New Role for Grb10 Signaling in the Pancreas

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After surgically removing the canine pancreas, Joseph von Mering and Oskar Minkowski linked the pancreas with diabetes for the first time (1). Their observation led to the use of purified pancreatic extracts to successfully treat diabetic patients some 30 years later, 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 health care 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 (*Insr*) gene 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 analyzed separately in several tissues, including skeletal muscle, white adipose tissue (WAT), liver, brain, and pancreas (rev. in 3–5). This work revealed roles for Insr signaling in noncanonical 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 insulin receptor substrate-1 and -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 due to, for instance, 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 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 postnatally, including in both canonical (muscle and WAT) and noncanonical (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 ~30% heavier than their wild-type sibs (14). In adulthood, *Grb10* knockout mice have increased muscle mass and reduced adipose compared with wild types (11,12,15). This "antidiabetic" phenotype of lean body proportions with an enhanced ability to clear blood glucose is very interesting but needs to be better understood.

In the article 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, knockdown 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. Discrepancies 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 knockdown.

Importantly, Zhang et al. (6) show that loss of pancreatic *Grb10* resulted in increased β-cell mass, with an associated increase in the number of insulin secretory granules,

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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

	Global *Grb10* knockout	Pancreas-specific *Grb10* knockout
Body weight	↑	→
Food intake	→	→
Adipose	↓	→
Skeletal muscle	↑	n.d.
Pancreas weight	↑	↑
β-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	11–13, 15	6

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 it is associated with increased insulin levels and secretion. →, no change; ↑, increased; ↓, decreased; n.d., not determined.

increased insulin secretion, and improved glucose tolerance, but without a significant change in insulin tolerance. These favorable changes in pancreatic β-cell physiology were replicated in mice challenged with a high-fat diet; 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 β-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 β-cell mass. The recently established link between Grb10 and mammalian target of rapamycin signaling is a promising advance (19,20), and data reported by Zhang et al. (6) showing increased mammalian target of rapamycin 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 the following: 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?

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