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Betatrophin

The long awaited circulating factor from the liver promoting β-cell replication?

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Regenerative therapy in diabetes with the capacity to reconstitute a functional β-cell mass sufficient for glycemic control holds the promise to effectively prevent the development of devastating late complications due to the unique ability of the β-cell to sense and regulate blood-glucose levels. An ability that cannot be mimicked by insulin replacement therapy or any other means of current treatment regiments for very large patient populations. Recently, Douglas A. Melton's group from Harvard University reported the identification of a circulating protein secreted from the liver under insulin resistant states which is sufficient to dramatically and specifically increase the replication rate of β-cells in the mouse resulting in an increased functional β-cell mass over time. They re-named the factor betatrophin and described a number of exciting features of this molecule which suggested that it could be a potential candidate for development as a regenerative medicine in diabetes.[1] The official name of the gene encoding mouse betatrophin is Gm6484, but it has been annotated a number of times under different names: EG624219[2,3], RIFL[4], Lipasin[5] and ANGPTL8.[6] The official human gene name is C19orf80, but it has also been annotated as TD26[7], LOC55908[8], as well as RIFL, Lipasin, ANGPTL8 and betatrophin.

Diabetes mellitus is a metabolic syndrome characterized by hyperglycemia caused by an insufficient functional β-cell mass resulting in inadequate levels of the hormone insulin. Current treatment aims to restore normoglycemia by insulin replacement, by enhancing β-cell function, by sensitizing tissues to insulin or by modulation of the glucose output from the liver. However, sufficient control over glycemia is difficult to achieve and the long-term consequence of hyperglycemia are devastating so called late complications including blindness, renal failure, neuropathy, amputations and increased risk of heart disease and stroke.

The 6th edition of The International Diabetes Federation's diabetes atlas states that 382 million people are living with diabetes today and this is expected to increase to 592 million people by 2035. Diabetes is causing 5.1 million deaths and costs USD 548 billion in healthcare spending globally in 2013 (http://www.idf.org/diabetes-atlas). It should thus be clear that diabetes poses a phenomenal burden on patients and on the health care budgets where the great majority of expenses are used to treat the late complications. Therapies preventing the development of late complications are therefore critically important to develop. It is hoped that regenerative therapies aimed at the reconstitution of the functional β-cell mass may be a novel avenue by which patients will regain much better control of glycemia, one of the most critical parameters in the prevention of late complications.

The most common form of diabetes, type 2 diabetes mellitus (T2DM), is characterized by peripheral insulin resistance and a failure of the endocrine pancreas to increase the functional β-cell mass to adequate levels for glycemic control. In rodent models of insulin resistance and type T2DM, it is well established that the β-cells compensate for increased demand

by increasing their numbers, and evidence for a circulating factor promoting β-cell replication in insulin resistant, normoglycemic states was provided more than 10 years ago by the group of Ronald Kahn.[9] The evidence was based on the observation that islets transplanted into the kidney capsule of insulin resistant (IRS1/INSR double heterozygous mice) or leptin signaling deficient obese (*ob/ob mice*) mice increased their proliferation rate markedly, resulting in increased islet mass in the grafts over the course of 8 weeks relative to islets grafted in the kidney of wild type mice. This observation—combined with studies from the same group demonstrating that a liver specific INSR knockout displays a large increase in the pancreatic β-cell mass[10] whereas muscle specific INSR knockout has normal glucose metabolism[11]—led the authors to suggest the liver as a possible source of the circulating factor.[9]

Recently, a number of papers have further advanced the thought that the liver and the endocrine pancreas is tightly connected through complex inter organ signaling mechanisms: in an elegant study by Junta Imai et al. it was shown that ERK signaling in the liver transmits a signal to the central nervous system which is relayed to the pancreas through the efferent vagus nerve resulting in β-cell proliferation.[12]

The observation that a liver-specific INSR knockout (the LIRKO mouse) results in increased β-cell mass was further studied by the group of Rohit N. Kulkarni: they used a parabiotic model where the circulation of mice is coupled by surgery thus allowing the study of the effect of circulating factors between mice of different genotypes. It was demonstrated that β-cells in wild-type mice joined to LIRKO mice increase their proliferation rate by approximately 3-fold, suggesting the existence of a circulating factor promoting this effect.[13] It was further shown that wild-type islets transplanted into the kidney capsule of LIRKO mice also increased their proliferation rate demonstrating that the effect on proliferation is uncoupled from the vagus innervation of the pancreas in this model.[13] Importantly, it was also shown that serum from LIRKO mice was able to induce specific β-cell replication in wild-type mice by interperitoneal serum injection and that LIRKO serum could increase mouse and human β-cell replication rates in vitro. Lastly it was shown that cell culture media conditioned with liver explants from LIRKO mice could increase the replication of mouse and human β-cells in vitro and it was suggested that hepatocytes were the origin of the signal based on media conditioned with primary LIRKO hepatocytes.[13] Together, these data provide compelling evidence for a circulating liver-derived factor in insulin resistant mice that is able to induce proliferation of mouse and human β-cells.

Interestingly, it was recently reported that a liver specific glucagon receptor knockout displays increased α-cell proliferation and hyperplasia similar to what has been previously reported for the global glucagon receptor knockout,[14] and that wild-type islets transplanted under the kidney capsule of glucagon receptor deficient mice or the liver specific knockout likewise increase their α-cell proliferation.[14,15] These data suggest the existence of a circulating factor induced in glucagon resistant states which can induce α-cell proliferation independent of a vagus nerve signal to the pancreas.

Together, these findings provide very strong evidence that the liver and the endocrine pancreas are coupled and that liver derived, circulating factors influences the pancreatic endocrine mass in the mouse. However, none of these studies have identified a candidate molecule exerting the effect on the endocrine pancreas.

Some evidence exists that hepatocyte growth factor (HGF) is a secreted liver factor and that serum HGF levels are increased under insulin resistant conditions. Interestingly, recombinant HGF is able to stimulate β-cell proliferation and mass expansion in mice whereas small molecule pharmacological blockade of HGF signaling prevents the effect on the β-cell.[16] However, although these findings are interesting, the receptor for HGF is the protooncogene C-Met, which may pose a problem from a drugability safety point of view.

Recently, Douglas A. Melton's group at Harvard University identified a novel gene which they named betatrophin which could be the missing factor driving β-cell replication in insulin resistant states in the mouse.[1] They developed an elegant pharmacological model of insulin resistance utilizing a peptide insulin receptor antagonist, S961, invented by Lauge Schäffer at Novo Nordisk A/S[17] which induces severe insulin resistance when infused in mice. They discovered that S961 continuously dosed by miniosmotic pumps induces a dramatic, immediate and dose dependent increase in the β-cell replication rate even at low doses not affecting blood glucose levels. At the highest dose, S961 induced a spectacular 12-fold increase in the proliferation rate and it was shown that the increased proliferation was associated with regulation of relevant cell cycle markers by quantitative RT-PCR on isolated islets. The β-cell area fraction increased 3-fold after 1 weekas a result of the increased proliferation and, importantly, the effect was shown to be specific as other tissues investigated including other pancreatic cell types were unaffected.

S961 did not promote β-cell replication in vitro, and it was thus hypothesized that it acts indirectly on the β-cell. The authors therefore conducted gene expression profiling on a number of tissues involved in metabolic regulation and identified betatrophin specifically upregulated in the liver and white adipose tissue after S961 infusion. Betatrophin contains a predicted N-terminal secretion signal and, indeed, it was demonstrated that cells transfected with expression plasmids encoding betatrophin secreted the protein to the culture media. Furthermore, it was shown that ectopic expression of betatrophin in the mouse liver led to detectable levels of serum betatrophin, and endogenous betatrophin could be detected in human serum samples confirming that betatrophin is a naturally circulating molecule. It was further shown that betatrophin was upregulated in leptin signaling deficient mice and in pregnancy, models of insulin resistance where β-cell replication is increased. In contrast, partial ablation of β-cells which also results in a β-cell proliferative response showed unchanged levels of betatrophin expression suggesting that betatrophin may be driving a specific physiological response but is not involved in regeneration following acute

β-cell injury. However, it is noteworthy that betatrophin serum levels are doubled in patients with long-standing type 1 diabetes.[18]

To investigate the effect of betatrophin in vivo, expression in the liver was achieved by hydrodynamic tail injection of expression plasmids encoding mouse and human betatrophin. This resulted in a specific 17-fold increase in the β-cell replication rate relative to control injected animals followed by a 3-fold increase in the β-cell mass and a 2-fold increase in the pancreatic insulin content after 8 days. The betatrophin injected mice displayed improved glucose tolerance and isolated islets after betatrophin plasmid injection-had normal glucose stimulated insulin secretion suggesting that the β-cells are normally functional.

Further data has been published by Melton and Yi in a patent application (WO 2012/1790997 A1) where it is reported that multiple daily doses of subcutaneous administered insulin receptor antagonist is sufficient to elicit a β-cell replication response in a dose-dependent manner. This response is also reported for diet-induced obese mice where insulin receptor antagonist treatment results in increased β-cell replication and mass. Interestingly, it is suggested that fragments of betatrophin can induce β-cell replication in mice following hydrodynamic delivery to the liver. However, the response appears to be in the order of 2-fold, much lower than that reported for full-length betatrophin in the study by Yi et al. The reported fragments appear to be non-overlapping and it would be interesting to investigate whether the non-overlapping fragments in combination has a synergistic effect on β-cell replication sufficiently to drive a response similar to that of native betatrophin

Betatrophin annotated as EG624219 was first knocked out in the mouse by homolog recombination in 2010 in a large phenotype screen of the effect of loss of secreted and transmembrane proteins.[3] The deposited phenotypical data (http://www.kompphenotype.org) was subsequently used to identify the gene (named RIFL) with some similarity to angiopoietin-like 3 (ANGPTL3) and with reported effects on lipid storage.[4] At the same time, it was reported that the same gene, named lipasin, was nutritionally regulated in white adipose tissue and that adenoviral gene transfer of lipasin resulted in increased serum triglycerides.[5] This was the opposite effect of that observed in the knockout where serum triglycerides drops (http://www.kompphenotype.org)[4] suggesting that the action of lipasin is required and is sufficient to regulate serum triglyceride levels. It was subsequently demonstrated that adenoviral gene transfer of ANGPTL8/betatrophin to the liver of mice resulted in increased serum triglycerides in an ANGPTL3-dependent manner. This was shown in parallel studies in wild-type and ANGPTL3-deficient mice. It was further demonstrated that ANGPTL8/betatrophin directly interacts with ANGPTL3 in the mouse facilitating the cleavage of ANGPTL3 *in* vivo and in vitro to generate an active N-terminal fragment which mediates the effect on the serum triglyceride levels.[6]

The study by Peng Yi et al.[1] did not investigate the effect of betatrophin on triglycerides, the consequence of loss-of-function of betatrophin on β-cell mass and glucose homeostasis, or the dependence of the effect on ANGPTL3. Furthermore, it was not reported whether recombinant betatrophin or serum from betatrophin overexpressing mice have effects on the proliferation rate of β-cells in vitro or in vivo. The importance of the pancreatic context was also not clarified, e.g., in kidney graft experiments. So while betatrophin certainly has very promising features as a potential regenerative drug candidate, further studies are required to elucidate the biology.

Some interesting follow-up studies have been published very recently addressing some of these points: Yan Wang et al. recently reported that mice deficient in betatrophin (ANGPTL8) display normal glucose metabolism suggesting that betatrophin is not required to maintain a functional β-cell mass in mice with normal insulin sensitivity. It remains to be investigated if betatrophin is required for the expansion of the β-cell mass in insulin resistant states. Furthermore, Yang Jiao et al. have just published a study with kidney transplanted mouse and human islets in the same S961 induced insulin resistance model used by Peng Yi et al.[1,19] They confirm, albeit in a NOD-Scid immunodeficient background, that S961 induced insulin resistance results in a marked increase in hepatic betatrophin expression and an increase in the proliferation of the pancreatic β-cells. Furthermore, they show that mouse islets transplanted under the kidney capsule in S961 mice also dramatically increase their proliferation rate demonstrating independence of the pancreatic context. However, when human islets are transplanted in the same mouse no induced β-cell proliferation is observed regardless of donor age ranging from 4 to 53years. This is an important finding challenging the idea of betatrophin as a human therapeutic protein and further studies are warranted. It should be noted that the mechanism of the induced β-cell proliferation remains unknown and it cannot be excluded, for example, that human betatrophin in humans may exert a positive effect although it is known that human betatrophin has similar effects on triglyceride levels in the mouse as mouse betatrophin suggesting evolutionary conservation of action. The study was restricted to 1 week and it was not investigated if human islets may show a different temporal response in the model compared with mouse islets. Furthemore, it is well documented that isolated human islets generally are very resistant to induced proliferation and the lack of a positive control in the study by Yang Jiao does not exclude the possibility that the specific protocol used for islet isolation and grafting produces an artifactual betatrophin resistance. Recently, a longitudinal study of the adaptation of human islets to a high-fat diet using kidney grafting in another immunodeficient background (nondiabetic $Rag2^{-/-}$) was reported.[20] In this model, healthy human islets responded to a high-fat diet by a tendency to an increased proliferation rate and a doubling of the β-cell volume after 12 wk. However, both a 12 wk endpoint analysis of proliferation by a BrdU pulse or Ki67 and 7 d cumulative BrdU incorporation studies failed to detect a statistically significant increase in the β-cell proliferation index and, generally, the proliferation index was found to be exceedingly low, suggesting that the mechanism underlying the observed increased β-cell mass of the

graft may be more likely due to β-cell survival or perhaps neogenesis. Nevertheless, it would be interesting to investigate the effect of S961 and betatrophin in this model.

Finally, since the liver/islet α- and β-cell interplay is uniquely involved in sensing and regulating levels of circulating nutrients an alternative to the mode of action of betatrophin could be indirect and related to S961/betatrophin-induced changes in circulating nutrients or metabolites. Although S961/betatrophin-effects are seen even before insulin-resistance leads to increased blood-glucose levels—this latter phenomenon could be speculated to be explained by a relative increased glucose-uptake in less resistant organs (e.g the β-cell). Dor et al. recently demonstrated that even subtle increases in glycolytic flux through the β-cells are coupled to an increased β-cell replication in the mouse. Here, the observed increase in rodent β-cell replication and regeneration following partial β-cell mass ablation is linked to an increased glucokinase activity, glucolytic-flux and active secretion.[21] Earlier studies by the Kulharni lab also links insulin-receptor signaling in the β-cell as essential for compensatory β-cell mass upregulation, e.g., in the LIRKO mouse, where β cell mass expansion is completely inhibited in the cross between LIRKO and bIRKO mice.[22] Thus β-cell-IR signaling activated by local insulin (or possibly IGF2[23]) coupled to increased β-cell glycolytic flux could possibly constitute a permissive signal to allow additional factors to induce proliferation. It is therefore tempting to speculate that the betatropin mediated effect is indirect—and possibly linked to the increased lipid/triglyceride circulation or other metabolites sensed by the β-cell. It would be informative to know the effect on β-cell mass of S961-treatment in the βIRKO mouse.

In summary, a number of important studies are required in order to further assess the potential of betatrophin for regenerative therapy in diabetes. However, it is encouraging that a single factor in circulation has been identified with such a potent effect on the mouse β-cell mass. It will be very interesting indeed how well these early studies may translate into other diabetes models and to human biology and it is also likely that other factors from the liver and elsewhere can be isolated which hopefully meet important drugability criteria in terms of efficacy and safety.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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