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REVIEW

Insulin-producing cells derived from stem/progenitor cells: therapeutic implications for diabetes mellitus

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Abstract

One of the most common diseases on pancreas is diabetes mellitus. The current treatment of exogenous insulin supply is not fully capable of achieving the tight control of glucose regulation, leading to long-term complications. Hence, recent success in islet transplantation-based therapies for diabetes mellitus and extreme shortage of pancreatic islets have motivated recent efforts to develop renewable sources of islet-replacement tissue. Of clinical interest, I will review the recent progress on stem cell-based strategies for diabetes in view of regenerative medicine.

Key words insulin producing cell (IPC)・pancreatic stem cell・diabetes mellitus・CD133・ES cells・iPS cells

A. Diabetes and pancreatic ß cells

The pancreas is a medically important organ and subject to many diseases. One of the most common is diabetes mellitus. Type 1 diabetes is caused by the autoimmune destruction of insulin producing ß cells in the pancreatic islets. In contrast, most common form, type 2 diabetes results from impaired ß cell function combined with insulin resistance in peripheral organs. The current treatment of exogenous insulin supply is not fully capable of achieving the tight control of glucose regulation, leading to long-term complications. Over the past few decade, pancreas or pancreas-kidney organ transplantation has been the most effective treatment for severe diabetic patients. Recently, successful pancreatic islet transplantation to reconstitute the insulin-producing ß cells has also emerged as a promising cell replacement therapy.[1] The advantage of islet transplantation is less invasive and repeatable treatment compared with whole organ transplantation. The clinical outcomes of islet transplantation from the Edmonton Center by Shapiro and colleagues showed that 10% of the 65 patients remained insulin-free for five-year after islet transplantation.[2] Although long-term insulin-free has not been achieved in most patients, islet transplantation is encouraging.

However, the chronic shortage of the organ donors has prevented a widespread use of islet transplantation like other organ or tissue transplantation. Hence, advances in cell-replacement strategy for diabetes and the shortage of transplantable pancreatic islets have focused on renewable sources of glucose responsive, insulin producing cells (IPCs). Of clinical interest, I will review the recent progress on stem cell-based strategies for diabetes.

B. Embryonic stem cells (ESCs)

The use of human embryonic stem cells (ESCs) attracts tremendous public attention due to their pluripotency and the ease of generating large numbers in culture. However, there are still many limitations in using ESCs as therapeutic tools, notably the lack of reliable methods for generating specific lineages, and the difficulty of purifying specifically differentiated progeny and avoiding immunological rejection of the transplanted cells.[3] Furthermore, once they are transplanted, it is almost impossible to control development of the transplanted ESCs into specific cell lineages. Despite these problems, the potential application of ESCs in regenerative medicine provides great

impetus to studies of the molecular genetic nature of the stem cell state. Figure 1 summarizes the strategy for regenerative medicine using ESCs. If IPCs or islet-like cells differentiation from patient-derived ESCs can be achieved, we promise to provide the immune-tolerant and customized cell therapy for diabetic patients.

1. Selection of nestin-positive cells to generate IPC from ESCs

At early stage of ESCs research to generate IPC, we and others focused on the selection of cells positive for nestin,[4-6] an intermediate filament protein that served as a neural stem/progenitor marker.[7] Islets are the principal source of insulin in humans, but in some invertebrate species such as Drosophila, brain neurons are the main source of circulating insulin.[8, 9] The similarities between islet cells and neurons are further underscored by the demonstration of insulin gene transcription in the vertebrate brain,[10] although it remains unclear whether these vertebrate neurons produce or secrete insulin protein.[11] Recent reports have shown that nestin is a marker for neural progenitors and pancreatic exocrine progenitor cells but not endocrine progenitor cells,[12, 13] suggesting that nestin-positive cells from ESCs likely generate neuronal cell types. Consequently,

ESC-derived IPCs may produce insulin at much lower level compared with pancreatic ß cells. This hypothesis is agreement with our previous report.[14] We have shown that the brain-derived human neural progenitor cells, exposed to a series of signals that regulate in vivo pancreatic islet development, form clusters of glucose-responsive IPCs.[14] We calculate that C-peptide content in IPC derived from human neural progenitor cells is approximately 0.3% of the level in isolated pancreatic ß cells. While much attention has been focused on the promise of ESCs, recent work suggests that neural stem cells, like ESCs, may have an unusually broad differentiation potential. For example, Gage and colleagues demonstrated the conversion of mouse neural stem cells to the endothelial lineage, indicating that plasticity is a bona fide property of cultured neural stem cells. These results were unexpected because endothelial cells and neuronal cells normally derive, respectively, from mesoderm and ectoderm, distinct embryonic germ layers.[15] Further investigation is necessary to determine whether ESC-derived nestin-positive cells may differentiate into fully functional IPCs.

2. Differentiation of ESCs following embryonic pancreas development

Embryonic pancreas development requires the stepwise activation of several signaling pathway (Fig. 2).[16, 17] To generate fully differentiated IPCs, attempts for mimicking embryonic endoderm development and pancreas specification are emerging. Recent reports have shown the efficient differentiation of pancreatic lineage from ESCs.[18, 19]

The first step from ESCs toward the pancreatic lineage is definitive endoderm (DE) formation. Previous studies implicated transforming growth factor (TGF)-ß and Wnt signals as critical signals for DE formation *in vivo*.[20, 21] Activin A, a member of the TGF-ß was thus used at high concentration to induce DE,[22] in combination with Wnt3a.[18, 19, 23, 24] More recently, we showed efficient differentiation of ESCs into mesoderm and endoderm using tetracycline trans-activator system to drive expression of *nodal*, the most notable endogenous molecule induce the formation of mesoderm and endoderm.[25] *Nodal* expressing cells differentiated toward a mesendodermal progenitor population, a transient tissue that gave rise to both the mesodermal and endodermal germ layer, more efficiently than exogenous activin treatment.[25] Notably, sustained *nodal* expression in mouse ESCs maintained Oct4 levels and prevented the

differentiation of DE cells. Up-regulation and subsequent down-regulation of nodal signaling resulted in the maturation of DE, which showed the expression including TTF-1, SftpC, albumin (foregut marker), pancreatic duodenal homeobox 1 (Pdx1), glucagon (midgut marker), IFABP and villin (hindgut marker). Unfortunately, we were unable to detect insulin gene expression in our system.

The next crucial step is the induction of pancreatic epithelium. Previous report has shown that suppression of sonic hedgehog (Shh) activity is required for the specification of pancreatic buds.[26] Addition of cyclopamine, inhibitor of Hedgehog signaling pathway by blockade of Smoothend,[27, 28] promotes the formation of pancreatic epithelium marked by the Pdx1 expression, the master gene for pancreas organogenesis.[18, 19] Retinoic acid induces development of primitive endodermal cells from a subset of embryonal carcinoma cell lines, and is an endogenous signal that directs development of posterior organs like the pancreas from embryonic endoderm.[14, 29, 30] Addition of retinoic acid promotes the commitment of the Pdx1-positive pancreas progenitors toward the endocrine lineage.[18, 19] However, ESC-derived hormone-producing endocrine cells have not yet achieved the full maturation.

3. Differentiation of IPCs by a genetic approach

Previous reports have shown pancreatic differentiation could be achieved through exogenous *Pdx1*, *Ngn3*, and *Pax4* expression in ESCs, although functional IPCs were not generated.[6, 31, 32] The level, timing, and duration of forced expression of these transcription factors are important.[25] Prolonged overexpression of *Pdx1* failed to induce the specification of IPCs.[33] In addition, precocious expression of *Ngn3* during pancreas development results in the predominant formation of α-cells at the expense of other endocrine cell types.[34, 35]

C. Induced pluripotent stem (iPS) cells

iPS cells have been derived from fibroblast by ectopic expression of the transcription factors *Oct4*, *Sox2*, c-*myc*, and *Klf4*.[36] iPS cells are molecularly and functionally highly similar to ESCs, including their ability to contribute to all tissues as well as the germ line in mice. We questioned and expected if iPS cells derived from somatic cells with a specific cell fate might be susceptible to differentiate toward

original somatic cells. Reprogramming is not only restricted to mesodermal derivatives, but is also possible with an endodermal cell types, including stomach, liver and pancreatic insulin-producing ß-cells.[37, 38] So far, there have been no evidence that iPS persist the susceptibility to differentiate toward original somatic cells. In addition, it remains to be shown that iPS cells have the same capacity as human ESCs for differentiation toward ß-cells. Recent report described that human iPS derived from skin fibroblasts indeed can give rise to IPCs.[39] Taken together, although ESCs or iPS cells hold a promising potential as a source of IPC, IPC clusters from these stem cells still have a high degree of cellular heterogeneity, tumor-forming potential, and low insulin levels compared with pancreatic islets. More recently in Japan, future speculations by researchers about the clinical implication of iPS cells have been published. It will take over ten years until the clinical cell replacement therapy for diabetes and liver failure is achieved, suggesting that we still have a major hurdle to differentiate ESCs or iPS toward fully matured endodermal cells.

D. Tissue specific pancreatic stem/progenitor cells

Recent studies in regenerative medicine have also focused on the isolation and characterization of repopulating tissue-specific stem/progenitor cells. However, only a few attempts have been made at the prospective isolation of pancreatic stem/progenitor cells, because of the lack of specific markers and the failure to develop a cell culture strategy to determine their capacity for self-renewal and multilineage potential.

1. Adult pancreatic stem/progenitor cells

Putative adult pancreatic stem/progenitor cells that clonally expand while expressing a low level of insulin and other pancreatic markers have been found in the mouse pancreas.[40, 41] However, their capacity for self-renewal and the ability to differentiate into functional islets remain undetermined. The use of adult stem cells isolated from patients can provide an excellent solution to immunological problems raised in cell therapies. The main problem with this approach is that adult stem cells are rare and hard to expand in culture. In contrast, Dor et al. reported that new ß-cells, after birth, could be generated by the replication of existing ß-cells, rather than by putative pancreatic stem cells.[42] Upon injury, insulin-producing ß-cells are also produced from

endogenous endocrine progenitors.[43] Taken together, the existence of adult pancreatic stem/progenitor cells and its signals to expand still remain controversial.

2. Embryonic pancreatic stem/progenitor cells

On the other hand, there is no doubt that pancreatic stem/progenitor cells exist in the developing pancreas. The pancreas develops from the posterior foregut, emerging as buds from the ventral and dorsal area of the gut tube. A key component and a central transcription factor of pancreatic development is Pdx1. Pdx1 was identified based on its ability to bind the insulin and somatostatin genes. In the early pancreas, Pdx1 is expressed throughout the epithelium, but then is suppressed in cells as they commit to the endocrine or ductal cell lineage. As endocrine cells begin to differentiate to the insulin producing ß cell lineage, Pdx1 reappears, and its expression in ß cells is known to be necessary for *de novo* insulin synthesis. Although it is thought that Pdx1-expressing epithelial progenitor cells give rise to endocrine, exocrine, and ductal cells,[44] evidence that isolated clonogenic cells are pancreatic stem/progenitor cells with characteristics indicating the capability for self-renewal and pluripotency still remains to

be achieved.

We have recently established the prospective isolation of putative pancreatic epithelial progenitor cells by sorting for cell surface markers.[45] First, the expression of stem cell markers in pancreatic buds was examined at mouse embryonic day (E) 11.5 and E13.5, when the majority of the epithelium consists of undifferentiated progenitor cells and we observed CD133 (mouse prominin1) expression on the apical membrane of the Pdx1-expressing pancreatic epithelial cells (Fig. 3). CD133 expression has been previously shown in putative stem/progenitor cells in brain,[46] kidney,[47] prostate,[48] and ES-derived progenitors.[49] More recently, CD133 expression has also been reported in neonatal, adult pancreatic ductal progenitor cells[50] and fetal islet progenitor cells.[51] Previous efforts to expand the number of ß cells and other pancreatic epithelial cells are limited by mesenchyme-derived fibroblast overgrowth in vitro culture. To rule out any other tissue-derived progenitor cells, including hematopoietic and neural progenitor cells, and to further establish an in vitro culture system, it is necessary to distinguish the epithelial cells from the surrounding mesenchymal cells. We observed that platelet derived growth factor receptor ß (PDGFRß, also known as CD140b) was expressed on

the mesenchymal cells within the embryonic pancreas (Fig. 3). We obtained subpopulations by fluorescence-activated cell sorting (FACS) using CD133 and PDGFRß and pancreatic stem/progenitor cells were enriched in $CD133^{high}$ $PDGFR\beta^{-}$ fraction by RT-PCR and DNA microarray (Fig 4, not shown). While we were unable to differentiate these cells toward all pancreatic cell lineage in culture, they differentiated all pancreatic cells, including ductal, endocrine, and exocrine cells after engraftment with mesenchymal cells.[45] It is noted that the concept of stem cells has been extended from hematopoietic stem cells to many other tissues until now, however, only rarely have stem cells been identified as clonogenic precursors that include in their progeny with both self-renewing and differentiated potential. Based strictly on this definition, stem cells reported in other tissues are not clonogenic. Of interest, an encouraging study has recently reported that single Lgr5 stem cells build crypt-villus structures in vitro.[52]

D. Stem/progenitor cells from other sources

The stem/progenitor cells of other sources also might be expanded and trans-differentiated into IPCs under specific conditions. These studies provide many

candidates, including human neural progenitor cells,[14] hepatic oval cells,[53] umbilical cord blood-derived stem cells,[54] placenta-derived multipotent stem cells,[55] and bone marrow-derived mesenchymal stem cells.[56, 57] Additional studies in vivo, including rescue of diabetic phenotype in mice, appear to be necessary to indicate the clinical benefit of using these stem/progenitor cells.

E. Conclusion and future

Cell replacement strategy for diabetic patients using stem/progenitor cells has become a promising therapy, because its capacity to self-renew and the potential to differentiate into pancreatic cell lineage. Advances in understanding the mechanism underlying pancreas development and regeneration, including specific intrinsic and extracellular signals that govern the switch between the self-renewal and differentiation of pancreatic stem/progenitor cells, is necessary for a further clinical benefit.

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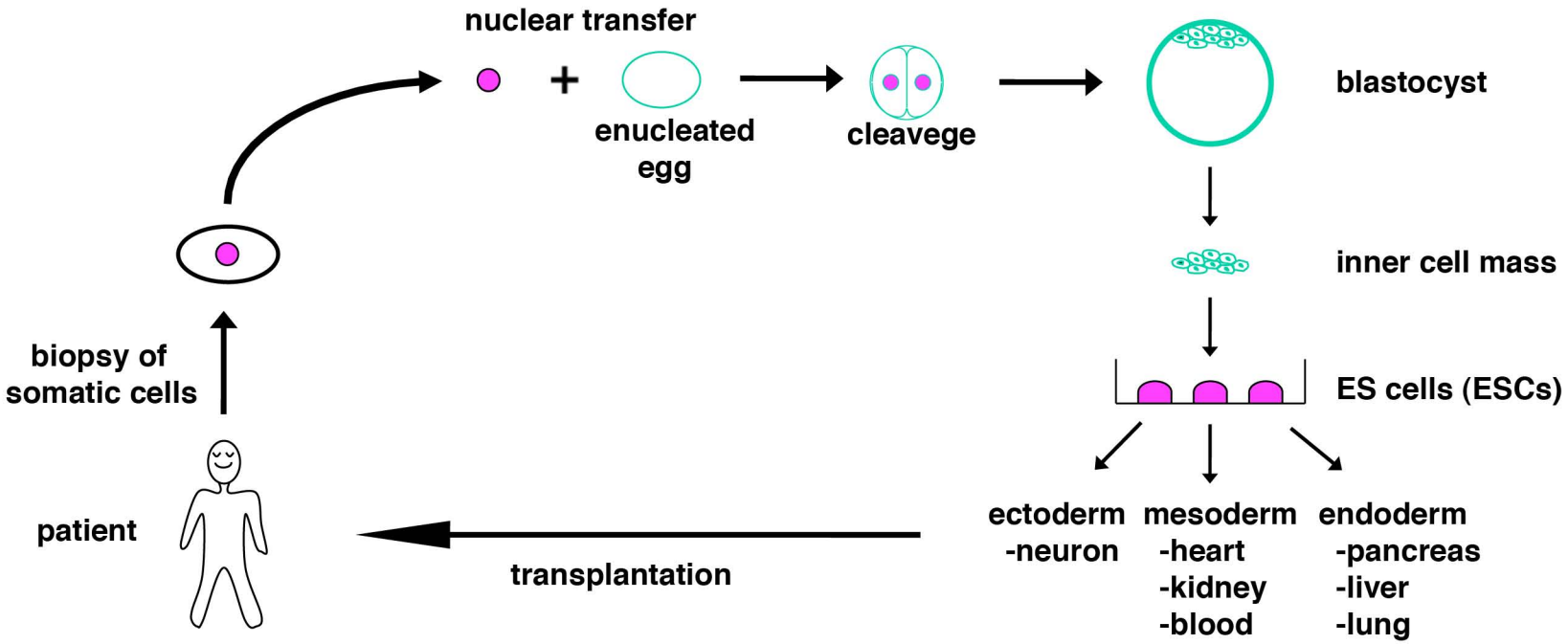
Figure legends

Fig. 1. The strategy for regenerative medicine using Embryonic Stem Cells (ESCs).

Fig. 2. Embryonic pancreas development and signaling pathway. 1.The definitive endoderm layer (yellow) and mesoderm and ectoderm layers (green) are derived from blastocyst. 2. definitive endoderm develop and form the gut tube. 3. The gut tube subsequently gives rise to budding of foregut (thyroid, lung, liver), midgut (pancreas), and hindgut (intestine). 4. After the induction of $Pdx1^{+}$ pancreatic epithelium, 5. Endocrine cells proliferate and mature toward islet of Langerhans, aggregates of insulin-producing ß cells. TGF-ß, Transforming growth factor-ß; RA, Retinoic acid; SHH, Sonic hedgehog; VEGF, vascular endothelial growth factor.

Fig. 3. CD133 is expressed on the $Pdx1^{+}$ pancreatic epithelium, whereas PDGFRß is expressed on the surrounding mesenchyme at E11.5 and E13.5 mouse embryonic pancreas.

Fig. 4. Prospective isolation of pancreatic stem/progenitor cells using CD133 and PDGFRß by FACS. Enriched pancreatic stem/progenitor cells in $CD133^{high}$ $PDGFR\beta^{-}$ fraction were confirmed by RT-PCR and microarray (not shown).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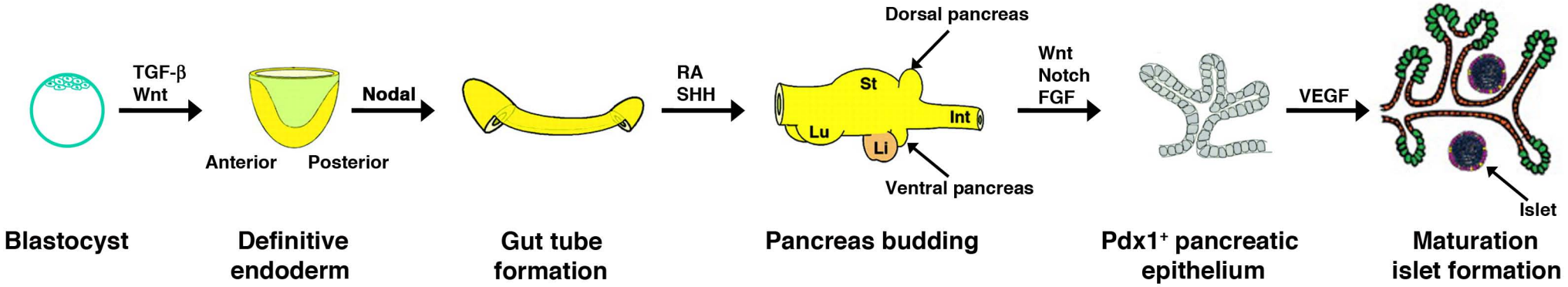
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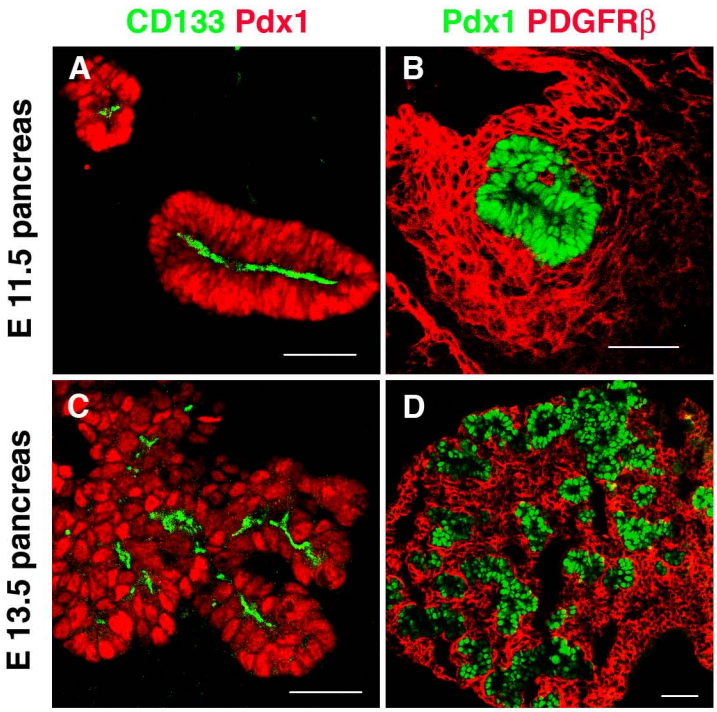
Maturation islet formation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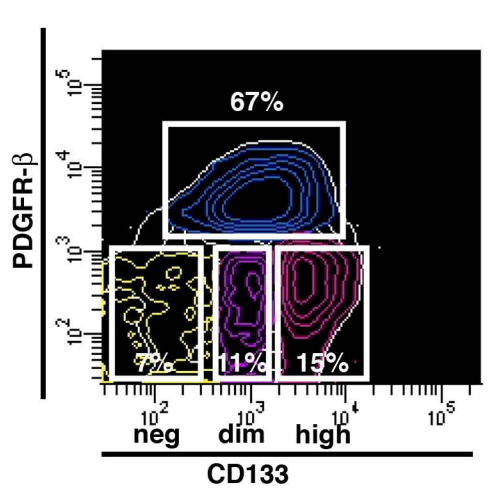
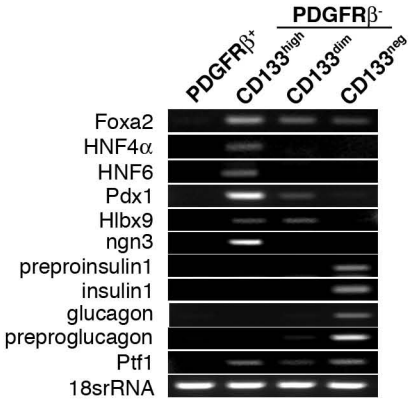

PDGFR-β
67%
7%
11%
15%
neg
dim
high
CD133


PDGFRβ-
PDGFRβ+
CD133high
CD133dim
CD133neg
Foxa2
HNF4α
HNF6
Pdx1
Hlbx9
ngn3
preproinsulin1
insulin1
glucagon
preproglucagon
Ptf1
18srRNA