Human expandable pancreatic progenitor–derived β cells ameliorate diabetes

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1.簡述論文的概要與重大發現

糖尿病在現今社會中日漸流行，而選擇做移植手術是目前最主要治療糖尿病的選擇之一。但此方法有所限制，會受限於移植物 β cells 供應短缺，與一些移植相關的免疫排斥等問題，所以如何能大規模，小成本的生產出 functional pancreatic β cells (ePP-β)，是現階段的主要障礙。而早在多年前，便已成功從人類多能幹細胞 hPSC 取得 beta cells。但從幹細胞分化到 β cells 的步驟繁瑣，耗時長。因此，希望能從 Pancreatic progenitors (PPs) 這步開始，來減少製備的時間。PPs 會主要表現 PDX1 、NKX6.1，這兩個基因。但繼代後會使這兩個基因表現下降，所以用化學篩選法找出能使基因表現提升的藥 I-BET151，在確認了添加藥後，會使 PPs 有穩定表現 PDX1 、NKX6.1，也能使細胞更穩定的擴增，不會發生突變。最後更將細胞移植入免疫缺陷的糖尿病鼠中，且成功控制了糖尿病鼠的血糖。總之，這樣的結果對生物醫學研究和再生醫學等邁出了顯著的一步。

2.對論文內容的疑問

Fig 1 的 flow 圖證明，加了 I-BET151 會讓 $PDX1^+$ $NKX6.1^+$基因 expression 上升，但沒說明控制組與加藥組細胞是否為同一代數，如果拿 passage 1 的細胞不添加 I-BET151 應該也能達到 63 %，又或者，細胞隨繼代數拉更長，儘管加了 I-BET151 讓基因表現增強，但總體來說，基因表現仍是下降的，如果有標示的話就更好了。

3.. 論文的缺點與評論

作者在 Fig 3 中比較 ePP-β，跟人類 islet 細胞的 insulin 分泌能力，想表示分化出的 ePP-β 細胞功能和人類真正的 islet 越來越類似，如果能在 insulin 分泌能力上，再比上一個 insulin–producing pancreatic β–like cells (SC-β cells) 的值就更好了。

DEVELOPMENTAL BIOLOGY

Human expandable pancreatic progenitor–derived β cells ameliorate diabetes

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An unlimited source of human pancreatic β cells is in high demand. Even with recent advances in pancreatic differentiation from human pluripotent stem cells, major hurdles remain in large-scale and cost-effective production of functional β cells. Here, through chemical screening, we demonstrate that the bromodomain and extraterminal domain (BET) inhibitor I-BET151 can robustly promote the expansion of PDX1^{+}NKX6.1^{+} pancreatic progenitors (PPs). These expandable PPs (ePPs) maintain pancreatic progenitor cell status in the long term and can efficiently differentiate into functional pancreatic β (ePP-β) cells. Notably, transplantation of ePP-β cells rapidly ameliorated diabetes in mice, suggesting strong potential for cell replacement therapy. Mechanistically, I-BET151 activates Notch signaling and promotes the expression of key PP-associated genes, underscoring the importance of epigenetic and transcriptional modulations for lineage-specific progenitor self-renewal. In summary, our studies achieve the long-term goal of robust expansion of PPs and represent a substantial step toward unlimited supplies of functional β cells for biomedical research and regenerative medicine.

INTRODUCTION

Diabetes mellitus represents a global health epidemic and affects millions of people worldwide. Islet transplantation holds great promises but is limited by shortage in supply of organ donors and immunosuppression issues associated with transplantation (*1*). Human pluripotent stem cells (hPSCs) can give rise to all cell types of the body (*2*, *3*). During the past two decades, stepwise differentiation protocols have been devised to guide the specification of hPSCs into definitive endoderm (DE), pancreatic progenitor (PP), endocrine precursor (EP), and pancreatic β–like cells (*4*–*17*). While these findings are highly encouraging, generating a large quantity of functional pancreatic β–like cells for disease modeling, drug screening, and cell-based therapy remains an extremely labor- and time-intensive process because of the multiple intermediate steps in directed differentiation.

A possible solution is to bypass the upstream steps starting with the hPSC source by initiating differentiation from renewable and expandable pancreatic progenitors (ePPs) that are developmentally more proximal to the β cells. However, robust expansion of human pancreatic progenitors has been challenging because the molecular mechanisms of human pancreatic progenitor self-renewal are poorly defined. In development, transcription factors (TFs) play an integral role, and the expression of key TFs is widely used to monitor the differentiation process and to evaluate cellular identity (*18*, *19*). During pancreatic development, expression of NKX6.1 follows that of PDX1 (*20*–*23*), and coexpression of PDX1 and NKX6.1 is widely used for defining and identifying pancreatic progenitors that can efficiently differentiate into functional pancreatic β–like cells (*5*–*8*). In recent years, several groups reported methods for culturing human endodermal derivatives at early developmental stages (*24*–*26*). In terms of pancreatic progenitors, Trott *et al.* (*27*) developed a culture condition that could expand PDX1-positive pancreatic progenitors, but unfortunately, these progenitors could not maintain the expression of NKX6.1. Pancreatic progenitors have also been expanded in three-dimensional (3D) culture (*28*–*30*). Ameri *et al.* (*31*) reported that genetic knockdown of cyclin-dependent kinase inhibitors *CDKN1A* and *CDKN2A* could increase the proliferation of glycoprotein 2–positive pancreatic progenitors, but here, application of the genetic methods may cause safety issues, which potentially limit its clinical usages. We previously demonstrated that pancreatic progenitors directly converted from human fibroblasts could be expanded in a chemically defined medium containing epithelial growth factor (EGF), basic fibroblast growth factor (bFGF), and A83-01 [a transforming growth factor–β (TGFβ) inhibitor], but the percentage of PDX1 and NKX6.1 double-positive cells was less than 20% (*32*). Whether this culture condition is suitable for human pancreatic progenitors directly differentiated from hPSCs has not been demonstrated so far. Thus, whether PDX1 and NKX6.1 double-positive pancreatic progenitors from hPSCs are competent for long-term expansion and, if yes, how to accomplish this goal are key questions in the field.

Synthetic chemical compounds provide useful tools to control cell fates and can also be used to decode the molecular mechanisms of biological processes (*33*, *34*). During the past decades, small molecules targeting specific signaling pathways are selected and applied on the basis of the knowledge learned from pancreatic developmental biology, and unbiased high-throughput chemical screening approaches have also been applied stepwise during the pancreatic differentiation process, resulting in the discovery of many effective small molecules for pancreatic differentiation (*5*, *35*–*38*). Small molecules have several advantages over the genetic methods, including that they are convenient to use, cost-effective, and can provide greater temporal control and be fine-tuned by varying their concentrations and combinations (*39*, *40*).

Here, we carried out a phenotypic chemical screen and identified that BET bromodomain inhibitor I-BET151 could significantly

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