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Accelerated Maturation of Human Stem Cell-Derived Pancreatic Progenitor Cells into Insulin-Secreting Cells in Immunodeficient Rats Relative to Mice

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SUMMARY

Pluripotent human embryonic stem cells (hESCs) are a potential source of transplantable cells for treating patients with diabetes. To investigate the impact of the host recipient on hESC-derived pancreatic progenitor cell maturation, cells were transplanted into immunodeficient SCID-beige mice or nude rats. Following the transplant, basal human C-peptide levels were consistently higher in mice compared with rats, but only rats showed robust meal- and glucose-responsive human C-peptide secretion by 19–21 weeks. Grafts from rats contained a higher proportion of insulin:glucagon immunoreactivity, fewer exocrine cells, and improved expression of mature β cell markers compared with mice. Moreover, ECM-related genes were enriched, the collagen network was denser, and blood vessels were more intricately integrated into the engrafted endocrine tissue in rats relative to mice. Overall, hESC-derived pancreatic progenitor cells matured faster in nude rats compared with SCID-beige mice, indicating that the host recipient can greatly influence the fate of immature pancreatic progenitor cells post-transplantation.

INTRODUCTION

Patients with type 1 diabetes suffer from a severe deficiency in insulin production by pancreatic islets as a result of immune-mediated destruction of pancreatic β cells. Insulin independence can be achieved by transplantation of cadaveric human islets (Shapiro, 2011), but because of the scarcity of donor tissue, the field is exploring the potential use of scalable human embryonic stem cell (hESC)-derived pancreatic cells as an alternative cell source. We have demonstrated previously that hESC-derived pancreatic progenitor cells develop over several months in vivo into insulin-secreting cells capable of reversing hyperglycemia in a mouse model of type 1 diabetes (Rezania et al., 2012, 2013; Bruin et al., 2013). Interestingly, the maturation process was accelerated when mice were exposed to chronic hyperglycemia but unaffected by exposure to long-term insulin therapy, short-term exendin-4 treatment, oral anti-diabetic medications, or high-fat diets (Bruin et al., 2013, 2015). In addition, we recently reported a revised differentiation protocol that generated glucose-responsive insulin-secreting cells in vitro and required a much shorter maturation period (~6 weeks) following transplantation to reverse hyperglycemia in mice (Rezania et al., 2014). Given the uncertainty surrounding the complex host environment and variables that may affect the maturation process in vivo, advancing the differentiation protocols in vitro prior to transplantation may be advantageous. Nevertheless, hESC-derived pancreatic progenitor cells are currently being tested for safety, tolerability, and efficacy in a phase 1/2 clinical trial by Viacyte (ClinicalTrials.gov identifier NCT02239354). Therefore, although newer differentiation protocols have been reported (Pagliuca et al., 2014; Rezania et al., 2014; Russ et al., 2015), it remains important to understand the development of pancreatic progenitor cells in vivo because clinical trials are underway in patients with diabetes.

There are several obvious differences between the preclinical transplant recipients tested to date (immunodeficient mice) and the target patient population, including the species, distinct metabolic profiles, and large size difference. Although rats are not directly comparable with humans, their physiology is reportedly more similar to humans than mice, particularly in terms of cardiovascular parameters (Davies and Morris, 1993). We have demonstrated previously that hESC-derived grafts were capable of robust glucose-stimulated insulin secretion (GSIS) after just 14 weeks in nude rats, whereas GSIS was not observed until after 30 weeks in similar studies with severe combined immunodeficiency (SCID)-beige mice (Rezania et al., 2012). However, these studies were performed at different facilities and with different batches of cells, so we could not make direct comparisons between species. Interestingly, others have reported that hESC-derived pancreatic progenitor cells did not efficiently differentiate into pancreatic endocrine tissue following transplantation in nude rats (Matveyenko et al., 2010). The authors speculated that the nude rat may be a less accommodating host environment compared with immunodeficient mice (Matveyenko et al., 2010). To address these conflicting observations, we

performed a carefully controlled study within a single research facility to directly compare the in vivo development of hESC-derived pancreatic progenitor cells from the same preparation and transplanted in parallel into either immunodeficient nude rats or SCID-beige mice.

RESULTS

hESC-Derived Insulin-Producing Cells Develop Faster and Function Better in Rats Than in Mice

Pluripotent H1 cells were differentiated into pancreatic progenitor cells over 14 days, resulting in a population containing 17% endocrine cells (synaptophysin+). Chromogranin+ endocrine cells, coexpressed NKX2.2 but were largely negative for NKX6.1, a sign of immaturity (Figure S1). The differentiated cells were ~80% PDX1+, ~50% NKX6.1+, ~18% PAX6+, and ~15% Ki67+ (Figure S1). Pluripotent cells (OCT3/4+) were not detected in the differentiated population at the time of transplantation (Figure S1). This preparation of pancreatic progenitor cells was then transplanted under the kidney capsule of either SCID-beige mice (~5 million cells) or nude rats (~7 million cells). To determine whether increasing the number of transplanted cells would affect maturation in vivo, we also compared glucose-stimulated human C-peptide secretion at 28 weeks following transplantation of either 5, 10, or 20 million hESC-derived progenitor cells in a separate study (Figure S2). Although the amount of human C-peptide tended to increase with the higher doses of cells, the degree of glucose-responsiveness (indicated by the C-peptide stimulation index) of hESC-derived cells did not differ between doses (Figure S2).

At the time of transplantation, nude rats weighed ~ ten times more than SCID-beige mice on average (Figure S3A), but rats were transplanted with less than two times the amount of cells (Figure S3B). Therefore, although the kidney grafts in rats were slightly larger than in mice (Figures S3C and S3E), they were not scaled proportionately relative to the profound difference in body weight and, consequently, blood volume of the host (Figure S3D). Interestingly, despite this discrepancy in dosing, plasma human C-peptide concentrations were indistinguishable between species after a meal challenge (Figure 1A). However, rats had significantly lower basal human C-peptide levels compared with mice between 19 and 32 weeks post-transplantation (Figure 1A), which translated into an improved stimulation index for meal-induced human C-peptide secretion (Figure S4). At just 19 weeks, hESC-derived cells from rats secreted significantly more human C-peptide under fed versus fasted conditions, whereas there was no meal response by engrafted cells in mice at any stage (Figure 1A; Figure S4). Blood glucose levels were similar between species after an overnight fast, but mice had consistently higher glucose excursions following a meal compared with rats at all ages examined, regardless of graft maturity (Figure 1B). Mice also experienced significantly higher glucose excursions during an oral glucose challenge relative to rats (Figure 1C). Glucose-stimulated human C-peptide secretion was observed in rats at 21 weeks post-transplantation, whereas human C-peptide levels dropped below baseline levels following the glucose challenge in mice (Figure 1D). There was a trend toward lower basal human C-peptide levels after a 6-hr fast in rats compared with mice, although this did not reach statistical significance ($p = 0.06$; Figure 1E). Under random-fed conditions, human insulin, glucagon, and GLP-1 levels were all significantly lower in rats relative to mice at 22 or 33 weeks post-transplantation (Figure 1F).

Kidney Capsule Grafts from Rats Contain More Mature β Cells Than Those Harvested from Mice

At 22/33 weeks post-transplantation, grafts from rats expressed significantly higher levels of mature β cell genes, including *INS*, *NKX6.1*, *MAFA*, *PAX6*, *ABCC8*, *PCSK1*, and *IAPP*, as well as significantly lower levels of the immature pancreatic endocrine marker *NEUROG3* and mature α cell genes, *GCG* and *ARX*, relative to grafts from mice (Figure 2). Furthermore, grafts from rats contained approximately three times more insulin relative to glucagon immunoreactivity, whereas glucagon immunoreactivity was almost twice as prevalent as insulin in grafts from mice (Figures 3A and 3C). Glucagon-positive cells in both species uniformly co-expressed the α cell transcription factor ARX (Figure S5A). Relative to the total endocrine area, grafts from rats were ~40%–50% insulin-positive, whereas grafts from mice were ~10%–25% insulin-positive (Figures 3A and 3C). There also appeared to be more pancreatic polypeptide (PP)-positive cells in grafts from rats, although cells expressing PP, somatostatin, or ghrelin were much less prominent and, therefore, not quantified (Figure S5B). At the gene level, there were no differences in *SST* or *PPY* expression between species, but grafts from mice had approximately two times more *GHRL* transcript compared with grafts from rats (Figure 2). Overall, hESC-derived progenitor cells developed more efficiently into the endocrine lineage in rats compared with mice, as indicated by the significantly reduced trypsin-positive area (Figures 3B and 3D), higher proportion of synaptophysin:trypsin immunoreactivity (Figures 3B and 3D), and decreased gene expression of the exocrine marker *PTF1A* in grafts from rats versus mice (Figure 2). The presence of non-endodermal germ layers was not detected in kidney capsule grafts from either species (Table S1), unlike grafts generated with our earlier differentiation protocol that, on occasion, contained regions of bone and cartilage tissues (Rezania et al., 2012).