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The undoing and redoing of the diabetic β-cell:

β-cell de-differentiation in type 2 diabetes mellitus and re-differentiation and recovery with β-cell rest: Matching clinical observations with experimental insights

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Abstract

A hallmark of type 2 diabetes (T2DM) is the reduction in functional β-cell mass, which is considered at least in part to result from an imbalance of β-cell renewal and apoptosis, with the latter being accelerated during metabolic stress. More recent studies, however, suggest that the loss of functional β-cell mass is not as much due to β-cell death but rather to de-differentiation of β-cells when these cells are exposed to metabolic stressors, opening the possibility to re-differentiate and restore functional β-cell mass by therapeutic intervention. In parallel, clinical observations suggest that temporary intensive insulin therapy in early diagnosed humans with T2DM, so as to "rest" endogenous β-cells, allows these patients to regain adequate insulin secretion and to maintain euglycemia for prolonged periods free of continued pharmacotherapy. Whether observations made in (mostly rodent) models of diabetes mellitus and in clinical trials are revealing identical mechanisms and therapeutic opportunities remains a tantalizing possibility. Our intention is for this review to serve as an overview of the field and commentary of this particularly exciting field of research.

β-cell dysfunction in Type 2 diabetes mellitus

Type 2 Diabetes Mellitus (T2DM) is predominantly characterized by a combination of impaired response to insulin action in target organs and inadequately timed and blunted insulin secretion in response to secretory stimulus. T2DM develops by progressive deterioration of glucose tolerance over several years (1, 2). While insulin resistance, once established, appears to remain fairly constant (2), functional β-cell failure is detectable very early - even before diabetes diagnosis (2) and shows a relentless progression despite pharmacotherapy (3-7). In large clinical trials, treatment of insulin resistance shows success with respect to outcomes but does not address the continued deterioration in β-cell function (8). Conversely, pharmacologically stimulating β-cell function – while temporarily improving insulin release and glycemic control - fails to halt the progression of β-cell functional failure and - in the case of some secretagogues (5) - may even accelerate β-cell failure (3-5, 8).

Several mechanisms underlying a reduction in functional β-cell mass have been proposed. Analysis of pancreas specimens from cadaveric human donors show an approximately 50% reduction in β-cell mass in humans who had been diagnosed with T2DM as compared to adequately matched controls (age, sex, weight) (9, 10). A deficit in β-cells is attributed to an imbalance in the rate of β-cell self-renewal and proliferation and loss by apoptosis (9, 11, 12) with a modest uptick in apoptosis observable in β-cells of humans with T2DM (9). It should be noted that with apoptosis being a rapid cellular event, low numbers of observed apoptotic cells may not adequately reflect the true nature of progressive β-cell loss through ongoing apoptosis. Further, β-cell mass at onset of disease remains an elusive parameter in these studies, leaving the uncertainty that a lower β-cell mass may be preexistent to diabetes onset. This is important as β-cell mass appears to be determined during the first few years of life (13, 14) and well before most individuals are diagnosed with T2DM, and individuals endowed with a relatively low β-cell mass at the start of their lives may lack sufficient reserve to adapt to metabolic demands such as obesity related insulin resistance (context-dependent β-cell failure) and be at increased risk of developing T2DM (13). Overall, the rather small increase in β-cell apoptosis in pancreata of humans with T2DM versus controls indicates that β-cell functional impairment - rather than outright β-cell loss – predominantly contributes to insufficient insulin secretion and glycemic control in T2DM (15). Furthermore, in this context it is important to note that mistimed and insufficient GSIS is found in at risk humans even before the development of elevated fasting glucose levels (2).

A pancreatic β-cell challenged with glucose responds with a compensatory increase in insulin secretion, and –at least in rodents – with β-cell proliferation and adaptive increase in β-cell mass. However, prolonged increases in glucose levels will paradoxically result in impaired β-cell function (8). This phenomenon termed glucotoxicity has been widely studied and described (15-18). According to these theories, a prerequisite for glucotoxicity to occur, however, is already elevated glucose levels and thus already dysfunctional β-cells. Thus, glucotoxicity leading to functional compromise of β-cells, while a clinical reality, is a secondary phenomenon that occurs after an initial decline in β-cell function has already led to suboptimal glycemic control. A roadmap of molecular events resulting in β-cell functional decline remains to be clearly outlined.

In patients with T2DM, β-cell function and insulin secretion improves after reducing excessively elevated glucose levels (3, 8, 19-21) suggesting that in diabetes, the β-cell may be overburdened and dysfunctional and that providing β-cells the opportunity to “rest” by exogenous insulin treatment allows β-cells to functionally recover.

Extending this notion, Weng et al (22) have in a carefully designed randomized clinical trial made the remarkable observation that in newly diagnosed T2DM in humans, aggressive intensive insulin therapy initiated immediately at time of diagnosis for a duration of 2-3 weeks results in recovery of β-cell function such that insulin therapy can be stopped and patients continue to remain euglycemic – in some cases for several months to 2 years – only with dietary management for diabetes mellitus (a condition termed “glycemic remission”). A wider survey of multiple studies using short-term intensive insulin treatment has indicated that approximately 40% of patients remain euglycemic and fee of antidiabetic pharmacotherapy at 24 months after the intensive insulin regimen (23, 24).

β-cell dysfunction and de-differentiation following experimental reduction of β-cell mass in rodents

Common observations in a variety of mouse models of T2DM are the reduction in the proportion of insulin-expressing cells (i.e. β-cells) accompanied by an increase in glucagon-expressing (i.e. α-cells) as well as an increase in islet cells, which are have little or no detectable hormone immunoreactivity (25-27).

Impaired β-cell function and reduced glucose stimulated insulin secretion (GSIS) has been observed in rat models following neonatal streptozotocin treatment or partial pancreatectomy to reduce β-cell mass. The partial pancreatectomy rat model that exhibits defective insulin secretion reveals changes in β-cell gene expression indicative of β-cell de-differentiation (25). Notably, the pancreatectomy model shows reduced expression of genes that are normally highly expressed in β-cells including Insulin I, II and GLUT2, glucokinase, mitochondrial glycerol phosphate dehydrogenase and pyruvate carboxylase, MafA, Pdx-1, Nkx6.1, Hnf3β, Hnf4a, Hnf1a (28) while genes ordinarily silenced in mature β-cells appear to be re-expressed (e.g. lactate dehydrogenase A, glucose 6 phosphatase and hexokinase I) (28, 29). When partially pancreatectomized hyperglycemic rats were rendered normoglycemic by phlorizin treatment, these changes in islet gene expression were absent, linking hyperglycemia to the loss of β-cell differentiation markers and supporting the idea that “glucotoxicity” may aggravate β-cell dysfunction and contribute to the loss of a fully differentiated state in β-cells (25, 30).

More recently, several groups have extended observations of selective conditional ablation of specific transcription factors in β-cells of adult mice (31-38). The sequential expression of an array of transcription factors regulates pancreas development and endocrine lineage specification. In adult mice, β-cell-selective ablation of individual transcription factors, which in concert partake in a co-dependent network of β-cell specific gene regulation, results in similar phenotypes of glucose intolerance, β-cell functional deficit and loss of insulin content in β-cells and mis-expression of other endocrine hormones in such “de-differentiated” β-cells (31-38). These studies have been recently reviewed (39) and will not be elaborated here in detail. Suffice it to recognize that metabolic conditions as are found in T2DM cause reduced β-cell expression of transcription factors that are required to maintain β-cell morphologic and functional identity. It appears that metabolic disruption of the function of only one of the key transcription factors renders the β-cell vulnerable to lose its morphologic and functional identity.

β-cell dysfunction and loss of insulin expression in mouse models of insulin resistance

Alarcon and Boland et al. (40) have examined the leptin signaling defective db/db mouse model of hyperphagia and obesity-linked T2DM.d db/db mutation in the C57Bl/6J background strain (C57Bl/6J$^{db/db}$) exhibit β-cell mass expansion (proliferation) in response to and to compensate for insulin resistance, whereas the db/db mutation in the C57BLKS strain (C57BLKS$^{db/db}$) lacks β-cell expansion and exhibits impaired glucose stimulated

insulin secretion (GSIS) and consequently more pronounced hyperglycemia in the fasting state as well as after a glucose challenge as compared to the C57Bl/6J$^{db/db}$ mice. The C57Bl/6J$^{db/db}$ strain is considered to represent an early stage of human T2DM, whereas the C57BLKS$^{db/db}$ represents a later stage of T2DM. Islets from -KS$^{db/db}$ as compared to -6J $^{db/db}$ mice exhibited a larger proportion of MafA positive cells lacking detectable insulin. This observation was interpreted as heterogeneity of β-cells. At the ultrastructural level, both mouse strains exhibited degranulation of β-granules, increased number of immature β-granules and expansion of the rough endoplasmic reticulum and Golgi apparatus in β-cells, while other pancreatic endocrine cells did not show any differences as compared to controls islets. Again the -KS$^{db/db}$ mice exhibited more pronounced changes as compared to -6J$^{db/db}$ mice. These changes were also accompanied by diminished GSIS from freshly isolated islets during *in vitro* perifusion studies (40).

A key important observation in these studies was that both -KS$^{db/db}$ and -6J$^{db/db}$ islets, when removed from their *in vivo* milieu marked by insulin resistance and cultured *in vitro* for 24 hours at normoglycemia, the ultrastructural changes rapidly reversed to normal structures and the abnormal GSIS recovered to match what is observed in control islets (40). Based on these observations, the *in vivo* insulin resistant environment induces morphologic and functional changes in β-cells, which are rapidly reversible when β-cells are transferred to an environment that relieves the β-cell from the increased metabolic demand.

β-cell-selective FoxO1 ablation model confirms a link between impaired β-cell function and β-cell dedifferentiation

Starting from studies indicating that FoxO1 is instrumental in maintaining β-cell functional integrity, Talchai et al. (41) observed that under euglycemic conditions, FoxO1 localized in the cytoplasm of β-cells, whereas in a variety of mouse models of T2DM, as hyperglycemia progresses, FoxO1 immunoreactivity localized to the nucleus. Further, as hyperglycemia worsened, nuclear FoxO1 immunoreactivity diminished in parallel with a loss of insulin immunoreactivity – absent clear markers of apoptosis, a large proportion of β-cells were found to be missing or “empty” of insulin.

Talchai et al extended these observations by somatic FoxO1 ablation specifically in β-cells. At baseline conditions, these mice were indistinguishable from control counterparts. However, when exposed to physiologic metabolic stressors such as multiple parturition or aging in males, mice lacking FoxO1 –as compared to control counterparts - showed a 30% reduction in β-cell mass (insulin positive cells) and a 50% increase in α-cell mass (glucagon positive cells). Islets isolated from these mice showed reduced *in vitro* glucose stimulated insulin secretion and increased glucagon secretion (41).

These studies confirmed the β-cell autonomous nature of FoxO1 mutation leading to an increased susceptibility towards a loss of insulin-producing cells and an increase in glucagon-producing cells. Remarkably, lineage tracing studies in multiparous mice lacking FoxO1 in their β-cells, revealed that when exposed to these physiologic metabolic stress, β-cells lose their phenotypic characteristics (insulin, Pdx1, MafA), express increased levels of the pro-endocrine marker neurogenin 3 (Ngn3) and exhibit multipotency markers (Oct4,

Nanog, L-Myc). Moreover, these lineage-tracing studies indicated that in these mice, some α-cells had emerged from previously dedifferentiated β-cells. Returning back to models of T2DM such as the leptin signaling defective db/db mouse and the severely insulin resistant mouse lacking the insulin receptor in muscle, adipose and brain, Talchai et al also found evidence of insulin-negative endocrine cells within islets, that expressed Ngn3 as well as the above-mentioned multipotency markers (41).

Collectively, these observations indicated that in multiple models of T2DM and metabolic stressors to β-cells, a loss of β-cells results from dedifferentiation of mature β-cells into a multipotent state, from which some cells likely differentiate towards an α-cell phenotype.

Kim-Mueller et al. (42) advanced these studies further by identifying the stem cell marker aldehyde dehydrogenase 1a3 to be increased in β-cells of a variety of diabetic mouse models, including mice lacking FoxO1, 3a and 4 selectively in β-cells. While adlh1a3 did not appear to confer the de-differentiated state of diabetic β-cells, it served as a marker, which allowed to selectively enrich de-differentiated β-cells by fluorescent sorting for further transcriptomic analyses, which reflected changes in gene expression pattern broadly consistent with oxidative and mitochondrial stress, and suggest that increased oxidiative damage to β-cells as a consequence to increased metabolic demand may be a principal driver of β-cell de-differentiation (42).

De-differentiation of unexcitable β-cells and re-differentiation during euglycemia following exogenous insulin therapy

Monogenic forms of neonatal diabetes mellitus due to mutations in the Kir6.2 subunit of the K_{ATP} channel as well as the T2DM associated polymorphism (e.g. E23K) in Kir6.2 show ATP insensitivity of the K_{ATP} channel, resulting in inexcitable β-cells, that lack typical membrane potential fluctuations required for physiologic stimulus secretion coupling (43). Remedi, Nichols et al. have examined mice, which allow conditional expression of a dominant ATP-insensitive mutant Kir6.2 in β-cells exhibit diabetes mellitus and lack insulin immunoreactivity in their islets (43). Based on lineage tracing studies in these mice, the underlying mechanism for absent insulin-containing islets was found not to be due to apoptosis, but due to loss of expression of mature β-cell markers (insulin, Pdx-1, MafA) in exchange for detectable expression of multipotency markers (Ngn3, Nanog, L-Myc) - consistent with β-cell dedifferentiation.

More strikingly, when these mice were treated with exogenous insulin to achieve euglycmia, their β-cells regained insulin, Pdx1, Nkx6.1 MafA expression while losing the multipotency markers (43). Lineage tracing studies confirmed that these newly insulin-expressing cells were identical to those that had de-differentiated at the start of the studies. These studies strongly suggest, that at least in model of Kir6.2 mutant β-cells, β-cells dedifferentiate, and when relieved from the metabolic stimulus (hyperglycemia), are able to regain their differentiated state – supporting the idea that “resting” β-cells may permit an over-extended and thus de-differentiated β-cell to regain its original mature phenotypic characteristics (43).

Using a different but similar mouse model of conditional β-cell specific expression of a dominant ATP-insensitive mutant Kir6.2, Brereton et al. (44) also observed a dramatic reduction of insulin-positive cells and increased glucagon-positive cells in islets with little of any change in cell turnover. In these studies, lineage tracing studies also suggested that some β-cells express glucagon, albeit retaining some β-cell characteristics – suggesting a loss of β-cell differentiation without a complete trans-differentiation into other cell phenotypes. Consistent with the observations outlined above, the phenotypic changes in β-cells in mice conditionally expressing the mutant Kir6.2 transgene could be prevented by insulin therapy (44) and reversed by treatment with sulphonylurea, which sufficiently restores Kir6.2 function despite misexpression of the ATP-insensitive mutant (44, 45).

Brereton et al have extended these initial findings and recently shown that β-cells from mice expressing dominant ATP-insensitive mutant Kir6.2 exhibit a change in metabolic gene expression as well as glucose handling. Glucose is diverted towards glycogen production and accumulation in β-cells (46). In contrast to their original observations, the investigators also report an increase in β-cell apoptosis in their extended studies. Remarkably, sulphonylurea treatment, which normalizes glycemia, is accompanied by a reversal of the observed glycogen accumulation in β-cells (46). The investigators also confirmed that cadaveric islet β-cells from humans diagnosed with T2DM also showed accumulation of glycogen.

Importantly, the investigators established a direct link between these changes in β-cells to hyperglycemia: 1) mice with inducible expression of glucokinase transgene carrying an activating mutation show increased glycolytic flux, which is also accompanied by glycogen accumulation in β-cells; and 2) insulinoma cells cultured *in vitro* in hyperglycemic conditions showed evidence of glycogen accumulation and increased activated caspase-3 levels and apoptosis. Pharmacologic reduction of glycogen accumulation by metformin treatment reduced glycogen content and apoptosis markers (46).

It should be noted that while these mouse models with a dominant mutation that renders Kir6.2 insensitive to ATP may represent an extreme model of neonatal diabetes, Kir6.2 mutations have in genome-wide association studies been linked to human T2DM, supporting the idea that the above observations made in Kir6.2 mutant mice may by extended to human T2DM.

In these mouse models it appears that early intervention to achieve euglycemia achieves re-differentiation and regain of function of de-differentiated β-cells, but that delayed attempts to achieve euglycemia may be met with a diminished number of β-cells resulting from progressive β-cell loss due to (glycogen-induced?) apoptosis (46).

β-cell de-differentiation in human islets: pro and contra

Chinti et al examined pancreas specimens from diabetic and non-diabetic organ donors. By defining endocrine lineage cells within islets as synaptophysin-positive (Syn+), these investigators observed in specimens from diabetics approximately 30% of endocrine islet cells to be hormone negative, while positive for aldehyde dehydrogenase 1A3, an endocrine

progenitor marker. Overall samples from diabetics had an approximately a 30% reduction in insulin-positive (β-) cells and a 60% increase in glucagon-positive (α-) cells. Furthermore, insulin secretion in response to glucose was markedly reduced in islets of diabetic donors as compared to controls (47).

Differentiation markers of mature β-cells FOXO1 declined in β-cells of type 2 diabetic donors. NKX6.1 and MAFA were found in nuclei of control β-cells, but distributed throughout the cell in β-cells from diabetic donors. β-cells with (aberrant) cytoplasmic NKX6.1 also showed increased in ALDH1A3. Further, approximately 40% of ALDH13 positive cells were hormone negative (47).

In summary, these observations reveal in islets of diabetic donors an increase hormone-negative cells that also lack FOXO1 expression and show cytoplasmic NKX6.1 and that express the endocrine progenitor marker ALDH1A3. Islets from such donors release less insulin upon glucose stimulus. Collectively the observations suggest the possibility of β-cell de-differentiation in humans with diabetes mellitus (47).

Dai et al transplanted human islets into immuno-incompetent mice before inducing an *in vivo* hyperglycemic state or insulin resistance (48). Transplanted human islets – in contrast to transplanted mouse islets – showed impaired insulin secretion when faced with hyperglycemia or insulin resistance. In this model system, human islets exposed to chronic insulin resistance showed a decrease antioxidant enzyme expression, an increase in superoxide and amyloid formation. Neither increased proliferation nor apoptosis were detectable in such transplanted human islets. NKX6.1 and MAFB were decreased during chronic insulin resistance, while only MAFB was decreased during chronic hyperglycemia. In these studies, FOXO1 expression, at least at the mRNA level was not changed in human islets. These studies did not specifically examine the presence of hormone-negative endocrine cells in transplanted human islets, albeit a reduction in β-cell NKX6.1 and MAFB suggest the possibility of early stages of dedifferentiation within the relatively short observation period of these studies (48).

The group of S. Efrat has established lineage tracing methods of human islets kept *in vitro* culture and observed proliferation and epithelium to mesenchymal transition (EMT) of endocrine cells as a mechanism of de-differentiation (49-51). Importantly, in such de-differentiated islet cells, the chromatin structure of key β-cell genes remains similar to those observed in mature normal human islets (52). Examination of key signaling pathways identified Notch, WNT and TGFβ pathways to induce both β-cell proliferation as well as de-differentiation (53-55). Importantly, inhibition of the key mediators of Notch, WNT and TGFβ induced re-differentiation of these expanded cells into insulin-producing cells expressing characteristic β-cell markers (53-55).

Extending this line of studies, Blum et al recently showed that *in vitro* treatment of islets extracted from mouse models of T2DM with a small molecule inhibitor of the TGFβ pathway can reverse, at least in part, the dedifferentiation of β-cells.

In contrast to the above observations, not all studies on human pancreas specimens support the presence of de-differentiated β-cells. Md Moin et al. (56) surveyed pancreas samples

from lean T2 diabetic and lean non-diabetic humans, and found only approximately 2% of β-cells in T2DM shoed evidence of some loss of mature β-cell markers. Based on these observations these authors suggest that de-differentiation may play a minor role in the pathogenesis of β-cell failure in T2DM (56). Jurgens et al. and Westermark et al have observed in human T2DM an increase in β-cell apoptosis, which was associated with an increase in islet amyloid deposits (57, 58). However, it remains unclear whether absence of insulin resistance, which may not be as pronounced in lean T2DM humans, may explain the lack of significant evidence for β-cell de-differentiation. Furthermore, difficulties in detecting β-cell de-differentiation in humans specimens may in part reflect the effects of anti-diabetic treatment (and thus improved glycemia), which the cadaveric donors likely received. As such, a natural history of the human endocrine pancreas during T2DM remains unknown.

Recent observations of single cell transcriptomic and proteomic analysis of human islets from non-diabetic and diabetic donors do not specifically detect dedifferentiated β-cells in diabetes mellitus (59-61). However, the methodology of these studies utilizes the presence of cardinal hormones to identify the different endocrine cell-types within islets, thereby excluding any hormone-negative cells in their initial reporting. A detailed analysis of the primary data interrogating the frequency of hormone-negative endocrine cells will hopefully be informative in the near future.

While some studies on human islets support the notion β-cell dedifferentiation during diabetes as an underlying mechanism of β-cell failure, studies in non-human primates have provided mixed results. Studies by McCulloch et al in baboons (62), treated with low dose streptozotocin to reduce β-cell mass by 40-50%, fasting hyperglycemia and impaired insulin secretion was detectable, as found in humans with T2DM. However, even after 5-46 months of observation no “empty” β-cells were reported by the investigators, arguing against the development of β-cell dedifferentiation in these animals (62). Conversely, Guardado-Mendoza et al. (63) observed that in baboons the extent of islet amyloid deposits was strongly associated with β-cell apoptosis, extent of α-cell proliferation and –mass, circulating glucagon levels and an index for insulin resistance, and inversely correlated with β-cell mass.

Fiori et al placed rhesus monkeys on a high fat/high sugar diet for 24 months, which induced increased GSIS and insulin resistance as compared to standard diet fed counterparts (64). Although dysglycemia did not occur during the observation period, the investigators noted a reduction in insulin-positive β-cells and an increase in glucagon-positive α-cells. The investigators did not find any evidence of β-cell apoptosis or α-cell proliferation. However, increased α-cell mass was not reflected in an increase in circulating glucagon levels or any correlation between glucagon levels and hyperglycemia. High fat/high sugar diet fed monkeys showed a reduction in mature β-cell transcription factors FOXO1, NKX6.1, NKX6.2 and PDX1 (64). These observations suggested in the diet-challenged monkeys β-cell de-differentiation with the possibility of insulin-positive β-cells converting to glucagon-positive cells. Remarkably, resveratrol supplementation prevented the changes in α- to β-cell ratio as well as in the reduction in β-cell defining transcription factor levels (64).

Conclusion and Outstanding questions

Based on the above summaries, β-cell de-differentiation is a phenomenon that is observable in a broad variety of experimental mouse models of diabetes mellitus, and appears to provide a pathogenic mechanism of β-cell failure. This failure may be reversed by alleviating the secretory drive on β-cells (i.e. achieving euglycemia by exogenous insulin therapy) and allowing β-cells to "rest" and re-differentiate at least transiently to a functional state. One study of diet induced insulin resistance in macaques suggests that β-cell dedifferentiation may also occur during diabetes pathogenesis in higher vertebrates. However, a study in baboons with reduction in β-cell mass (but without increasing insulin resistance) did not reveal any evidence of β-cell de-differentiation.

Clinical observations using early and temporary intensive insulin therapy in T2DM may be consistent with a pathogenic mechanism of β-cell de-differentiation before therapy onset and re-differentiation after a resting period during intensive insulin therapy, albeit alternate mechanisms may be at play. Additional mechanisms involving amyloid deposits and β-cell apoptosis are certainly not ruled out at least as parallel manifestations in the diabetic islet. Studies on pancreas tissue from humans with T2DM are too few at this time to definitely confirm or refute the possibility of the existence of de-differentiated β-cells in human T2DM. In addition to the need to increase information on whether human β-cells behave similarly to those in mouse models, several outstanding questions remain in this exciting time as diabetes pathogenesis and its treatment are being reevaluated:

1. During diabetes pathogenesis, which metabolic pathways in β-cells lead to loss of FoxO1 and β-cell de-differentiation?
2. Are there subsets of β-cells, which are particularly vulnerable to apoptosis versus de-differentiation when exposed to physiologic or pathologic metabolic stressors?
3. Are there non-β-cell (systemic, environmental) signals that increase β-cell susceptibility to de-differentiation when exposed to physiologic metabolic stressors?
4. Do islet amylin deposits, which thus far have been linked to β-cell apoptosis, participate in the overall phenomenon of β-cell de-differentiation?
5. Do recent gene and protein expression studies of human islet cells indicate the existence of de-differentiated β-cells in islets of humans with diabetes mellitus?
6. Do variations in certain diabetes susceptibility genes increase the propensity of β-cell dedifferentiation?
7. Is intensive insulin therapy in early T2DM that leads to a "cured" episode paralleled with a re-differentiation and functional mobilization of de-differentiated β-cells?
8. Does bariatric surgery contribute towards returning de-differentiated β-cells to a functional state?

9. Does β-cell de-differentiation occur in T1DM and is a de-differentiated β-cell protected or invisible to immune assault?
10. Are there clinical biomarkers that will identify pre-diabetics who will benefit from pre-emptive therapy to prevent β-cell de-differentiation?
11. Are there clinical biomarkers that will identify diabetics with de-differentiated β-cells that could be re-differentiated?
12. How will attempts to stimulate β-cell proliferation in humans interplay with β-cell de-differentiation and re-differentiation?

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