Defining the underlying defect in insulin action in type 2 diabetes

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Abstract

Insulin resistance is one of the earliest defects in the pathogenesis of type 2 diabetes. Over the past 50 years, elucidation of the insulin signalling network has provided important mechanistic insights into the abnormalities of glucose, lipid and protein metabolism that underlie insulin resistance. In classical target tissues (liver, muscle and adipose tissue), insulin binding to its receptor initiates a broad signalling cascade mediated by changes in phosphorylation, gene expression and vesicular trafficking that result in increased nutrient utilisation and storage, and suppression of catabolic processes. Insulin receptors are also expressed in non-classical targets, such as the brain and endothelial cells, where it helps regulate appetite, energy expenditure, reproductive hormones, mood/behaviour and vascular function. Recent progress in cell biology and unbiased molecular profiling by mass spectrometry and DNA/RNA-sequencing has provided a unique opportunity to dissect the determinants of insulin resistance in type 2 diabetes and the metabolic syndrome; best studied are extrinsic factors, such as circulating lipids, amino acids and other metabolites and exosomal microRNAs. More challenging has been defining the cell-intrinsic factors programmed by genetics and epigenetics that underlie insulin resistance. In this regard, studies using human induced pluripotent stem cells and tissues point to cell-autonomous alterations in signalling super-networks, involving changes in phosphorylation and gene expression both inside and outside the canonical insulin signalling pathway. Understanding how these multi-layered molecular networks modulate insulin action and metabolism in different tissues will open new avenues for therapy and prevention of type 2 diabetes and its associated pathologies.

Keywords

Cell-autonomous; Insulin action; Insulin resistance; iPS cells; Phosphorylation; Review; The metabolic syndrome; Tissue crosstalk; Type 2 diabetes

Introduction

The ground-breaking discovery of insulin 100 years ago [1] turned diabetes from a death sentence into a manageable condition. However, it soon became clear that most individuals with diabetes are not insulin deficient, but rather have increased insulin levels and are resistant to exogenous insulin [2]. Major breakthroughs in understanding insulin action and insulin resistance came in the early 1970s, with the demonstration of the existence of insulin receptors on the membrane of cells [3] and the subsequent recognition of their intrinsic tyrosine kinase activity [4]. Here, we review current and evolving concepts of the mechanisms of insulin signalling and how these are modified by extrinsic and intrinsic factors that underlie insulin resistance in type 2 diabetes.

Defining the insulin signalling network

Although key components involved in insulin signal transduction are present in virtually every cell, the biological outcomes following activation or disruption of this pathway are highly dependent on the cell type and physiological context (Fig. 1). In skeletal muscle, insulin promotes glucose transport and utilisation, stimulates glycogen synthesis and inhibits protein catabolism (Fig. 1a). In adipose tissue, insulin promotes glucose transport and lipogenesis and inhibits lipolysis (Fig. 1b). In liver, insulin action inhibits glucose production and fatty acid oxidation and stimulates glycogen synthesis and lipogenesis (Fig. 1c). In addition to these direct cellular effects, insulin can also regulate metabolism indirectly. For example, insulin suppression of lipolysis in fat and inhibition of protein catabolism in muscle reduces substrate supply for gluconeogenesis in the liver [5, 6]. In states of insulin resistance, all or only some of these pathways may be altered, with the exact combination leading to differing clinical presentations.

Insulin and IGF-1 receptors

Insulin and IGF-1 regulate growth and metabolism through binding to their cognate receptors on the cell surface. The insulin receptor and IGF-1 receptor (IGF1R) are highly homologous heterodimers composed of two α and two β subunits stabilised by disulfide bonds. These subunits are derived from single-chain proreceptors (encoded by the *INSR* and *IGF1R* genes), which are processed to the mature $\alpha_2\beta_2$ receptor tetramer. The α subunits are completely extracellular and create the insulin binding sites through their three-dimensional inverted-V structure, while the transmembrane β subunits contain an intracellular tyrosine kinase domain that is required for catalytic activity and signal transduction [4, 7]. Alternative splicing of exon 11 in the *INSR* mRNA results in a shorter insulin receptor isoform (insulin receptor isoform A [IR-A]), which is predominantly expressed in neurons and less-differentiated cellular progenitors, and a longer isoform (insulin receptor isoform B [IR-B]), which is predominant in mature cells and tissues with prominent roles in glucose, lipid and protein metabolism. Both IR-A and IR-B display similar affinity for insulin, while IR-A has higher affinity for IGF-1 and IGF-2 than IR-B [8].

Functionally, the insulin receptor and IGF1R are members of the family of receptor tyrosine kinases. Despite their high degree of homology and many shared downstream signalling pathways, activation of each receptor results in different physiological outcomes, with the

insulin receptor primarily regulating metabolic functions and IGF1R being more involved in mitogenesis and growth. Some of these functional differences are explained by distinct cellular distribution but, even in the same cell type, these receptors exert differential effects. Studies have demonstrated that these receptor-specific effects depend on differences in both the extracellular and intracellular domains of these receptors and their relative affinity for different intracellular substrates, with the insulin receptor favouring phosphorylation of IRS proteins and IGF1R favouring phosphorylation of src homology 2 (SH2) domain containing transforming protein (SHC) [9, 10].

Studies using x-ray crystallography and cryo-electron microscopy have shown that insulin/ IGF-1 binding to the extracellular domains of the insulin receptor/IGF1R involves both the N- and C-terminal regions of the α-subunit, leading to conformational changes that bring together the intracellular β-subunit within each receptor [11, 12]. This leads to activation of the intrinsic tyrosine kinase, resulting in transphosphorylation of the β-subunits and phosphorylation of multiple tyrosine residues within the receptors themselves and their immediate substrates.

Insulin receptor substrates

For metabolic action, the two most important substrates are IRS1 and IRS2. Structurally, IRS proteins are defined by phosphotyrosine binding (PTB) and pleckstrin-homology domains located in the N-terminal region, which are required for their interaction with phosphorylated insulin receptor and targeting to the plasma membrane, and by multiple tyrosine residues in the mid- and C-terminal regions, which are phosphorylated by activated insulin receptor and serve as docking sites for proteins containing SH2 domains [13]. Genetic ablation in mice has shown a predominant role of IRS1 in insulin signalling in skeletal muscle and adipose tissue [14, 15] and IRS2 in liver, pancreatic beta cells and neurons [16]. Consistent with their complementary roles, MS-based phosphoproteomic studies of IRS1 and IRS2 knockout in pre-adipocytes have revealed regulation of distinct signalling pathways downstream of each substrate and suggest that IRS1/2 do not fully compensate for each other [17].

In addition to signalling through canonical substrates and downstream elements, the insulin receptor and its signalling are also regulated, both positively and negatively, by interaction with membrane and intracellular proteins. These include glycosylphosphatidylinositol (GPI)-linked proteins, such as glypican-4 [18], membrane pyrophosphatases and phosphodiesterases, such as ectonucleotide pyrophosphatase/phosphodiesterase 1 (PC-1) [19], α-arrestin adaptors [20] and, even, transcription factors [21] and cell-cycle regulators [22].

Downstream insulin signalling

The critical step linking insulin receptor activation to downstream metabolic functions of insulin is the binding of class IA phosphoinositide 3-kinase (PI3K) to tyrosine-phosphorylated IRS proteins, leading to the formation of phosphatidylinositol (3,4,5)-triphosphate (PIP_3). Downstream effects of PIP_3 lead to activation of 3-phosphoinositide dependent protein kinase (PDK)1 and subsequent activation of a variety of kinases, of which

Akt1–3 are the best studied, but which also include p70 ribosomal S6 kinase (S6K), serum- and glucocorticoid-induced protein kinase (SGK) and protein kinase C (PKC) isoforms [23].

PI3K is a lipid kinase consisting of a catalytic subunit (either p110α, p110β or p110δ, encoded by the *PIK3CA*, *PIK3CB* and *PIK3CD* genes, respectively) and a regulatory subunit (either p85α [and its splice variants p55α and p50α], p85β or p55γ, encoded by *PIK3R1*, *PIK3R2* and *PIK3R3* genes, respectively) [24]. The binding of SH2 domains in the regulatory subunits to phosphotyrosines on IRS proteins reduces the constitutive inhibitory effects exerted on the catalytic subunits, leading to increased kinase activity towards phosphatidylinositol 4,5-bisphosphate (PIP$_2$) in the plasma membrane, resulting in PIP$_3$ formation. PIP$_3$ then serves as a docking site for proteins containing pleckstrin-homology domains, including Akt, PDK1 and the mechanistic target of rapamycin complex (mTORC) 2 component mitogen-activated protein kinase associated protein 1 (SIN1), which represent critical steps in downstream signalling. Thus, Akt undergoes PDK1-dependent phosphorylation at T308 within the kinase domain and mTORC2-dependent phosphorylation at S473 in a C-terminal hydrophobic motif, resulting in full kinase activation [25]. In addition to mTORC2, the Akt S473 residue is phosphorylated by DNA-dependent protein kinase (DNA-PK) [26].

A wide range of insulin's metabolic actions are linked to Akt-dependent phosphorylation: Akt-induced phosphorylation inactivates glycogen synthase kinase-3 (GSK3)α/β, allowing dephosphorylation and activation of glycogen synthase, which increases glycogen synthesis [27]; phosphorylation of forkhead box (FOX)O transcription factors results in their nuclear exclusion, thus, inhibiting their effect on the expression of gluconeogenic genes in the liver [28, 29] and autophagy genes in muscle [30]; phosphorylation of tuberous sclerosis 2 (TSC2) and the 40 kDa proline-rich Akt substrate (PRAS40) leads to activation of mTORC1, resulting in stimulation of protein synthesis and suppression of autophagy [31, 32]; phosphorylation of TBC1 domain family member 1/Akt substrate of 160 kDa (TBC1D4/AS160) regulates trafficking of intracellular GLUT4 vesicles to the plasma membrane and increases glucose uptake [33, 34]. In addition, PI3K/Akt signalling plays a role in cell survival, proliferation and cytoskeleton organisation. While some of these actions occur through phosphorylation of targets, such as GSK3, FOXO1 and mTORC1, Akt also directly phosphorylates proteins in the apoptotic pathway (B cell lymphoma 2 [BCL2]-associated agonist of cell death [BAD], X-linked inhibitor of apoptosis [XIAP] and BCL2-interacting mediator of cell death [BIM]) and regulates cell division through phosphorylation of cyclin-dependent kinase 2 (CDK2) and the cell cycle arrest protein cyclin-dependent kinase inhibitor 1B (p27) [25].

Regulation of gene expression

The pleiotropic effects of insulin action on cell growth and metabolism result from a complex interaction between rapid phosphorylation-dependent signalling [35, 36] and slower changes in gene expression [37]. For example, the effect of insulin on glucose transport in skeletal muscle and adipocytes is dependent on the movement of pre-existing vesicles containing GLUT4 glucose transporters to the plasma membrane [38] and is dependent on AS160 phosphorylation by Akt [39], while glycogen synthesis and glycolytic

and oxidative glucose metabolism are supported by increased mRNA expression of glycogen synthase 1 [40], hexokinase 2 [41] and many components of the mitochondrial electron transport chain [42]. Insulin also regulates several key mechanisms involved in gene expression, with the regulation of mRNA transcription being the best studied [43]. This important aspect of insulin action is accomplished by insulin-induced changes in phosphorylation, expression, processing and translocation of a variety of transcription factors, leading to stimulation or inhibition of gene transcription.

FOX proteins represent a large family of transcription factors, of which FOXOs (FOXO1, FOXO3, FOXO4 and FOXO6) are the most well-characterised regulators of downstream insulin signalling. Here, the effect of insulin is one of negative regulation (Fig. 2a). Upon insulin stimulation, FOXOs undergo multisite phosphorylation by Akt and/or SGK kinase. This creates interaction sites for FOXOs with phosphoserine-binding 14-3-3 proteins, resulting in their retention in the cytoplasm and decreased transcriptional activity in the nucleus [44, 45]. Thus, insulin-induced phosphorylation of FOXOs results in reduced hepatic gluconeogenesis [46], inhibition of muscle autophagy and protein degradation [30, 47] and regulation of adipocyte differentiation [48]. Because of the negative nature of insulin action on FOXO-regulated gene expression, FOXO deletion can reverse many of the metabolic abnormalities caused by insulin resistance resulting from deletion of the insulin receptor/IGF1R [30, 49] or deletion of Akt1/Akt2 [50] or those caused by streptozotocin-induced diabetes [51].

Another emerging class of FOX proteins that act in insulin signalling are the FOXK1 and FOXK2 transcription factors [21, 52]. In contrast to FOXOs, which are turned off by insulin, FOXKs display increased nuclear localisation and transcriptional activity following insulin stimulation (Fig. 2a) and exhibit complex regulation. In the basal state, GSK3 phosphorylates FOXKs leading to increased interaction with 14-3-3 proteins and nuclear exclusion (Fig. 2b); this is reversed by insulin-induced Akt and mTORC1 activation [21, 52]. In hepatocytes, FOXKs regulate genes involved in the cell cycle, apoptosis and lipid metabolism [21], while in adipocytes and muscle, FOXKs promote glucose transport and lactate production by stimulation of glycolytic metabolism and inhibition of mitochondrial pyruvate oxidation [53].

In addition to phosphorylation, insulin also regulates the expression and processing of transcription factors. For example, sterol regulatory element binding proteins (SREBP) 1 and 2 are important regulators of triacylglycerol and cholesterol synthesis and are synthesised as precursors that reside in the endoplasmic reticulum (ER). Upon activation of Akt/mTOR pathways by insulin, these are transported to the Golgi apparatus where membrane-bound transcription factor protease, site 1/2 (SP1/SP2) mediate proteolytic cleavage, releasing the active forms of SREBP1/2, which then migrate into the nucleus and regulate the transcription of genes involved in lipid synthesis and transport [54].

A re-emerging concept in insulin control of gene expression is the possibility of direct effects of the insulin receptor itself. Studies from over 40 years ago showed binding of insulin to nuclear preparations [55]. The significance of such findings has only come to light by recent studies demonstrating interactions between the insulin receptor and FOXK1 [21]

and interactions of the insulin receptor with RNA polymerase II (Pol II) on DNA in the nucleus [56]. Indeed, chromatin immunoprecipitation followed by sequencing (ChIP-seq) analysis of HepG2 hepatocytes revealed ~4000 peaks bound by the insulin receptor, many overlapping with Pol II sites on promoters. These occur in genes involved in a variety of cellular functions including lipid metabolism, translation and immunity, as well as genes involved in pathophysiological states, such as diabetes.

Insulin resistance as a central component of type 2 diabetes and the metabolic syndrome

Type 2 diabetes affects more than 400 million adults worldwide and its prevalence continues to increase at epidemic rates, thus posing one of the greatest public health challenges to society [57]. This is the result of both genetic and environmental factors. While it remains debated whether insulin resistance and relative beta cell failure constitute the primary defect in type 2 diabetes [58, 59], a 25 year prospective longitudinal study of people at high genetic risk of developing type 2 diabetes has demonstrated that insulin resistance precedes and predicts disease development [60]. Likewise, family studies have shown that glucose tolerant offspring of parents with type 2 diabetes show insulin resistance, while loss of first-phase insulin secretion was observed in those that developed impaired glucose tolerance [61]. Clamp and MRI studies have revealed skeletal muscle as a primary site of insulin resistance in the offspring of parents with type 2 diabetes, with the muscle of these individuals exhibiting reduced glucose uptake and reduced glycogen synthesis before hyperglycaemia develops [62]. This impaired glucose metabolism has been attributed to a number of defects, including decreased glucose transport [63], lower rates of insulin-induced ATP production [42] and reduced expression of genes involved in mitochondrial function [64, 65]. The major question that remains is what are the fundamental defects leading to insulin resistance and how do cell-intrinsic vs cell-extrinsic factors contribute to these defects? Cell-extrinsic factors include circulating or paracrine molecules (such as hormones, cytokines, lipids and metabolites) that are released from a cell or tissue other than the target cell/tissue, or absorbed by the intestine from the diet or microbiome action. Conversely, cell-intrinsic factors are those that persist after removal or normalisation of all extrinsic factors. These are most likely due to genetic or epigenetic effects, but may or may not be in the insulin signalling pathway itself. For example, a genetic defect that changes intracellular ATP or Mg^{2+} concentrations or membrane fluidity could affect insulin receptor- or kinase-mediated signalling, creating a state of insulin resistance. How each of these might contribute to insulin resistance in type 2 diabetes is discussed in the following sections.

Extrinsic factors in the pathogenesis of insulin resistance

In type 2 diabetes, most attention has focused on extrinsic factors contributing to insulin resistance, including the role of adipose tissue, circulating metabolites, inflammatory signals and the gut microbiome [66–68] (Fig. 3). In states of overnutrition, increased levels of circulating fatty acids and ectopic lipid accumulation in muscle and liver contribute to insulin resistance through the release of intermediate metabolites, such as diacylglycerols (DAG) and ceramides, that activate members of the novel PKC family (PKC δ, ɛ, θ), leading

to increased Ser/Thr phosphorylation of the insulin receptor and IRS proteins and resulting in reduced tyrosine phosphorylation [69–73]. While IRS1 is the best studied substrate in the context of insulin resistance, Ser/Thr phosphorylation also impairs IRS2 signalling [74]. In addition, fatty acids activate Toll-like receptor 4 (TLR4) to promote activation of c-Jun N-terminal kinase (JNK) and inhibitor of κB kinase (IKK), which also increase Ser/Thr phosphorylation of IRS1, thus, reducing insulin action [75, 76]. Accumulation of ceramides can also activate protein phosphatase 2A (PP2A) and PKCζ, inhibiting Akt2. Adipose tissue expansion is also associated with increased adipose tissue inflammation and hypoxia [77], promoting recruitment of proinflammatory macrophages [78] that secrete cytokines, such as TNF-α and IL-6, which further worsen insulin resistance by activation of the TNF-α receptor (TNFR) and other cytokine receptors [79]. Cytokine signalling induces expression of suppressor of cytokine signalling (SOCS) proteins, such as SOCS1 and SOCS3, which directly bind to the insulin receptor via SH2 domains and prevent IRS1/2 tyrosine phosphorylation, as well as promoting their ubiquitination and proteasomal degradation [80]. Finally, increases in reactive oxygen species (ROS) and ER stress also occur in target tissues in states of insulin resistance, leading to activation of JNK, IKK isoforms and other Ser/Thr kinases [81, 82].

Circulating branched-chain amino acids (BCAAs) and aromatic amino acids (isoleucine, leucine, valine, phenylalanine and tyrosine) are also associated with insulin resistance [67], and lowering BCAA levels can improve insulin sensitivity, at least in mice [83]. It is suggested that BCAAs exert these effects by activation of mTORC1, again altering Ser/Thr IRS1/2 phosphorylation [84]. Gut microbiota may also play a role in regulating BCAA supply, as well as the production of short-chain fatty acids and other metabolites, which, in turn, have an impact on systemic insulin sensitivity [85]. Thus, there are multiple potential extracellular mediators of insulin resistance, which appear to act through increasing IRS1/2 Ser/Thr phosphorylation.

Understanding how changes in Ser/Thr phosphorylation of IRS proteins regulate insulin signalling is, however, challenging because there are over 50 Ser/Thr sites on IRS1/2 and many kinases can be involved [86]. In any case, these extrinsic factors appear to act primarily as 'progression factors' in disease pathogenesis since none of these kinases has been linked genetically to human type 2 diabetes (although PKCδ has been linked to insulin resistance in mice [87]). Since insulin itself regulates many of these same Ser/Thr phosphorylation sites with both positive and negative effects, depending on dose and duration of stimulation, it is possible that IRS1/2 Ser/Thr phosphorylation is a normal feedback mechanism that is subsumed by these metabolic stresses to inhibit insulin signalling. Recently, we and others have shown that adipose tissue can also crosstalk with other tissues through secretion of exosomal microRNAs (miRNAs) [88, 89]; however, how this fits in the regulation of insulin sensitivity at a signalling level remains to be determined.

Intrinsic factors and cell-autonomous insulin resistance

In vitro approaches, where cells are cultured under controlled conditions, provides an opportunity to minimise the influence of extrinsic factors and isolate cell-autonomous determinants of insulin resistance, which are more closely linked to the genetic and

epigenetic alterations underlying type 2 diabetes. Skeletal muscle biopsies and primary cultured myoblasts derived from people with type 2 diabetes show insulin resistance and several metabolic defects. These include impaired insulin signalling at the level of IRS1-associated PI3K activity [90] and Akt/GSK3 phosphorylation [91, 92] and decreased glucose uptake and glycogen synthesis rates [93, 94]. However, primary cell models have limited usefulness for the definition of molecular mechanisms underlying insulin resistance due to limits in expandability and ability for screening using RNA interference (RNAi), chemical genetics or CRISPR. Induced pluripotent stem cells (iPSCs) represent a step forward in this direction since these cells have unlimited potential for expansion and differentiation into multiple lineages, allowing cells from patients to be used for mechanistic studies, large-scale 'omics' and gene-editing approaches. Such iPSC modelling has been applied to severe insulin resistance caused by insulin receptor mutations [95–97] and other forms of genetically determined type 2 diabetes and obesity [98, 99].

Recently, we have applied the iPSC technology to study signalling defects that underlie skeletal muscle insulin resistance in type 2 diabetes [100]. Importantly, iPSC-derived myoblasts (iMyos) from individuals with type 2 diabetes show defects in insulin signalling at the level of Akt/GSK3/FOXO1 phosphorylation and decreased insulin-stimulated glucose uptake and mitochondrial respiration, similar to the defects observed in the muscle in type 2 diabetes. Global phosphoproteomics using LC-MS/MS revealed that these defects are part of a large multi-dimensional network of signalling changes involving ~1200 Ser/Thr phosphorylation sites on 725 proteins. Only a small proportion of these abnormalities are in classical insulin-regulated phosphorylations that define critical nodes in insulin action [101]. More importantly, type 2 diabetic iMyos show a large degree of perturbations in pathways outside of the canonical insulin signalling pathway and not regulated by insulin (Fig. 4). These include up- and downregulation of phosphorylation on several Rho GTPases, proteins involved in cytoskeleton remodelling and vesicle trafficking, and many nuclear proteins involved in gene transcription, mRNA splicing and/or processing and chromatin remodelling. These findings clearly open our view to a wider definition of mechanisms of insulin resistance at the molecular and cellular level that needs to be taken into account in understanding the pathogenesis of type 2 diabetes.

A major challenge going forward will be to identify the molecular defect(s) that drive these signalling changes. Possibilities include kinases and phosphatases, a wide range of co-regulators of the activity of kinases and phosphatases, redox balance, ionic milieu, scaffolding proteins and other factors. While these alterations could represent some form of metabolic memory or epigenetic regulation due to altered DNA methylation [102, 103], this seems unlikely since genetic reprogramming of iPSCs is known to erase most epigenetic marks [104]. Likewise, while genome-wide association studies (GWAS) have collectively identified over 500 independent SNPs associated with type 2 diabetes [105], few of these are in proteins active in insulin signalling. Furthermore, although some SNPs may fall into regulatory regions acting on adjacent or even distant genes, most of these GWAS variants occur in non-coding regions of the genome [106, 107]. While there has been some progress in linking GWAS variants to alterations in beta cell function, insulin sensitivity and energy balance [108, 109], even taken together, all GWAS loci account for only a small fraction of

the strong familial clustering of type 2 diabetes, leaving understanding the primary defect a major challenge.

Advances in profiling technologies have led to a greater appreciation of the potential role of non-coding RNAs, especially miRNAs and long non-coding RNAs (lncRNAs), in the control of cellular metabolism. Indeed, studies using in vitro models, as well as tissues from rodent models of and humans with obesity and type 2 diabetes, have revealed a network of altered miRNAs targeting the insulin receptor, as well as the IRS/PI3K/Akt pathways, thus, contributing to metabolic abnormalities [110]. For example, miR-29a and miR-29c are elevated in skeletal muscle of individuals with type 2 diabetes and *ob*/*ob* mice, and ectopic expression of miR-29a and miR-29c in muscle cells is sufficient to cause insulin resistance and impaired glucose transport [111]. While some miRNA changes in type 2 diabetes may result from tissue crosstalk through exosomal delivery [112], miRNA profiling of cultured myoblasts from donors with type 2 diabetes also revealed some modest, but significant, changes compared with control donors [113]. Similar to miRNAs, some lncRNAs are also regulated by insulin and other physiological cues [37, 114], and are dysregulated in type 2 diabetes [115], resulting in abnormal insulin signalling [116]. The finding of altered phosphorylation and gene expression of factors involved in mRNA splicing in iMyos [100] and skeletal muscle biopsies from individuals with type 2 diabetes [117] could provide another link between genetic regulation and the insulin resistance of type 2 diabetes.

Conclusions and perspectives

Insulin and IGF-1 signalling is present in virtually every cell of the body and plays a central role in the control of metabolism, growth and differentiation. In spite of significant progress, understanding the primary driver of altered insulin receptor signalling in type 2 diabetes, obesity and the metabolic syndrome represents a continuing challenge. Integrating multiple 'omics' layers into a unique disease signature and translating these findings into novel and personalised therapies is an important challenge for the next decade.

Funding

Abbreviations

AS160	Akt substrate of 160 kDa
BCAA	Branched-chain amino acid
BCL2	B cell lymphoma 2
ER	Endoplasmic reticulum
FOX	Forkhead box
GSK3	Glycogen synthase kinase-3

GWAS	Genome-wide association studies
IGF1R	Insulin-like growth factor 1 receptor
IKK	Inhibitor of κB kinase
iMyo	Induced pluripotent stem cell-derived myoblast
iPSC	Induced pluripotent stem cell
IR-A	Insulin receptor isoform A
IR-B	Insulin receptor isoform B
JNK	c-Jun N-terminal kinase
lncRNA	Long non-coding RNA
miRNA/miR	MicroRNA
mTORC	Mechanistic target of rapamycin complex
PDK	3-Phosphoinositide dependent protein kinase
PI3K	Phosphoinositide 3-kinase
PIP$_3$	Phosphatidylinositol (3,4,5)-triphosphate
PKC	Protein kinase C
Pol II	RNA polymerase II
SGK	Serum- and glucocorticoid-induced protein kinase
SH2	Src homology 2
SOCS	Suppressor of cytokine signalling
SREBP	Sterol regulatory element binding

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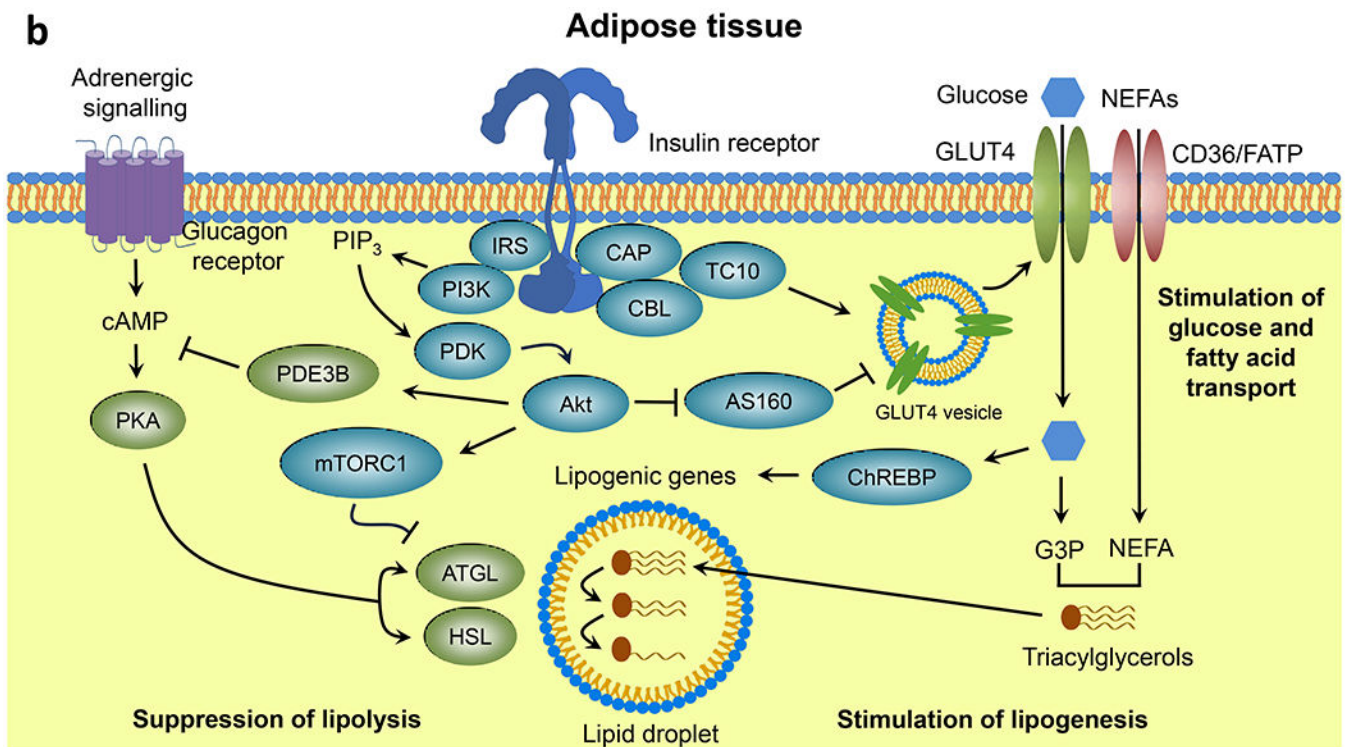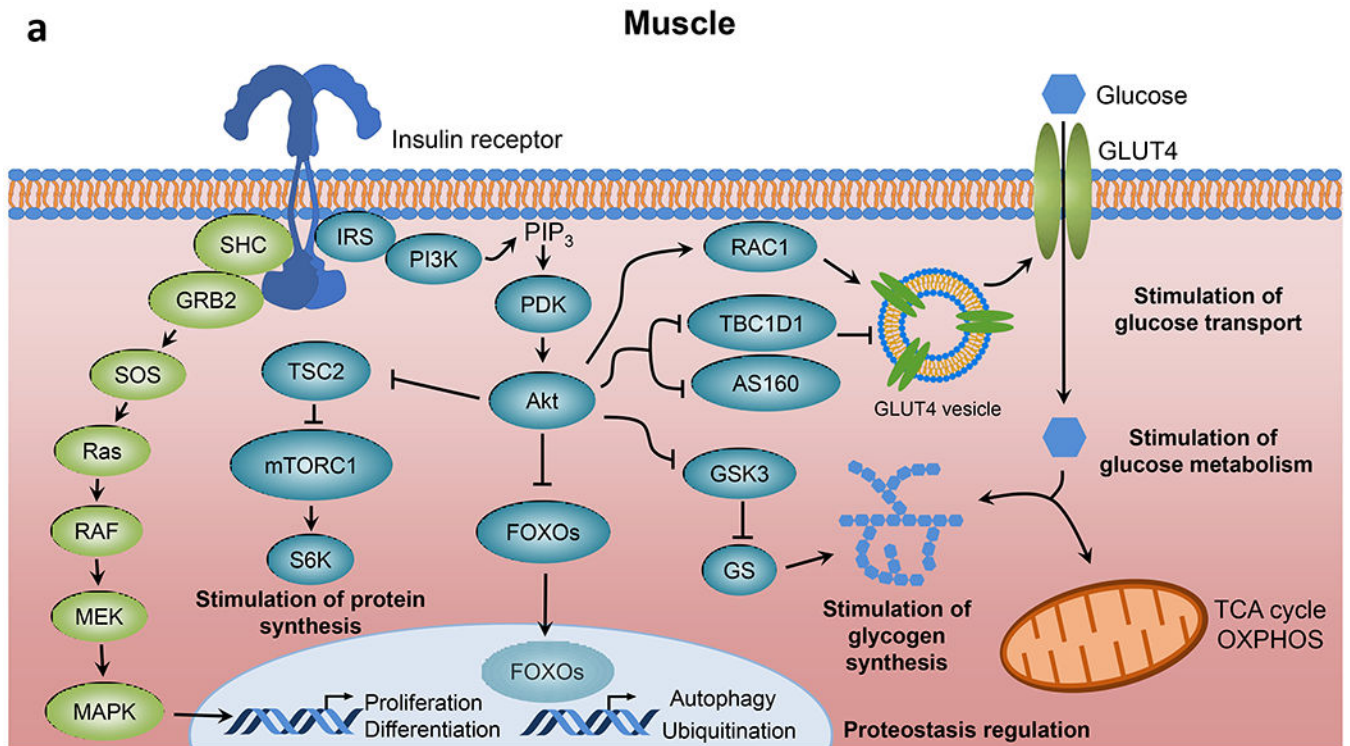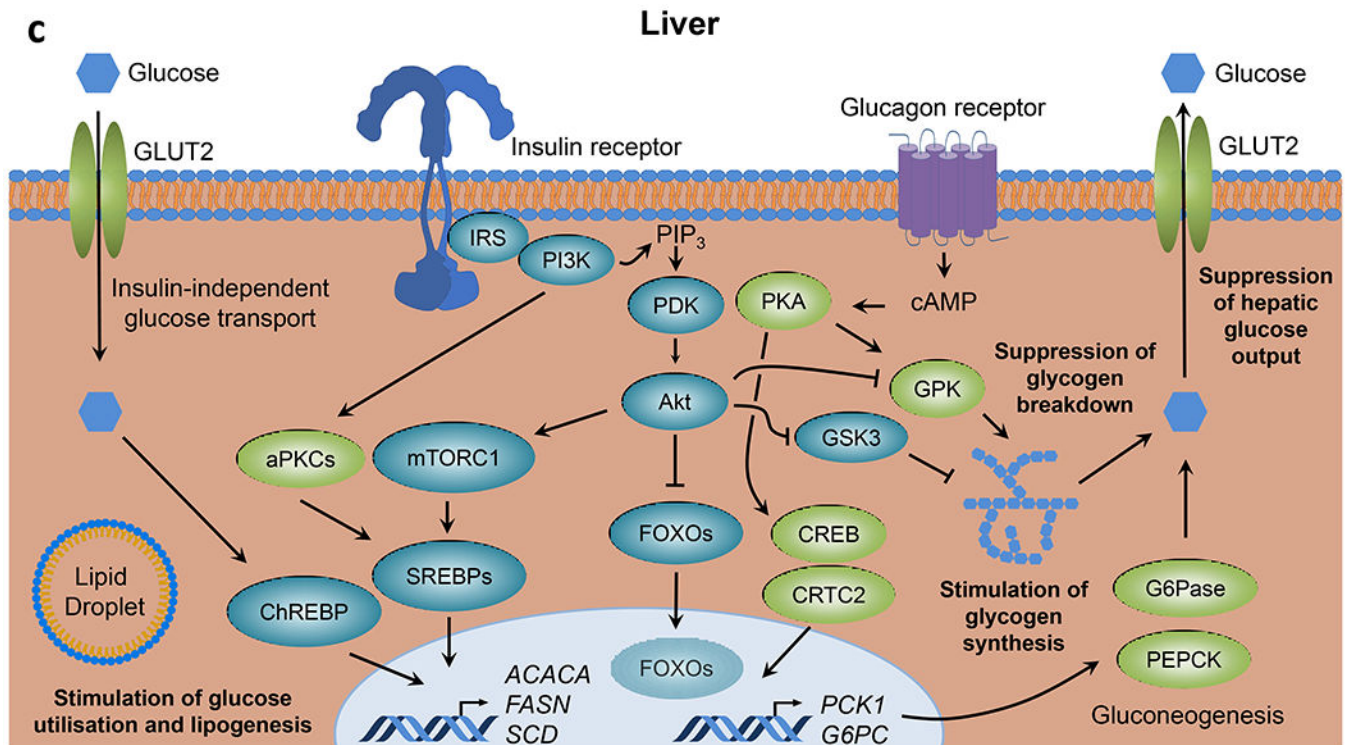
Fig. 1.
Insulin signalling in classical tissues. Insulin binding to the insulin receptor leads to activation of intrinsic tyrosine kinase activity and multisite insulin receptor and IRS phosphorylation. Tyrosine-phosphorylated IRS serves as docking sites for PI3K leading to PIP_3 formation and PDK-dependent Akt activation, which in turn promotes nutrient utilisation, storage and other anabolic processes, and concomitantly suppresses catabolic pathways in (**a**) skeletal muscle, (**b**) adipose tissue and (**c**) liver. aPKC, atypical PKC; ATGL, adipose triglyceride lipase; CAP, Cbl-associated protein; CBL, Cbl proto-oncogene; ChREBP, carbohydrate-responsive element binding protein; CREB, cAMP responsive element binding protein; CRTC2, CREB-regulated transcription coactivator 2; FATP, long-chain fatty acid transport protein; G3P, glyceraldehyde 3-phosphate; G6Pase, glucose-6-phosphatase; GRB2, growth factor receptor bound protein 2; GS, glycogen synthase; HSL, hormone-sensitive lipase; MAPK, mitogen-activated protein kinases; MEK, MAPK kinase; OXPHOS, oxidative phosphorylation; PDE3B, phosphodiesterase 3B; PHK, phosphorylase kinase; PKA, protein kinase A; RAC1, RAC family small GTPase 1; RAF, RAF proto-oncogene serine/threonine kinase; Ras, Ras GTPase; SHC, SH2 domain containing transforming protein; S6K, p70 ribosomal S6 kinase; SOS, son of sevenless homolog; TBC1D1, TBC1 domain family member 1; TC10, Rho-related GTP binding protein RhoQ; TCA, tricarboxylic acid; TSC2, tuberous sclerosis 2. This figure is available as part of a downloadable slideset.
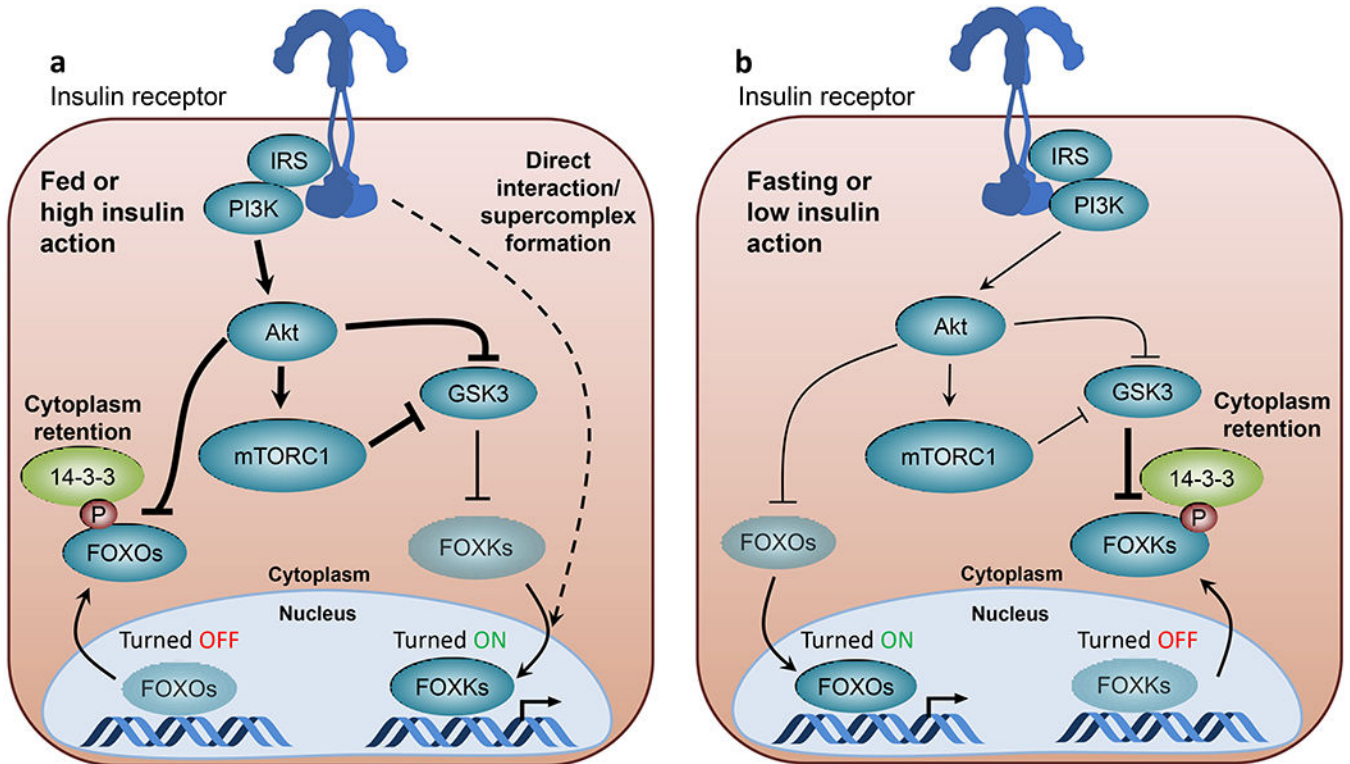
Fig. 2.
Reciprocal regulation of FOX transcription factors by insulin. (**a**) Under feeding or other conditions where insulin action is high, FOXOs are phosphorylated by Akt on serine residues, creating interaction sites for 14-3-3 proteins, leading to cytoplasmic retention and inhibited transcriptional activity. Under these conditions, increased Akt and mTORC1 activity inhibits GSK3 signalling and relieves FOXKs from inhibitory GSK3-mediated phosphorylation, leading to increased nuclear translocation and FOXK transcriptional activity. Since FOXKs and the insulin receptor co-immunoprecipitate together [21], it is possible that some of FOXK's transcriptional effects are due to insulin receptor–FOXK complexes in the nucleus (dotted line). (**b**) Under fasting or other conditions of insulin deficiency or insulin resistance, low activity of PI3K/Akt/mTORC1 pathways results in FOXO hypophosphorylation leading to increased nuclear localisation and transcriptional activity. Under these conditions, increased GSK3 activity leads to increased FOXK phosphorylation and interaction with phosphoserine-binding 14-3-3 proteins, resulting in cytoplasmic retention and decreased transcriptional activity. Line thickness indicates strength of signalling activity, with thicker lines indicating stronger signalling activity. Faded shading of text boxes indicates lower FOXO/FOXK abundance at the nucleus or cytoplasm within in each state, and arrows between the nucleus and cytoplasm indicate the direction of translocation. This figure is available as part of a downloadable slideset.
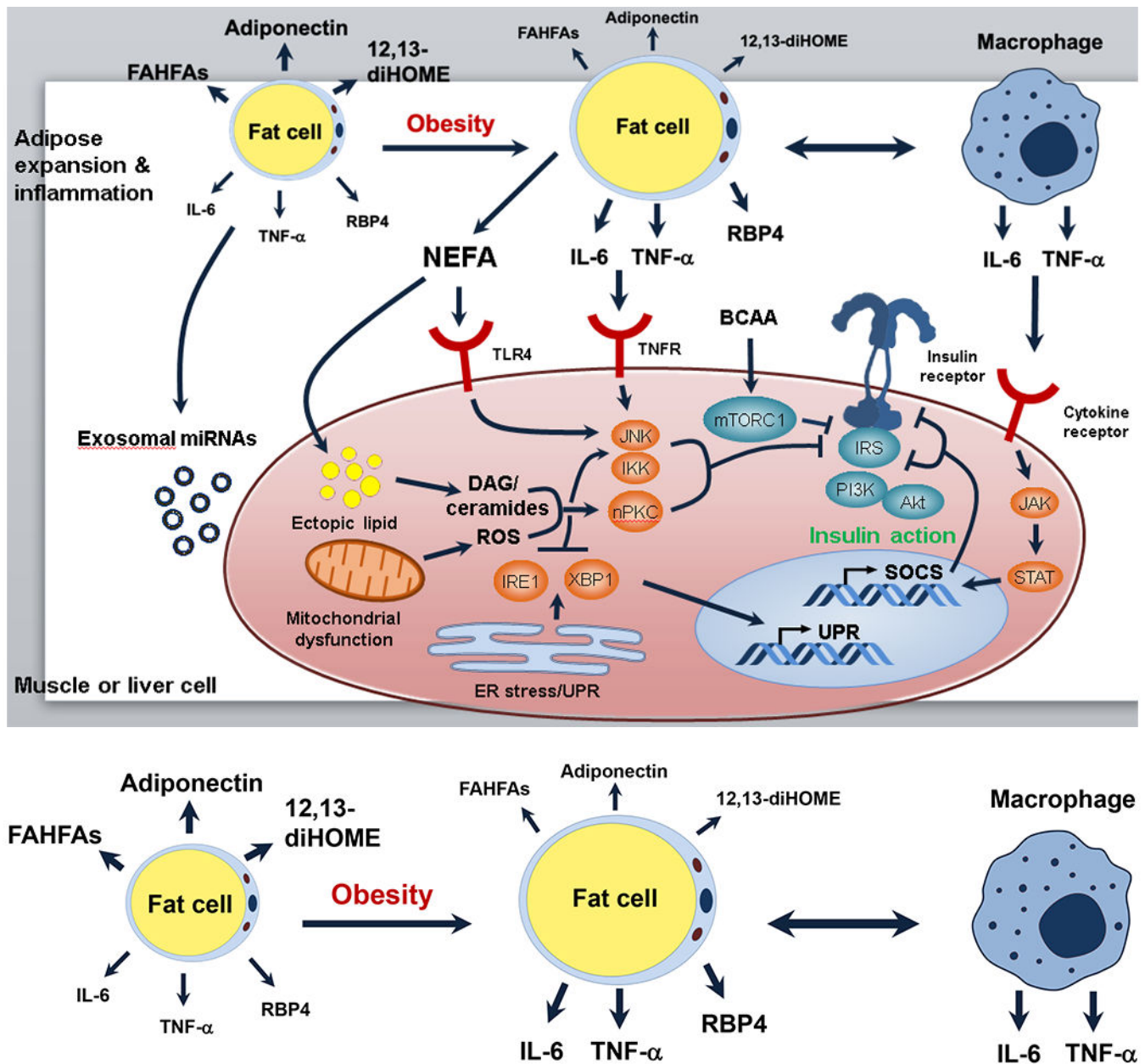
Fig. 3.
Extrinsic factors contributing to insulin resistance. Several environmental factors may
lead to systemic changes affecting multiple tissues and contributing to impaired insulin
signalling. Obesity negatively correlates with circulating levels of adiponectin [118]
and signalling lipids with beneficial properties, such as 12,13-dihydroxy-9Z-octadecenoic
acid (12,13-diHOME) [119] and branched fatty acid esters of hydroxy fatty acids
(FAHFAs) [120]. Overnutrition leads to adipose tissue expansion and increased release
of cytokines and other inflammatory mediators (e.g., retinol binding protein 4 [RBP4])
by macrophages and adipocytes themselves; these mediators bind to cytokine receptors
on metabolic tissues and downregulate proximal insulin signalling due to activation of
Ser/Thr kinases (e.g. JNK, IKK and novel PKCs [nPKCs]) and increased IRS serine

phosphorylation, and due to increased transcription of SOCS proteins, which interfere with IRS tyrosine phosphorylation. Adipose tissue insulin resistance is associated with ectopic lipid accumulation, mitochondrial dysfunction and reactive oxygen species (ROS) generation, and ER stress in insulin-sensitive tissues. All of these mechanisms contribute to activation of Ser/Thr kinases and IRS serine phosphorylation. Adipose tissue expansion in obesity may also have an impact on systemic metabolism through altered release of exosomal miRNAs. Insulin signalling proteins are shown in blue and intracellular mediators of cytokine receptors and other stress signals are shown in orange. Changes in adipocyte lipid/cytokine release during obesity are indicated font/arrow size, with bigger font/thicker arrows representing increased release and smaller font/thinner arrows representing decreased release. DAG, diacylglycerol; IRE1, inositol-requiring enzyme 1; JAK, Janus kinasePI3K, phosphoinositide 3-kinase; STAT, signal transducer and activator of transcription; TLR4, Toll-like receptor 4; TNFR, TNF-α receptor; UPR, unfolded protein response; XBP1, X-box binding protein 1. This figure is available as part of a downloadable slideset.
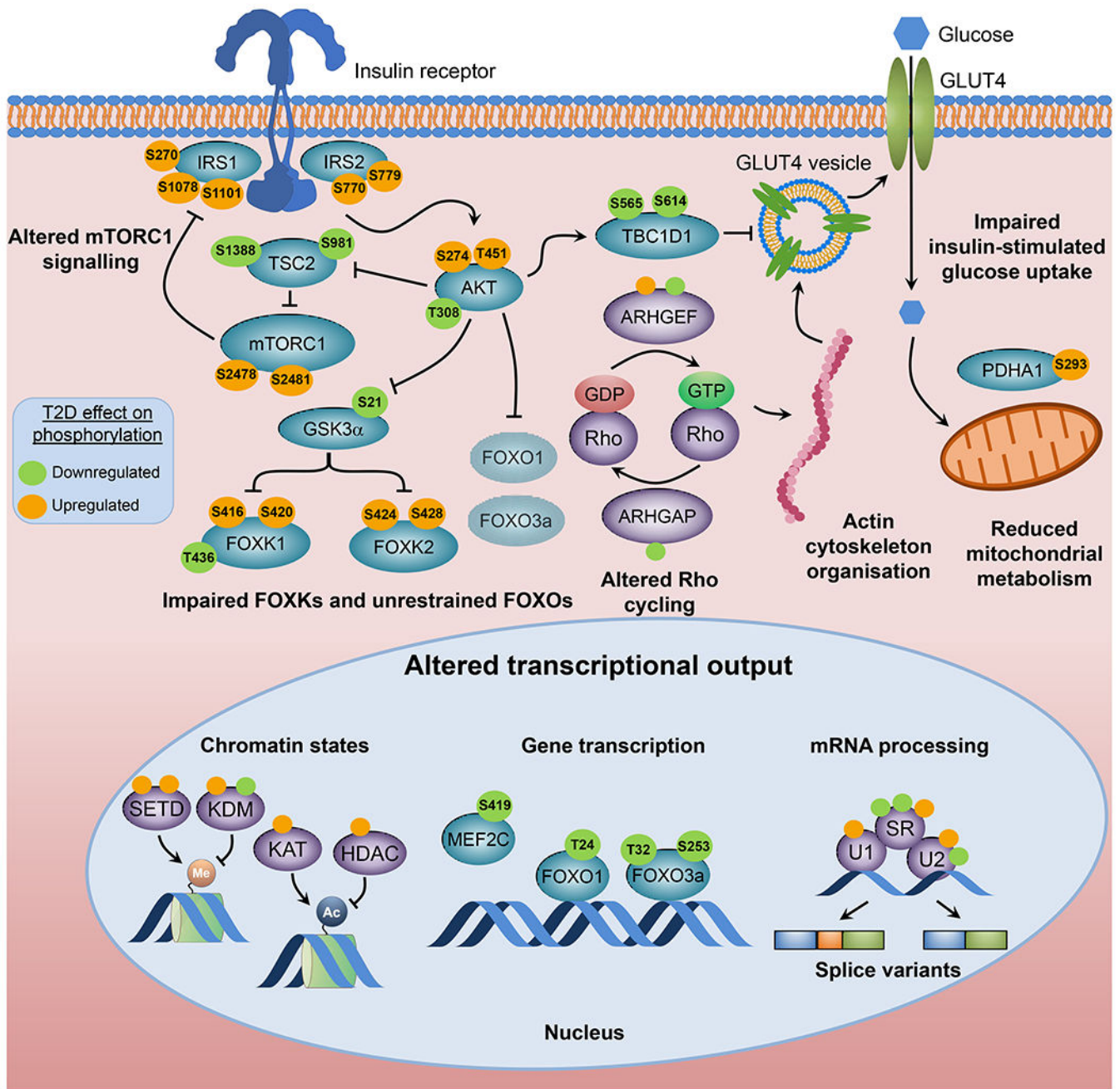
Fig. 4.

Intrinsic factors contributing to insulin resistance. Cell-autonomous insulin resistance is associated with defects in glucose transport, mitochondrial metabolism and insulin signalling. Global phosphoproteomics of iMyos from individuals with type 2 diabetes reveal a network of signalling defects that underlie skeletal muscle insulin resistance [100]. Proteins linked to insulin action and metabolism are indicated in blue and site-specific effects of type 2 diabetes evidenced by increased and decreased basal phosphorylation are shown in orange and green, respectively. Groups of multiple proteins of the same category are shown in purple and non-labelled circles indicate groups of up- or downregulated

phosphosites. All signalling events are derived from MS-based phosphoproteomics except for Akt^{T308}, GSK3α^{S21} and FOXO1^{T24}/FOXO3^{T32}, which are from immunoblot analysis. Faded shading of text boxes indicates lower cytoplasm abundance. Ac, acetyl group; ARHGAP, Rho GTPase activating protein; ARHGEF, Rho guanine nucleotide exchange factor; HDAC, histone deacetylase; KAT, lysine acetyltransferase; KDM, lysine demethylase; Me, methyl group; MEF2C, myocyte enhancer factor 2C; PDHA1, pyruvate dehydrogenase E1 subunit alpha 1; SETD, SET domain containing histone lysine methyltransferase; SR, serine- and arginine-rich splicing factor; T2D, type 2 diabetes; TBC1D1, TBC1 domain family member 1; TSC2, tuberous sclerosis 2; U1, U1 small nuclear ribonucleoprotein complex; U2, U2 small nuclear ribonucleoprotein complex. This figure is available as part of a downloadable slideset.