.

Indeed, differentiation of embryonic stem cells (ESCs) into insulin-producing cells has been a highly competitive research area for over a decade. Throughout this period, substantial progress occurred in terms of directing differentiation of ESCs into pancreatic progenitors (D'Amour et al., 2006). However, the "Holy Grail" remained promoting complete maturation of human ESCs (hESCs) into insulin-secreting cells, an effort that proved difficult to achieve.

This said, Baetge and colleagues from ViaCyte were able to differentiate hESCs into definitive endoderm, primitive insulin-containing cells, and ultimately glucose-responsive insulin-secreting cells that self-organized as functional islet-like clusters (Kroon et al., 2008). For reasons unclear, the final maturation step required transplantation into mice in order to yield bona fide insulin-secreting cells. These cells, combined with an encapsulation device, formed the basis for a phase I/II clinical trial (NCT02239354), which is now underway. However, uncertainties regarding the means for in vivo maturation, the theoretical potential for teratoma, and the insulin-secretion properties of ViaCyte cells remain outstanding.

Recently, two groups independently reported achievement of the elusive milestone: in vitro protocols having the *potential* to yield insulin-secreting cells in quantities of therapeutic value for T1D. The first emanated from BetaLogics (Johnson and Johnson, Raritan, NJ) along with Timothy Kieffer and colleagues (Rezania et al., 2014); the second, from the research group of Doug Melton (Pagliuca et al., 2014). Starting with either hESCs or human induced pluripotent stem cells, both groups induced definitive endoderm and early pancreatic progenitors (Figure 1). The BetaLogics/Kieffer group then used TGFß, a GSK3β inhibitor, FGF7, a PKC activator, BMP receptor inhibition, and vitamin C, followed by TGFß thyroid hormone, a BMP receptor inhibitor, and a gamma secretase inhibitor. This protocol generated insulin- and insulin/glucagon-coexpressing cells that lacked the ability to dynamically secrete insulin. They then performed a small-molecule screen and identified R428 (a tyrosine kinase inhibitor of AXL) as a molecule capable of inducing β cell maturation. Melton and colleagues employed KGF (a FGF family member), SANT (a hedgehog inhibitor) and retinoic acid, followed by molecules that influence various signals including wnt, activin, hedgehog, EGF, TGFß, thyroid hormone, retinoic acid, and a gamma secretase inhibitor. The resulting cells from both groups appear remarkably similar in that they express β cell transcription factors (PDX1 and NKX6.1) and produce cells that contain substantial insulin, secreted in response to glucose. Xenotransplantation studies revealed the cells were capable of rescuing diabetes in mice.

Both groups completed initial steps to assess function of their insulin-secreting cells; however, the extent to which these cells are representative of "functionally mature" adult β cells remains unclear. Using static incubation studies, both groups show that their cells secrete insulin in response to glucose. Rezania et al. (2014) take their characterization further, reporting that their cells appear to display a very small and gradual response to glucose in a dynamic cell perfusion assay. Although difficult to determine the significance of this response, calcium imaging studies reveal that at least some of their cells (5%–10%) responded to glucose with a modest rise in intracellular Ca^{2+}. Pagliuca et al. (2014) report that a much higher percentage of cells respond to glucose with increased Ca^{2+} (~50%), but the resulting curves do not match the prototypical kinetics of a human islet

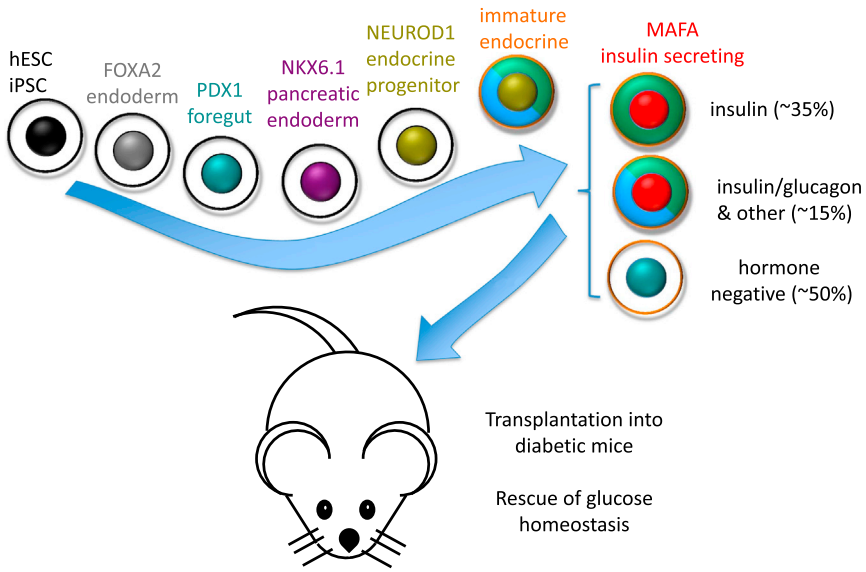

Figure 1. Differentiation of hESCs into Insulin-Secreting Cells
Rezania et al. and Pagliuca et al. show that sequential addition of various signaling molecules progressively gives rise to endoderm (FOXA+), foregut (PDX1+), pancreatic endoderm (NKX6.1), endocrine progenitors (NEUROD1+), immature endocrine cells (mostly chromogranin+ hormone− but also some insulin+ and some insulin+glucagon+), and then mature insulin-secreting cells (MAFA+). (MAFA expression analysis performed by Rezania et al., 2014.) Cells are capable of controlling blood glucose when transplanted into the kidney capsule of diabetic mice.

response (Kindmark et al., 1991). Gene expression analysis by Pagliuca et al. indicates that their cells are closer to adult rather than fetal β cells.

Clearly, a more detailed functional analysis of both groups' cells is required and should include: (1) analysis of key functional proteins and metabolic enzymes responsible for glucose sensing and secretory coupling; (2) metabolomics analysis of mitochondrial activity and secretory coupling factors; (3) mechanistic studies of the electrical and exocytotic machinery; (4) assessment of secondary stimulatory pathways; and (5) precise characterization of actual glucose sensitivity (both graded and dose-response).

While it can easily be recognized that additional functional characterization of these cells is desirable, this belies an even larger issue. Although inroads have been made in recent years in our understanding of human β cell function (Rorsman and Braun, 2013), the field has yet to define the properties of a fully functional healthy human β cell. Both groups describe their respective insulin-producing cells as "functional" largely based on similarity to static insulin secretion assays in comparison to responses of isolated human islets (which is an approximately 2-fold increase in insulin output after glucose stimulation). This response seems quite modest, and given that human islet stimulation indices have been reported from two to ten, or even higher (Shapiro et al., 2000), it becomes pertinent to ask the following question: what is the insulin secretion "gold standard" for human islets in vitro? The answer does not appear to be straightforward. Most qualitative human islet assessments were designed to predict and evaluate transplantation success. It is less clear whether such protocols represent ideal methodologies that define key functional parameters to compare against those of stem-cell-derived β cells. Specialized isolation protocols aimed at targeting a lower yield of higher quality human islets may prove beneficial to generate a gold standard of insulin secretion. In short, a continued focus on defining key basic mechanisms that influence adult human β cell function are paramount and should be pursued through strengthened interaction between islet isolation facilities, β cell biologists, and stem cell scientists.

Based on the early success of xenotransplantation studies, these cells, with encapsulation technologies, have been proposed for transplantation studies in diabetes patients (Ledford, 2014). While such advances form the basis for optimism in terms of therapeutic development, the potential for risk (whether real or theoretical) does exist. These risks would include, but not be limited to the following: (1) inadequate control of postprandial glucose excursions; (2) formation of teratoma, pancreatic adenocarcinoma, or hormone-secreting tumor (insulinoma, glucagonoma, or pancreatic neuroendocrine cancer); (3) hyperfunctioning or constitutive functioning of the graft of insulin or other hormones resulting in intermittent or chronic hypoglycemia; (4) provocation of systemic autoimmunity; and (5) HLA sensitization, a facet of importance if an individual who previously received allogeneic hESCs required subsequent cadaveric organ transplant. These risks must be carefully assessed when evaluating stem cell therapies for T1D. Nevertheless, these studies represent a positive step forward (multiplied by two) toward the development of surrogate insulin-producing cells capable of improving the lives of those with T1D. However, until evidence is provided that they represent "true" human β cells, it seems prudent to take time to pause until more reliable measures of β cell phenotypes are defined.

REFERENCES

Atkinson, M.A., Eisenbarth, G.S., and Michels, A.W. (2014). Lancet *383*, 69–82.

D'Amour, K.A., Bang, A.G., Eliazer, S., Kelly, O.G., Agulnick, A.D., Smart, N.G., Moorman, M.A., Kroon, E., Carpenter, M.K., and Baetge, E.E. (2006). Nat. Biotechnol. *24*, 1392–1401.

Kindmark, H., Köhler, M., Nilsson, T., Arkhammar, P., Wiechel, K.L., Rorsman, P., Efendić, S., and Berggren, P.O. (1991). FEBS Lett. *291*, 310–314.

Kroon, E., Martinson, L.A., Kadoya, K., Bang, A.G., Kelly, O.G., Eliazer, S., Young, H., Richardson, M., Smart, N.G., Cunningham, J., et al. (2008). Nat. Biotechnol. *26*, 443–452.

Ledford, H. (2014). Nature *514*, 281.

Pagliuca, F.W., Millman, J.R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J.H., Peterson, Q.P., Greiner, D., and Melton, D.A. (2014). Cell *159*, 428–439.

Rezania, A., Bruin, J.E., Arora, P., Rubin, A., Batushansky, I., Asadi, A., O'Dwyer, S., Quiskamp, N., Mojibian, M., Albrecht, T., et al. (2014). Nat. Biotechnol., in press. Published online September 11, 2014. http://dx.doi.org/10.1038/nbt.3033.

Rorsman, P., and Braun, M. (2013). Annu. Rev. Physiol. *75*, 155–179.

Shapiro, A.M., Lakey, J.R., Ryan, E.A., Korbutt, G.S., Toth, E., Warnock, G.L., Kneteman, N.M., and Rajotte, R.V. (2000). N. Engl. J. Med. *343*, 230–238.