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The maintenance of normal carbohydrate metabolism requires an intricate interplay between insulin action and insulin secretion. Type 2 diabetes usually results from a combination of peripheral insulin resistance and defects in β-cell function. Analysis in humans has identified specific genetic defects associated with early-onset forms of diabetes, but a common molecular mechanism for the majority of cases of type 2 diabetes has yet to be identified. Recent results from a number of murine knockout and transgenic models suggest that disruption of insulin/insulin-like growth factor (IGF)-1 signaling mechanisms, and in particular, alteration in the function of insulin-receptor substrate (IRS) proteins, might contribute to defects in both peripheral insulin action and β-cell function. These studies identify a potential common final pathway to explain the cellular and physiological defects observed in type 2 diabetes. While the exact molecular nature of such defects in humans has yet to be identified, components of the insulin/IGF signaling network and its downstream effector molecules are attractive therapeutic targets for the rationale treatment of this disease.

Genetic and physiological studies in diverse organisms from humans to C. elegans provide strong evidence that the insulin/IGF-signaling system is an evolutionarily conserved mechanism integrating fuel metabolism with growth, development, reproduction, and longevity (1). At a cellular level, insulin/IGF-signaling regulates multiple processes, including carbohydrate and lipid metabolism, gene transcription, DNA synthesis, anti-apoptosis, and cell proliferation. Insulin and insulin-like growth factors (IGF-1 and IGF-2) bind to members of the insulin receptor tyrosine kinase family, including the type a and b insulin receptors (IRa and IRb) and the IGF-1 receptor (IGF1r). Insulin and IGF-1 are relatively specific agonists for the insulin and IGF-1 receptors, respectively; however, IGF-2 provides cross-talk between these receptors as it binds with high affinity to the IGF1r, and to IRa that predominates during fetal development (Fig. 1). In postnatal life, insulin is an essential metabolic signal, whereas IGF-1 and IGF-2 promote cell proliferation, differentiation and survival; however, as IGF-1 receptor signals promote pancreatic β-cell survival and expansion, the IGF1/2 might play important roles in the pathogenesis of type 2 diabetes. Whereas the conventional view asserts that diabetic complications arise from the deleterious effects of chronic hyperglycemia, type 2 diabetes might be best understood as a global disorder of insulin/IGF1 signal transduction that ultimately dysregulates gene expression and cell function in a wide range of tissues. The use of transgenic mice provides surprising new insights into the pivotal role of insulin/IGF signal transduction mechanisms in the pathogenesis of type 2 diabetes. Understanding the molecular basis of insulin/IGF signaling might provide a common molecular platform to rectify abnormal insulin action and insulin secretion.

Multisystem insulin resistance, an essential element in diabetes

Insulin resistance is a common element in type 2 diabetes, which is thought to have its greatest impact in certain insulin “target tissues,” including muscle, adipocytes, and liver; insulin resistance in muscle is frequently cited as the culprit in type 2 diabetes (2, 3). As expected, disruption of the insulin receptor in humans and mice is lethal shortly after birth, owing to extreme hyperglycemia, and other poorly characterized developmental defects (4, 5). By contrast, disruption of the insulin receptor gene in specific target tissues using the Cre-loxP system yields viable mice that rarely develop diabetes. Mice without insulin receptors in skeletal muscle never develop diabetes, suggesting that insulin sensitivity in skeletal muscle might not be a primary cause of diabetes (6). However, these mice display elevated fat mass, serum triglycerides, and FFA, suggesting that insulin resistance in muscle contributes to the altered fat metabolism associated with type 2 diabetes. Overexpression of dominant negative dysfunctional insulin receptors in muscle also fails to cause diabetes, although lipid metabolism is perturbed and glucose intolerance ensues; this approach might be more severe because it inhibits both insulin and IGF-1 receptor function (7). Mice lacking hepatic insulin receptors also fail to develop diabetes, as compensatory hyperinsulinemia apparently maintains fasting glucose within a normal range (8); however, these mice display a complex metabolic phenotype, including glucose intolerance and reduced serum triglycerides and FFA.

Multisystem insulin resistance might be an essential element in the pathogenesis of type 2 diabetes, either exacerbating usual compensatory mechanisms or directly interfering with insulin secretion. Although the later possibility is generally ignored, the disruption of insulin receptors in pancreatic β-cells reveals an unexpected role for insulin signaling during insulin secretion. Without insulin receptors, glucose-stimulated insulin secretion is reduced in β-cells, and glucose intolerance develops with age, although diabetes does not occur (9, 10). Combined insulin resistance in β-cells and hepatic/muscle might be an important component of type 2 diabetes. Thus, tissue specific insulin receptor disruption highlights the integrated nature of insulin-regulated metabolism.
The IRS-proteins coordinate the insulin signaling cascade

Recent studies on the mechanisms of insulin/IGF action reveal common signaling pathways that are coordinated by the insulin receptor substrate family of proteins (IRS-proteins). Members of the IRS-protein family are tyrosine phosphorylated by the receptors for insulin and IGF-1, as well as certain cytokines receptors coupled to Janus kinases (11). At least four IRS-proteins occur in mammals: IRS-1 and IRS-2 are widely expressed; IRS-3 is restricted to adipose tissue, b-cells, and possibly liver; and IRS-4 is expressed in the thymus, brain, and kidney. Disruption of each IRS-protein in mice suggests that IRS-1 and IRS-2 coordinate essential effects of insulin/IGF upon peripheral metabolism, b-cell function, reproduction, and murine growth and development (9, 12, 13). By contrast, IRS-3 and IRS-4 might have redundant roles in insulin/IGF action; however, in combination with IRS-1 disruption, a role of IRS-3 in adipose function is revealed.1

IRS-proteins have a conserved amino terminus composed of adjacent pleckstrin homology and phosphotyrosine-binding domains that mediate coupling to activated receptor tyrosine kinases. By contrast, the COOH-terminus of each IRS-protein contains a set of tyrosine phosphorylation sites that act as on/off switches to recruit and regulate various downstream signaling proteins. Each tyrosine phosphorylation site is surrounded by a unique amino acid motif that binds and activates specific effector proteins, including enzymes (PI 3-kinase; a phosphotyrosine phosphatase SHP2; and Src-like kinase, fyn) and adapter proteins (Grb-2, nck, crk, and others) (Fig. 1).

Fig. 1. The IRS-protein-dependent insulin/IGF-1 signaling cascade. The insulin/IGF family consists of three hormones: insulin, IGF-1, and IGF-2. These hormones bind three receptors: insulin receptor, IGF-1 receptor, and mannose-6-phosphate receptor (M6Pr). The insulin receptor is the primary target for insulin during development and adult life. The IGF-1 receptor is the primary target for IGF-1. IGF-2 binds to the type A insulin receptor during embryonic development and binds the IGF-1 receptor in adults. IGF-2 also binds to the mannose-6-phosphate receptor, which targets the IGF-2 for degradation instead of signaling. Activation of the insulin receptor or the IGF-1 receptor mediates signals primarily via the cytoplasmic proteins IRS-1 and IRS-2, which mediate somatic cell growth and metabolism. Activation of the receptors for insulin and IGF-1 stimulate tyrosine phosphorylation of the IRS-proteins, which bind PI 3-kinase, Grb2/SOS and SHP-2, and other SH2-proteins. The Grb2/SOS complex binds p21^ras to activate the MAPK cascade (Erk1/Erk2), which phosphorylates and activates transcription factors like elk1 and c-fos. The activation of PI 3-kinase by IRS-protein produces phospholipids (PI 3,4P2 and PI 3,4,5P3) which activates a variety of downstream kinase cascades, including mTOR → PHAS/p70^s6k/eIF4→protein synthesis; PKCs/PKB→GSK3, which might promote glucose uptake and glycogen synthesis; PKB→BAD/FKHRL1, which might promote cell survival. PI 3,4,5P3 is dephosphorylated by the action of PHAS, and SHIP2 converts PI 3,4,5P3 to PI 3,4P2.

tases, including pTEN, which hydrolyzes the 3'-phosphate and SHIP2 which hydrolyzes the 5'-phosphate from PI-3,4,5-P₃ (Fig. 1). Products of the PI 3-kinase recruit serine kinases to the plasma membrane, including the phospholipid-dependent kinases (PDK1/2) and protein kinase B (akt/ PKB) (Fig. 1). During colocalization, the PDKs phosphorylate and activate akt/PKB, which might contribute directly to the regulation of various biological responses (Fig. 1). Together with other PI 3-kinase-dependent serine kinases, akt/PKB promotes glucose transport, protein synthesis, glycogen synthesis, cell proliferation, and cell survival in various cells and tissues (11, 14, 15).

The regulation of PI 3-kinase by IRS-proteins is an important site of signal redundancy and diversity. In cell-based assays, IRS-1 and IRS-3 activate PI 3-kinase more strongly than IRS-2, whereas IRS-4 barely activates PI 3-kinase (16, 17). However, in murine liver, IRS-2 appears to play a major role in the regulation of PI 3-kinase (12). In addition to the diversity of the IRS-proteins, the PI 3-kinase itself exists as multiple isoforms, owing to the differential expression and dimerization of multiple regulatory subunits (p85α, p85β, p55PIK, p55α, or p50α) and catalytic subunits (p110α, p110β, or p110δ) (18). Disruption in mice of p85α reveals the redundancy and selectivity at this important signaling step. Unexpectedly, mice lacking p85α display increased insulin sensitivity and hypoglycemia due to increased glucose transport in skeletal muscle and adipocytes; insulin-stimulated PI3K activity associated with IRS-proteins is mediated through p85α, which augments the generation of PI-3,4,5-P₃ in adipocytes. Thus, IRS-proteins and PI 3-kinase provide an important coordinate step in the regulation of insulin signaling cascade.

**IRS-proteins integrate growth and development**

IRS-1 plays a predominant role in somatic growth as deletion of irs1 gene in mice reduces embryonic and neonatal growth 40%, whereas deletion of irs2 barely reduces prenatal and postnatal growth by 10%. Additionally, the size of [irs1⁻/⁻, irs2⁻/⁻] mice is reduced 40%, whereas the size of [irs1⁻/⁻, irs2⁺/⁺] mice is reduced 70%, confirming that IRS-1 is the principal element that mediates somatic growth. IRS-2 also promotes growth, but it cannot fully replace IRS-1 in this process, suggesting that the signaling pathways mediated by IRS-1 and IRS-2 overlap incompletely.

**IRS-proteins mediate the effects of insulin on peripheral carbohydrate metabolism**

The complete disruption of the irs-1 gene in mice causes peripheral insulin resistance with nearly normal glucose homeostasis, owing largely to lifelong compensatory hyperinsulinemia (13, 19, 20). By contrast, mice lacking irs-2 gene develop progressive diabetes during the first 10 weeks of life (12). Hepatic insulin resistance is a major component of this process, but the development of overt diabetes results from a concomitant failure of β-cell development and compensation.

IRS-1 appears to exert its greatest effect on metabolism by regulating insulin signals in muscle and adipose tissue as demonstrated by both in vivo clamp experiments and in vitro tissue culture studies, whereas it plays a lesser role in mediating insulin’s effects on the liver metabolism (6, 10, 12, 20–22). To compare the impact of irs1 or irs2 gene disruptions on basal and insulin-stimulated carbohydrate and lipid metabolism in vivo, we studied 18-h fasted chronically catheterized mice during basal and euglycemic (~5 mm), hyperinsulinemic (5 mU·kg⁻¹·min⁻¹) clamp (90–210 min) conditions. Both IRS1⁻/⁻ and IRS2⁻/⁻ mice are markedly insulin resistant compared with wild-type mice as reflected by the absence of insulin on the rate of glucose appearance. Most of the decreased insulin responsiveness in the IRS1⁻/⁻ mice is related to decreased insulin-stimulated peripheral glucose uptake, whereas insulin resistance in IRS2⁻/⁻ mice is attributed to both decreased peripheral glucose uptake and unsuppressed hepatic glucoseogenesis (Previs, S. F., Ren, D. Withers, M. F. White, and G. I. Shulman, in preparation). Moreover, insulin poorly suppresses lipolysis in IRS2⁻/⁻ mice. These data suggest important tissue specific roles for IRS1 and IRS2 in mediating insulin’s effects on carbohydrate and lipid metabolism in vivo, with IRS-2 being important in liver and muscle, and IRS-1 having a major role in muscle.

To further refine these observations upon the interactions of IRS-1 and IRS-2 with insulin receptor in the regulation of metabolism, we created combined heterozygous mutations for the insulin receptor, IRS-1 and IRS-2 (23). Insulin sensitivity was estimated by the relative activity of PI 3-kinase in muscle and liver extracts, and compared against the fed serum insulin levels (Fig. 2). Mice lacking a single allele of each protein [ir/irs1/irs2]⁻/⁻ display significant hepatic and skeletal muscle insulin resistance with marked hyperinsulinemia; serum glucose levels are only slightly elevated (Fig. 2). Less drastic disruption of these signaling elements, as in ir⁺/⁻, [ir/irs1]⁻/⁻ or [ir/irs2]⁻/⁻ mice, attains better insulin-stimulated activation of PI 3-kinase and normal serum glucoses.
Fig. 3. Model: The relative importance of IRS-1/IRS-2 and insulin/IGF signaling in mammalian physiology. Insulin released by the β-cells of the islets mediates metabolic signaling in peripheral target tissues such as muscle, adipose, and liver. IRS-1 is relatively more important in mediating the insulin signal in muscle and adipose tissue, while IRS-2 signaling dominates in the liver. However, these signals are also important for β-cell function. IGF-1 receptors in the pancreatic islets might promote islet and β-cell growth and survival, especially to compensate for peripheral insulin resistance. By contrast, insulin receptors and IRS-1 might enhance glucose stimulated insulin secretion.

cose levels with lower insulin levels (Fig. 2). These genetic experiments might reflect the balance between insulin secretion and insulin action ordinarily encountered during compensation for peripheral insulin resistance.

Nevertheless, less than half of the [ir/irs1/irs2+/−] mice develop diabetes at 6 months of age in spite of hyperinsulinemia, which suggests that other genetic or environmental factors influence successful compensation. Diabetes is 2-fold less frequent in double heterozygous mice, either [ir/irs1]+/− or [irs2]+/−; however, the pathophysiology in each case is different: [ir/irs1]+/− mice develop severe insulin resistance in skeletal muscle and liver with β-cell hyperplasia, whereas [irs2]+/− mice display hepatic insulin resistance and modest β-cell hyperplasia; moreover, diabetic [irs2]+/− mice generally display lower insulin levels than measured in [ir/irs1]+/− mice. These studies reveal that defects in common signaling elements in different tissues may underlie the metabolic phenotype in type 2 diabetes and that oligogenic interactions might cause this disease.

The insulin/IGF signaling system regulates β-cell function

IRS-2 plays a special role in carbohydrate metabolism as it mediates both peripheral insulin action and pancreatic β-cell function. This relation is dramatically revealed in [irs1+/−/irs2+/−] mice. Although very small at birth, these lean mice are insulin-resistant and become glucose intolerant with age, but β-cell compensation is robust and diabetes never develops (13). By contrast, the larger [irs1+/−/irs2−/−] littermates fail to compensate for peripheral insulin resistance and die by 30 days of age with few β-cells and extreme hyperglycemia. Apparently the presence of a single allele of irs-2 promotes sufficient β-cell expansion to compensate for severe peripheral insulin resistance, whereas IRS-1 fails. This striking distinction reveals IRS-2 signaling as a rational target in the treatments for diabetes.

To delineate the signals that activate IRS-2 pathways in β-cells, we analyzed fetal igf1r−/− mice, or postnatal igf1r+/− and [igf1r+/−/irs2−/−] mice. Islets are reduced in size in igf1r+/− mice, and develop poorly in igf1r−/− mice as a reduced number of α-cells and β-cell fail to form typical islet structures. The igf1r−/− mice die at birth, whereas igf1r+/− mice survive with reduced β-cell mass, and never develop diabetes; however, combined with an irs2 disruption, [igf1r+/−/irs2−/−] mice die at 30 days of age with severe hyperglycemia, owing to small islets containing a reduced number of α-cell and nearly no β-cells. These results suggest provisionally that IGF1→IRS2 signaling pathway might be critical for both the embryonic development and postnatal growth of β-cells and reveal an important interface between the insulin and IGF signaling pathways.

Conclusions

In summary, the murine models described here have proved highly informative about the role of insulin signaling mechanisms and have given new insights into the pathogenesis of the diabetic phenotype. They reveal the important role of insulin/IGF signaling through the IRS-proteins in both peripheral tissues and β-cells (Fig. 3). Insulin receptor and the IRS-proteins promote both peripheral insulin action and promote β-cell survival and glucose-sensitive insulin secretion. Thus, failure of the IRS-protein signaling system might cause both insulin resistance and impaired compensatory β-cell insulin secretion. So far, significant mutations in these core signaling elements have not been found in type 2 diabetes; however, dysregulation of diverse signaling pathways that regulate the function of the IRS-proteins might play significant roles in the development of diabetes. For the moment, the interaction of the insulin and IGF-1 receptors with the IRS-proteins provides common ground in our searches for the causes and treatments of diabetes.

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References


12. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF 1998 Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904


