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Molecular Basis of Insulin Resistance: The Role of IRS and Foxo1 in the Control of Diabetes Mellitus and Its Complications

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Abstract

Insulin/IGF-1 signaling plays a central role in control of cellular metabolism and survival, while insulin receptor substrate (IRS) protein -1 and -2 and downstream PI-3 kinase→Akt→Foxo1 signaling cascade play key roles in many functions of insulin/IGF-1. Dysregulation of this branch of signaling cascades may provide a mechanism for insulin resistance as we observed in cells, animals, and even humans. Targeting this branch of IRS→Foxo1 signaling may provide us with fundamental strategies for drug development in the future.

Introduction

More than one-third U.S. adults now have metabolic syndrome, a variety of cardiometabolic risk factors including insulin resistance, obesity, dyslipidemia, and hypertension, which threaten public health due to their unprecedented prevalence [1]. Insulin resistance is the primary contributor to metabolic and cardiovascular disorders and to the development of type 2 diabetes [1, 2]. Two-thirds of patients with type 2 diabetes die of cardiovascular complications or heart failure. In the patients with type 1 diabetes who suffer from pancreatic β -cell dysfunction and insulin deficiency in childhood, insulin injections are the primary therapy used to alleviate hyperglycemia and to reduce the risk of cardiovascular dysfunction (DCCT trial) [3, 4]; however, in patients with type 2 diabetes, insulin therapy increases body weight and cardiovascular risks, even promoting heart attack in adults (ACCORD trial) [4]. Thus, understanding the mechanisms responsible for insulin action and resistance pertaining to cardiovascular complications is critical for the development of new strategies for the treatment of type 2 diabetes, reducing the risk of cardiovascular dysfunction.

Molecular Basis of Insulin Signaling

The discovery of insulin and subsequent signaling studies have been so revolutionary in metabolic research that the Nobel Prizes in 1923, 1958, 1964, and 1977 were awarded to the scientists studying in insulin purification methods, its DNA and protein sequences, and immunological assays. Insulin, a major hormone which counteracts the concerted action of a

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number of hyperglycemia-inducing hormones, plays a key role in regulating glucose and lipid metabolism for fuel storage, in addition to affecting food intake, cardiovascular growth, bone formation, and longevity (Fig. 1). During the postprandial state when insulin secretion from pancreatic β -cells increases, insulin promotes glucose uptake and utilization and the synthesis of protein and fatty acids, while it suppresses fatty acid oxidation in “classic” insulin responsive tissues, including liver, muscle, brain and fat, as well as “non-classic” insulin responsive tissues, such as pancreatic β -cells, vascular endothelium, and bone osteoblasts. In a fasting state when insulin falls, tissues respond to other hormones, such as glucagon in the liver, using fatty acids generated by adipocyte lipolysis as a major substrate for ATP generation in order to conserve glucose. The substrate preferences for ATP production during the transition between fasted and postprandial states are tightly controlled by insulin under physiological conditions [5], whereas this adaptive transition is largely blunted in the tissues of patients with insulin resistance and type 2 diabetes mellitus.

In the last decade, with the creation of insulin receptor and insulin receptor substrate -1 and -2 (IRS1 and IRS2) genetically-engineered mouse models, further breakthroughs in our understanding of insulin-dependent control of energy metabolism and nutrient homeostasis have been achieved. For example, mice lacking insulin receptor were born with slight growth retardation, but they rapidly developed hyperglycemia and hyperinsulinemia, followed by diabetic ketoacidosis and early postnatal death [6]. Reconstitution of insulin receptor in the liver, β -cells, and brain prevented diabetes as well as postnatal death [7, 8], suggesting that liver, β -cells, and brain are the major organs contributing to the incidence of diabetes. Furthermore, mice lacking insulin receptor in either liver or brain developed hyperglycemia and insulin resistance [9, 10], while mice lacking insulin receptor in either skeletal muscle or white adipose tissue exhibited nearly typical blood glucose homeostasis [11, 12].

Insulin receptor substrate-1 and -2 (IRS1 and IRS2)

Upon binding to the cell surface receptor, insulin activates insulin receptor (IR), a heterotetrameric glycoprotein consisting of two extracellular α -subunits (135 kDa) and two transmembrane β -subunits (95 kDa). IR acts as an allosteric enzyme in which the α -subunit inhibits the tyrosine kinase activity of the β -subunits. Insulin binding to the α -subunit results in the stimulation of tyrosine kinase activity in the β -subunits, which leads to autophosphorylation at Tyr1158, Tyr1162, and Tyr1163, the first step in IR activation. Activated IR directly phosphorylates tyrosine residues on several substrates including insulin receptor substrate (IRS) protein 1-4, Shc, Grb-2-associated protein (Gab1), Dock1, Cbl, and APS adaptor proteins, all of which provide specific docking sites for other signaling proteins containing SH2 domains, leading to activation of both Ras $\rightarrow$ MAP kinases and PI-3K $\rightarrow$ Akt signaling cascades [13]. Activation of Ras $\rightarrow$ MAP kinases regulates the effect of insulin on mitogenesis and cellular growth, while activation of PI-3K generates phosphatidylinositol (3,4,5)-triphosphate (PIP3) which activates 3-phosphoinositide dependent protein kinase-1 and -2 (PDK1 and PDK2/Rictor-mTOR), which mediate the metabolic and pro-survival effects of insulin. PDK1 and PDK2 in turn activate protein kinase Akt (also called PKB) by inducing phosphorylation at T³⁰⁸ and S⁴⁷³, respectively [14, 15]. Akt phosphorylates downstream targets including inhibitors of macromolecular synthesis, such as glycogen synthase kinase-3 β (Gsk3 β , glycogen synthesis), tuberous sclerosis protein-2 (Tsc2, protein synthesis), and *forkhead* transcription factor Foxo1 (gene transcription). Akt phosphorylates Foxo1 at S²⁵⁶ and inhibits transcriptional activity of Foxo1, which regulates a variety of physiological functions, such as energy metabolism [16, 17] and myocardial growth [18-20]. Akt $\rightarrow$ Foxo1 phosphorylation mediates many biological responses to insulin and it serves as an indicator of insulin sensitivity [21, 22].

Systemic IRS1 knockout mice displayed growth retardation and peripheral resistance to insulin and insulin-like growth factor-1 (IGF-1) mainly in skeletal muscle; however, they avoided the development of diabetes due to IRS2-dependent pancreatic β -cell growth and compensatory insulin secretion [23]. Systemic IRS2 null mice displayed metabolic defects in liver, muscle, and adipose tissues and they developed diabetes owing to pancreatic β -cell failure [24]. Recently, it was demonstrated that the deletion of both IRS1 and IRS2 genes in the liver of mice (L-DKO mice) prevented activation of hepatic Akt $\rightarrow$ Foxo1 phosphorylation and resulted in the development of diabetes [22, 25], and that deletion of both IRS1 and IRS2 in cardiac and skeletal muscle caused heart failure and death in animals at the age of 2 to 3 weeks [26]. These results indicate that IRS1 and IRS2 are major mediators of insulin/IGF-1 action, required to support physiological functions in many organs.

Forkhead transcription factor Foxo1 signaling

A key IRS downstream effector in insulin signaling cascades, Foxo1, a member of O-class of forkhead/winged helix transcription factors, was first identified as an Akt substrate in hepatocytes involved in mediating the effect of insulin on suppressing transcription of genes encoding IGF-binding protein-1 (IGFBP-1), phosphoenolpyruvate carboxykinase (Pck1), or glucose-6-phosphatase (G6pc), all of which have promoter regions containing at least a consensus Foxo1 binding site or an insulin response element (CAAAACAA) sensitive to PI3K $\rightarrow$ Akt activation [16]. We established that Foxo1 has three Akt phosphorylation sites at T24, S256 and S319 [27]. Phosphorylation of these residues, triggered by S256, promotes Foxo1 nuclear to cytoplasmic translocation and degradation [28-30], thereby inhibiting transcriptional activity of Foxo1 by removing Foxo1 from basal gene transcriptional machinery. This provides a molecular link by which Foxo1 integrates extracellular ligand reception with gene transcriptional control [16]. Additionally, other members of the O-class forkhead family include Foxo3 and Foxo4 that have the conserved Akt phosphorylation motif -RXXRXXS/T (R- arginine, X- any amino acid, and S/T, phosphorylation site of serine or threonine by Akt). With insulin resistance and diabetes, dephosphorylation of Foxo proteins at the conserved Akt phosphorylation sites promotes Foxo1 stability, enhances Foxo transcriptional activity, and contributes to the incidence or progression of hyperglycemia, since the negative regulation of Foxo1 from Akt activation is insufficient. Indeed, deletion or suppression of Foxo1 gene in the liver of L-DKO mice and db/db mice rescued the hyperglycemic diabetes [17, 25]. Collectively, these results suggest that IRS1/2 $\rightarrow$ Akt $\rightarrow$ Foxo1 signaling cascades govern hepatic glucose production by targeting multiple genes in a metabolic pathway, at least those involved in gluconeogenesis. Of note is that Foxo1 is required for the completion of early mouse embryogenesis for sufficient vascular angiogenesis [31]. In addition, Foxo1 ortholog *Daf-16* gene in the worm *Caenorhabditis elegans* enhances longevity, potentially by increasing anti-oxidative gene expression [32]. In rodents, Foxo1 activation following IRS2 deficiency in the brain is thought to contribute to an increase of longevity, while it promotes obesity and diabetes [33]. Thus, Foxo1 activation likely plays a detrimental role resulting in metabolic disease development and progression.

Hepatic insulin resistance and hyperglycemic control

The liver is a key organ which controls blood glucose and lipid homeostasis. Deletion of either IRS1 or IRS2 in mouse liver maintained glucose homeostasis, but deletion of both IRS1 and IRS2 blocked insulin or feeding-induced Akt and Foxo1 phosphorylation, leading to unrestrained gluconeogenesis in hepatocytes, which resulted in hyperglycemia with a reduction in hepatic lipogenesis and blood lipid concentrations [22]. Therefore IRS1 and IRS2 have a redundant role in mediating insulin action in hepatocytes, at least for the

activation of Akt→Foxo1 signaling cascades. Moreover, a high-fat diet severely impaired IRS2 expression and phosphorylation in hepatocytes of liver-specific IRS1 null mice, such that the mice developed diabetes [22]. Overfeeding and metabolic stresses, including high levels of free fatty acids or inflammatory cytokines, enable activation of a number of endogenous protein kinases, such as mTOR, JNK, and IKK β , which can phosphorylate IRS1 and IRS2 at serine and threonine residues and which mediate IRS protein ubiquitination and degradation, resulting in insulin resistance [34]. This provides a molecular link between environmental stimuli and activation of intracellular protein kinases that cause insulin resistance (Fig.1), likely contributing to the prevalence of diabetes over last decades.

Cardiac insulin resistance and heart failure

Deletion of both IRS1 and IRS2 in the heart of mice diminished cardiac Akt and Foxo1 phosphorylation, resulting in heart failure and death of animal at the age of 7 to 8 weeks (Qi and Guo, unpublished data). Similarly, deletion of both IRS1 and IRS2 in both skeletal and cardiac muscle caused heart failure and diminished Akt and Foxo1 phosphorylation in skeletal muscle; but, the mice displayed normal blood glucose homeostasis and insulin sensitivity [26], confirming that insulin resistance in skeletal muscle is not necessary for disrupting systemic glucose homeostasis in mice, but cardiac muscle requires either IRS1 or IRS2 for maintenance of endogenous Akt activity and Foxo1 inactivation to promote cardiac function and survival. Moreover, cardiac Foxo1 overexpression not only caused heart failure in mice [20], but also was observed in human samples of failed hearts [18].

In diabetics, insulin is believed to be a strong inducer of insulin resistance [35], and we recently showed that hyperinsulinemia in cardiomyocytes is sufficient for induction of insulin resistance through degradation of both IRS1 and IRS2 via activation of p38 α MAP kinase, revealing a fundamental mechanism for heart failure caused by insulin resistance, type 2 diabetes mellitus or chronic pathological stresses (Qi and Guo, unpublished data).

Central nervous system (CNS) insulin resistance and obesity

Deletion of IRS2 in the hypothalamus caused hyperglycemia and obesity in mice, indicating that insulin signaling in the brain is key in controlling glucose homeostasis and obesity [33, 36], while deletion of IRS1 in the hypothalamus has minimal impacts on glucose homeostasis and the development of obesity (Guo and White MF, unpublished data). Similar to the action of leptin, an adipocyte-derived hormone that inhibits food intake through central nervous system leptin-receptor neurons by activating the Jak2→Stat3 signaling cascade [37, 38], insulin in the brain also reduced food intake by activation of PI3K via IRS2 and by inactivation of Foxo1, which can be independent of the Jak2→Stat3 pathway [33]; however, both leptin and insulin promoted IRS2 tyrosine phosphorylation and PI3K activation in the brain [39], and IRS2 deletion in leptin receptor-expressing neurons caused diabetes and obesity in which Foxo1 inactivation completely reversed the metabolic dysfunction [40].

Hypothalamic neurons expressing Agouti—regulated peptide (Agrp) encourage food intake (orexigenic), such as during fasting state. Foxo1 stimulates orexigenic Agrp expression, an effect that was reversed by leptin delivery, in which activation of Stat3 squelches Foxo1 occupancy on the Agrp promoter region [41]. Foxo1 deletion in Agrp neurons of mice resulted in reduced food intake, increased leanness and decreased hepatic glucose production, involving the suppression of a G-protein-coupled receptor Gpr17, a Foxo1 target gene in Agrp neurons [42]. By antagonizing the effect of Agrp, hypothalamic neurons expressing pro-opiomelanocortin (Pomc) discourage food intake (anorexigenic), such as during a fed state. Foxo1 deletion in Pomc neurons also resulted in reduced food intake and

body weight by increasing obesity susceptibility protein carboxypeptidase E (Cpe) and subsequent β -endorphin production that mediates anorexigenic effects in mice [43].

In addition to the classic insulin responsive tissues, liver and brain, where insulin controls glucose homeostasis, insulin also plays active roles in glucose homeostasis in non-classic insulin responsive tissues, such as pancreatic β -cells, endothelium of blood vessels, and bone (Table 1).

Insulin resistance in pancreas and β -cell regeneration

Deletion of IRS2 from the whole body in mice caused diabetes due to pancreatic β -cell failure [24], while Foxo1 inactivation in the IRS2 null mice prevented β -cell apoptosis and diabetes [44], indicating that IRS2 $\rightarrow$ Foxo1 signaling or Foxo1 inactivation is required for β -cell survival. On the other hand, deletion of IRS2 in β -cells triggered β -cell repopulation or regeneration, leading to restoration of insulin secretion and prevention of diabetes in aged mice [36], suggesting that Foxo1 activation following IRS2 inactivation in β -cells promotes β -cell regeneration or differentiation. Indeed, Foxo1 inactivation in β -cells resulted in reduced β -cell mass, hyperglycemia, and hyperglucagonemia, owing to dedifferentiation of β cells into progenitor-like cells or pancreatic α -cells [45].

Insulin resistance in vascular endothelium and glucose homeostasis

Insulin resistance in vascular endothelium stimulates vasoconstriction, promotes atherosclerosis, and impairs systemic insulin sensitivity and glucose homeostasis. Inactivation of insulin receptor in vascular endothelium diminished insulin-induced eNOS phosphorylation and blunted aortic vasodilating responses to acetylcholine and calcium ionophore in normal mice [46], and accelerated atherosclerosis in apolipoprotein E in null mice [47]. Vascular endothelium deficient in IRS2, or both IRS1 and IRS2, reduced Akt phosphorylation and eNOS phosphorylation of endothelium, and impaired skeletal muscle glucose uptake, leading to systemic insulin resistance [48]. Foxo activation following deficiency of IRS2 or both IRS1 and IRS2 may play key roles in stimulating endothelial cell dysfunction. In fact, deletion of Foxo1, Foxo3, and Foxo4 in endothelium enhanced eNOS phosphorylation, reduced inflammation and oxidative stress of endothelial cells, and prevented atherosclerosis in high-fat diet or low-density lipoprotein receptor null mice [49]. Endothelium-targeted deletion of insulin receptor or Foxo genes in mice barely disrupted glucose homeostasis [46, 47, 49], but we recently showed that endothelium-targeted deletion of the transcription factor related transcriptional enhancer factor-1 (RTEF-1) increased blood glucose levels and insulin resistance and that RTEF-1 has potential for interaction with the insulin response element and Foxo1 in the cells [50]. Thus, vascular endothelium is a tissue that potentially regulates glucose homeostasis.

Insulin resistance in the bone and glucose homeostasis

Insulin promotes bone formation and differentiation of osteoblasts that synthesize osteocalcin, a bone-derived insulin secretagogue, which regulates insulin secretion and then systemic glucose homeostasis. Mice lacking insulin receptor in osteoblasts had reduced bone formation and increased peripheral adiposity, while they exhibited insulin resistance mainly due to reduced gene expression and activity of osteocalcin [51, 52]. These studies suggest that insulin in osteoblasts may stimulate osteocalcin via suppression of Foxo1 during bone remodeling and then they go on to control glucose homeostasis. Foxo1 inhibits osteocalcin expression and activity by increasing expression of Esp, a protein tyrosine phosphatase that inhibits bioactivity of osteocalcin by favoring its carboxylation. Moreover, osteoblast-specific Foxo1 null mice have increased osteocalcin expression and insulin production and reduced blood glucose concentrations [53]. Collectively, these studies suggest that bone

serves as an endocrine organ to control glucose homeostasis via a bone-pancreas crosstalk, in which Foxo1 plays a key role in how insulin action regulates osteocalcin expression and activity in osteoblasts.

Conclusion and Prospect

Akt inactivation and Foxo1 activation following suppression of IRS1 and IRS2 provide a fundamental mechanism for insulin resistance, which occurs in both classic and non-classic insulin responsive tissues, impairing systemic glucose homeostasis and body weight control. The regulatory mechanisms of IRS→Akt→Foxo1 cascades should be further explored under different cellular and environmental contexts. Of note is that current anti-diabetic therapeutics, such as glucagon-like peptide (GLP), thiazolidinediones, (TZD) and metformin, can significantly affect this pathway directly or indirectly (Fig.1). Targeting IRS1 and IRS2 by activating the Akt→Foxo1 signaling cascade and associated protein kinases will be critical for therapeutic intervention in the pursuit of a treatment for diabetes, obesity, cardiovascular disorders, and associated metabolic syndrome in the future.

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Physiological functions of insulinMolecular, cellular, and biochemical mechanisms

Figure 1.
Physiological functions of insulin and its molecular basis at the molecular, cellular, and biochemical levels

Table 1

Summary of phenotype due to conditional IRS1/2 knockout and Foxo1/3/4 knockout mice using the Cre-LoxP genetic approaches

Tissue-specific IRS or Foxo null mice genotype	Phenotype	Cre-mice	Ref.
Liver IRS1 ^{-/-}	Normal glucose; severe insulin resistance on high-fat diet	Alb-cre	22, 25
Liver IRS2 ^{-/-}	Normal glucose	Alb-cre	22, 25
Liver IRS1 ^{-/-} :: IRS2 ^{-/-}	Hyperglycemia; insulin resistance	Alb-cre	22, 25
Liver Foxo1 ^{-/-}	Reduced blood glucose	Alb-cre	17
Liver Foxo3 ^{-/-}	Normal glucose	Alb-cre	17
Liver Foxo4 ^{-/-}	Normal glucose	Alb-cre	17
Liver Foxo1 ^{-/-} :: Foxo3 ^{-/-} :: Foxo4 ^{-/-}	Reduced blood glucose; increased triglycerides and hepatic steatosis	Alb-cre	17
Liver Foxo1 ^{-/-} :: IRS1 ^{-/-} :: IRS2 ^{-/-}	Protect hyperglycemia from hepatic IRS1 and IRS2 deficiency	Alb-cre	25
Skeletal & Cardiac muscle IRS1 ^{-/-} ::IRS2 ^{-/-}	Normal glucose; normal insulin; die 2 weeks after birth	MCK-cre	26
Cardiac Foxo1 ^{-/-}	Prevent heart failure from high-fat diet	αMhc-cre	19
Cardiac Foxo3 ^{-/-}	No prevent heart failure from high-fat diet	αMhc-cre	19
Cardiac Foxo1 ^{-/-} :: Foxo3 ^{-/-}	Prevent heart failure from high-fat diet	αMhc-cre	19
Hypothalamic & β-cell IRS2 ^{-/-}	Obesity; hyperglycemia; insulin resistance	Rip-cre	33, 36
Hypothalamic (AGRP neuron) Foxo1 ^{-/-}	Leanness, reduced food intake; increase insulin and leptin sensitivity	Agrp-cre	41, 42
Hypothalamic (POMC neuron) Foxo1 ^{-/-}	Leanness, reduced food intake; increase insulin and leptin sensitivity	Pomc-cre	43
Leptin receptor neuron IRS2 ^{-/-}	Obesity; hyperglycemia; insulin resistance	Lep-R-cre	40
Leptin receptor neuron Foxo1 ^{-/-} ::IRS2 ^{-/-}	Leanness; protect obesity and hyperglycemia from IRS2 deficiency	Lep-R-cre	40
Pancreatic β-cell Foxo1 ^{-/-}	Reduced β-cell regeneration; β-cell dedifferentiated into progenitor-like cells or α-cells; hyperglucagonemia; hyperglycemia	Ins2-cre	45
Endothelium IRS1 ^{-/-} ::IRS2 ^{-/-}	Reduced Akt and eNOS phosphorylation; impaired skeletal muscle glucose uptake and insulin resistance	Tie2-cre	48
Endothelium Foxo1 ^{-/-} ::Foxo3 ^{-/-} ::Foxo4 ^{-/-}	Increased eNOS phosphorylation; reduced inflammation and oxidative stress of endothelium; prevented atherosclerosis	Tie2-cre	49
Bone osteoblasts Foxo1 ^{-/-}	Increased osteocalcin and insulin production; reduced blood glucose concentration	Collagen I-cre	53