Commentary: A new role for Grb10 signaling in the pancreas

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Words: 1165

Figures: 0

Tables: 1

Following surgical removal of the canine pancreas, Joseph von Mering and Oskar Minkowski were the first to link the pancreas with diabetes (1). This observation led to the successful treatment of diabetic patients some 30 years later using purified pancreatic extracts, then to the identification and application of subcutaneous insulin delivery, as well as more recent islet transplantation therapies (2). Despite these life-saving advances diabetes is still a global healthcare problem. However, there remains much to learn about the mechanisms involved in glucose homeostasis. Insulin receptor signaling is the central molecular pathway and remains the subject of intense research activity. Mouse knockout models have played a pivotal role in bridging the gap between our understanding of the relevant molecular mechanisms and glucose homeostasis in the whole animal (3-5).

In this issue, Zhang et al (6) report on the pancreas-specific disruption of the *Grb10* signaling adaptor protein gene. This builds on a long tradition of mouse knockout experiments targeting components of the insulin signaling pathway. Seminally, germline or "global" disruption of the insulin receptor gene (*Insr*) revealed a subtle effect of Insr signaling on fetal growth (7) as well as perinatal lethality due to acute diabetic ketoacidosis (8; 9). The physiological effects of gene disruption can be difficult to interpret when they involve such a severe phenotype and/or when signaling is altered within multiple insulin-sensitive tissues. To circumvent these problems "conditional" or tissue-specific mouse knockouts can be generated using Cre-lox technology, an approach used to great effect within the glucose homeostasis field. In a series of elegant experiments the effects of Insr ablation have been analysed separately in several tissues, including, skeletal muscle, white adipose, liver, brain and pancreas (reviewed in(3-5)).

This work revealed roles for Insr signaling in non-canonical insulin-responsive tissues, such as liver, brain and endocrine pancreas that were more prominent than had previously been appreciated (4). A number of genes acting downstream of the Insr have also been the subject of mouse knockout experiments. Broadly speaking, knockouts resulting in impaired signaling, such as those for Irs-1, Irs-2 and Akt2, were associated with insulin resistance or diabetes, whereas knockouts that disrupted an inhibitor of insulin signaling (e.g. PTP1B) led to increased insulin sensitivity. These experiments also revealed myriad subtleties, for instance, due to redundancies between related factors and to the relative importance of individual factors in specific tissues (3-5).

The Grb10 signaling adaptor functions to inhibit signaling through receptor tyrosine kinases including the Insr and insulin-like growth factor type 1 receptor (Igf1r) (10). *Grb10* germline knockout mice have elevated Insr/Igf1r downstream signaling, at least in skeletal muscle and white adipose tissue (WAT), without an increase in circulating insulin levels, and have an enhanced ability to clear a glucose load from the circulation (11-13) (Table 1). Expression of Grb10 is widespread during fetal development but more restricted post-natally, including in both canonical (muscle and WAT) and non-canonical (pancreas and brain) insulin-responsive tissues (11; 12). Grb10 is known to inhibit both fetal and placental growth, such that *Grb10* knockout mice are at birth approximately 30% heavier than their wild type sibs (14). In adulthood Grb10 knockout mice have increased muscle mass and reduced adipose compared to wild types (11; 12; 15). This "anti-diabetic" phenotype, of lean body proportions with an enhanced ability to clear blood glucose, is very interesting but needs to be better understood.

In the paper by Zhang et al (6), the first report of a conditional *Grb10* knockout, *Grb10* was abolished in pancreas by crossing a "floxed" Grb10 allele with transgenic mice that express Cre-recombinase under the control of a pancreas-specific *Pdx1* gene promoter. Global *Grb10* knockout resulted in significantly increased growth of many tissues, including pancreas (11-15). Zhang *et al*. (6) confirm that *Grb10* is expressed in pancreatic islets of adult mice and show that pancreas-specific *Grb10* knockout resulted in a substantial increase in pancreas tissue weight. This observation is consistent with the established role for Grb10 as an inhibitor of tissue growth (14; 16) and indicates that Grb10 participates in a local growth control mechanism, consistent with its intracellular signaling function. More work will be required to uncover the relative importance of Grb10 in regulating pancreas growth during development, versus tissue maintenance in adulthood. Under control of the *Pdx1* promoter, Cre recombinase is expressed from the earliest stages of pancreas development (17), resulting in deletion of the *Grb10* floxed allele in both exocrine and endocrine tissue. Expression of Grb10 is not readily detected in exocrine pancreas (6; 11). However, in a separate study, knock-down of Grb10 levels in adult mouse pancreas using viral delivery of a short hairpin RNA targeting *Grb10* resulted in increased apoptosis of both endocrine and exocrine tissue (18), supporting a role for Grb10 in promoting cell survival in both compartments. Disrepancies between the two studies (6; 18) will need to be resolved, but could be due to differences in the techniques used and the timing of *Grb10* knockout or knock-down.

Importantly, Zhang et al. (6) show that loss of pancreatic *Grb10* resulted in increased beta-cell mass, with an associated increase in the number of insulin secretory granules, insulin secretion and improved glucose tolerance, but without a significant change in insulin tolerance. These favourable changes in pancreatic beta-cell physiology were replicated in mice challenged with a high fat diet and, moreover, pancreas-specific *Grb10* knockout ameliorated the effects of streptozotocin-induced diabetes. This fuels the suggestion that inhibition of Grb10 might offer a means of increasing beta-cell mass in type 1 and type 2 diabetes. In this context, it is interesting to compare the outcomes of pancreas-specific with global *Grb10* knockouts (Table 1). Mice lacking Grb10 in all tissues had increased lean tissue mass, with no significant change in circulating insulin levels, despite having an enlarged pancreas, and exhibited improvements in both glucose tolerance and insulin sensitivity (11; 12). Collectively, the global and tissue-specific knockout experiments indicate a role for Grb10 in coordinating endocrine pancreas function with that of the canonical insulin-sensitive tissues, suggesting there may be additive therapeutic benefits from targeting Grb10 function at both sites. However, if therapeutic molecules are to be developed then a greater understanding is needed of the signaling pathways that Grb10 acts on *in vivo*. Zhang et al. (6) provide evidence of increased Insr/Igf1r signaling in islets lacking Grb10 expression but also point out that this is not necessarily the cause of the increased beta-cell mass. The recently established link between Grb10 and mTOR signaling is a promising advance (19; 20) and data reported by Zhang et al. (6) showing increased mTOR signaling in *Grb10* knockout islets is an early indication that the link has physiological significance.

The conditional *Grb10* knockout mice have illuminated the pancreatic role of Grb10 (6) and will undoubtedly continue to aid in unraveling the intricacies of Grb10 signaling function, allowing key questions to be addressed, including: What are the tissue-specific roles of Grb10 in the regulation of Insr signaling? What are the relative contributions of altered signaling versus body proportions to the physiological changes seen in *Grb10* knockout mice?

Author Contributions

AW conceived and wrote the manuscript.

Acknowledgements

Thanks to Professors Geoff Holman and David Tosh of the University of Bath for helpful comments on the manuscript. Andrew Ward is the sole author and guarantor of this manuscript.

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	Global *Grb10* knockout	**Pancreas-specific *Grb10* knockout**
Body weight	↑	→
Food intake	→	→
Adipose	↓	→
Skeletal muscle	↑	n.d.
Pancreas weight	↑	↑
Beta-cell mass	n.d.	↑
Insulin levels	→	↑
Insulin sensitivity	↑	n.d.
Glucose clearance	↑	↑
Insr/Igf1r signaling in skeletal muscle and WAT	↑	n.d.
Insr/Igf1r signaling in islets	n.d.	↑
References	12, 13, 15	6

Table 1. Phenotypic comparison of adult mice with either global or pancreas-specific *Grb10* knockout alleles illustrates differences in body composition, insulin signaling and glucose-regulated metabolism, relative to wild type controls. Pancreas weight is increased in both models. Global knockouts have insulin levels appropriate for their body weight, and enhanced glucose clearance is associated with increased insulin sensitivity and enhanced Insr/Igf1r signaling in peripheral tissues. Pancreas-specific

knockouts also exhibit enhanced glucose clearance, but in this case associated with increased insulin levels and secretion. Key: →, no change; ↑, increased ↓, decreased; n.d., not determined.