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Identification and expansion of pancreatic stem/progenitor cells

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Abstract

Pancreatic islet transplantation represents an attractive approach for the treatment of diabetes. However, the limited availability of donor islets has largely hampered this approach. In this respect, the use of alternative sources of islets such as the *ex vivo* expansion and differentiation of functional endocrine cells for treating diabetes has become the major focus of diabetes research. Adult pancreatic stem cells/progenitor cells have yet to be recognized because limited markers exist for their identification. While the pancreas has the capacity to regenerate under certain circumstances, questions where adult pancreatic stem/progenitor cells are localized, how they are regulated, and even if the pancreas harbors a stem cell population need to be resolved. In this article, we review the recent achievements both in the identification as well as in the expansion of pancreatic stem/progenitor cells.

Keywords: stem/progenitor cell - islet - proliferation - transdifferentiation - heart failure - growth factor

Introduction

Stem cells have the ability to self-renew and to differentiate along multiple lineages. During development, stem cells divide to yield distinct subpopulations giving rise to non-self-regenerating progenitors with restricted differentiation potential, which finally differentiate into functionally mature cells. After birth, either stem cells or progenitor cells play central roles in the maintenance, repair, and reconstitution of tissues in response to homeostatic or regenerative signals [1]. Because of these unique characteristics, stem cells hold great therapeutic potential and have

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become one of most fascinating topics in biological research. Adult stem cells have been defined in tissues with high turnover, including skin and gut [2]. Recently, stem cells have also been identified in tissues with low regenerative potential and turnover, such as the brain [3] and the kidney [4]. The existence of pancreatic stem cells has been elusive because specific markers for their identification are not available. Hence, stem cell research in the pancreas has become one of the most controversial issues in the diabetes field. The present review will focus on the current knowledge of our understanding of the existence of pancreatic stem/progenitor cells and the factors important for their expansion.

The pancreas is composed of the exocrine and endocrine tissues. The exocrine tissue is organized into acini, which produce digestive enzymes, and secretory ducts, which deliver enzymes produced by pancreatic acinar cells into the duodenum. The endocrine tissue (the pancreatic islets of Langerhans) is composed of four different cell types, α, β, δ and PP cells. These cells produce the hormones glucagon, insulin, somatostatin, and pancreatic polypeptide (PP), respectively [5, 6]. Under physiological conditions, the pancreatic islets control blood glucose homeostasis. Diabetes mellitus ensues as a consequence of the body's inability to respond normally to high blood glucose levels. Type I diabetes occurs when a person's immune system specifically attacks and destroys the insulin-producing beta cells of the pancreatic islets of Langerhans [7]. While routine insulin therapy can provide the patients with their daily insulin requirement, optimal glucose levels are not maintained and therefore leaving diabetics susceptible to debilitating complications such as hypoglycemia, retinopathy, and cardiovascular diseases. An ideal therapy is the transplantation of functional beta cells into diabetic patients to restore normal glucose regulation. This therapy has proven successful clinically [8]. However, major difficulties include the shortage of donor tissue and recurring autoimmunity. Type II diabetes mellitus results from either the inability of pancreatic beta cells to secret sufficient insulin or development of a state of insulin resistance in target tissues. Reduction of beta cell mass is critical in the genesis of type II diabetes. In type II diabetic subjects, the beta cell mass show a 40-60% reduction [9, 10]. Interestingly, the beta cell mass changes seem to be specific for the beta cells, because the non-beta cells mass (α, δ, and PP) were unchanged [11]. Thus, cell engineering of non-beta cells or the selective expansion of stem/progenitor cells offer the greatest potential for the development of an abundant source of functional beta cells to treat the insulin deficiency wrought by diabetes.

Experimental models of adult pancreatic regeneration

Evidence of the existence of a stem/progenitor cell in the pancreas rests mainly on the phenomenon of islet neogenesis, which can be experimentally induced. Cellophane wrapping of the hamster pancreas induces a trophic stimulus that leads to ductal proliferation and the development of a new population of beta cells that is capable of reversing streptozocin-induced diabetes [12, 13]. Another model of regeneration is that of partial pancreatic duct ligation [14]. In this model, the islet neogenesis occurs from ductal complexs which have been considered a proliferating stem-cell compartment. Other models of adult regeneration include partial pancretectomy [15, 16] in which 85-90% of the pancreas is removed. In this case, beta cell neogenesis was found in the remnant pancreas. In addition, in the streptozotocin (STZ) induced diabetes model, the STZ specifically destroys the beta cells and induces the regeneration of pancreas, especially in neonates [17, 18]. In addition, we have documented the continuous development of new endocrine cells in the pancreas of interferon-gamma (IFN-γ) transgenic mice, in which the IFN-γ is expressed under the control of the human insulin promoter. IFN-γ transgenic mice demonstrate dramatic proliferation of epithelial cells and continuous neogenesis of islet cells from a progenitor cell population localized in the ducts through a process similar to that occurring during embryonic islet morphogenesis [19–21]. This model is therefore very useful for studies of adult pancreatic regeneration.

Where do adult pancreatic stem cells/progenitor cells exist?

The neogenesis of islets from duct epithelial cells occurs during normal embryonic development and in very early postnatal life [5, 22]. As noted

above, the beta cell mass in the adult pancreas possesses the ability to undergo limited regeneration following injury. Therefore it is believed that like other tissues, stem/progenitor cells must also exist in the pancreas to participate the regeneration and repair process.

There is some evidence to suggest that pancreatic stem/progenitor cells reside within pancreatic ductal cells, where they can differentiate and migrate to form new islets during both organogenesis and regeneration [20, 23]. In addition, endocrine differentiation has been reported in human pancreatic duct cells [24, 25] and culture of mouse pancreatic ductal epithelial cells generated functioning islets containing α-, β-, and δ- cells [26]. Indeed, when these intro-generated islets were transplanted into diabetic NOD mice, diabetes was reversed. Furthermore, a recent study demonstrated that fetal pancreatic ductal cells could differentiate into insulin-producing cells [27]. Transplantation of the pseudo-islets prepared from the beta cells derived from ductal cells reversed STZ- induced diabetes in nude mice. These data suggest that duct cells are a source of pancreatic progenitor cells. While progenitors of insulin producing cells may exist in ductal cells, duct cells have a differentiated phenotype, and thus it is difficult to support the notion that they are true stem cells. For this reason, people usually recognize them as progenitor cells.

There is also some support for the notion that pancreatic stem/progenitor cells reside inside the islet or acinar tissue. Guz *et al* has reported that after administration of STZ, two kinds of presumptive progenitor cells exist inside the islets, one expressing Glu-2 and the other expressing insulin and somatostatin, suggesting the possibility that the progenitor cells may exist inside the islet and might potentially include the insulin-producing cell itself [28]. A distinct population of nestin-positive cells resides in the rat and human islets and has been reported as hormone-negative immature cells that are multi-potential and can proliferate extensively in vitro and appear to be multipotent [29, 30]. Another study demonstrated that a population of small cells that are positive for PDX-1, synaptophysin, insulin, glucagon, somatostatin, pancreatic polypeptide, alpha-fetoprotein and Bcl-2, but negative for cytokeratin 19 and nestin in human and canine pancreas serve as progenitors contributing to islet growth [31]. Insulin secretion studies demonstrated that these cells secrete insulin in a glucose-responsive fashion. Although this study does not provide evidence of the proliferation and differentiation potential of these fascinating cells, their immature morphology, along with their small size and quiescence has lead to the hypothesis that these cells may serve as progenitors contributing to islet growth. Furthermore, Suzuki *et al* indicated that a possible pancreatic stem /progenitor cell candidate that expresses the receptor for hepatocyte growth factor (HGF) c-Met resides in the developing and adult mouse pancreas. In adult these cells are expressed in the duct as well as some of the acinar cells. These cells form colonies *in vitro* and differentiate into multiple pancreatic lineage cells from single cells [32].

Pancreatic transdifferentiation - stem cell plasticity

Traditionally, adult stem cells are thought to be restricted in their potential to differentiate and regenerate to the tissues in which they reside. However, this concept has been changing due to new evidence suggesting that adult stem cells not only reside locally in specific niches, but may also be recruited from the circulation to actively participate in the regeneration of various tissues. The ability of multipotential adult stem cells to cross lineage boundaries and to give rise to cells not normally found in the organs or tissues of residence is called transdifferentiation [33]. For example, bone marrow stroma cells can generate skeletal muscle [34] and neural stem cells can give rise to blood cells [35] cardiac muscle [36] and oval hepatocytes [37]. Transdifferentiation of adult ductal cells [24, 26], acinar cells [38–40] and even cells that do not reside within the pancreas such as liver [41–44] and intestine [45] have been suggested as an alternative mechanism by which beta cell neogenesis can occur. Adenovirus-mediated uptake of PDX-1 into liver cells *in vivo* can induce a beta cell-like phenotype that produces sufficient biologically active insulin to alleviate the hyperglycemia associated with streptozotocin-induced diabetes in rats [46]. Liver cells that overexpress PDX-1 can become pancreatic cells both *in vitro* and *in vivo* [42]. In addition, oval cells (hepatic stem / progenitor cells)

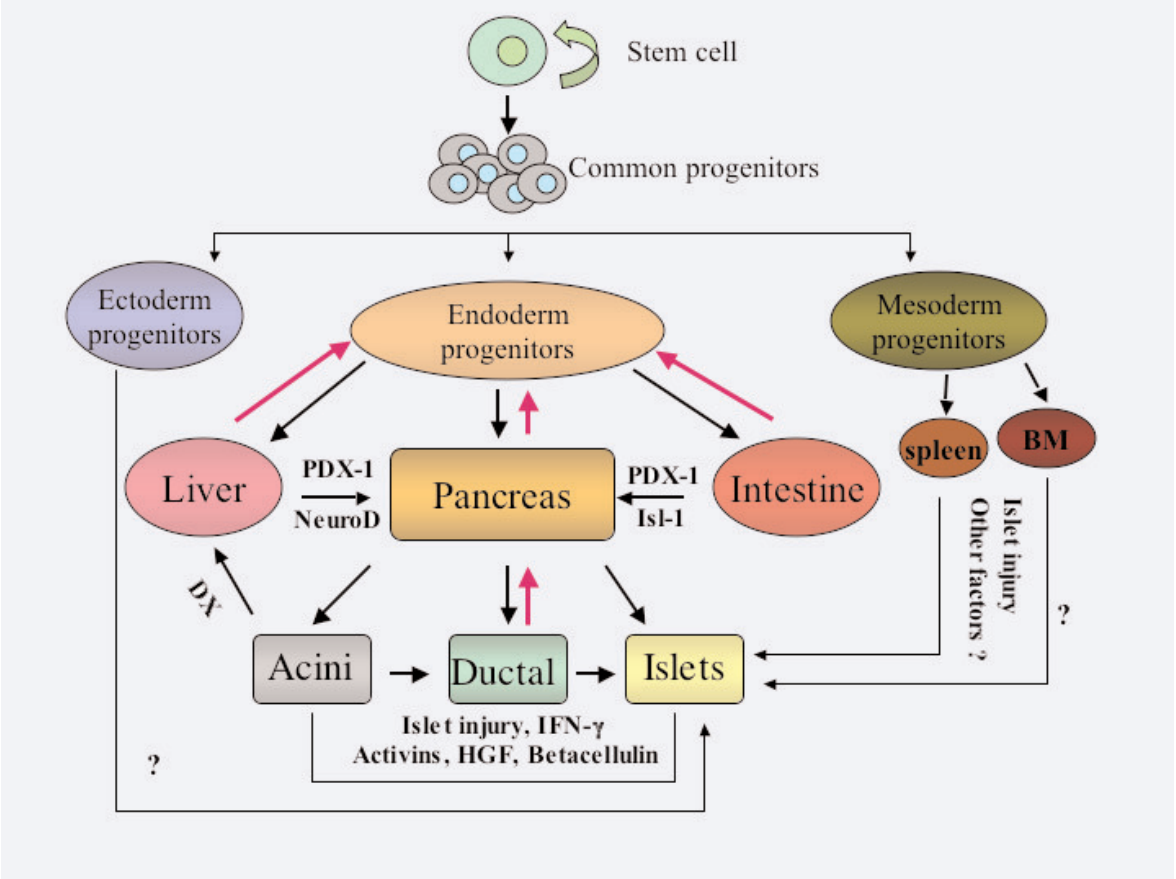

Fig. 1 A simple diagram depicting the possible mechanisms for pancreatic transdifferentiation. Cells from liver, intestine, and pancreas have common progenitor cells in the endoderm during development. Upon stimulation by cytokines or growth factors such as activins, HGF, EGF, TNF-α, or IFN-γ, or during islet injury, these cells can be reprogrammed or dedifferentiated and then transdifferentiated. In addition, mature tissues, both in the endoderm and in the mesoderm might harbor a small number of pluripotent stem cells that have more lineage plasticity to differentiate into completely distinct phenotypes from that found normally in that tissue under specific circumstances.

can differentiate into insulin-producing cells that can reverse hyperglycemia in diabetic NOD-SCID mice [41]. Recently, Kojima *et al* reported that NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice [44]. Previous studies have shown that activin A and betacellulin (BTC) or hepatocyte growth factor (HGF) convert amylase-secreting pancreatic AR42J cells into insulin-producing cells [38, 47], whereas dexamethasone treatment can convert AR42J cells and pancreatic cultures from mouse embryo [48] and acinar cells into hepatocytes [49]. This transdifferentiation is associated with induction of the transcription factor C/EBP β (CCAATenhancer binding protein) [48]. A recent study also demonstrated that treatment with leukaemia inhibitory factor (LIF) and epidermal growth factor (EGF) can transdifferentiate exocrine tissue into functional beta cells [50].

Besides the endodermal origin of transdifferentiation, Ianus and colleague showed that bone marrow cells can serve as pancreatic stem cells [51]. Splenocytes can transdifferentiate into islet and ductal epithelial cells within the pancreas where they mature into fully functional islet cells responsible for restoring normoglycemia [52]. However, recent reports suggest that transdifferentiation is in fact a cell fusion phenomenon. For example, adult

Table 1 Factors regulating proliferation and differentiation of pancreatic progenitor cells.

Factors	*In vivo* studies	*In vitro* studies	Reference
GLP-1	Stimulates the proliferation of β-cells and the differentiation of ductal progenitor cells.	Induces differentiation of pancreatic progenitor cells into insulin-producing cells	72–76
Exendin-4	Stimulates the proliferation of ductal progenitor cells and β-cells.	Promotes the differentiation of insulin-positive cells in ES cultures; induces the differentiation of AR42J cells into endocrine cells.	62, 74, 76
FGF2, FGF7(KGF), FGF10	Regulate the growth and differentiation of endoderm; induces the proliferation of ductal and intra-islet progenitor cells.	Stimulate proliferation of ductal epithelial cells.	77–84
EGF	Expands the pool of embryonic epithelial precursors; inhibit differentiation of β-cells.	Stimulates the proliferation of ductal epithelial cells.	82, 85–88
BTC	Regulates growth and differentiation of fetal pancreas; promotes neogenesis of β-cells from the progenitors inside islets and the ductal epithelial cells	Stimulates ductal cell growth, converting AR42J cells into insulin-producing cells	38, 89–92
HGF	Promotes β-cell proliferation; increases β-cell mass in fetal and regenerating pancreas.	Promotes proliferation of pancreatic progenitors and β-cells; converts AR42J cells into insulin-producing cells.	47, 93–98
Activins	Promote β-cell differentiation in fetal pancreas and adult regenerating pancreas	Promotes the differentiation of insulin-positive cells in ES cultures; Inhibits growth of AR42J cells and converts AR42J cells into endocrine cells; increases insulin-positive cells in ES cultures.	27, 38, 47, 62, 99, 100
Follistatin	Promotes proliferation of pancreatic progenitor cells in adult regenerating pancreas.	Promotes the growth of ductal progenitor cells.	100
GDF11	Inhibits the endocrine progenitor cell formation in embryonic pancreas and stimulates β-cell maturation and size.	n.d.	108
SDF-1a/CXCR4	Promotes the proliferation of ductal progenitor cells.	Stimulates ductal progenitor cell migration.	105
Conophylline	Induces differentiation of fetal pancreas.	Induces differentiation of AR42J cells and increases β-cell formation from progenitor cells.	109, 110

n.d.: not determined

murine bone marrow cells can fuse spontaneously with embryonic stem cells [53], mouse neural progenitor cells can fuse with pluripotent embryonic stem cells [54] and cell fusion between adult stem

cells and epithelial cells is a frequent event [55]. *In vivo* studies also suggest that transdifferentiation of circulating hematopoietic stem cells (HSCs) is an extremely rare event [33] and may be caused by cellular fusion [56, 57]. However, in the study of Ianus and colleagues, the bone marrow cells that selectively express the reporter gene-enhanced green fluorescent protein (EGFP) under the control of insulin promoter were transplanted in recipient mice and gave rise to EGFP-positive insulin-producing cells in pancreatic islets. These bone marrow-derived cells expressed islet related transcription factors and were functionally responsive to glucose. Furthermore, by using the CRE-LoxP reporter system, the authors suggested that *in vivo* cell fusion is an unlikely explanation for the "transdifferentiation" of bone marrow-derived cells into differentiated cell phenotypes. Another study also demonstrated that adult bone marrow cells are capable of transdifferentiating into a pancreatic lineage *in vitro* and transplantation of these cells could correct the hyperglycemia *in vivo* [58]. Mathews and colleagues, on the contrary, reported that bone marrow-derived endothelial progenitor cells (EPCs) are recruited to the pancreas in response to islet injury and contribute to the survival and function of islet, but there is no evidence of transdifferentiation of bone marrow cells into insulin-producing cells [59]. This suggests that the improvement of beta cell function is induced by the up-regulated neovascularization induced by EPCs. It is clearly that the plasticity of bone marrow cells needs to be clarified.

The mechanism of transdifferentiation is not understood. One possibility might be that cells from liver, intestine, and pancreas have common progenitor cells from the endoderm or adjacent area during development, and upon certain stimulation, such as tissue damage, cytokines, or growth factors, they can be reprogrammed or dedifferentiated and transdifferentiated into a completely distinct phenotype which is found normally in that tissue. An alternative explanation might be that mature tissues harbor a small number of pluripotent stem cells that have more lineage plasticity than previously thought (Fig. 1). Since not all of the cells in a given tissue will transdifferentiate upon stimulation, we need to clarify the characteristics of the cell subpopulation sensitive to transdifferentiation. We also need to determine if bone marrow or hepatocytes can transdifferentiate into beta cells whether there are other cell types of islet such as α cells, PP cells and δ cells. Finally, we need to understand what factors make cells from one tissue differentiate into a tissue of a different type as well as what signals are necessary for the transdifferentiation to be triggered. Although many questions still need to be addressed, transdifferentiation represents a valuable approach to generate "self" surrogate beta cells that are suitable for replacing impaired islet cell function in diabetics. We can imagine that if cells from tissues outside of the pancreas can be to develop into insulin-producing cells that are responsive to physiologic signals, they will provide an additional source of beta cells for transplantation.

In summary, islet stem/progenitor cells might not only in the ductal epithelium but also within the islets itself, and might even arise from exocrine acinar cells or from cells outside of the pancreas which posses remarkable plasticity to dedifferentiate and transdifferentiate.

Differentiation of embryonic stem (ES) cells into insulin-producing cells

The therapeutic potential of transplantation of insulin-producing beta cells has stimulated interest in using pluripotent embryonic stem (ES) cells to generate insulin secreting cells *in vitro*. Studies have shown that human ES cells can be differentiated into insulin-producing cells *in vitro* and if this method is further improved, may lead to the formation of an unlimited source of cells suitable for transplantation [60]. Mouse ES cells can also be differentiated into insulin-producing cells; expression of Pax-4 in mouse embryonic stem cells promotes the differentiation of nestin-positive progenitor and insulin-producing cells, which lead to the formation of islet-like clusters [61]. Cells in the islet-like clusters show glucose-dependent insulin release at their terminal stage. The authors also suggested that nestin and cytokeratin 19 are normally expressed by ES cells preceding differentiation into C-peptide/insulin producing cells, whereas islet-like clusters at the terminal stage are nestin-negative. Another study showed that mouse ES cells can be directed to differentiate into insulin-producing cells with the stimulation of activin B and exendin 4 [62], and transfection of Nkx6.1 can direct the

differentiation of mouse ES cells into insulin-producing cells [63]. These studies open a new avenue for developing strategies to differentiate ES cells into insulin-producing cells *in vitro*.

Like other emerging fields, stem cell research is surrounded by controversies. A recent study suggested that mouse ES cells readily differentiate into extra-embryonic endoderm *in vitro*, raising the possibility that the insulin-producing cells might be extra-embryonic endoderm rather than the assumed authentic embryonic endodermal endocrine cells [64]. Another report also showed that the immunoreactivity to insulin results from the uptake of exogenous insulin in vitro rather than from the differentiated cells themselves [65]. In spite of these reports, researchers are optimistic that embryonic stem cells provide a potential source for beta cell replacement. If we can direct ES cells into insulin-producing cells *in vitro*, and further lead them form pseudo-islets, which are more efficient for transplantation, an effective treatment diabetes will be available to affected individuals.

Do pancreatic stem/progenitor cells really exist in the adult pancreas?

A recent study raised a very critical question about the existence of pancreatic adult stem cells. Using a genetic lineage-marking technique, Dor and colleagues have demonstrated that beta cell turnover under normal condition as well as during regeneration induced by partial pancreatectomy results from duplication of pre-existing beta cells themselves, but is not the contributions of non-beta cells, such as duct cells or "stem" cells. This challenges the prevailing hypothesis in the research field of adult pancreatic stem cells [66]. However, certain caveats of the study are not addressed. The partial pancreatectomy model used in this study showed only very low regeneration and might not have been enough to induce the pancreatic regeneration from a pancreatic stem cell population. A more efficient method of pancreatic regeneration such as 90% pancreatectomy or the IFN-γ transgenic model might be better models for this study. Other concerns with the study raised by Zaret is whether the recombinase gene used is a little "leaky", producing some marked non beta cells that escaped detection [67]. Despite these concerns, this study by Dor et al suggests that beta cells themselves might be the optimal cell type for the generation of more beta cells. If beta cells could be induced to replicate at a higher rate, this might prove beneficial in maintaining normoglycaemia, since the beta cell mass is a major determinant of the total amount of insulin that can be secreted by the pancreas. Therefore, research on the proliferation of beta cells and the molecular basis of beta cell replication should be given more attention than ever before.

Regulation of expansion and differentiation of pancreatic stem/progenitor cells

While the control of pancreas development by the critical transcription factors is an active area of research in many labs [68, 69], non-transcription factors that control the proliferation and differentiation of pancreatic progenitor cells are also attracting researchers' attention. Understanding these factors will not only help to increase regeneration of the progenitor cells themselves but will also be applicable to the expansion of beta cell mass in transplanted islets.

Glucagon-like peptide-1 (GLP-1) is an incretin and is produced by the L-cells of the intestine [70]. GLP-1 has been reported to induce proliferation of the insulinoma (INS-1) cell [71] and to stimulate the differentiation and proliferation of beta cells in the Goto-Kakizaki rat, a model of type II diabetes [72]. GLP-1 increases beta cell mass by inducing the differentiation and neogenesis of ductal progenitor cells into islet endocrine cells [73]. Exendin-4, a long-acting GLP-1 agonist, stimulates both the differentiation of beta cells from ductal progenitors and the proliferation of beta cells in the pancreatectomized adult rat [74]. *In vitro* studies also showed that GLP-1 induces differentiation of islet PDX-1 positive pancreatic ductal cells into insulin-secreting cells [75]. GLP-1 and exendin 4 convert pancreatic AR42J cells into glucagon-and insulin-producing cells [76].

Fibroblast growth factor (FGF) 10 was shown to be capable of inducing the proliferation of the pancreatic epithelium in a direct fashion *in vitro*, and

can therefore be used in amplifying the pool of progenitor cells [77]. FGF10 is also reported to maintain the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis [78]. Recent studies suggested that FGF10 stimulates proliferation and blocks the differentiation of pancreatic epithelial cells [79, 80]. Keratinocyte growth factor (KGF), also called FGF 7, is a member of the heparin-binding fibroblast growth factor family that has been demonstrated to enhance the proliferation of pancreatic ductal cells *in vivo* [81]. Transgenic mice expressing KGF in their beta cells showed increased ductal cell proliferation [82]. Furthermore, KGF is capable of inducing human fetal beta cell expansion by activating ductal cell proliferation and their subsequent differentiation into beta cells. Hence, beta cell mass is increased in transplanted human fetal pancreatic cells [83]. Another study also suggested that KGF and nicotinamide could induce the proliferation and differentiation of intra islet precursor cells [84].

Epidermal growth factor (EGF) is induced in the adult regenerating pancreas [85]. Over-expression of EGF in islet induces disorganized islet growth [82]. EGF also stimulates ductal cell growth *in vitro* [86]. EGF has the ability to expand the pool of embryonic pancreatic epithelial precursor cells, while it also suppressed their differentiation into endocrine tissue [87]. Administration of EGF/gastrin in diabetic mice significantly increased beta cell mass and yeilded a greater number of BrdU labelled beta cells [88].

Betacellulin (BTC), a member of EGF family, was originally isolated from an insulinoma cell line [89]. BTC has been shown to play a role in regulating growth and differentiation of endocrine precursor cells of the fetal pancreas [90] and to convert amylase-secreting pancreatic AR42J cells into insulin-producing cells [38]. BTC has also shown to stimulate the regeneration of pancreatic beta cells and induce the neogenesis of beta cells from progenitors located in or by the pancreatic duct in animal models of diabetes [91]. Glucose metabolism has also been improved by BTC, which promotes the conversion of intra islet precursor cells to beta cells in streptozotocin-treated mice [92].

Hepatocyte grwoth factor (HGF) has been demonstrated to promote beta cell proliferation and regeneration of fetal and adult islets [93–95]. Transgenic mice that overexpress HGF in islets are resistant to the STZ-induced diabetes and have enhanced beta cell proliferation and increased islet mass [96]. Together with Activin A, HGF can induce the differentiation of AR42J cells into insulin-producing cells [47]. *In vitro* studies showed that HGF promotes beta cell proliferation with the support of a fibrin matrix [97]. Recent studies demonstrated that HGF mediates differentiation of immature cell types into insulin-expressing cells [98]. Together, the above data suggests that GLP-1, exendin-4, FGFs, BTC and HGF are useful to promote neogenesis of beta cells from the progenitor cells inside the islets and pancreatic ductal cells.

Activins, members of the transforming growth factor-beta (TGF-β) superfamily, are important in the differentiation of several distinct types of cells, as well as in governing embryonic axial patterning and the function of foregut-derived organs. Activin A promotes beta cell differentiation in human fetal pancreatic cells in the STZ induced diabetic mouse [99]. Activin A converts AR42J cells into endocrine cells [38, 47]. Treatment with activin A and BTC coverts ductal cells from neonatal rats into insulin producing cells [27]. We have demonstrated that the expression of both activins and their receptors are increased during adult pancreatic regeneration in the IFN-γ mouse [100]. Interestingly, activins are also up-regulated in duct cells following partial pancreatectomy and streptozotocin (STZ) injection, suggesting that activins might be involved in the initiation of beta cell neogenesis following distinct stimuli in adulthood [101, 102]. Of particular importance, the cellular inhibitors of activin signaling, such as follistatin [103] and Cripto [104] are also present within a subset of the epithelial compartment. Administration of the natural antagonist of activins, follistatin, enhanced duct cell expansion in the regenerating pancreas. The proliferation of epithelial duct cells was increased 1.8 fold compared to the control transgenic mice. However, the subsequent differentiation into pancreatic beta cells was inhibited compared to that of the control mice. These results demonstrated that homeostasis of growth and terminal differentiation requires a precise context-dependent regulation of activin signaling, and that follistatin participates in this process by promoting expansion of precursor cells during pancreas growth. These data also suggested that follistatin might be useful for *in vitro* expansion of

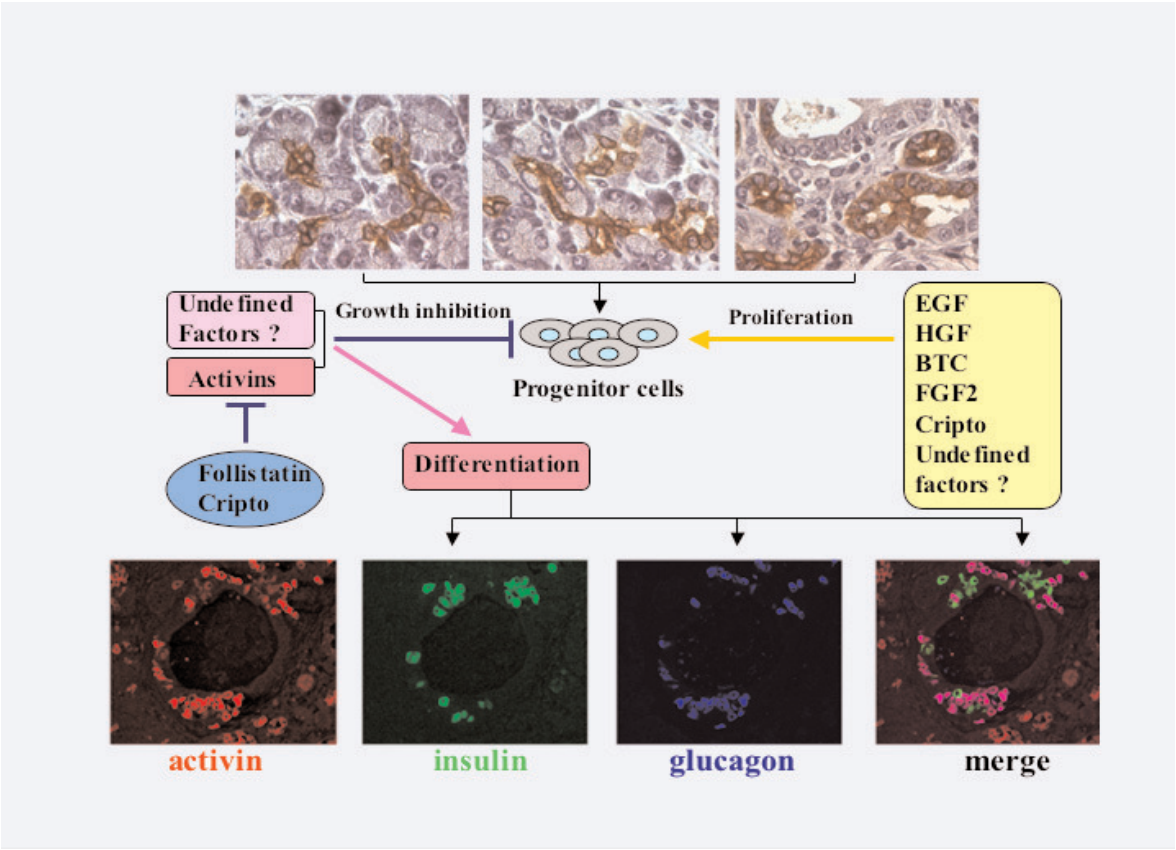

Fig. 2 A schematic diagram for endocrine differentiation from ductal progenitor cells in the adult regenerating pancreas. The centroacinar and ductal cells in the top 3 panels are stained for the A subunit of activins (brown). The centroacinar cells grow into small ducts, and the small ducts grow into large ducts, and the endocrine cells differentiate from the progenitor cells residing in the duct cells. The balance between growth and differentiation is maintained by activins and follistatin, Cripto and other growth factors such as, EGF, HGF, BTC, FGFs and etc.

pancreatic progenitor cells [100]. Therefore, elective blocking of activin signaling might be used in the expansion of progenitor cells. Together with other data, such as EGF [87] and FGF10 [79, 80] which expand the pool of embryonic pancreatic progenitor cells but at the same time inhibit endocrine cell differentiation, we conclude that cell proliferation might act as a repressor of endocrine cell differentiation, raising the possibility that if we can repress the differentiation of endocrine cells, cell proliferation will be increased. The balance between growth and differentiation of pancreatic progenitor cells might therefore be controlled by growth factors such as EGF, FGFs, Cripto, activin, and follistatin (Fig. 2).

Other factors such as the stromal cell-derived factor-1alpha (SDF-1alpha)/CXCR4 ligand-receptor axis [105] is also critical for progenitor cell survival and migration. Vascular endothelial growth factor (VEGF) is also known to stimulate ductal cell replication and insulin production [106, 107]. Growth differentiation factor 11(GDF11), also know as BMP11, recently was reported to negatively regulate the production of pancreatic islet precursor cells and is required for regulating islet size, beta cell maturation, and beta cell mass [108]. Conophylline, a vinca alkaloid extracted from the tropical plant Ervatamia microphylla, has been reported to induce differentiation of pancreatic AR42J cells [109] *via* a similar pathway to activin A. As it is a smaller molecule than the activins, better penetration of tissues is expected. Conophylline induces the differentiation of fetal rat pancreas in organ culture and of pancreatic progen-

itor cells and increases the formation of beta cells both *in vitro* and *in vivo* [110]. This suggests that Conophylline may be useful for the promotion of differentiation of pancreatic progenitor cells. Table 1 summarizes recent studies on the factors that are important for the expansion and differentiation of pancreatic progenitor cells.

While many factors have been shown to be important for the growth and differentiation of pancreatic progenitor cells, the relationships between these factors and transcription factors important for the development of pancreas are poorly understood. GLP-1 is known to stimulate the growth and differentiation of beta cells by inducing the expression of the pancreatic and duodenal homeobox gene (PDX1) which is required for pancreas development [73]. Exendin-4 induces the differentiation of human pancreatic duct cells into endocrine cells by activating adenylyl cyclase and MAP kinase activity which, in turn, might lead to activation of PDX-1 and HNF3b [111]. We have previously reported that the expression of transcription factors Pax4 [112] and neurogenin 3 (Ngn 3) [113], which are critical for islet development, were induced and significantly increased during the differentiation of AR42J cells by activin and HGF. Activin A therefore regulates the expression of neurogenin 3 that is critical for the differentiation of AR42J cells [114]. In addition, in probing the mechanism by which KGF induces differentiation of ductal precursor cells into beta cells, Movassat *et al* suggested that the mechanism of KGF on differentiation might be partially through the stimulation of activin expression [83]. This was based on the previous work showing that KGF upregulated the expression of activin A in skin [115]. It is clear that further studies on these important subjects will open a wider avenue for our understanding of the mechanism of differentiation and expansion of pancreatic progenitor cells.

Conclusion and perspectives

Because of its great therapeutic potential, stem cell research has become one of the most promising fields in biomedical research today. Despite the conflicts and difficulties encountered in this area of research, the possibility that insulin-producing cells can be induced *in vitro* inspires us to deepen our understanding of basic stem cell biology. Reports outlined in this review enumerate examples of the identification and expansion of adult pancreatic stem/progenitor cells. At the moment, however, we understand very little about the regulatory mechanisms and the signaling pathways that direct the balance between growth and differentiation. Further studies in this area will have an impact on our understanding of stem/progenitor cell differentiation. Conditions that activate the proliferation and differentiation of stem/progenitor cells in the central nervous system have been identified [116]. We are optimistic that with all of these models of pancreatic regeneration in adults, and the enthusiastic efforts of researchers in the diabetes field, this goal will be achieved in the near future.

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