Pancreatic Stem Cell Research Literatures

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Abstract: The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

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Key words: stem cell; pancreatic; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

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Danner, S., J. Kajahn, et al. "Derivation of oocyte-like cells from a clonal pancreatic stem cell line." Mol Hum Reprod. 2007 Jan;13(1):11-20. Epub 2006 Nov 17.

Adult pancreatic stem cells (PSCs) are able to differentiate spontaneously in vitro into various somatic cell types. Stem cells isolated from rat pancreas show extensive self-renewal ability and grow in highly viable long-term cultures. Additionally, these cells express typical stem cell markers such as Oct-4, nestin and SSEA-1. Although differentiation potential is slightly decreasing in long-term cultures, it is possible to keep cell lines up to passage 140. Clonal cell lines could be established from different passages and showed similar characteristics. Remarkably, one clonal cell line, generated from passage 75, showed deviant properties during further culture. Clonal cells formed aggregates, which built tissue-like structures in suspension culture. These generated 3D aggregates produced permanently new cells at the outside margin. Released cells had remarkable size, and closer examination by light microscopy analysis revealed oocyte-like morphology. A comparison of the gene expression patterns between primary cultures of passages 8 and 75, the clonal cell line and the produced oocyte-like cells (OLCs) from tissue-like structures demonstrated some differences. Expression of various germ cell markers, such as Vasa, growth differentiation marker 9 and SSEA-1, increased in the clonal cell line, and OLCs showed additionally expression of meiosis-specific markers SCP3 and DMC1. We here present a first pilot study investigating the putative germ line potential of adult PSCs.

Guo, X. M. and C. Y. Wang "[Recent advances in islet transplantation and pancreatic stem cell research]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2003 Feb;25(1):83-7.

This paper reviewed recent advances in pancreatic islet transplantation research, including islet isolation, purification, culture, cryopreservation and immunoisolation. Latest progresses in induction of pancreatic stem cell and embryonic stem cell to differentiate into insulin-producing islets were also introduced. On the basis of the present situation and future development of islet transplantation-based therapies for diabetes, the author thought that allogeous islet transplantation is a main choice for type I diabetes today and pancreatic stem cell transplantation for tomorrow.

Han, W., X. He, et al. "Establishment of a porcine pancreatic stem cell line using T-REx() system-inducible Wnt3a expression." Cell Prolif. 2015 Jun;48(3):301-10. doi: 10.1111/cpr.12188. Epub 2015 Apr 20.

OBJECTIVES: Porcine pancreatic stem cells (PSCs) are highly valuable in transplantation applications for type II diabetes. However, there are still many problems to be solved before they can be

used in the clinic, such as insufficient cell number availability and low secretion level of insulin. It has been reported that Wnt3a plays pivotal roles during cell proliferation and differentiation. Here, we have aimed to establish an ideal research platform using the T-REx() system, to study mechanisms of Wnt3a during PSC proliferation and differentiation. MATERIALS AND METHODS: Construction of the recombinant plasmid and cell transfection were used for establishment of a porcine PSC line. Related gene expressions were examined using quantitative real-time PCR (QRT-PCR), western blotting, immunostaining and flow cytometry. BrdU incorporation assay and cell cycle analysis were used to investigate Wnt3a roles in PSCs. RESULTS: Wnt3a-expressing clones regulated by T-REx() were successfully obtained. Wnt3a and GFP expression were strictly regulated by Dox in a time- and dose-dependent manner. Furthermore, we found that Wnt3a-expressing porcine PSCs induced by Dox exhibited raised proliferative potential. After Dox stimulation, expression of PCNA, C-MYC and active beta-catenin were higher, but were down-regulated after Dkk1 addition. CONCLUSION: We established a porcine PSC line that dynamically expressed Wnt3a, and we found that Wnt3a promoted PSC proliferative potential. This inducible expression system thus provides an important tool for further study on porcine PSC development and differentiation.

Kuise, T., H. Noguchi, et al. "Establishment of a pancreatic stem cell line from fibroblast-derived induced pluripotent stem cells." Biomed Eng Online. 2014 May 27;13:64. doi: 10.1186/1475-925X-13-64.

BACKGROUND: For cell therapies to treat diabetes, it is important to produce a sufficient number of pancreatic endocrine cells that function similarly to primary islets. Induced pluripotent stem (iPS) cells represent a potentially unlimited source of functional pancreatic endocrine cells. However, the use of iPS cells for laboratory studies and cell-based therapies is hampered by their high tumorigenic potential and limited ability to generate pure populations of differentiated cell types in vitro. The purpose of this study was to establish a pancreatic stem cell line from iPS cells derived from mouse fibroblasts. METHODS: Mouse iPS cells were induced to differentiate into insulin-producing cells by a multi-step differentiation protocol, which was conducted as described previously with minor modifications. Selection of the pancreatic stem cell was based on morphology and Pdx1 expression. The pancreatic potential of the pancreatic stem cells was evaluated using a reverse transcription PCR, real-time PCR, immunofluorescence, and a glucose challenge test. To assess potential tumorigenicity of the pancreatic stem cells, the cells were injected into the quadriceps femoris muscle of the left hindlimb of nude mice. RESULTS: The iPS-derived pancreatic stem cells expressed the transcription factor--Pdx1--a marker of pancreatic development, and continued to divide actively beyond passage 80. Endocrine cells derived from these pancreatic stem cells expressed insulin and pancreatic genes, and they released insulin in response to glucose stimulation. Mice injected with the pancreatic stem cells did not develop tumors, in contrast to mice injected with an equal number of iPS cells. CONCLUSION: This strategy provides a new approach for generation of insulin-producing cells that is more efficient and safer than using iPS cells. We believe that this approach will help to develop a patient-specific cell transplantation therapy for diabetes in the near future.

Noguchi, H., K. Oishi, et al. "Establishment of mouse pancreatic stem cell line." Cell Transplant. 2009;18(5):563-71.

beta-Cell replacement therapy via islet transplantation is a promising possibility for the optimal treatment of type 1 diabetes. However, such an approach is severely limited by the shortage of donor organs. Pancreatic stem/progenitor cells could become a useful target for beta-cell replacement therapy in diabetic patients because the cells are abundantly available in the pancreas of these patients and in donor organs. In this study, we established a mouse pancreatic stem cell line without genetic manipulation. The duct-rich population after islet isolation was inoculated into 96-well plates in limiting dilution. From over 200 clones, 15 clones were able to be cultured for over 3 months. The HN#13 cells, which had the highest expression of insulin mRNA after induction, expressed PDX-1 transcription factor, glucagon-like peptide-1 (GLP-1) receptor, and cytokeratin-19 (duct-like cells). These cells continue to divide actively beyond the population doubling level (PDL) of 300. Exendin-4 treatment and transduction of PDX-1 and NeuroD proteins by protein transduction technology in HN#13 cells induced insulin and pancreas-related gene expression. This cell line could be useful for analyzing pancreatic stem cell differentiation. Moreover, the isolation technique might be useful for identification and isolation of human pancreatic stem/progenitor cells.

Pattou, F., J. Kerr-Conte, et al. "[Human pancreatic stem cell and diabetes cell therapy]." Bull Acad Natl Med. 2000;184(9):1887-99; discussion 1899-901.

Cell therapy offers today important perspectives for the treatment of type 1 diabetes. The current utilization of primary human islets of Langerhans nevertheless forbids all hope of

developing this treatment on a large scale. The recent description of the persistence of stem cells capable of proliferating and differentiating in the adult pancreas offers an attractive alternative for the production in vitro of homologous insulin-secreting cells. We first reproduced in vitro from human islet preparations the proliferation of ductal epithelial structures and their progressive organization. Thereafter, we focused on the description of a reproducible source of human ductal cells by the transdifferentiation of exocrine preparations. More recently we described in these exocrine derived ductal cells the the expression the of insulin promoter factor-1 (IPF-1/otherwise known as PDX-1), a transcription factor essential for the differentiation of ductal cells into endocrine cells during both development and pancreatic regeneration. If the proliferation and differentiation of these cells is confirmed, this approach could lead to the description of an abundant source of human pancreatic stem cells for the production ex vivo of human insulin secreting cells and may even allow autologous cell therapy, in the absence of immunosuppression.

Rivas-Carrillo, S. D., J. Kanamune, et al. "Endothelial cells promote pancreatic stem cell activation during islet regeneration in mice." Transplant Proc. 2011 Nov;43(9):3209-11. doi: 10.1016/j.transproceed.2011.09.082.

OBJECTIVES: Diabetes is the clinical consequence of the loss of the majority of the beta-cell population and failure to regenerate new pancreatic beta cells. The current therapies based on beta-cell replacement have failed to achieve beta-cell renewal and thus, long-term insulin freedom. We have hypothesized that early rejection of endothelial elements within the islet grafts may seriously hamper islet regeneration in both native and islet grafts. METHODS: In the present study, we analyzed the role of endothelial cells to activate pancreatic stem cells during islet regeneration. Mice were pretreated with or without endothelial pharmacological ablation of endothelial cells, followed by an acute beta-cell injury using a single intraperitoneal injection of streptozotocin. We performed comparative morphometric analyses of recovered pancreata on days 3, 7, 10, and 30 after streptozotocin injury, staining with bromodeoxyuridine (BrdU) for representative cell types, beta cells, endothelial elements, and stem cells. Blood glucose levels were measured continuously after the injury to monitor the capacity for metabolic control. RESULTS: Morphometric analyses revealed an increasing number of cells over time to be stained with a stem cell and BrdU markers among animals only injured with streptozotocin but not with endothelial ablation. Notably, on day 10, stem cell markers were dramatically decrease nearly to basal levels, with appearance of numerous insulin-positive cells. Intact vessels with cobblestone-shaped endothelial elements were observed in direct proportion to the better outcomes, both by morphometric and by metabolic parameters. In contrast, fewer insulin-positive cells were observed in pancreata that had been ablated of endothelial cells showing extensive collapse of endocrine functions. CONCLUSIONS: We observed that endothelial elements promoted stem cell proliferation and islet regeneration after a beta-cell insult. We believe that preservation of endothelial cells positively affects the process of pancreatic regeneration.

Taylor-Fishwick, D. A. and G. L. Pittenger "Harnessing the pancreatic stem cell." Endocrinol Metab Clin North Am. 2010 Dec;39(4):763-76. doi: 10.1016/j.ecl.2010.08.008. Epub 2010 Oct 13.

Building on the elaborate research studies that have helped map out key decision points in the process of pancreas development, reprogramming of pluripotent embryonic stem cells or induced pluripotent stem cells offers the possibility of overcoming restrictions on tissue supply associated with transplantation of donor islets. In a healthy pancreas, the beta-cell mass can exhibit significant plasticity, as reflected in the normal adaptive response in beta-cell mass to offset the metabolic challenge associated with pregnancy and obesity. In this article, alternative strategies and potential sources of pancreatic stem cells are considered.

Xiao, M., L. An, et al. "Establishing a human pancreatic stem cell line and transplanting induced pancreatic islets to reverse experimental diabetes in rats." Sci China C Life Sci. 2008 Sep;51(9):779-88. doi: 10.1007/s11427-008-0109-6. Epub 2008 Aug 24.

The major obstacle in using pancreatic islet transplantation to cure type I and some type II diabetes is the shortage of the donors. One of ways to overcome such obstacle is to isolate and clone pancreatic stem cells as "seed cells" and induce their differentiation into functional islets as an abundant transplantation source. In this study, a monoclonal human pancreatic stem cell (mhPSC) line was obtained from abortive fetal pancreatic tissues. Pancreatic tissues were taken from abortive fetus by sterile procedures, and digested into single cells and cell clusters with 0.1% type IV collagenase. Cultured in modified glucose-low DMEM with 10% fetal bovine serum (FBS), these single cells and cell clusters adhered to culture dishes, and then primary epidermal-like pancreatic stem cells started to clone. After digesting with 0.25% trypsin and 0.04% EDTA, fibroblasts and other cells were gradually eliminated

and epithelioid pancreatic stem cells were gradually purified during generations. Using clone-ring selection, the mhPSCs were obtained. After addition of 10 ng/mL epidermal growth factor (EGF) in cell culture medium, the mhPSCs quickly grew and formed a gravelstone-like monolayer. Continuously proliferated, a mhPSC line, which was derived from a male abortive fetus of 4 months old, has been passed through 50 generations. More than 1 x 10(9) mhPSCs were cryo-preserved in liquid nitrogen. Karyotype analysis showed that the chromosome set of the mhPSC line was normal diploid. Immunocytochemistry results demonstrated that the mhPSC line was positive for the pdx1, glucagon, nestin and CK19, and negative for the insulin, CD34, CD44 and CD45 protein expression. RT-PCR revealed further that the mhPSCs expressed transcription factors of the pdx1, glucagon, nestin and CK19. Also, in vitro induced with beta-mercaptoethanol, the mhPSCs differentiated into nerve cells that expressed the NF protein. Induced with nicotinamide, the mhPSCs differentiated into functional islet-like clusters, as identified by dithizone staining, which expressed the transcription factor of the insulin and secreted the insulin and C-peptide. Furthermore, the transplantation of mhPSCs-induced pancreatic islets into the subcapsular region of the kidney in streptozotocin-induced diabetic rats could reduce blood glucose levels and prolong the life time.

Xiao, M., L. L. An, et al. "[Research on differentiation features of the monoclonal human pancreatic stem cell]." Fen Zi Xi Bao Sheng Wu Xue Bao. 2008 Dec;41(6):457-64.

The in vitro and in vivo differentiation features of the monoclonal human pancreatic stem cell (mhPSC) line derived from the pancreatic tissues of a male abortive fetus at 4 month-old were studied. The mhPSCs were plated in culture dishes that had been coated with 0.1% gelatin in phosphate-buffered saline without calcium and magnesium. After proliferated for 3 days, the mhPSCs were induced in modified high-glucose Dulbecco's Modified Eagles's Medium for 25 days. The changes of the cell morphology were observed by phasecontrast microscope during inducement course. The results of the mhPSCs in vitro induced to differentiate into functional pancreatic islets were identified using dithizone staining, RT-PCR and stimulation-glucose secreting insulin and C-peptide radioimmunoassay. The mhPSCs suspension was separately injected under the groin hypoderm of male nude mice. On the 30th day, the grafts were taken off. The immunochemistry reactions were performed by the SP method. The in vivo differentiation ability of the mhPSCs in nude mice was assessed. In vitro proliferation culture, the mhPSCs adhesively grew and showed polygon epithelioid morphology. After proliferation a layer, the mhPSCs showed the gravelstone-like. During in vitro directional inducement, the mhPSCs gradually turned from polygon to round, suspended to grow and assembled pancreatic islets-like clusters. On the 15th inducement day, only a few cells of pancreatic islet-like clusters were induced into the beta cells that became crimson with dithizone staining. However, till the 25th inducement day, most cells of pancreatic islet-like clusters had differentiated into the beta cells, as identified by dithizone staining, which expressed transcription factor of insulin. Respectively stimulated with different concentration glucose, the induced pancreatic islets not only secreted insulin and C-peptide, but also the secretion volumes of the insulin and C-peptide were markedly increased after the stimulation with higher concentration glucose ($0.01<P<0.05$). In the in vivo differentiation experiment, all nude mice which were injected by the mhPSCs displayed a teratoma-like with white color and rich blood vessels. Immunohistochemistry showed that the teratoma-like expressed the proteins of the pdx1, insulin, glucagon, CK, MBP and NF. This indicated that the mhPSCs not only could be in vitro induced into functional islet-like clusters, but also could in vivo differentiate into the pancreatic islets, epithelium and neural cells.

Xiao, M., L. L. An, et al. "[Establishment of the isolation and culture system for monocloning human pancreatic stem cell]." Fen Zi Xi Bao Sheng Wu Xue Bao. 2008 Dec;41(6):450-6.

The present study aimed to establish an isolation and culture system for the monoclonal human pancreatic stem cells and monoclonal human pancreatic stem cell line. Some factors which would influence the proliferation of the monoclonal human pancreatic stem cells were assessed. Pancreatic tissues, taken from abortive fetuses by sterile procedures, were dissected into 1 mm3 segments and digested with 0.1% type IV collagenase. The isolated cells were grown in media containing low glucose Dulbecco's Modified Eagles's Medium supplemented with 10% fetal bovine serum (FBS), 3.7 g/L sodium pyruvate, 0.08 g/L penicillin and 0.1 g/L streptomycin. These cells were further digested with 0.25 g/L trypsin and 0.4 g/L EDTA for propagation. The monoclonal human pancreatic stem cells were selected by clone-ring, and further proliferated after addition of 10 ng/mL epidermal growth factor (EGF) in culture media. The cell chromosome set was determined by karyotype analysis. The growth curve was made by the 3-(4, 5)-dimethylthiahiazo (-z-yl)-3, 5-di-phenytetrazoliumromide (MTT) method. The results showed that pancreatic tissues were digested to

many single cells and cell clusters with collagenase. Adherently cultured, primary epidermal-like pancreatic stem cells grew clonally. After several times of dissociation and propagation, pancreatic stem cells were gradually purified during generations. Using clone-ring selection, the monoclonal human pancreatic stem cells were obtained. Continuously propagated, a monoclonal human pancreatic stem cell line which was derived from a male abortive fetus of 4 month-old had been passed through 50 generations. Karyotype analysis demonstrated that the chromosome set of the monoclonal human pancreatic stem cell line was normal diploid. Growth curve revealed that monoclonal human pancreatic stem cells grew slowly in initial 1-4 days. Then, they entered the logarithmic growth period in next 5-6 days. Their proliferation was speeded up by supplementation with 15% FBS in culture media, which was even more quickly after addition of the 15 ng/mL EGF or 10 ng/mL insulin growth factor II (IGF-II). The result identified that the monoclonal human pancreatic stem cell line could be obtained by the cell isolation and culture system.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

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