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# 5′-AZA induces Ngn3 expression and endocrine differentiation in the PANC-1 human ductal cell line

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ABSTRACT

Neurogenin 3 is necessary for endocrine cell development in the embryonic pancreas and has been shown to induce transdifferentiation duct cells from adult pancreas toward a neuro-endocrine phenotype. Here we discovered that the demethylating agent 5′-Azadeoxycytidine (AZA) induced Ngn3 expression and endocrine differentiation from the PANC-1 human ductal cell line. The expression of markers specific to mature islet cells, i.e., glucagon and somatostatin, was also observed. In addition, we demonstrated that growth factors (betacellulin and soluble factors released during pancreas embryogenesis) increased the level of maturation. Our studies revealed that the PANC-1 model system may provide a basis for elucidating the ductal/endocrine differentiation.



## Introduction

The global prevalence of diabetes has spurred efforts to generate large numbers of insulin-producing beta cells for replacement therapy [1,2]. Promising strategies include expansion of existing beta cells and conversion of either pancreatic or non-pancreatic adult stem/progenitor cells to beta cells [3,4]. Although beta cells proliferate in vivo [5] and in vitro for mouse and rat cells [6], fully differentiated human beta cells from donors with the typical age range used for transplantation failed to expand in vitro [7]. Putative adult pancreatic stem/progenitor cells represent an attractive area of investigation. Based initially on morphological and histological observations, Xu et al. demonstrated recently the existence of endogenous progenitors in injured adult mouse pancreas showing that the adult pancreas retains the potential to reactivate its embryonic mode of beta cell development [8]. Expression studies and lineage tracing experiments demonstrated that these endocrine progenitor cells emerge from the pancreatic duct.

Endocrine and exocrine cells originate from a precursor epithelial cell during pancreatic organogenesis [9,10]. Various different factors are required to achieve the mature phenotype characteristic of islet beta cells. It has been shown that all endocrine cells derived from Ngn3, a member of the basic helix-loop-helix transcription factor family, positive cells and Ngn3 deficiency abolished islet cell differentiation [11–14]. Thus, Ngn3 expression has been proposed as an exclusive marker for islet progenitors in both embryonic and adult pancreata. As proof of concept, it has been demonstrated that ectopic expression of Ngn3 in adult human or porcine pancreatic ductal cells can induce differentiation to an endocrine phenotype [15,16].

Recent study demonstrated the role of epigenetic mechanisms in pancreatic lineage development [17]. In the present study, we investigated the ability of HDAC inhibitors and demethylating agent 5′-Azadeoxycytidine (AZA) to induce Ngn3 expression and endocrine differentiation from the PANC-1 human ductal cell line. We showed here that AZA treatment was able to induce Ngn3 expression and endocrine differentiation. Thus, the PANC-1 model system may provide a basis for elucidating the ductal/endocrine differentiation.

## Materials and methods

*Materials.* Trichostatin A (TSA) and 5′-Azadeoxycytidine were obtained from Sigma (St. Quentin-Fallavier, France) and dissolved in ethanol and DMSO, respectively. Activin A, Activin B, betacellulin were also obtained from Sigma and prepared as the manufacturer's guidelines.

*Cell culture.* PANC-1 human ductal cell line was purchased from ATCC and cultured in Dulbecco's modified Eagle's medium Glutamax-1, high glucose (Invitrogen) supplemented with 10% fetal calf

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serum (PAA laboratories Gmbh) plus 100 U of penicillin and 100 μg of streptomycin per ml (Invitrogen).

*RNA preparation and real-time PCR.* Total RNA was prepared using RNeasy Minikit (Qiagen). Purified RNA was adjusted to 1 μg/μl and its integrity was assessed with the Agilent RNA 6000 chips coupled with the Agilent 2100 Bioanalyzer (Agilent Technologies), by visualizing the 18S and 28S ribosomal ribonucleic acid (rRNA). Reverse transcription (RT) was performed using random hexamers as recommended by the manufacturer (Applied Biosystems). cDNAs were analyzed by PCR amplification using the TaqMan PCR master mix (Applied Biosystems) and a mix of RPLO primers and probes. The different probes were purchased from Applied Biosystems (assay on demand kit). Reactions (40 cycles) and data analysis were carried out with an ABI Prism 7700 (Perkin-Elmer).

*Immunocytochemistry.* PANC-1 cells were grown in 6-well plates and fixed for 20 min in ice-cold ethanol 80% at 4 °C and permeabilized for 15 min in 0.5% Triton X-100. Cells were then incubated sequentially with an anti-Chromogranin A antibody (Dako) and a secondary antibody as described by the manufacturer (dako). The cells were then examined using a leica microscope.

## Results

### *AZA induced Ngn3 expression and endocrine differentiation in the PANC-1 human ductal cell line*

We first investigated the ability of HDAC inhibitors and demethylating agents to induce Ngn3 expression and endocrine differentiation. For this, PANC-1 cells were cultured for 3 days in the presence of 10 nmol/l of TSA or 1 μmol/l of AZA then 2 days in normal media. As shown in Fig. 1A, quantitative RT-PCR analysis revealed that Pdx1 was expressed in treated and untreated cells. However, no Ngn3 expression was detected in control cells and a strong Ngn3 induction (50-fold increase) was observed after AZA treatment (Fig. 1B).

To understand the potential functional significance of Ngn3 expression, we next determined if the cells underwent endocrine differentiation using Chromogranin A (CgA) as a marker. CgA is the major member of the granin family of acidic secretory glycoproteins that are expressed in all endocrine and neuro-endocrine cells [18]. As shown in Fig. 1C, a 12-fold increase in the expression of CgA was observed after AZA treatment. In accordance with this observation, immunocytochemical analysis showed a positive staining for CgA after AZA treatment.

Taken together, these results demonstrate that the demethylating agent AZA was able to induce Ngn3 expression and neuro-endocrine differentiation from the ductal human cell line PANC-1.

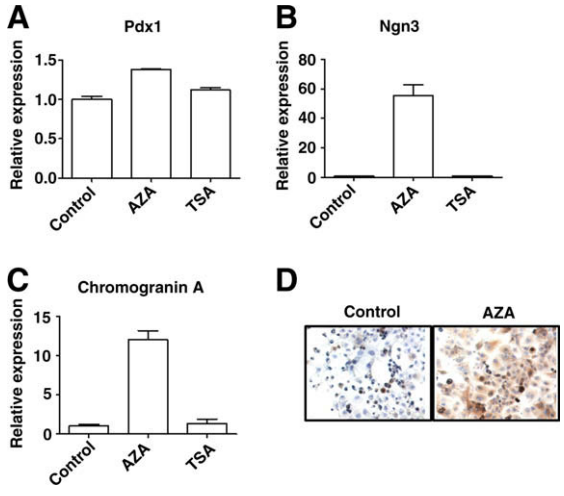


**Fig. 1.** Induction of Ngn3 expression in PANC-1 cells treated with AZA. PANC-1 cells were cultured for 3 days in the presence of 1 μmol/l AZA or 50 nmol/l TSA then for an additional 3 days in normal media. mRNA levels of expression of Pdx1 (A), Ngn3 (B) and CgA (C) were assessed by quantitative RT-PCR analyses. All experiments were carried out in triplicate. (D) Immunocytochemical analysis for detection of CgA in PANC-1 cells cultured in the absence or presence of AZA.

### *Alteration in the expression of transcription factors in PANC-1 cells treated with AZA*

All islet cells are believed to originate from pluripotent progenitor cells during both infancy and adulthood. Islet cell differentiation is influenced by many transcription factors that have highly cell-specific expression patterns [19]. Quantitative PCR analysis was used to detect expression of these transcripts after 3 days of AZA treatment and 4 days in normal media.

As shown previously, the level of Pdx1 mRNA expression was not modified upon AZA treatment (Fig. 2A). On the contrary, no Ngn3 expression was detected in control cells but subsequently expressed after 2 days AZA treatment after which its levels increased progressively (Fig. 2B). NeuroD has been shown to be a direct target of Ngn3. NeuroD expression could not be detected until day 4 where we observed a 10-fold induction until day 7. To follow the differentiation into the different endocrine cell types, we next analyzed the expression of Pax4, Nkx6.1 and Brn4. Virtually no expression of Pax4 could be detected. However, we observed an increase in the level of Nkx6.1 expression starting from day 2 until day 4 which did not decrease with time afterward (Fig. 2E). Brn4 expression was detected after 2 days after which its level increased progressively thereafter (Fig. 2D).

We also followed the expression of several genes expressed in mature endocrine cells, insulin, glucagon, somatostatin and PP. Somatostatin expression was observed after 2 days and increased progressively until day 7 (Fig. 3A) whereas glucagon expression was detected only at day 7. However, no expression of insulin and PP were detected after AZA treatment and subsequent culture.

These data indicate that, in addition to induce Ngn3 expression, AZA treatment of PANC-1 ductal cell line was able to induce the sequential expression of genes required for pancreas development as well as genes expressed in mature endocrine cells.

### *Influence of different differentiation factors on PANC-1 after AZA treatment*

Many growth and differentiation factors have been investigated to examine whether they induce the differentiation of pancreatic progenitors into insulin-producing beta cells or other lineages. The pancreatic ductal cells or embryonic stem cells from mice could be efficiently differentiated into insulin secreting cells by activin A treatment in vitro [20–22]. The same effect was observed for betacellulin and nicotinamide [23–26]. HDACs inhibitors like TSA have recently been shown to impact on endocrine differentiation and to amplify specific cellular subtypes [17]. We finally focused on the ability of soluble factors released during pancreas embryogenesis to influence on PANC-1 differentiation. For this, we used conditioned medium from pancreatic rudiments at e16.5 and cultured for 10 days [27,28].

To assess the differentiation potential of these factors, we cultured the PANC-1 cells 3 days in the presence of AZA to induce Ngn3 expression then cultured the cells for an additional 5 days with the different factors. None of these compounds were able to induce Pax4 expression or impact on Pdx1, Ngn3 and NeuroD expression (Fig. 4A–C). However, quantitative RT-PCR analysis revealed that activin B, betacellulin, e16.5 and TSA were able to in-

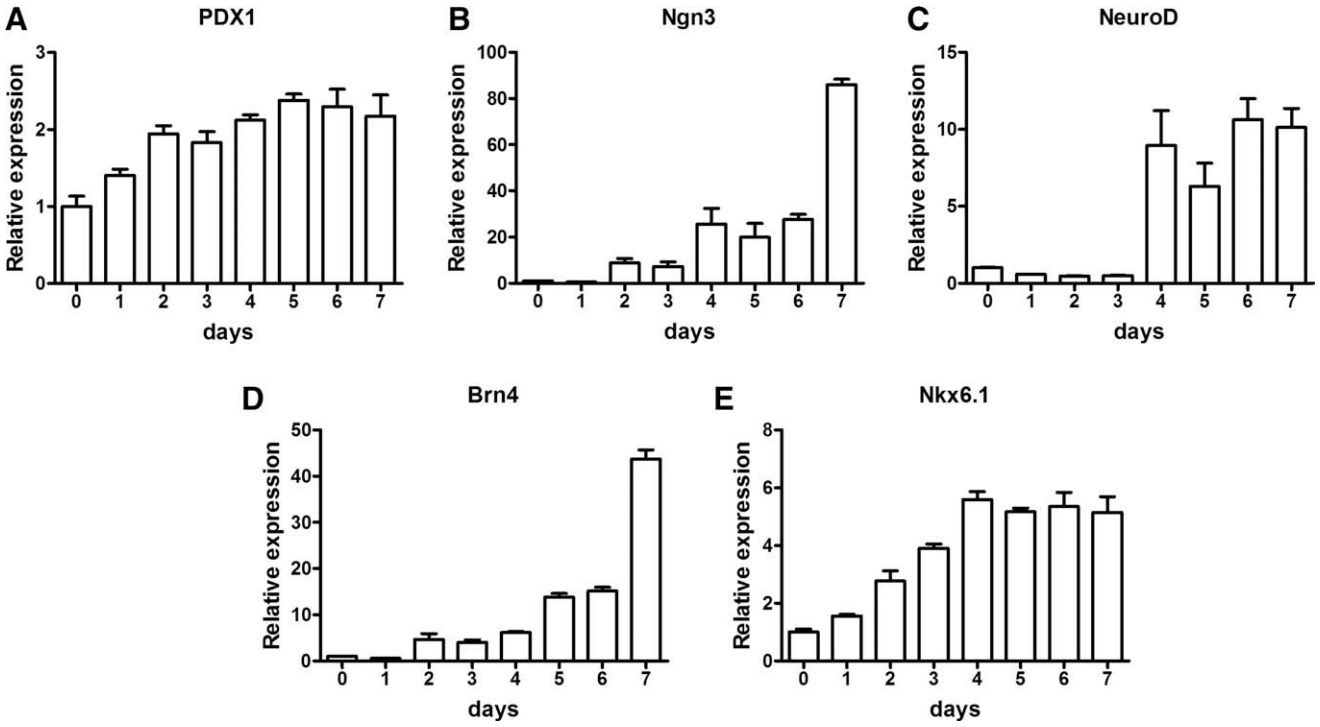


**Fig. 2.** Effect of AZA treatment on expression of several key markers of endocrine differentiation. PANC-1 cells were cultured for 3 days in the presence of 1 μmol/l AZA then for an additional 3 days in normal media. mRNA levels of Pdx1 (A), Ngn3 (B), NeuroD (C), Brn4 (D) and Nkx6.1 (E) were assessed by quantitative RT-PCR analyses. All experiments were carried out in triplicate.

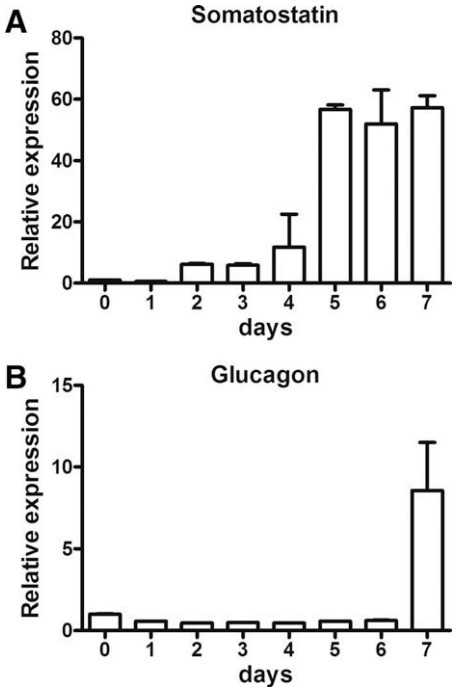


**Fig. 3.** Increased PP and glucagon expression after AZA treatment. PANC-1 cells were cultured for 3 days in the presence of 1 μmol/l AZA then for an additional 3 days in normal media. mRNA levels of PP (A) and glucagon (B) were assessed by quantitative RT-PCR analyses. All experiments were carried out in triplicate.

crease the level of Brn4 mRNA expression (between 2- and 2.5-fold increase). Further analyzes demonstrated that none of these factors induced insulin and PP mRNAs expression. Interestingly, we observed a strong increase in somatostatin mRNA expression in the presence of e16.5 (3-fold induction compared to AZA treatment) and glucagon expression with betacellulin and e16.5 (4-fold increase) (Fig. 4E and F).

Thus, these results demonstrate that a combination of differentiation or growth factors could effectively stimulate the differentiation of PANC-1 cells after AZA treatment.

## Discussion

In the present study, we show that the PANC-1 human ductal cell line differentiates into endocrine lineage after AZA treatment. Previous reports showed that pancreatic ductal cell lines like PANC-1 were able to differentiate into endocrine cell lineage and insulin-like positive cells after stable expression of Pdx1 and exposure to GLP-1 [29] or defined serum-free media [30]. However, these studies failed to detect Ngn3 expression, a critical factor in embryogenesis of the endocrine pancreas. Using the demethylating agent 5′-Azadeoxycytidine, we have been able to induce a strong Ngn3 expression and trigger endocrine differentiation from the PANC-1 human ductal cell line. This result suggests that Ngn3 promoter or other transcription factor promoters necessary for Ngn3 expression are methylated in PANC-1 cells. It is most unlikely that the observed endocrine differentiation requires the participation of Pdx1 since this factor is already expressed in PANC-1 cells. This result is in agreement with previous studies demonstrating that ectopic expression of Ngn3 is sufficient to induce endocrine differentiation from different cell lines or duct primary cells [15,16,31].

The mechanism by which Ngn3 induces PANC-1 cell differentiation after AZA treatment seems to involve NeuroD1, Nkx6.1 and Brn4 activation. It has been shown that, in embryonic progenitors, the expression of these genes depends on Ngn3 activity [13,32]. During embryonic development, Ngn3 appears transiently in pancreatic epithelial cells [32,33]. In PANC-1 cells, Ngn3 expression constantly increased after AZA treatment and induces predominantly the formation of glucagon- and somatostatin- and not insu-

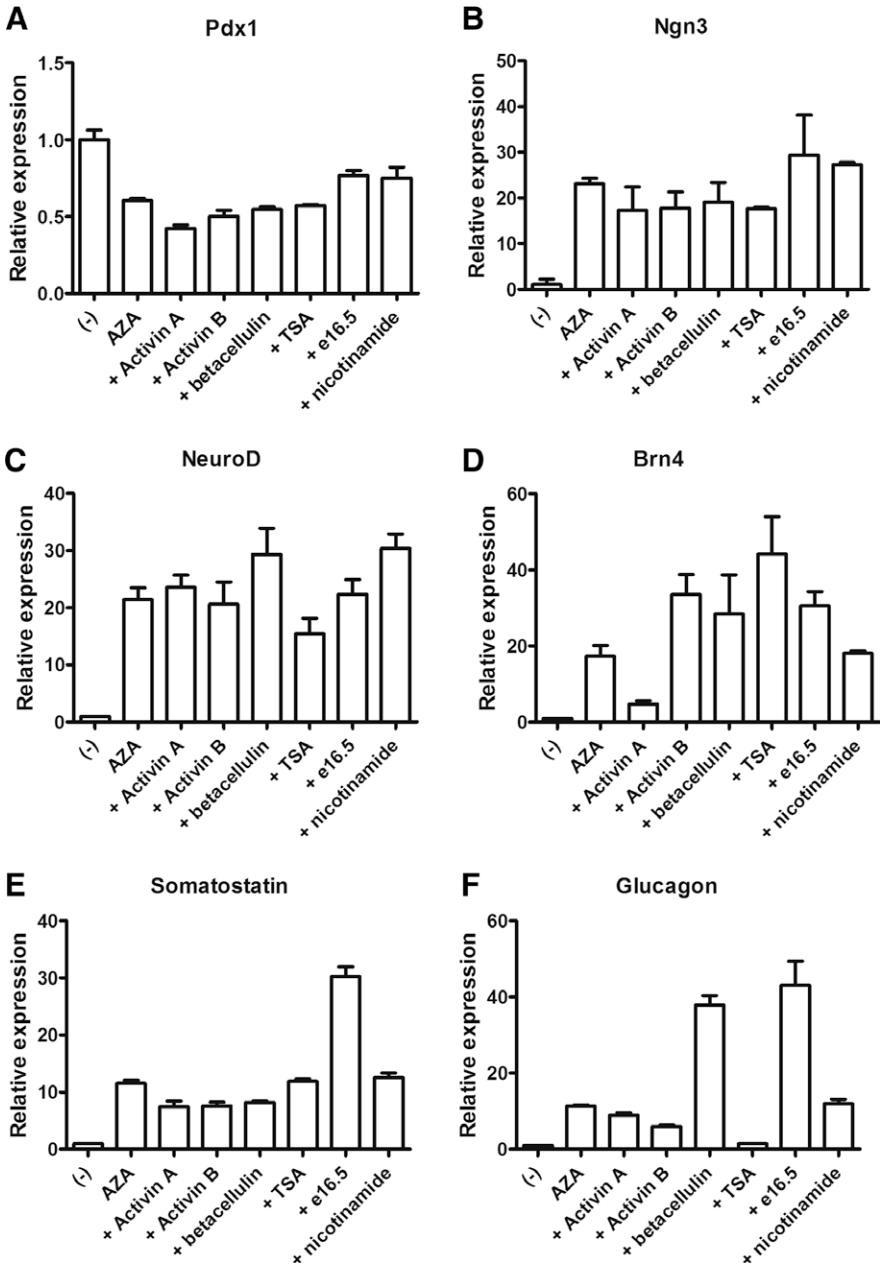


**Fig. 4.** Effect of differentiation factors on PANC-1 cells differentiation after AZA treatment. PANC-1 cells were cultured for 3 days in the presence of 1 μmol/l AZA then for an additional 5 days in the presence of activin A (50 ng/l), activin B (50 ng/l), betacellulin (10 ng/l), TSA (10 nmol/l), nicotinamide (5 mmol/l) and conditioned media from e16.5 (50%). mRNA levels of expression of Pdx1 (A), Ngn3 (B) and NeuroD (C), Brn4 (D), somatostatin (E) and glucagon (F) were assessed by quantitative RT-PCR analyses. All experiments were carried out in triplicate.

lin and PP-expressing cells. These results are not surprising since others have reported similar findings resulting from Ngn3 overexpression. Ectopic Ngn3 expression was found to induce premature differentiation into glucagon- and somatostatin-producing cells when introduced into early chicken endoderm [34] and in neonatal pig pancreatic precursor cells [16]. In support of this explanation, we observed first a strong induction in Brn4 expression that possesses an important role in the determination of alpha cell lineage [35]. Second, the transcriptional factor Pax4, an essential regulator in the determination of beta cell lineage, is not induced in PANC-1 cells [36,37]. It is worth notice that ectopic Ngn3 expression in mPAC cells or human duct cells failed to elicit Brn4 and glucagon expression suggesting that the different cell types may express inhibitory factors or lack of positive regulators for the different lineage [15,31].

Cell proliferation and differentiation during pancreatic development is controlled by permissive signals [38,39]. In addition, several differentiation factors or molecules have been shown to direct pancreatic cell differentiation in vitro. In this study, we demonstrate that betacellulin and soluble factors released from the embryonic pancreas may increase to some extent the somatostatin and glucagon expression but did not impact on cell lineage. These results are somewhat different from previous reports. For example, nicotinamide – a poly(ADP-ribose) synthetase inhibitor – is known as a potent inducer of endocrine differentiation in cultured fetal pancreatic cells [25,40]. Similarly, betacellulin has been shown to promote beta cell differentiation [23–25]. Finally, HDAC inhibitors increased the pool of beta cells in embryonic pancreases cultured in vitro [17]. Two explanations can account for the lack of effect on PANC-1 cellular pancreatic lineage after AZA treatment. First,

it has been shown that extracellular signals must take place in a specific range of time. For example, FGF10 is required for the proliferation of early pancreatic progenitors but endocrine differentiation was strongly inhibited in transgenic mice overexpressing FGF10 or in pancreatic epithelium grown with FGF10 [41–43]. Second, as stated previously, there seems to be intrinsic differences between the differentiation program that cells can adopt and that remains to be elucidated. It would be interesting to investigate AZA treatment effects on others pancreatic ductal cell lines.

Taken together, our data indicate that (1) Ngn3 expression is strongly induced after AZA treatment of the human pancreatic ductal cell line; and (2) endocrine differentiation is induced in vitro. Thus, the PANC-1 model system may provide a basis for elucidating the ductal/endocrine differentiation. Knowledge gained from these studies could be applied for the generation of endocrine cells from adult human ducts.

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## References

[1] D. Mineo, A. Pileggi, R. Alejandro, C. Ricordi, Point: steady progress and current challenges in clinical islet transplantation, Diabetes Care 32 (2009) 1563–1569.
[2] M.H. Khan, D.M. Harlan, Counterpoint: clinical islet transplantation: not ready for prime time, Diabetes Care 32 (2009) 1570–1574.
[3] A. Inada, S. Bonner-Weir, E. Toschi, How can we get more beta cells?, Curr Diab. Rep. 6 (2006) 96–101.
[4] T. Nir, Y. Dor, How to make pancreatic beta cells – prospects for cell therapy in diabetes, Curr. Opin. Biotechnol. 16 (2005) 524–529.
[5] Y. Dor, J. Brown, O.I. Martinez, D.A. Melton, Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation, Nature 429 (2004) 41–46.
[6] A. Lechner, J.F. Habener, Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E259–E266.
[7] G. Parnaud, D. Bosco, T. Berney, F. Pattou, J. Kerr-Conte, M.Y. Donath, C. Bruun, T. Mandrup-Poulsen, N. Billestrup, P.A. Halban, Proliferation of sorted human and rat beta cells, Diabetologia 51 (2008) 91–100.
[8] X. Xu, J. D'Hoker, G. Stange, S. Bonne, L.N. De, X. Xiao, C.M. Van de, G. Mellitzer, Z. Ling, D. Pipeleers, L. Bouwens, R. Scharfmann, G. Gradwohl, H. Heimberg, Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas, Cell 132 (2008) 197–207.
[9] G. Gu, J.R. Brown, D.A. Melton, Direct lineage tracing reveals the ontogeny of pancreatic cell fates during mouse embryogenesis, Mech. Dev. 120 (2003) 35–43.
[10] L.C. Murtaugh, D.A. Melton, Genes, signals, and lineages in pancreas development, Annu. Rev. Cell Dev. Biol. 19 (2003) 71–89.
[11] S.E. Schonhoff, M. Giel-Moloney, A.B. Leiter, Neurogenin 3-expressing progenitor cells in the gastrointestinal tract differentiate into both endocrine and non-endocrine cell types, Dev. Biol. 270 (2004) 443–454.
[12] G. Gu, J. Dubauskaite, D.A. Melton, Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors, Development 129 (2002) 2447–2457.
[13] G. Gradwohl, A. Dierich, M. LeMeur, F. Guillemot, Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas, Proc. Natl. Acad. Sci. USA 97 (2000) 1607–1611.
[14] S. Wang, J. Hecksher-Sorensen, Y. Xu, A. Zhao, Y. Dor, L. Rosenberg, P. Serup, G. Gu, Myt1 and Ngn3 form a feed-forward expression loop to promote endocrine islet cell differentiation, Dev. Biol. 317 (2008) 531–540.
[15] Y. Heremans, C.M. Van de, V.P. in't, G. Gradwohl, P. Serup, O. Madsen, D. Pipeleers, H. Heimberg, Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3, J. Cell Biol. 159 (2002) 303–312.
[16] G. Harb, Y. Heremans, H. Heimberg, G.S. Korbutt, Ectopic expression of neurogenin 3 in neonatal pig pancreatic precursor cells induces (trans)differentiation to functional alpha cells, Diabetologia 49 (2006) 1855–1863.
[17] C. Haumaitre, O. Lenoir, R. Scharfmann, Histone deacetylase inhibitors modify pancreatic cell fate determination and amplify endocrine progenitors, Mol. Cell. Biol. 28 (2008) 6373–6383.
[18] G.N. Hendy, S. Bevan, M.G. Mattei, A.J. Mouland, Chromogranin A, Clin. Invest. Med. 18 (1995) 47–65.
[19] J. Jensen, Gene regulatory factors in pancreatic development, Dev. Dyn. 229 (2004) 176–200.
[20] M.K. Park, C. Han, K.H. Lee, S.H. Hong, H.S. Kim, Y.J. Lee, I.K. Jeong, J.H. Noh, T.Y. Yang, M.S. Lee, K.W. Kim, M.K. Lee, Effects of activin A on pancreatic ductal cells in streptozotocin-induced diabetic rats, Transplantation 83 (2007) 925–930.
[21] L. Li, Z. Yi, M. Seno, I. Kojima, Activin A and betacellulin: effect on regeneration of pancreatic beta-cells in neonatal streptozotocin-treated rats, Diabetes 53 (2004) 608–615.
[22] U. Frandsen, A.D. Porneki, C. Floridon, B.M. Abdallah, M. Kassem, Activin B mediated induction of Pdx1 in human embryonic stem cell derived embryoid bodies, Biochem. Biophys. Res. Commun. 362 (2007) 568–574.
[23] X.R. Zhan, X.Y. Li, X.M. Liu, J.H. Zhou, Y.L. Yang, R. Yi, J. Zhang, B.F. Yang, Generation of insulin-secreting cells from adult rat pancreatic ductal epithelial cells induced by hepatocyte growth factor and betacellulin-delta4, Biochem. Biophys. Res. Commun. 382 (2009) 375–380.
[24] Y. Yoshikumi, H. Ohno, J. Suzuki, M. Isshiki, Y. Morishita, H. Ohnishi, H. Yasuda, M. Omata, T. Fujita, H. Mashima, Up-regulation of JAM-1 in AR42J cells treated with activin A and betacellulin and the diabetic regenerating islets, Endocr. J. 55 (2008) 757–765.
[25] P. Vaca, G. Berna, R. Araujo, E.M. Carneiro, F.J. Bedoya, B. Soria, F. Martin, Nicotinamide induces differentiation of embryonic stem cells into insulin-secreting cells, Exp. Cell Res. 314 (2008) 969–974.
[26] M. Zhao, S.A. Amiel, M.R. Christie, M. Rela, N. Heaton, G.C. Huang, Insulin-producing cells derived from human pancreatic non-endocrine cell cultures reverse streptozotocin-induced hyperglycaemia in mice, Diabetologia 48 (2005) 2051–2061.
[27] P. Vaca, F. Martin, J.M. Vegara-Meseguer, J.M. Rovira, G. Berna, B. Soria, Induction of differentiation of embryonic stem cells into insulin-secreting cells by fetal soluble factors, Stem cells 24 (2006) 258–265.
[28] G.K. Brolen, N. Heins, J. Edsbagge, H. Semb, Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing beta-cell-like cells, Diabetes 54 (2005) 2867–2874.
[29] H. Hui, C. Wright, R. Perfetti, Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells, Diabetes 50 (2001) 785–796.
[30] A.A. Hardikar, B. Marcus-Samuels, E. Geras-Raaka, B.M. Raaka, M.C. Gershengorn, Human pancreatic precursor cells secrete FGF2 to stimulate clustering into hormone-expressing islet-like cell aggregates, Proc. Natl. Acad. Sci. USA 100 (2003) 7117–7122.
[31] R. Gasa, C. Mrejen, N. Leachman, M. Otten, M. Barnes, J. Wang, S. Chakrabarti, R. Mirmira, M. German, Proendocrine genes coordinate the pancreatic islet differentiation program in vitro, Proc. Natl. Acad. Sci. USA 101 (2004) 13245–13250.
[32] V.M. Schwitzgebel, D.W. Scheel, J.R. Conners, J. Kalamaras, J.E. Lee, D.J. Anderson, L. Sussel, J.D. Johnson, M.S. German, Expression of neurogenin3 reveals an islet cell precursor population in the pancreas, Development 127 (2000) 3533–3542.
[33] A. Apelqvist, H. Li, L. Sommer, P. Beatus, D.J. Anderson, T. Honjo, A.M. Hrabe de, U. Lendahl, H. Edlund, Notch signalling controls pancreatic cell differentiation, Nature 400 (1999) 877–881.
[34] A. Grapin-Botton, A.R. Majithia, D.A. Melton, Key events of pancreas formation are triggered in gut endoderm by ectopic expression of pancreatic regulatory genes, Genes Dev. 15 (2001) 444–454.
[35] M.A. Hussain, C.P. Miller, J.F. Habener, Brn-4 transcription factor expression targeted to the early developing mouse pancreas induces ectopic glucagon gene expression in insulin-producing beta cells, J. Biol. Chem. 277 (2002) 16028–16032.
[36] B. Sosa-Pineda, K. Chowdhury, M. Torres, G. Oliver, P. Gruss, The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas, Nature 386 (1997) 399–402.
[37] C. Dohrmann, P. Gruss, L. Lemaire, Pax genes and the differentiation of hormone-producing endocrine cells in the pancreas, Mech. Dev. 92 (2000) 47–54.
[38] S.K. Kim, M. Hebrok, Intercellular signals regulating pancreas development and function, Genes Dev. 15 (2001) 111–127.
[39] S.K. Kim, M. Hebrok, D.A. Melton, Notochord to endoderm signaling is required for pancreas development, Development 124 (1997) 4243–4252.
[40] T. Otonkoski, G.M. Beattie, M.I. Mally, C. Ricordi, A. Hayek, Nicotinamide is a potent inducer of endocrine differentiation in cultured human fetal pancreatic cells, J. Clin. Invest. 92 (1993) 1459–1466.
[41] A. Bhushan, N. Itoh, S. Kato, J.P. Thiery, P. Czernichow, S. Bellusci, R. Scharfmann, Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis, Development 128 (2001) 5109–5117.
[42] G.A. Norgaard, J.N. Jensen, J. Jensen, FGF10 signaling maintains the pancreatic progenitor cell state revealing a novel role of Notch in organ development, Dev. Biol. 264 (2003) 323–338.
[43] F. Miralles, L. Lamotte, D. Couton, R.L. Joshi, Interplay between FGF10 and Notch signalling is required for the self-renewal of pancreatic progenitors, Int. J. Dev. Biol. 50 (2006) 17–26.