Molecular mechanisms of insulin resistance in type 2 diabetes mellitus

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Free fatty acids are known to play a key role in promoting loss of insulin sensitivity in type 2 diabetes mellitus but the underlying mechanism is still unclear. It has been postulated that an increase in the intracellular concentration of fatty acid metabolites activates a serine kinase cascade, which leads to defects in insulin signaling downstream to the insulin receptor. In addition, the complex network of adipokines released from adipose tissue modulates the response of tissues to insulin. Among the many molecules involved in the intracellular processing of the signal provided by insulin, the insulin receptor substrate-2, the protein kinase B and the forkhead transcription factor Foxo 1a are of particular interest, as recent data has provided strong evidence that dysfunction of these proteins results in insulin resistance in vivo. Recently, studies have revealed that phosphoinositide-dependent kinase 1-independent phosphorylation of protein kinase Cε causes a reduction in insulin receptor gene expression. Additionally, it has been suggested that mitochondrial dysfunction triggers activation of several serine kinases, and weakens insulin signal transduction. Thus, in this review, the current developments in understanding the pathophysiological processes of insulin resistance in type 2 diabetes have been summarized. In addition, this study provides potential new targets for the treatment and prevention of type 2 diabetes.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder in man, currently affecting over 170 million people world-wide and, potentially, over 365 million in the year 2030[1]. Type 2 DM is rapidly emerging as one of the greatest global health challenges of the 21st century. This looming epidemic is also expected to trigger a steep rise in the complications associated with diabetes, such as ischemic heart disease, stroke, neuropathy, retinopathy, and nephropathy. Besides β cell failure, the major pathophysiological event contributing to the development of type 2 DM is the resistance of target tissues to insulin, which is usually associated with abnormal insulin secretion. Clinically, the term “insulin resistance” implies that higher-than-normal concentrations of insulin are required to maintain normoglycemia. On a cellular level, it defines the inadequate strength of insulin signaling from the insulin receptor downstream to the final substrates of insulin action involved in multiple metabolic and mitogenic aspects of cellular function[1].

The pathogenesis of type 2 diabetes involves abnor-
malities in both insulin action and secretion. Although the precise pathophysiological sequence which leads to insulin resistance is still largely unknown, recent studies have contributed to a deeper understanding of the underlying molecular mechanisms. This review deals with the mechanisms related to type 2 diabetes. A detailed understanding of these basic pathophysiological mechanisms is critical for the development of novel therapeutic strategies to treat diabetes.

NORMAL INSULIN SIGNALING

The insulin receptor (IR) is a heterotetramer consisting of two α subunits and two β subunits that are linked by disulphide bonds. Insulin binds to the α subunit of the insulin receptor and activates the tyrosine kinase in the β subunit. Once the tyrosine kinase of insulin receptor is activated, it promotes autophosphorylation of the β subunit, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity. Most of the metabolic and antiapoptotic effects of insulin are mediated by the signaling pathway involving the phosphorylation of the insulin receptor substrate (IRS) proteins, and the activation of the phosphatidylinositol (PI) 3-kinase, Akt (also known as protein kinase B), the molecular target of rapamycin (mTOR), and p70 S6 kinase. The insulin receptor tyrosine kinase phosphorylates the IRS proteins, and phosphotyrosine residues on IRS proteins become good targets for the p85 regulatory subunit of PI3-kinase. The activated PI3-kinase generates 3′-phosphoinositides [phosphatidylinositol-3,4,5-trisphosphate (PIP3)] and phosphatidylinositol-3,4-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-trisphosphate (PIP3)].

PKB

Downstream from PI3-kinase, the serine/threonine kinase Akt (also called PKB), triggers insulin effects on the liver, such as glycogen synthesis and the suppression of hepatic glucose production. Akt plays an important role by linking glucose transporter (GLUT4), the insulin-dependent glucose transporter protein, to the insulin signaling pathway. It activates GLUT4 which moves to the cell surface to transport glucose into the cell. Recent data from PKB knockout animal models offer a clearer answer to the question of whether PKB is required for normal glucose homeostasis. While disruption of PKB/Akt1 isoform in mice did not cause any significant perturbations in metabolism, mice with a knock-out of the PKB (Akt2) isoform show insulin resistance, ending up with a phenotype closely resembling type 2 diabetes in humans.

CONSEQUENCES OF IRS MUTATIONS

In humans, rare mutations of the IRS-1 protein are associated with insulin resistance. Disruption of the IRS-1 gene in mice results in insulin resistance, mainly of muscle and fat. Recent results are obtained by studying IRS in knockout mice. Heterozygous knockout mice lacking a single allele of IRS-1 gene lack any significant phenotype, whereas homozygous disruption of the IRS-1 gene results in a mild form of insulin resistance. IRS-1 homozygous null mice (IRS-1−/−) do not show a clear diabetic phenotypic expression, presumably because of pancreatic β cell compensation. IRS-2−/− mice, on the other hand, developed diabetes as a result of severe insulin resistance paired with β cell failure.

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INCREASE IN SERINE PHOSPHORYLATION OF IRS PROTEINS

Recent studies have demonstrated hyper-serine phosphorylation of IRS-1 on Ser612, Ser616, and Ser632 in several insulin-resistant rodent models as well as in young lean insulin-resistant offspring of type 2 diabetic parents. Further evidence for this hypothesis stems from recent studies in a muscle-specific triple serine to alanine mutant mouse (IRS-1 Ser → Ala612, Ser → Ala616, and Ser → Ala632), which has been shown to be protected from high-fat diet-induced insulin resistance in vivo. Based on in vitro studies, serine phosphorylation may lead to dissociation between the insulin receptor/IRS-1 and/or IRS-1/PI3-kinase, preventing PI3-kinase activation or increased degradation of IRS-1. Furthermore, there are data linking IRS dysfunction in skeletal muscle to adipocyte biology and lipotoxicity. For example, circulating free fatty acids (FFA) and the adipokine tumour necrosis factor (TNF) may increase serine phosphorylation of IRS proteins, thereby causing impaired insulin signal transduction.
The fasting hyperglycemia in patients with type 2 diabetes is the clinical correlate of the increased glucose production by the liver because of insulin resistance. This is the result of the lack of inhibition of the two key gluconeogenic enzymes, PEPCK and G6Pase, catalytic subunit. There is increasing evidence that Foxo-proteins are critically involved in the insulin dependent regulation of gluconeogenic gene expression and insulin-resistance \[38,39\]. Studies in hepatoma cells \[40,41\] suggest that transcription of reporter genes containing insulin response elements from the PEPCK and G6Pase promoters are regulated by forkhead box protein o (Foxo)-1 and 3. Furthermore, Foxo1 is phosphorylated in an insulin-responsive manner by Akt. Reduced activity of Akt2 results in decreased phosphorylation of Foxo protein, allowing it to enter the nucleus and activate the transcription of these rate-controlling enzymes of gluconeogenesis \[40,42\].

**PI3 KINASE**

A molecular mechanism that may potentially lead to insulin resistance is a disruption in the balance between the amounts of the PI3-kinase subunits \[43\]. The PI3-kinase family is divided into three different classes, of which class 1a \[44\] exists as heterodimers, consisting of a regulatory subunit p85, which is tightly associated

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**Figure 1** Insulin signaling pathway showing binding of insulin with the insulin receptor leading to the activation of glucose transporter 4 which imports glucose into the cell. Binding of insulin to the IR activates PI3-k which produces PIP3 and PI3, 4, 5P3. These serve as docking sites for PDK1 which then mediates activation of PKB. Activated PKB can regulate transcription of target genes-PEPCK and G6Pase via Foxo-1. Increased free fatty acids may cause serine phosphorylation of IRS proteins, which in turn decreases IRS-tyrosine phosphorylation, impairing downstream effectors. pY: phosphorylated tyrosine; IR: insulin receptor; IRS: insulin receptor protein; PI3-k: phosphatidylinositol 3-kinase; PDK1: phosphoinositidedependent kinase 1; PKB: protein kinase B; Foxo-1: forkhead box protein o; PEPCK: phosphoenolpyruvate carboxykinase; G6Pase: glucose-6-phosphatase; FFA: free fatty acids; PIP2: phosphatidylinositol-3,4-bisphosphate; PIP3: phosphatidylinositol-3,4,5-tris-phosphate; GLUT4: glucose transporter 4.

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**Table 1** Causes of insulin receptor substrate-1 serine phosphorylation

| mTOR | p70S6 kinase |
|------|--------------|
|      | Amino acids  |
|      | Hyperinsulinemia |
|      | JNK          |
|      | Stress       |
|      | Hyperlipidemia |
|      | Inflammation |
|      | IKK          |
|      | Inflammation |
|      | TNF-α        |
|      | Obesity      |
|      | Inflammation |
|      | Mitochondrial dysfunction |
|      | PKCα         |
|      | Hyperglycemia |
|      | Diacylglycerol |
|      | Inflammation |

mTOR: molecular target of rapamycin; JNK: c-Jun N-terminal kinase; IKK: IkB kinase; TNF: tumour necrosis factor; PKC: protein kinase C.
PKC

The underlying mechanism of FFA-induced impairment of insulin signals is still unclear. The molecular mechanism underlying defective insulin-stimulated glucose transport activity can be attributed to increases in intramyocellular lipid metabolites such as fatty acyl CoAs and diacylglycerol, which in turn activate a serine/threonine kinase cascade, thus leading to defects in insulin signaling through the Ser/Thr phosphorylation of the insulin receptor substrate-1. Diacylglycerol (DAG) has been shown to increase in muscle during both lipid infusions and fat feeding and it is also a known activator of novel PKC isoforms. Some of the PKC isoforms represent such signaling molecules. PKC isoforms are classified as classical (cPKCs), novel (nPKCs), and atypical (aPKCs). cPKCs are activated by Ca\(^{2+}\) and DAG, nPKCs are activated only by DAG and aPKCs respond to neither Ca\(^{2+}\) nor DAG. Among all these PKC isoforms, nPKCs are said to have a modulatory role in insulin signaling. Recent reports also demonstrate a link between nPKCs and FFA induced insulin resistance; lipid infusion in rats and humans impaired insulin-stimulated glucose disposal into the muscle and concomitantly activated PKC\(\theta\) and PKC\(\delta\). The latter has been shown to be a possible candidate for phosphorylation of the IR on serine residue, resulting in defects in the insulin signaling pathway and imposing insulin resistance.

Clearly, the IR is one of the major targets in FFA-induced impairment of insulin activity. Studies performed in vivo have suggested that glucose uptake rather than intracellular glucose metabolism is the rate-limiting step for fatty acid-induced insulin resistance in humans. This indicates a mechanism in which accumulation of intracellular fatty acids or their metabolites results in an impairment of signaling through the IRS/PI3-kinase and a decrease in the recruitment of GLUT4 transporters to the cell membrane.

PDK1 can directly phosphorylate all PKCs including nPKCs. The PKC\(\epsilon\) isotype has been shown to be related to insulin resistance. PKC\(\epsilon\) has also shown PDK1-independent phosphorylation due to FFA. This may be due to constitutive phosphorylation of PKC\(\epsilon\) by FFA in a PDK1-independent manner. It was shown that myristic acid incubation of HEPG2 cells causes myristoylation of PKC\(\epsilon\) which results in constitutive phosphorylation of PKC\(\epsilon\) at thr566/ser729 in the kinase domain required for PKC\(\epsilon\) activity. This phosphorylation was totally independent of PDK1, which was demonstrated by the researchers by using PDK1 knockout cells. In the same way, addition of palmitate to skeletal muscle cells or adipocytes may affect palmitoylation of PKC\(\epsilon\), resulting in constitutive phosphorylation of PKC\(\epsilon\). Taken together, it is clear that FFA causes PDK1-independent phosphorylation of PKC\(\epsilon\), which in turn translocates to the nucleus, and its time of entry into the nucleus coincides with the inhibition of IR gene transcription.

MOLECULAR MECHANISM OF INHIBITION OF IR GENE TRANSCRIPTION

In order to understand the molecular basis of the regulation of IR gene expression, the promoter region of the human IR gene has been identified and studied by several groups. Two unique AT-rich sequences, C2 and E3, within the IR gene promoter have been identified, and both these sequences are positively regulated by transcription factor HMGAI (earlier known as HMGI-Y). HMGAI interacts with the AT-rich regions and regulates transcriptional activation of many genes by modifying DNA conformation, which permits recruitment of transcriptional factor to the transcription start site. HMGAI induces transcriptional activation of the human IR gene by permitting the recruitment of SP1 and EBP, the ubiquitously expressed transcription factors, to the promoter region. A recent report demonstrates that a genetic flaw which reduces the intracellular expression of HMGAI protein can adversely affect IR expression in cells and tissues from subjects with insulin resistance and type 2 diabetes. There is also a possibility that activated PKC\(\epsilon\) phosphorylates HMGAI, which inhibits its mobilization to the promoter region IR gene. It has been shown that phosphorylation of the HMGAI protein reduces its DNA-binding ability. Without the mobilization of HMGAI to the IR promoter there is no recruitment of additional transcription factors to the promoter region of the IR gene and therefore no expression of the IR gene.
PGC-1
The PPARγ co-activator-1 (PGC-1) has been recognized as playing a major role in glucose homeostasis of the organism. Work mainly by Spiegelman’s group demonstrated the crucial role of PGC-1 in the regulation of GLUT4 gene expression in muscle cells. They showed that PGC-1 powerfully induces the expression of the endogenous GLUT4 gene in cultured myotubes, resulting in expression comparable to that seen in muscle in vivo. In addition, PGC-1, a factor integrating the effects of glucocorticoids and cAMP on gluconeogenic gene expression in the liver is also regulated by Akt and Foxo-1. PGC-1 may also play a role in the regulation of genes involved in the process of oxidative phosphorylation which commonly show reduced expression in the muscles of diabetic patients.

OTHER CAUSES OF INSULIN RESISTANCE
Mitochondrial dysfunction
It has been known for many years that severe mitochondrial dysfunction can result in diabetes. In a study using 31P MRS, it was found that in healthy lean elderly volunteers with severe muscle insulin resistance, there is a 40% reduction in the rates of oxidative phosphorylation activity associated with increased intramyocellular and intrahepatic lipid content. This study suggests that an acquired loss of mitochondrial function associated with aging predisposes elderly subjects to intramyocellular lipid accumulation, which results in insulin resistance. Further, it was found that mitochondrial density was reduced by 38%, intramyocellular lipid content was increased by 60% and serine phosphorylation of IRS-1 was increased by 50% in the young insulin-resistant offspring of type 2 diabetes parents.

Adipokines
Insulin has three major target tissues-skeletal muscle, adipose tissue and the liver. Not only is IR overexpressed in the cells of these tissues, but these are also the three places where glucose is deposited and stored; no other tissue can store glucose. About 75% of insulin-dependent postprandial glucose disposal occurs into the skeletal muscle; it is therefore the major target organ. Patients suffering from insulin resistance and type 2 diabetes frequently display signs of abnormal lipid metabolism, increased circulatory concentration and elevated deposition of lipids in the skeletal muscle. Increase in plasma FFA reduces insulin-stimulated glucose uptake, whereas a decrease in plasma lipid content improves insulin activity in the skeletal muscle cells, adipocytes and liver. Studies have shown that raising plasma fatty acids in both rodents and humans abolishes insulin activation of IRS-1-associated PI3-kinase activity in skeletal muscle where IRS-1 is most prevalent. Lipid-associated insulin resistance has also been shown to be linked to GLUT4 translocation defects.

Adipose tissue also acts as an endocrine organ producing adipokines which modulate glucose homeostasis. Currently, those most intensely discussed are TNF-α, leptin, adiponectin and resistin. At a molecular level, TNF-α increases serine phosphorylation of IRS-1 and down-regulates GLUT4 expression, thereby contributing to insulin resistance. Furthermore, mice lacking functional TNF-α were protected from obesity-induced insulin resistance. The role of leptin in regulating food intake and energy expenditure is well established. Humans with leptin deficiency or leptin receptor mutations are severely obese. In addition, it has direct effects on insulin sensitivity and may also reverse insulin resistance in mice with congenital lipodystrophy. Adiponectin has insulin-sensitizing effects, as it enhances inhibition of hepatic glucose output as well as glucose uptake and utilization in fat and muscle. The expression of adiponectin is decreased in obese humans and mice. Thus, in humans, adiponectin levels correlate with insulin sensitivity. Because of its insulin-antagonistic effects, the adipokine resistin has attracted a lot of primarily preclinical research interest. Resistin decreases insulin-dependent glucose transport in vitro and increases fasting blood glucose concentrations and hepatic glucose production.

CONCLUSION
In this review, we have summarized current developments contributing to our understanding of insulin resistance, and to the pathogenesis of type 2 diabetes. Among the many molecules involved in the intracellular processing of the signal provided by insulin, IRS-2, PKB, the Foxo protein and p85 regulatory subunit of PI-3 kinase have attracted particular interest, because their dysfunction results in insulin resistance. The identification of signaling defects and an understanding of the complex relationship of the different factors modulating insulin sensitivity is an important prerequisite for the development of novel and more specific anti-diabetic compounds. By elucidating the cellular and molecular mechanisms responsible for insulin resistance, these studies provide potential new targets for the treatment and prevention of type 2 diabetes.

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July 15, 2010 | Volume 1 | Issue 3 |
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