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Generation of glucose-sensitive insulin-secreting beta-like cells from human embryonic stem cells by incorporating a synthetic lineage-control network

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ABSTRACT

We previously reported novel technology to differentiate induced pluripotent stem cells (IPSCs) into glucose-sensitive insulin-secreting beta-like cells by engineering a synthetic lineage-control network regulated by the licensed food additive vanillic acid. This genetic network was able to program intricate expression dynamics of the key transcription factors Ngn3 (neurogenin 3, OFF-ON-OFF), Pdx1 (pancreatic and duodenal homeobox 1, ON-OFF-ON) and MafA (V-maf musculoaponeurotic fibrosarcoma oncogene homologue A, OFF-ON) to guide the differentiation of IPSC-derived pancreatic progenitor cells to beta-like cells. In the present study, we show for the first time that this network can also program the expression dynamics of Ngn3, Pdx1 and MafA in human embryonic stem cell (hESC)-derived pancreatic progenitor cells and drive differentiation of these cells into glucose-sensitive insulin-secreting beta-like cells. Therefore, synthetic lineage-control networks appear to be a robust methodology for differentiating pluripotent stem cells into somatic cell types for basic research and regenerative medicine.

1. Introduction

Type-I diabetes (T1D) is a consequence of autoimmune destruction of pancreatic beta cells, and so patients depend upon exogenous insulin for control of their blood glucose levels (Atkinson and Eisenbarth, 2001; Nathan, 2012). Although insulin injections reduce the hyperglycemic excursions in patients, failure to achieve correct dosing and timing can result in diabetic complications (Calcutt et al., 2009; Nathan, 1993). Transplantation of human pancreas or islets into patients can be curative, but there is severe shortage of donors (Bouwens et al., 2013; Shapiro et al., 2017) and the procedure involves the risk of transplant rejection, as well as life-long immunosuppressive medication (Ricordi and Strom, 2004). Therefore, an attractive approach to overcome these issues would be to use patient-derived pancreatic beta cells.

Beta cell development in an organism involves the orchestration of gene expression by transcriptional and translational regulation, epigenetic regulation, and intercellular communication (Arda et al., 2013; Shih et al., 2012; Watada et al., 2000; Xie et al., 2015). Studies of pancreatic development in mice identified the dynamics of three key transcription factors, Ngn3 (OFF-ON-OFF), Pdx1 (ON-OFF-ON) and MafA (OFF-ON), as being decisive for the specification and maturation of pancreatic progenitor cells into pancreatic beta cells (Gradwohl et al., 2000; Johansson et al., 2007; Nelson et al., 2007; Nishimura et al., 2009). We considered that these complex dynamics could be mimicked by introducing rationally designed synthetic circuits to regulate gene expression (Auslander et al., 2016; Xie et al., 2016). The level of control that can be achieved with synthetic circuits is sufficient to enable targeted differentiation of patient-derived stem cells into somatic cell types for cell therapy in a 'quality by design' framework (Lipsitz et al., 2017; Saxena et al., 2016).

Capitalizing on these design principles, we have recently reported the differentiation of human induced pluripotent stem cells (hIPSCs) into beta-like cells using a synthetic biology-inspired lineage-control network (Saxena et al., 2016). Here, to demonstrate the generalizability and robustness of this method, as well as to enhance our understanding of cell fate decisions during beta cell development, we implemented the same genetic network for the differentiation of human embryonic stem cell (hESC)-derived pancreatic progenitor cells into glucose-sensitive insulin-secreting beta-like cells.

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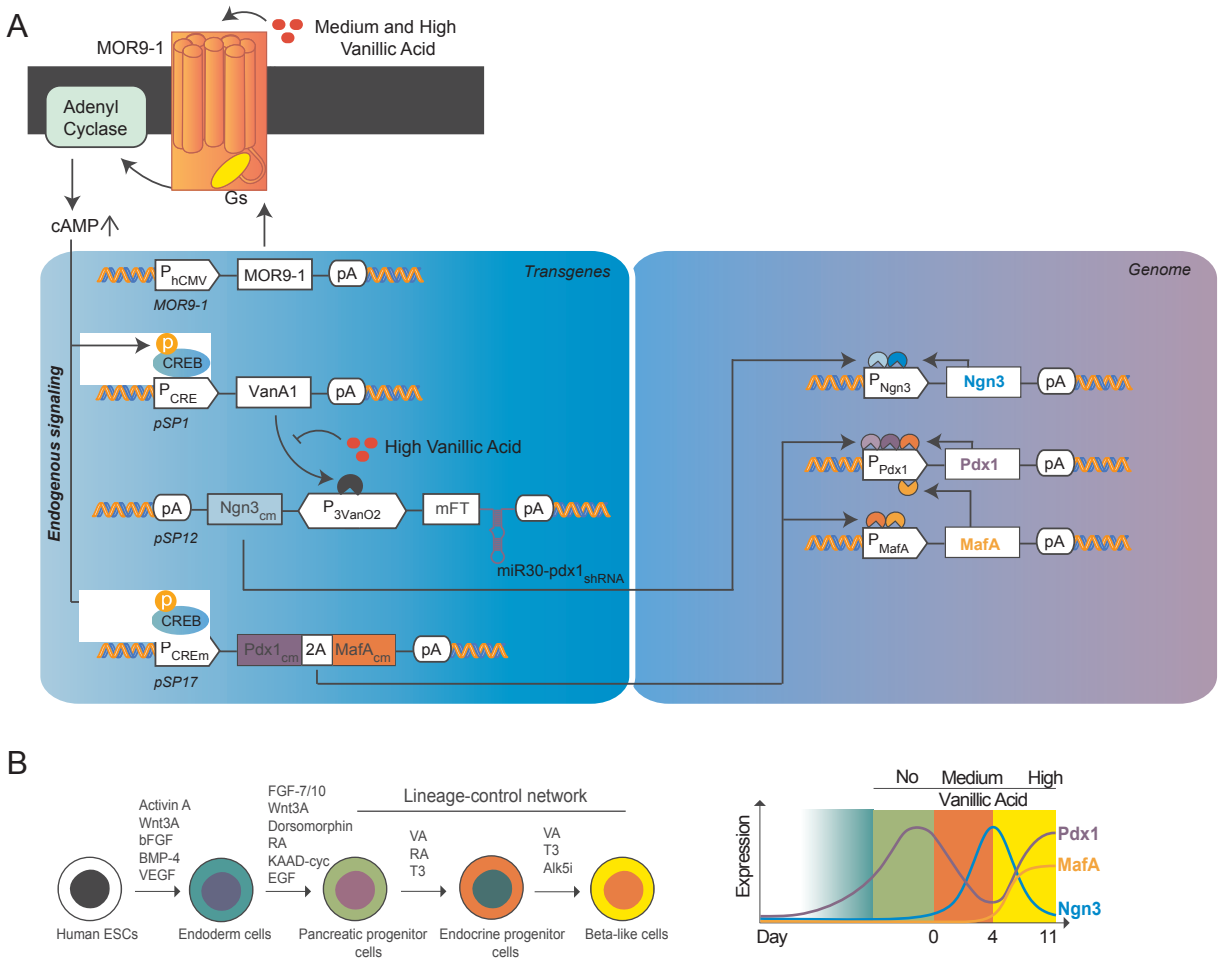

Fig. 1. Differentiation of hESC-derived pancreatic progenitor cells to beta-like cells using a synthetic lineage-control network. (A) Addition of medium concentration of vanillic acid activates the G-protein-coupled receptor (MOR9-1), leading to activation of adenyl cyclase via the G protein (Gs) and subsequent increase in cellular cyclic AMP (cAMP) levels. Thereby, the endogenous cyclic AMP-response element-binding protein (CREB) is activated by phosphorylation, triggering the expression of vanillic acid-dependent transactivator (VanA1) from the promoter P_{CRE}. Upon binding of VanA1 to its cognate promoter (P_{3VanO2}), codon-modified Ngn3 ($Ngn3_{cm}$), fluorescent timer protein (mFT) and a microRNA targeting Pdx1 (miR30-$Pdx1_{shRNA}$) are simultaneously expressed. Higher concentrations of vanillic acid inhibit the binding of VanA1 to P_{3VanO2}, thus repressing the expression of $Ngn3_{cm}$, mFT and miR30-$Pdx1_{shRNA}$. Thereafter, CREB activates a modified P_{CREm} with lower sensitivity which triggers expression of codon-modified $Pdx1_{cm}$ and $MafA_{cm}$. All codon-modified genes ($Ngn3_{cm}$, $Pdx1_{cm}$, MafAcm) transcriptionally activate their genomic counterparts (Ngn3, Pdx1, MafA) through positive feedback loops. (B) hESCs were differentiated into pancreatic progenitor cells using a standard growth-factor/chemical-based method. Transfection of plasmids encoding the lineage-control network into hESC-derived pancreatic progenitor cells triggers programmed expression dynamics of endogenous transcription factors Ngn3 (OFF-ON-OFF), Pdx1 (ON-OFF-ON) and MafA (OFF-ON) in a vanillic acid-controlled manner (the color code matches the cellular phenotypes and the expression dynamics). Abbreviations: ActA: activin A; Alk5i: Alk5 inhibitor II; bFGF: basic fibroblast growth factor; BMP4: bone morphogenetic protein 4; KAAD-Cyc: KAAD cyclopamine; EGF: epidermal growth factor; FGF7/10: fibroblast growth factor 7/10; RA: retinoic acid; T3: triiodothyronine; VA: vanillic acid; VEGF: vascular endothelial growth factor; Wnt3A: wingless-type MMTV integration site family, member 3A.

2. Materials and methods

2.1. Culture and differentiation of hESCs

The hESCs (WA09, WiCell Research Institute, Wisconsin, USA) used for differentiation were cultivated in Geltrex-coated 12-well culture plates (Invitrogen, Lucerne, Switzerland) containing mTESR media (STEMCELL Technologies, Grenoble, France). The cells were enzymatically passaged by detaching them into clumps of 200–400 cells using 1 U ml^{-1} dispase (STEMCELL Technologies). The differentiation of hESCs into pancreatic progenitor cells was performed according to our previously published protocol (Fig. 1B) (Saxena et al., 2016) with the following modification: Noggin was replaced with Dorsomorphin (Sigma-Aldrich, Buchs, Switzerland) during stage two (primitive gut tube cells) of differentiation.

The key plasmids used in this study are pCI-MOR9-1 (P_{hCMV}-MOR9-1-pA), pSP1 (P_{CRE}-$VanA_1$-pA), pSP12 (pA-$Ngn3_{cm}$←P_{3VanO2}→mFT-miR30-$Pdx1_{shRNA}$-pA), pSP17 (P_{CREm}-$Pdx1_{cm}$-2A-$MafA_{cm}$-pA), pSP26 (P_{hINS}-DsRed-Express-pA) and pEGFP-N1 (Clontech, California, USA), as described previously (Saxena et al., 2016). The pancreatic progenitor cells were transfected with 3 µg of total DNA (pCI-MOR9-1, pSP1, pSP12, pSP17, pEGFP-N1, pSP26) for the network-based differentiation or (pEGFP-N1, pSP26) for growth-factor/chemical-based differentiation, in accordance with our earlier report (Saxena et al., 2016). The transfection efficiency was determined by FACS-mediated analysis of pEGFP-N1-transfected pancreatic progenitor cells (Supplementary data, Fig. S1).

2.2. FACS-mediated-analysis and cell sorting

FACS-mediated-analysis and cell sorting was performed using a Becton Dickinson LSRII Fortessa flow cytometer (Becton Dickinson,