Generation of functional β-cell mass,

Searching for the best way to cure diabetes

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Molecular Imaging and Electron Microscopy
Review recent literature (2000-2010)

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Summary

Diabetes is becoming a global epidemic. Because the main therapeutic actions have many side effects it is necessary to develop new sources of insulin-producing β-cells. Induction of β-cell mass could be achieved via proliferation of preexisting β-cells or generation of new β-cells out of progenitor cells.
To purchase, proliferation of preexisting β-cells, activation of transcription factors E2F and Cdk4, or activation of CRF receptor 1, could achieve this goal. Transcription factor E2F in co-infection with anti-apoptotic growth factor Akt and CRFR1 in a glucose-dependent manner.
Also generation of new β-cells out of progenitor cells could be achieved via activation of transcription factors. From ductal cells to β-cell and α- to β-cell the transcription factors INSM1 and Pax4 are used, respectively. INSM1 in combination with islet transcription factor NeuroD1 and Pdx-1. Stimulation of the incretin hormone GLP-1 is another way to achieve ductal to β-cell differentiation.
The experiments show great opportunities for new kinds of diabetes treatment. Nowadays the GLP-1 analog Exendin4 is a treatment with great outcomes in experiments with rodents but is not as operative in humans to induce β-cell proliferation. This phenomenon could be due to ageing of β-cells or difference in species. Proliferation of β-cells seems to decline with age. The decline in β-cell proliferation with age correlates with increased expression of the cell cycle regulator p16Ink4a in islet cells. In contrast, compared islet transplantation of young and adult donors do not show any significance difference in β-cell replication in response to hyperglycemia.
However, older mice with a more limited regeneration capacity would serve as a better model for assessing therapies for humans.

Introduction

Diabetes is becoming a global epidemic, more than 200 million people worldwide have diabetes. In 2005 an estimate of 1.1 million people died from diabetes, and it will double between 2005 and 2030 without more knowledge to cure this disease (WHO.org). Failure of the pancreatic β-cells to be able to produce enough insulin to meet the metabolic demand of peripheral tissues such as liver, fat, and muscle results in diabetes. Overtime diabetes can damage the hart, blood vessels, eyes, kidneys, and nerves (who.org). A reduction in β-cell function and mass leads to hyperglycemia in both type 1 and type 2 diabetes. Islet transplantation is a treatment for diabetes but has been limited by the availability of donor islets and the toxicity of immunosuppressive drug regimes. It will be necessary to develop new sources of insulin-producing β-cells for replacement therapies. Research has continued to focus on methods to generating β-cells from alternative sources (Oliver-Krasinski et al, 2008). The efficiency of differentiation into functional β-cells must be improved, this review will outline two mechanisms to increase β-cell mass 1) proliferation of preexisting β-cells, and 2) plasticity; generation of β-cells via stem/progenitor cells. In recent studies all these mechanisms obtained great outcomes, but there is one thing they overlooked, aging.

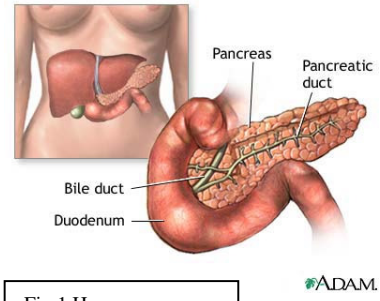

Fig.1 Human pancreas

The pancreas is located behind the liver and is where the hormone insulin is produced (fig1). The pancreas is organized in islets of Langerhans. The pancreas consist of two different types of glandular tissue, the exocrine and endocrine cells, that secrete enzymes into the intestine and hormones into the bloodstream, respectively. The exocrine pancreas is an acinar gland, arranged in acinis (fig 2A,B). The endocrine cells are grouped in the islet of Langerhans (Slack et al, 1995). The islet of Langerhans contain 5 cell subtypes, α, β, δ, ε, and PP cells, secreting glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively (Collombat et al, 2006).
Most studies use the pancreas of the rat to experiment with. The rat and human pancreas are not identical (fig 2 C). In figure 2 you see the differences, glucagon producing cells situated at the periphery and the insulin producing cells in the centre of the islet. In the human pancreas there is more random segregation. The green cells illustrate the PP cells which are located at the ventral part of the pancreas in human and rat.

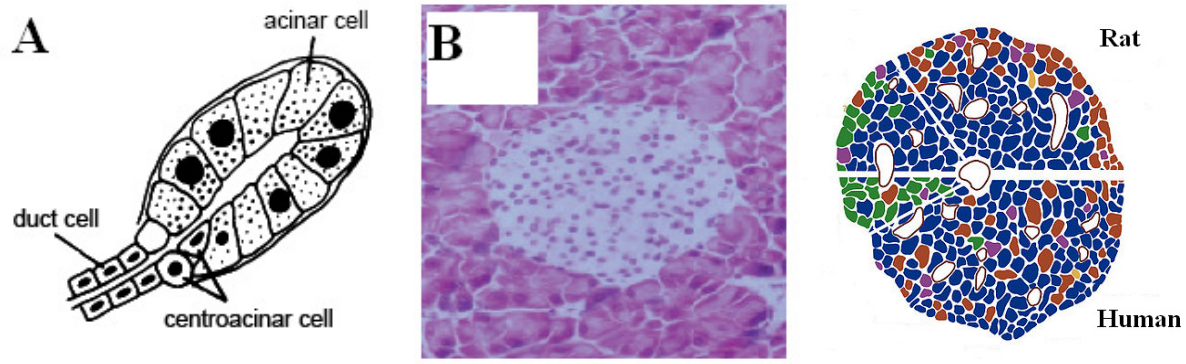

Fig 2. A exocrine pancreas, B dark tissue exocrine pancreas, light tissue endocrine pancreas, C difference between human and rat pancreas, β- and α-cells random and β-cells in the centre / α-cells on periphery, respectively.

To obtain a rat with the pathogenesis of diabetes in research, it needs to be injected with a dose of β-cell toxin streptozotocin (STZ). STZ is an alkylating agent that affects pancreatic islets, inducing diabetes in rat.
In pancreatic β-cell research the genes of interest are conditionally or ectopically expressed. The expression of a gene in a cell type in which it is usually inactive is ectopically expressed, this can be achieved by introducing a transgene with a modified promoter into the target cell.
To identify the origin of the obtained β-cell, from a pre-existing β-cell or due to cell differentiation, lineage tracing is used. This technique identifies precursors of mature cell types in vivo, cells are labeled or marked so that one can identify their progeny later during development. For example, in the mouse pancreas lineage tracing reveals that Pdx1- expressing progenitors in the early embryo give rise to all pancreatic cells (Gu et al, 2003). To examine β-cell replication BrdU labeling is used in most of the studies. BrdU labeling allows the calculation of total population of β-cell that incorporated BrdU over the entire period of the study. These BrdU-positive cells include both the original β-cells that are dividing and the regenerated daughter β-cells that may or may not be dividing (Chen et al, 2009).

Development of mouse pancreas

Recently it has been discovered that there are two types of β-cells, quiescent and proliferating β-cells that could reconstitute β-cell mass. They arise from different pancreatic endodermal tissue the ventral and the dorsal bud (Hesselson et al, 2009). To get a better understanding of these two types of β-cells there will be a short overview of the development of the mouse (since most of the studies are accomplished in mouse and rats) pancreas (fig 3). However, in humans it is generally accepted that only neogenesis and anti-apoptosis, and not proliferation may contribute to increasing β-cells mass. In rodents, all three processes are considered to be able to contribute to increase β-cells mass (Tornehave et al, 2008). The salivary glands, the liver, the trachea, the lungs, and the pancreas all develop from the wall of the originally simple digestive tract, and grow to become systems of branching tubes that open into the gut or pharynx. The endoderm forms only the secretory cells of the pancreas (molecular biology of the cell H22). The endoderm from which the pancreas arises, begins as a flat sheet of cells. Specification of the pancreas starts at embryonic day 8.5 with the expression of Pdx1 (a homeobox gene that regulates pancreatic growth and development of all pancreatic lineages) in two ventral domains preceding the expression of Pdx1 in the single dorsal

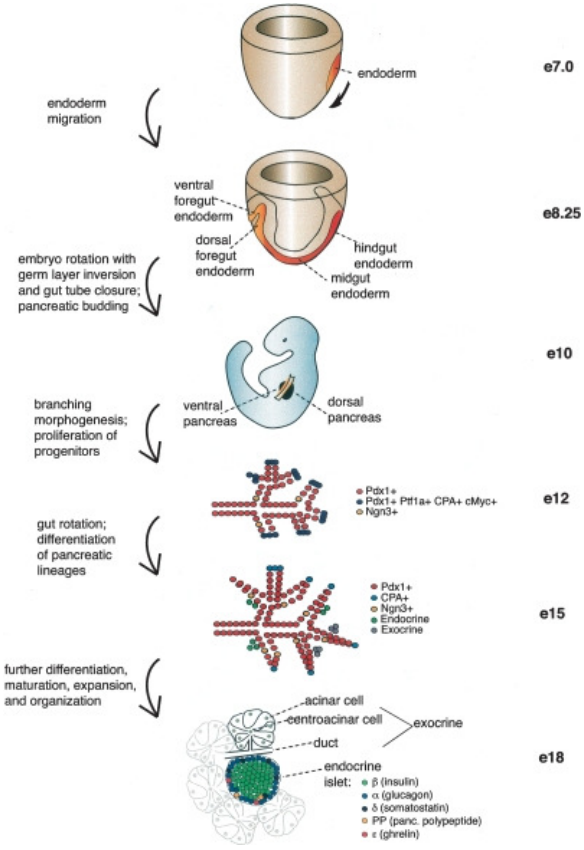

Fig 3. Mouse pancreatic organogenesis through endoderm migration, specification, pancreatic budding, branching morphogenesis, and differentiation of pancreatic lineages.

domain. Mesenchyme accumulates around the ventral and dorsal domain. At embryomicday 9.5 epithelium will branch into the mesenchyme. Recent evidence located the multipotent progenitor cells at tips of the branching network. The hormone expressing cells begin to emerge at embryomicday 13.5 (Oliver-krasinski et al, 2008). Some endocrine cells, are evident at embryomicday 9.5, these early cells lack some maturation markers and express hormones like insulin at low levels during primary transition. Their function is unclear. Fully differentiated β-cells arise at embryomicday 13.5, the secondary transition, and the proliferation potential of dorsal and ventral derived β-cells differences. The dorsally derived β-cell (DBC) stay quiescent and the ventrally derived β-cell (VBC) proliferate actively. The DBC may be responsible for the insulin secretion to support growth during the development of the embryo and VBC may differentiate in fully

functional β-cells, this specialization could be the explanation for the existence of primary and secondary transition states of β-cells (Hesselson et al, 2009). The latter could be important to understand the last unknown factors that will be required to obtain functionally β-cells out of embryonic stem cells. Analysis of the multifunctional bone morphogenetic protein (BMP) signaling pathway has been implicated in inducing liver progenitors in fish and mouse, because Pdx1/expressing progenitors give rise to intestine, stomach, and all pancreatic lineages (ductal, acinar, and endocrine cells), the role of Bmp signaling during the formation of β-cells, remains unclear. Chung et al 2009, demontrated that Bmp signaling is required for the outgrowth of the ventral pancreas and need to be suppressed for the induction of β-cells in zebrafish. As in mammals, the pancreas in zebrafish originates from multiple buds and the endorine cells derive from both the early forming dorsal and the late-forming ventral buds. Dorsal bud/derived β-cells are sensitive to Bmp signaling specifically during gastrulation and early stages. In contrast, ventral pancreatic cells, which require early Bmp signal to form, do not produce β-cells at a stage when the ventral bud-derived extrapancreatic duct is the main source of new endocrine cells. These data should help to optimize in vitro and in vivo differentiation protocols of pancreatic prognitors into β-cells for the treatment of type 1 diabetes (Chung et al, 2009).

Proliferation of preexisting β-cells

Lee et al, 2010, demonstrated that Cdk4 regulates recruitment of quiescent β-cells to reconstitute β-cell mass. Cdk4 is a cyclin-dependent kinase that plays a role in the cell cycle. The cell cycle machinery receives signals to control quiescence, proliferation, differentiation, senescence, and apoptosis. This cycle is divided in four stages, G1, S, G2, and M. Chromosome duplication starts at the S stage and separation of the cells in the M stage. Retinoblastoma protein negatively regulates the passage from G1 to S phase by inhibition of transcription factors like E2F, and Cdk's promotes S phase progression. Cdk4 catalyzes the recruitment of quiescent cells within the islets and the ductal epithelium to participate in the regenerative process. By using mice aged 6-8 weeks with active Cdk4 they figured out which of the two possibilities 1) proliferation of active β-cells, or 2) recruitment of quiescent cells into active cell population, enhance cell proliferation. The Bayesian model probability for the given data showed that a smaller quiescent cell population is an explanation of the induced proliferation rate (Fig. 4).

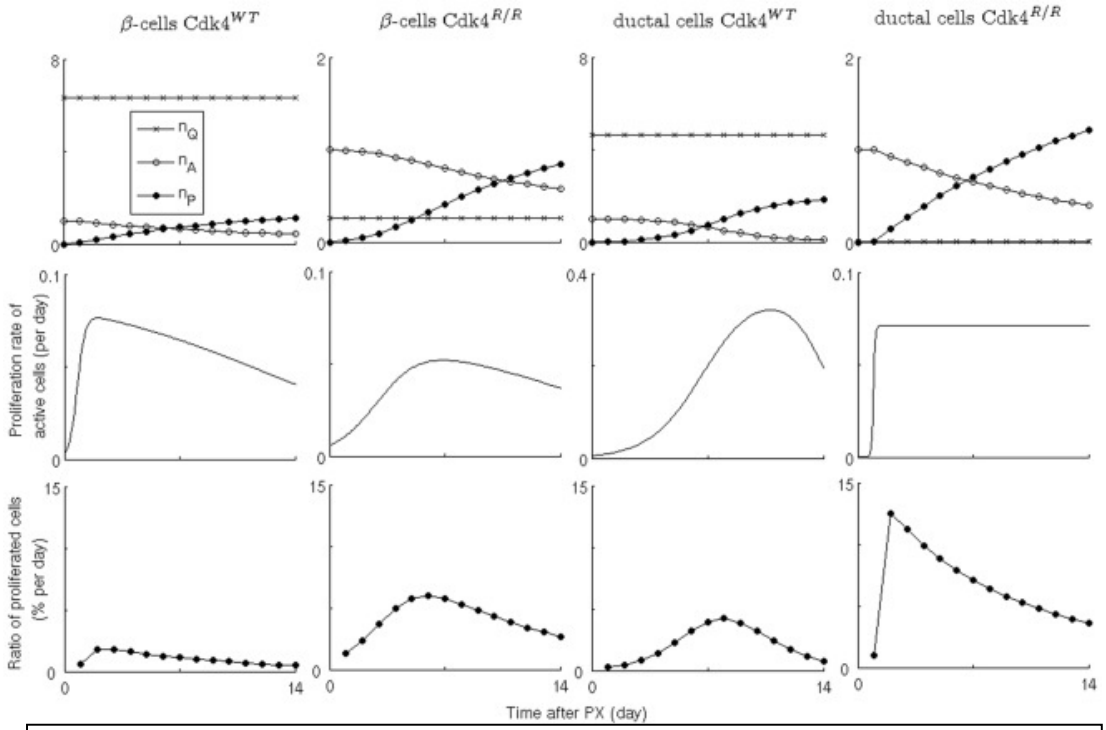

Fig 4. proliferation of β-cells and ductal epithelial cells of CDK4 wildtype and CDK4 dominant allel mice after pancreatectomy. Quiescent, active, and proliferated cell numbers (Nq, Na, Np)

In the wild type mice (genetically modified Cdk4 loci) there is no alteration in the level of quiescent cells after partial pancreatectomy, but still an induction of the proliferation. In the active Cdk4 mice there is a stronger induction of proliferation and a lower level of quiescent cells. Here, they neglected multiple proliferation events of the active cell because such events are rare (Lee et al, 2010).

This data shows a Cdk4 induced β-cell proliferation by self-repliction in mice aged 6 to 8 weeks. If it is due to re-entry of quiescent cells could not be determined. *It is suggested by a mathematical model which neglects multiple proliferation events of the active cell since it is rare. However, even if the proliferation is not due to quiescent β-cells it still occurs and could be of benefit in the development for treatment for diabetes type II.*

Grouwels et al, 2010, shows another way to improve the β-cell mass from preexisting cells via an ectopic expression of E2F1, previously mentioned as an transcription factor to passage the S phase, inhibited by retinoblastoma protein.
Over expression of E2F1, mediated by adenovirus, increased the proliferation of isolated β-cells but also enhanced cell death. E2F1 will increase β-cell proliferation but also creates a negative feedback loop which contribute to G1 arrest, E2F1 over expression in primary fibroblasts does not lead to S-phase entry but promotes senescence and apoptosis. Co-infection with Akt adenovirus, a growth factor with anti-apoptotic effects, suppressed β-cell deaths, and in addition increased β-cell proliferation, thus over expression of E2F1 cannot induce β-cell proliferation in the absence of an anti-apoptotic factor (fig. 5).

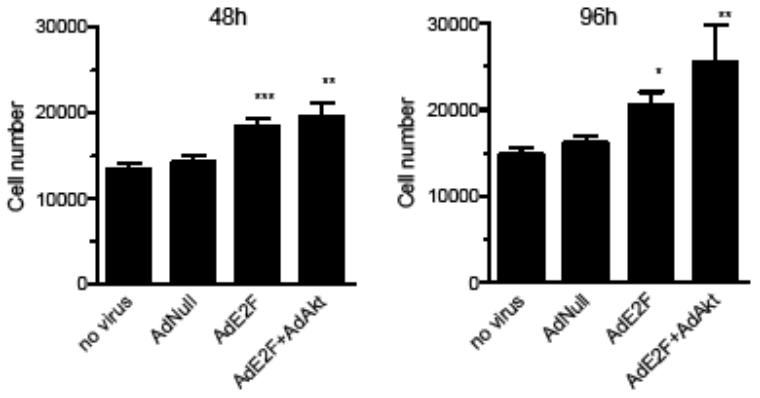

Fig 5.
E2F1 expression induces β-cell proliferation and death. Detection of mitotic marker in insulin expressing β-cells.

Not only the proliferation activity is increased also the insulin content in β-cells with a more potent glucose-induced insulin release in vivo. More insulin is synthesized in β-cells, less insulin is released, and hyperglycemia is normalized, this suggests an increased insulin sensitivity (fig. 6).

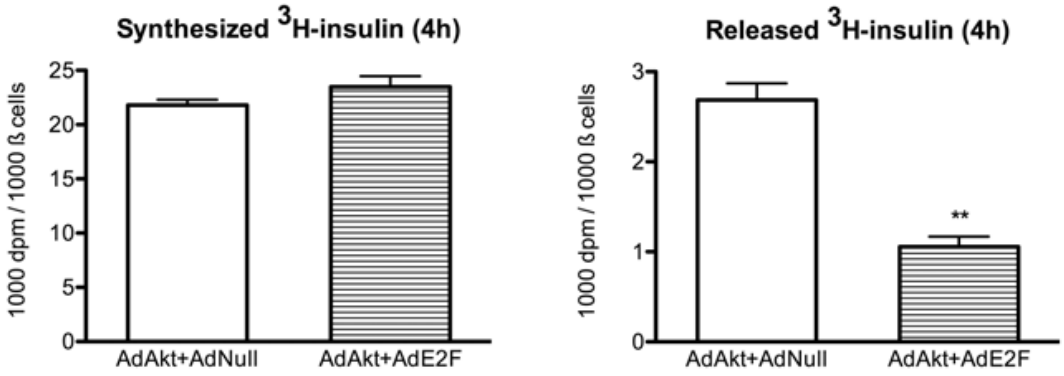

Fig 6. insulin content increases and insulin release decreases in proliferating β-cells

However, the consequences of over expression of E2F1 in β-cells are unknown, targeting this cell-cycle factor would need to be very much controlled, to prevent oncogenesis (Grouwels et al, 2010).
Grouwels et al, may open perspectives for cell therapy in diabetes by increasing the number of β-cells that enter replication, just like Lee et al, 2010. The proliferation of β-cells is not enough to cure diabetes, it is necessary to obtain functional β-cells. Both in vitro and in vivo studies are accomplished in rat and mice aged 8 weeks. In vitro E2F1/Akt β-cells did proliferate but blocked glucose induced insulin release. Since in vitro did not correspondent with in vivo experiments the E2F1 action remains unsettled.

Huising et al, 2010, discovered another way to induce β-cell proliferation via activation of the CRFR1 located at the β-cells. CRFR1 is the abbreviation of corticotropin-releasing factor receptor type 1, a receptor mediated by CRF hormone also known as the stress hormone. Originally familiar with the hypothalamus-pituitary-adrenal axis where it acts on the CRFR1 which stimulates the release of glucocorticoids that antagonize the actions of insulin (Chrousos et al, 2000). There have been suggestions that CRF can affect the endocrine pancreas. Li C et al 2003, demonstrated that the CRF paralogue urocortin 3, Ucn 3, is expressed in β-cells where it reacts to high levels of glucose. Ucn 3 is a selective CRFR2 agonist (Li et al, 2003).Huising et al, 2010, revealed CRF-mediated effects on the endocrine pancreas, the receptors involved, and the pancreatic cell type in which they are expressed.
The identification of the cell type in which the CRFR1 is expressed is achieved by the comparison of the amount of CRFR1 transcript in the total RNA of three different islet cells in vitro. The MIN6 cells, αTC1 cells, and TU6 cells representing β, α, and δ lineages, respectively. The highest level of CRFR1 transcript were found in the MIN6 cells.
After activation of the CRFR1 via CRF stimulates the insulin secretion in a glucose-dependent manner. The effect of CRF on insulin secretion is more abundant in synergy with high glucose (fig. 7a). The CRF acts on the CRFR1 receptor and not on the CRFR2 receptor since no effect is found of induced insulin secretion after coadministration of CRFR1-selective antagonist antalarmin. The induction of insulin could be the result of a higher expression of insulin in β-cells or due to the proliferation of β-cells. Western immunoblotting revealed after activating CRFR1, the presence of Erk1/2 in β-cells, which phosphorylate gene regulatory proteins in the nucleus. In this way the MAPK signaling pathway transfer signals from the cell surface to the nucleus and alters the pattern of gene expression. Genes activated by this pathway could stimulate cell proliferation.To test whether the activation of CRFR1 induces proliferation of β-cells, they measured the incorporation of 5-thynyl-2'-deoxyuridin (EdU) in β-cells in vitro. EdU can be coupled via click chemistry to the DNA, when the cells proliferate the daughter cell will incorporate EdU. The alkyne group of the EdU will react with the dye-conjugated azide (Diermeier-Daucher et al, 2009). The activation of CRFR1 increased the incorporation of EdU in insulin-positive cells and was blocked by antalarmin (fig. 7b). Briefly, CRFR1 activation stimulates insulin secretion and β-cell proliferation during conditions with elevated glucose conditions. This response is very similar of the actions

of GLP-1 (glucagon-like-peptide-1) an incretin, which potentiate insulin secretion only during elevated glucose conditions (Huising et al, 2010). The role of incretins in maintaining functional β-cell mass will be discussed later.
Two different papers, Li et al 2003 and Huising et al 2009, report CRFR activation which stimulates glucose-dependent insulin secretion. Huising et al, experiments where done on mice aged 8 to 12 weeks and no age is known of the rats used in Li et al experiments. *Althought, the CRF paralogues Ucn 3 and agonist ovine CRF, activates CRFR2 and CRFR1, respectively. It will be interesting if the CRF paralogues Ucn1, which activate both CRF receptors, induces a more significant β-cell proliferation and insulin secretion. Because of the glucose stimulating effect of CRF via the HPA axis it will be important to find a way in activating pancreatic CRF receptors exclusively. This needs futher examination.*

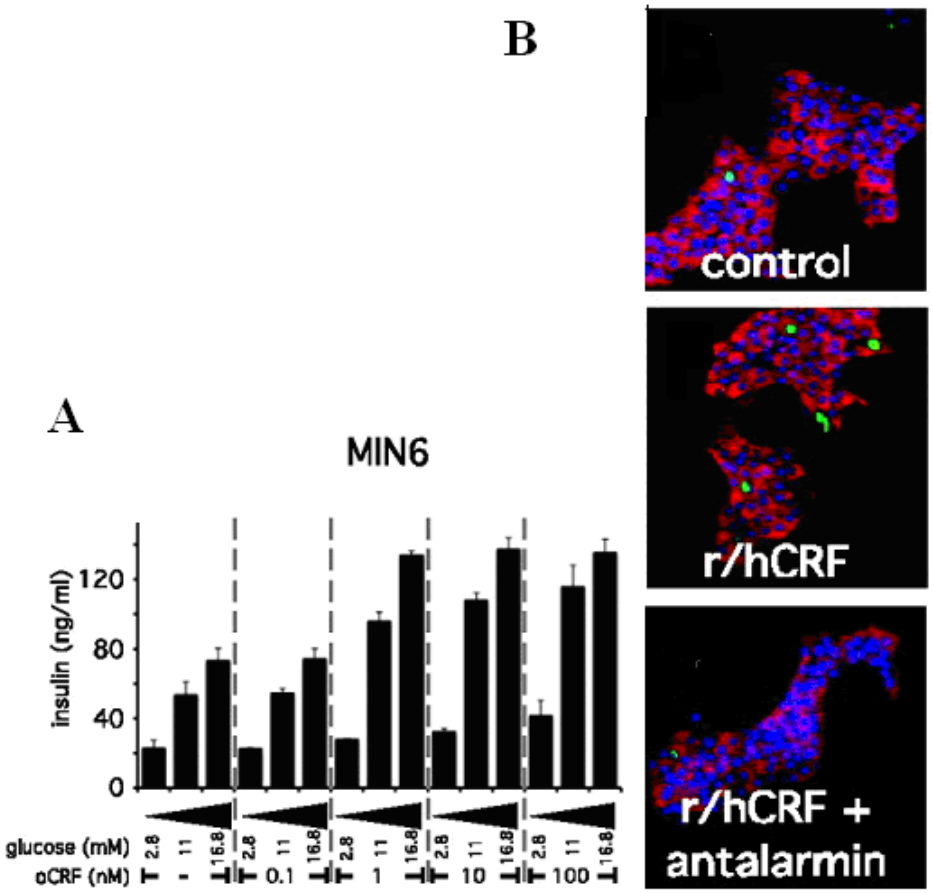

Fig 7. A, activation of CRFR1 by ovine CRF induces insulin transcription and is enhanced by high glucose concentrations. B, stimulation of rat neonatal islet cells with rat/human CRF increased the nuclear incorporation of EdU into insulin positive cells. Antalarmin blocked this incorporation.

Plasticity; generation of β-cells via stem/progenitor cells

Tissue renewal often involves the proliferation of pre-existing cell types. However, many tissues in our body have the potential to reactivate a process of cell plasticity. Embryonic stem cells are pluripotent and have the potential to differentiate into any lineage, unfortunately it is still difficult to achieve directed differentiation of human ES cells to endocrine pancreatic lineage.
When pancreatic damage occurs like 70% pancreactomy, it will be compensated predominantly through proliferation of pre-existing cells (Strobel et al, 2007). When there is to much damage and proliferation is insufficient, cells can become progenitor-like and transdifferentiate into pancreatic endocrine cells. The term transdifferentiation refers to a change in a cell from one differentiated state to another (Lee et al 2009). We need to explore sources that can be used for the generation of new β-cells. To understand which other cell types can be β-cell progenitor-like it requires knowledge of the epigenome. All human body cells contain the insulin gene, only a few pancreatic islets show insulin gene expression. Cells from the human fetal pancreatic islets show epigenetic marks that indicate an active promoter conformation. Cells that are more committed to differentiate into endocrine pancreatic fate are pancreatic duct cells, islet-derived precursor cells, and acinar cells (Baeyens et al, 2009, Dalvi et al, 2009).

Pancreatic duct cell trans-differentiation

Zhang et al, 2010, have focused on a novel transcription factor, insulinoma-associated antigen-1 (INSM1/IA-1), in neuroendocrine differentiation. INSM1 is a zinc-finger transcription factor, (a zinc-finger is a small molecule that uses zinc ions to stabilize their folds and function as an interaction module that binds DNA, RNA, or other proteins) only expressed in pancreatic β-cells during early development. INSM1 regulates neuroD1 (expressed in all pancreatic endocrine cells including earliest glucagon cells at embryonicday 9.5), insulin, and INSM1 itself. During islet differentiation there will be a feedback network stimulated by neurogenin 3 (Ngn3) and NeuroD1 for proper development.
To examine if INSM1 could contribute to reprogramming the pancreatic duct cell differentiation into islet-like cells, it will be ectopic expressed to promote Panc-1 cell, exocrine pancreatic cells, differentiation.
INSM1 up-regulates two islet transcription factor (ITFs), Pax6 and Nkx6.1 (required for the maintenance of islet cell number, morphology, and hormone gene expression) but is also down regulates NeuroD1, Pdx-1 and Ngn3. Without enough NeuroD1 and induction of Pdx-1 the Panc-1 cell differentiation seems to stop. INSM1 seems to have a dual functional role; ectopic expression of INSM1 lowers the numbers of cells entering G2/M fase and causes cell cycle arrest. The induction of the cell cycle arrest by ITFs facilitate the switch towards the endocrine differentiation. INSM1 alone or NeuroD1/Pdx-1, already increases the insulin expression level, but all together were most effective and significant (Fig. 8a,b). This result supports the idea that INSM1 with ITFs as NeuroD1 and Pdx-1 can promote Panc-1 cell differentiation into functional β-cells. It is likely that

INSM1 contributes to β-cell differentiation by inhibiting duct cell proliferation (Zhang et al, 2010).

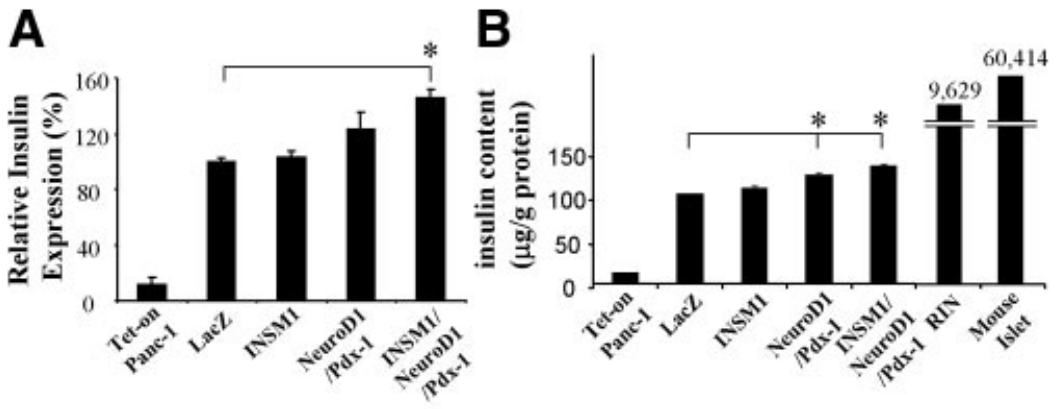

Fig 8. insulin expression levels induced by ITFs during Panc-1 cell differentiation. A) Insulin mRNA levels. B) insulin content.

Panc-1 is a tumor cell line from a human carcinoma of the exocrine pancreas. The outcome that exocrine pancreas differentiate in insulin producing cells does not exclude the trans-differentiation of other exocrine cells besides duct cells. *However, INSM1 does promote insulin gene expression. In vivo experiments of pancreatic tissues from transgenic mice which overexpress INSM1, and STZ treated transgenic mice, would give a better indication if INSM1 could contribute to insulin secretion instead of carcinoma cell line.*

Islet derived precursor cells plasticity

Zhang et al, 2010, demonstrated differentiation of pancreatic duct to β-cell by induction of the transcription factor ISNM1 and Ngn3. Next to these, the transcription factors Arx and Pax4 promote the endocrine fates. In mice lacking Arx the β-cell genesis was favored over α-cell genesis while the total endocrine cell content remained normal. The opposite was observed in mice lacking Pax4. It's indicating an inhibitory effect between Arx and Pax4. The finding that forced expression of Arx in adult β-cells induced their fate into α- or PP-cells (Collombat et al, 2007) suggests that the opposite conversion could be achieved, the generation of β-cells from other endocrine cells.
Collombat et al 2009, generated mice conditionally expressing the Pax4 gene in glucagon expressing cells (POE). They noticed the induction of α-cell differentiation into β-cells in two different stadia, in embryogenesis and young mice. During embryogenesis the α-cell induces a β-cell fate by the ectopic expression of Pax4, and in the young mice, with STZ induced depletion of β-cells, the ectopic expression of Pax4 mediated a regeneration of β-cell mass out of α-cells, with a normalization of the glycemia and an extended life span. The first experiment, embryogenesis, produced rats with normal sized islets of Langerhans and morphology, suggesting that Pax4 acts on the specification of endocrine progenitor cells by promoting the induction of the β-cell fate. Based on the lineage-

tracing in the POE mice, the expansion of the β-cell mass was attributed to the neogenesis of β-cells through α-cell differentiation upon Pax4 ectopic expression, instead of the self-renewal capacity of the β-cell. This process is also dependent on ITF's, a Ngn3-dependent process, like the Panc-1 cell differentiation into β-cells. Ngn3 is required for α-cell neogenesis due to the loss of α-cells after acquisition of β-cells out of α-cells. This experiment suggest that the forced expression of Pax4 in cells with glucagon expression domains like α-cells led to the development of islets of Langerhans mostly composed of functional β-cells.
However, it is of more interest if these cells also replace lost β-cells in diabetic mice. This experiment is assessed in diabetic mice with more than 95% β-cell loss after STZ treatment. The POE mice at different ages where injected with a dose of β-cell toxin STZ. A high mortality rate was found in mice older than 4 weeks, as compared to the younger mice. These mice obtained a normalization in blood glucose level after a peak in glycemia and survival rates (Fig. 9).

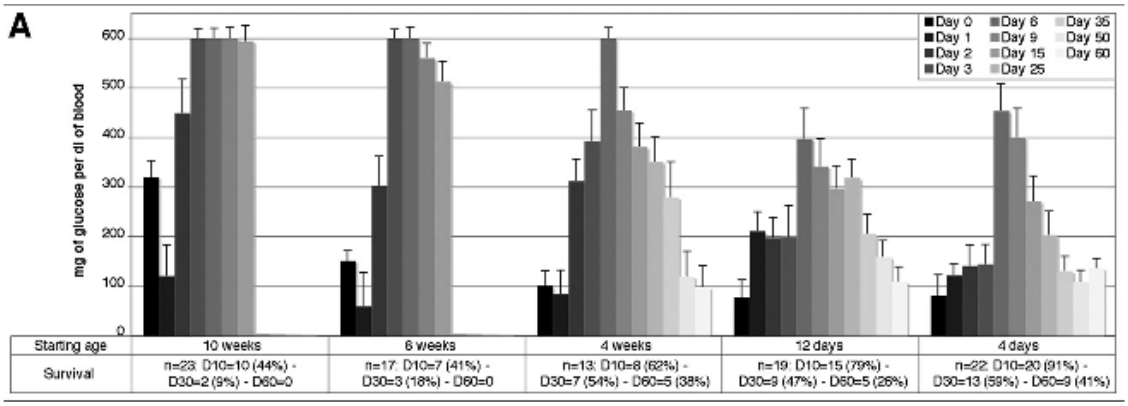

Fig 9. Pax4 ectopic expression promotes the reconstruction of the insulin-expressing cell mass after β-cell toxin STZ injection. After 4 weeks of STZ injection no recovery is induced by Pax4. In younger animals a steady recovery leads to normoglycemia.

Pax4 ectopic expression in β-cells does not induce their proliferation. It has been analyzed in POE mice expressing Pax4 in insulin producing cells instead of glucagon producing cells, and they did not reveal an increase in β-cell numbers.
In POE mice expressing Pax4 in glucagon producing cells, the cells which adopting a β-cell phenotype were detected adjacent to duct structures.
Collombats results suggest that the ectopic expression of Pax4 in α-cell can reverse the β-cell loss in STZ mice through the induction of α-cell neogenesis and their subsequent differentiation into normal β-cells (Collombat et al,2009, Dalvi et al, 2009).
This experiment is done only in vivo and not in vitro. It would be interesting if Pax4 expression generates β-cells, in vivo. Transplantation experiment with the same donor as recipient mice would create new options for therapy in humans.

Acinar cell transdifferentiation

Recently, the ability of pancreatic duct-like cells to differentiate into islet cells is observed (Zhang et al 2010, Bayens et al 2009, Dalvi et al 2009). Lee et al 2009, demonstrate an indirect way to regenerate new β-cells by transforming acinar to ductal cells. These cells can be used to regulate adult pancreatic differentiation toward developing therapy for diabetes type II.

Lee et al 2009, create two types of pancreatic-duct like prognitor cells by transdifferentiation from rat prancreatic acinar cells. Isolated acinar cells express high levels of the acinar markers amylase and P48, but not cytokeratin (CK) a ductal cell marker. After 5 days of culture the opposit is seen, and after 3 months, CK19 was expressed significantly and constantly. To rule out the possibility of islet and/or ductal cell contamination, BrdU labelling and western blotting of betacatenin, which is expressed in islet cells, not in acinar cells, is done. The results suggest that the increased population originated from acinar cells. Single cells where isolated and cloned, and among these clones, two morphologically distinct cell lines where chosen, YGIC4 and YGIC5. The two YGIC cell lines expressed pancreatic-development related genes, these genes suggested that YGIC cell lines had characteristics of pancreatic precursor cells. Both the cell lines express GLP-1 receptor protein, an incretin which stimulates insulin gene expression. After treatment with GLP-1 the insulin gene expression increased, and the expression of the ductal cell marker CFTR decreased, indicating differentiation into insulin-producing β-cell population with loss of ductal characteristics, only in the YGIC5 cell line. The GLP-1 expression needs to be derived with a Zinc-ion inducible promoter for differentiation into insulin-producing cells. Two types of YGIC5 cell lines, with and without GLP-1, where transplanted into STZ diabetic rats and control rats without STZ treatment. Only, the transplantation with GLP-1-YGIC5 cell line in the STZ diabetic rat shows a significant decrease in glucose and increase in insulin (Table 1).

Table 1. Experimental Design

Animal groups	*Transplantation*	*STZ treatment*	*$ZnCl_2$ treatment*	*Body weight (g)*	*Plasma glucose (mg/dL)*	*Plasma insulin (ng/mL)*
Group 1	Diabetic GLP-1/YGIC5 (n = 8)	+	+	285.38 ± 19.41	184.43 ± 35.52**	2.12 ± 0.56**
	Diabetic GLP-1/YGIC5 (n = 8)	+	–	281.25 ± 9.84	506.25 ± 44.28	0.84 ± 0.45
Group 2	Diabetic Mock/YGIC5 (n = 8)	+	+	270.40 ± 8.41	543.00 ± 12.73	0.94 ± 0.44
	Diabetic Mock/YGIC5 (n = 8)	+	–	298.20 ± 14.67	532.00 ± 2.83	0.52 ± 0.03
Group 3	Diabetic (n = 4)	+	+	267.00 ± 22.20	550.00 ± 67.88	1.10 ± 0.17
	Diabetic (n = 4)	+	–	298.20 ± 14.67	499.00 ± 63.64	0.82 ± 0.47
Group 4	Control (n = 4)	–	+	319.75 ± 16.89	107.50 ± 2.12	2.66 ± 0.57
	Control (n = 4)	–	–	277.00 ± 63.78	93.500 ± 0.71	2.27 ± 0.68

Lee et al 2009, demonstrated two distributions; the transdifferentiation of acinar cells to ductal cells result in two cell lines YGIC5 and YGIC4, and the differentiating YGIC5 cells into insulin-producing β-cells by GLP-1 stimulation. However, the presence of unknown prognitor cells inside the acinar granules that differentiate into duct-like cells could also be a possibility (Lee et al, 2009).
YGIC5 cell lines with GLP-1 expression are transplanted in STZ treated 8-week-old male rats, intravenously. Glucose levels in these rats were almost normalized 16 days after transplantation, it will be of more importance if this normalization remains for months in rats. Allready insulin injection are a treatment to cure diabetes induced problems, a new strategy must not just conform to increase functional β-cell mass, the treatment must contribute to a better standard of living. It could be a option to repeat this experiment in a long-term study.

Senescence in β-cells

Lineage tracing experiments show that new β-cells can arise from proliferation of preexisting β-cells or trans-differentiation to new β-cells (Huising et al, Zhang et al, 2010). However, both the capacity of regeneration and the mechanism involved can differ depending on the experimental model. Most of the studies are carried out on rodents at relatively young ages. In the experiments previously described the rodents aged 4 to 8 weeks approximately. Tanigawa K et al, 1997, demonstrated the effect of aging on β-cell function and replication in rat pancreas after 90% pancreatectomy and revealed an increase in insulin content in 1-month-old rats but not in rats that were 5 or 15 months old (Tanigawa et al, 1997). Figure 9 shows us an increase in mortality in mice with 95% β-cell loss older than 4 weeks (Collombat et al, 2009). Partial pancreatectomy in young mice is followed by extensive regeneration of β-cells through β-cells replication, in adult no β-cell regeneration occurs. It is not clear whether this different outcome is a species difference or age-dependent. The rate of β-cell proliferation gradually declines with aging in rats to a steady state by 7 months of age. The decline in β-cell proliferation with age correlates with increased expression of the cell cycle regulator p16Ink4a in islet cells. P16ink4a inhibits CDK4-cyclin D2 complex and can inhibit cell cycle progression and regeneration of islet cells. However, the mechanisms that regulate the increase in p16Ink4a with aging are not known (Tschen et al, 2009). Tschen et al, 2009, examined the capacity of β-cell renewal after high-fat diet or GLP-1 analog exendin-4, and after toxin administration in young (6-week-old) and old (7-8 month-old) mice. The results suggest, that the older mice have a limited capacity in β-cell renewal because of age-related accumulation of p16Ink4a. The polycomb group protein Bmi1 is suggested to play a role in the regulation of p16Ink41 as a suppressor and could serve as a biological marker. In old mice β-cell mass does not increase after a high-fat diet and the levels of Bmi1 and p16Ink4a are low and high respectively (fig.10a/b). Activation of the Bmi1 in distinct cell types within the pancreas will shed light on the compartment that regulates β-cell mass (Tschen et al, 2009).

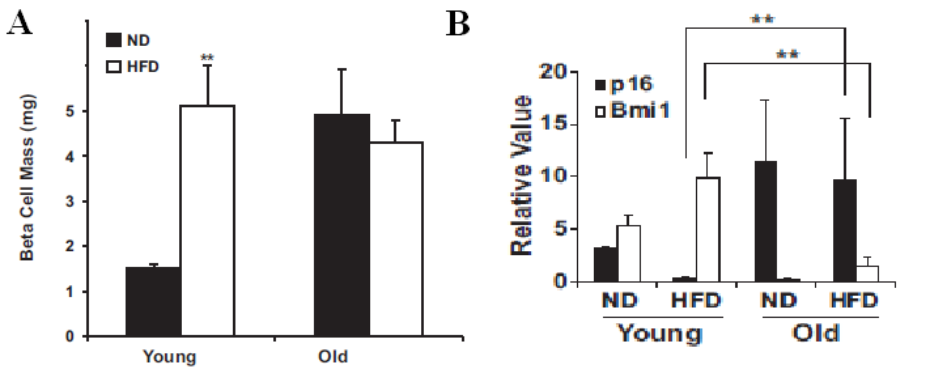

Fig. 10, A) β-cell mass increases after high fat diet in young mice, no significant difference occurs after high fat diet in old mice. B) increased β-cell proliferation in high fat diet young mice correlates with increased Bmi1 levels.

Wong et al, 2009, suggest the role of p38MAPK in the regulation of p16ink4a expression. The cell cycle inhibitor Cdkn2a is a tumor suppressor locus with the product p16ink4a. Partial inactivation of p38MAPK is sufficient to prevent aging-induced activation of multiple cell cycle inhibitors as Cdkn2a, and improves proliferation and regeneration in the islets of old mice with p38MAPK dominant negative allel (p38AF/+). Figure 11 shows the capacity of islet proliferation at 3 and 30 days after STZ injection in 10-12 month old mice. The p38AF/+ mice shows a significant higher level of proliferation than the wildtype mice.

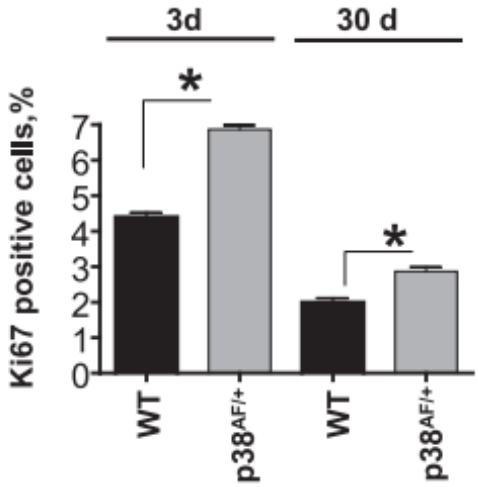

Fig. 11 islet proliferation based on Ki67 staining was analyzed in 10/12 month old wild-type and p38 homozygous mutant mice at 3 and 30 days after STZ injection.

A physiological way to regulate P38MAPK signaling with aging could be done through regulation of Wip1 phosphatase. Wip1 phosphatase is a potent regulator of p38. Wip1 deficiency results in a p38-dependent up regulation of Cdkn2a genes and impairs islet proliferation (fig 12a/b).
Activation of Cdkn2a genes could be disrupted in several ways to improve β-cell proliferation in old mice, p38MAPK allel inactivation, Bmi1 activation, and Wip1 over expression. However, it is important to fully control this mechanism because Cdkn2a is a tumor suppressor, and suppressing Cdkn2a must be done without promoting tumor genesis (Wong et al,2009).

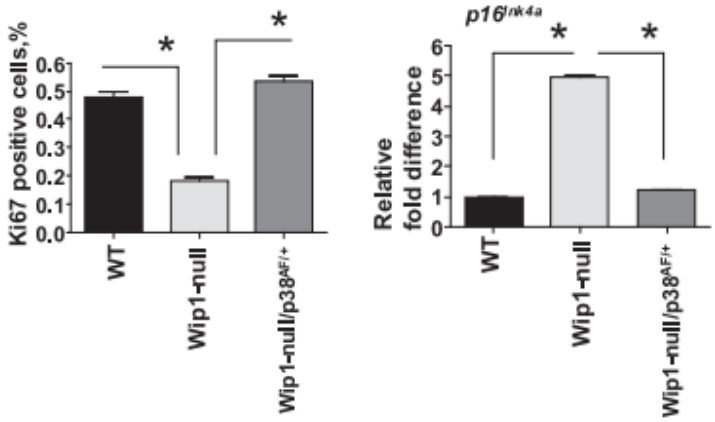

Fig. 12 dose dependent effect of WIP1 on islet proliferation

A) analysis of Ki67-positive cells in islet of 4 to 6 month old mice.
B) Analysis of Cdkn2a gene expression in islet of 4 to 6 month old mice.

Older mice with a more limited regeneration capacity would serve as a better model for assessing therapies for humans (Tschen et al, 2009 and Rankin et al, 2009).
Tyrberg et al 1996, demonstrated more than 10 years ago that adaptive β-cell proliferation is also restricted with age, basal replication rates in human pancreatic and cultured human islets decline with donor age (Tyrberg et al, 1996). Chen et al 2009, investigate the effect of ageing on β-cell regeneration under hyperglycemia using a islet transplantation model. This islet transplantation model characterize and compare the growth potential of islet β-cell from young and aged donors in response to long (1-3 weeks) hyperglycemia. They examined the β-cell replication under hyperglycemia by two parameters; BrdU labeling and detection of the presence of proliferation-associated protein Ki67 which represent the cells undergoing replication at the time of sample retrieval. Long term BrdU treatment may slightly suppress islet cell proliferation.
The main purpose of this study was to understand the total regenerative responses of β-cells over a prolonged period of time under a consistent level of hyperglycemia. Therefore, graft samples of mice at different stages of hyperglycemia were examined. The donor mice aged 3 to 24 months and the recipient mice were 3 months of age.
The results provide evidence that hyperglycemia induces β-cell replication that may result in increased islet mass. They took immunofluorescence images of islet graft sections of young and adult donors under normoglycemia and prolonged highglycemia (3-21 days) immunostained with antibodies against BrdU or Ki67 and insulin. The positive cells were counted and show no statistical difference of percent β-cells between the young versus aged donor islet groups were detected at the time points examined (fig 13 a/b). The data reveal that β-cells from aged mice replicate in respons to hyperglycemia after transplantation at a capacity and frequencies not significantly different than that of the young adult ones (Chen et al, 2009).

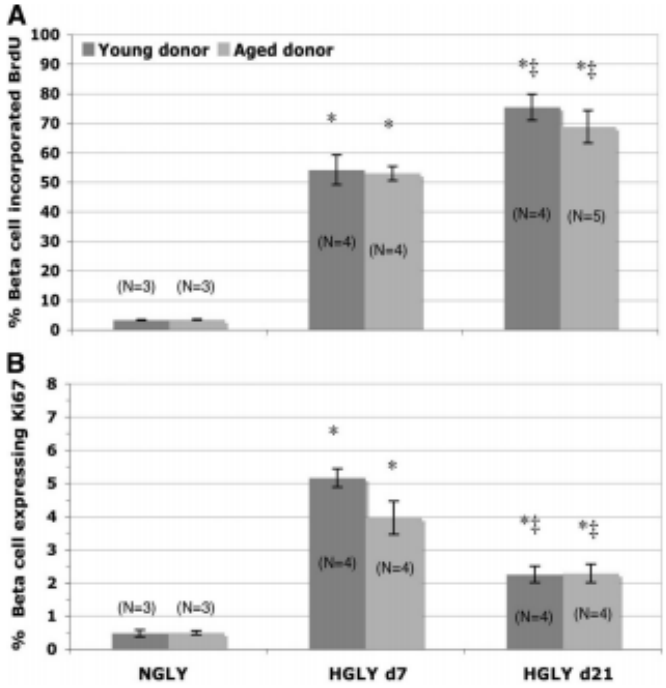

Fig. 13
A) measurment of β-cell replication with BrdU
B) measurment of β-cell replication with Ki67
no difference in β-cell expression between young and aged donor islet groups.

Wong et al 2009, examined whether aging reduces β-cell proliferation, while Chen et al 2009, examined if older pancreatic donor tissue would proliferate in respons to hyperglycemia. In times of hyperglycemia in aged pancreas, β-cell mass or insuline secretion must increase to lower glycemia. Not all adult humans develop diabetes, therefore it is important to analyze healthy aged rodents to understand the mechanism how they maintain normoglycemia, plausible without proliferation of the β-cells.

Conclusion/Discussion

Normal adult islets do not proliferate in a culture system this limits the islet supply merely to donor pancreas. It is essential to generate islets in culture from either inducing islet proliferation and/or trans-differentiation from precursors or other available cell types (Zhang et al, 2009).

In the proliferation of pre-existing β-cells reports mentioned in this review three ways to enhance β-cell mass and function are described. Transgene Cdk4 mice, which oppose cell cycle inhibition, did reconstitute β-cell mass with insulin expression. However, it is not known if these mice could normalize glycemia.
Double transgene E2F1 mice displayed an improved glucose tolerance. After STZ treatment blood glucose levels in double transgenic mice remained significantly lower than in the control mice, monitored for one month. In vitro adenovirus-mediated expression of E2F1 in β-cells, showed unexpected β-cell proliferation, but did not provide glucose-stimulated insulin secretion.

In vivo these two ways improve STZ-mediated diabetes in mice. Althought, it will be difficult to use them as a novel therapeutic target. To express these genes in diabetic patients, gene therapy has to be refined.
I prefer the third way to be the best new therapeutic target to proliferate pre-existing β-cells, which purpose the activation of the CRF receptor 1. Both in vitro and in vivo it evokes glucose-induced insulin secretion. Like E2F1 gene CRFR1 activation contradicts the non-proliferating activity in vitro. The therapeutic drug would be a hormone to activate CRFR1. There is only one negative side effect which remains to be solved, the activation of CRFR1 on the anterior pituitary.

Transplantation would be a better treatment, nowadays it is difficult because of the shortage of donors and the toxicity of immunosuppressive drug regimes. Transplantation of one cell-line would solve both problems. Since, it was supposed that pre-existing β-cells do not proliferate in vitro it would be better to purchase β-cells out of other endocrine or exocrine pancreatic cells. This review out lines three ways to induce plasticity,1 pancreatic duct cell transdifferentiation, 2 islet derived precursor cells plasticity, and 3 acinar cell transdifferentiation.

1. The transcription factor INSM1 differentiates exocrine pancreatic cell-line into functional β-cells. INSM1 combined with islet transcription factors NeuroD1 and Pdx-1 shows a significant increase of differentiation into β-cells and insulin expression. This study does not examine transplantation of the cell line and whether the obtained β-cells could normalize glycemia. They used a pancreatic carcinoma cell-line that would not be a safe option to transplant. If it is possible to obtain an exocrine non-carcinoma pancreatic cell-line, INSM1 would be an appealing therapy.
2. Transgene mice which express Pax4 in β-cells, convert progenitor cells into α and subsequently β-cells. Conversion occurs in the developing pancreas and in the pancreas of STZ induced diabetic mice in ectopic expressed Pax4 transgene mice in vivo. No in vitro experiment is done. It would be an interesting study when STZ induced diabetic mice evoke trans-differentiation of α-cells into β-cells in the pancreatic tissue after adenovirus-mediated Pax4 expression. If it occurs, transplantation would be an option.
3. Since it is still unclear I prefer again the third way to be the best new therapeutic target, too which purpose ductal cell differentiate into islet-like insulin producing cluster when exposed to GLP-1. Isolated acinar cells attained ductal cell characteristics in culture in vitro. After treatment with human GLP-1, insulin gene expression increased in the acinar to ductal cell-line. Transplantation of the GLP-1 induced cell-line in STZ-induced diabetic rats lowered hyperglycemia.

The preferred target ways involve hormones. Tscheng et al 2009 reported an age dependent decline in β-cell proliferation. The CRFR1 and the GLP-1 transplantation experiments were accomplished on 8-12 weeks of age mice and 6-8 weeks of age rats, respectively. They did not took aging into account which should be done in a following study. On the other hand Chen et al 2009, did not find any difference in β-cell expression in young and aged donor islet groups. To find a cure for Diabetes type I, it is of importance to obtain functional β-cells, in that case I prefer the GLP-1 method of Lee et al, 2009. In diabetes type II β-cell failure and insulin resistance could be a result of

obesitas. In that case transplantation does not solve the problem immediately and the CRFR1 activation method of Huising et al, 2009 seems to me a better solution otherwise it is necessary to undergo twice a transplantation.

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