CRISPR-editing of hESCs allows for production of immune evasive cells capable of differentiation to pancreatic progenitors for future type 1 diabetes therapy

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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease resulting in the demise of pancreatic beta cells. Patients living with T1D require frequent exogenous insulin injections to manage their chronic condition. Replacement of pancreatic islets with cadaver-derived islets has proven as a successful functional cure for some T1D patients allowing them to be independent of exogenous insulin. However, the limited supply of human islet donors along with the challenges associated with immunosuppression have restricted widespread access to islet transplantation. Moreover, lack of patient compliance with strict adherence to life-long immune suppression regimens also negatively impacts islet graft function. Human embryonic stem cells (hESCs) represent a renewable and expandable cell source with a demonstrated capacity to differentiate into insulin-producing cells for treating insulin-dependent diabetes. Stepwise directed differentiation approaches that mimic pancreatic development represent a viable approach to generate engraftable pancreatic lineages that can survive, mature, and effectively control blood glucose in rodent models. In order to protect these transplanted pancreatic cells from immune rejection, hESCs can be genetically modified ex vivo to prevent allogeneic-rejection. In particular, we generated edited clonal hESC lines that lack the *ß2-microglobulin* (*B2M*) gene, a required component of the major histocompatibility complex class I (MHC-I), and express a transgene encoding programmed death-ligand 1 (*PD-L1*) to further protect them from autoimmune T-cell attack as shown previously in mouse studies. Here, we describe the generation of such universal donor hESCs that appear immune evasive and retain competence to differentiate to pancreatic endodermal precursor cells (PEC) *in vitro*.
Materials and methods: CyT49 hESCs were genome edited with CRISPR-Cas9 to knock out the *B2M* gene and insert the *PD-L1* cDNA into the same locus via homology directed repair. The edited cells were then single-cell cloned to generate cell lines which were tested for B2M/PD-L1 and pluripotency marker expression and karyotypic stability. The resulting edited donor hESCs were successfully stage-wise differentiated to PEC cells. Flow cytometry and targeted RNA-sequencing were used to assess pancreatic lineage phenotypes. These modified PEC cells were then tested for their ability to trigger an *in vitro* T-cell response and susceptibility to natural killer (NK)-mediated cell killing.
Results: B2M negative/PD-L1 positive CyT49 cells were successfully generated and displayed a normal karyotype and pluripotency marker expression. Upon differentiation, the edited clonal cells showed morphological appearance, cell marker expression by flow cytometry (~40-50% NKX6.1/PDX1 double positive and CHGA negative), and gene expression characteristics of pancreatic endoderm cells equivalent to unedited cells. These edited PEC cells were evaluated in T-cell and NK-cell killing proliferation/lysis assays as well as in humanized rodent models.
Conclusion: Genome edited immune evasive stem cells were generated and differentiated to PEC, suggesting these cells may serve as a future therapy to treat diabetes in the absence of immunosuppression.
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