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PEDF attenuates insulin-dependent molecular pathways of glucose homeostasis in skeletal myocytes

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ABSTRACT

Pigment epithelium-derived factor (PEDF) is an anti-angiogenic serpin associated with insulin resistance in metabolic disorders such as diabetes, metabolic syndrome, obesity and polycystic ovarian syndrome. While the mechanism of PEDF induced-insulin resistance of metabolic disorders has been attributed to its inflammatory and lipolytic effects, little evidence exists to support a direct role of PEDF in mediating insulin resistance. Here, we seminally provide evidence that PEDF can inhibit insulin signal transduction governing glucose homeostasis from the receptor to the effector phosphorylation through Akt/PKB-dependent and -independent pathways in mouse and human skeletal muscle cell lines. PEDF attenuates the insulin-dependent molecular axes of glucose metabolism. Exposure of skeletal myocytes to PEDF attenuates insulin-dependent insulin receptor autophosphorylation, tyrosine phosphorylation of insulin receptor substrate 1, and dual loop phosphorylation-activation of Akt. PEDF significantly inhibits the downstream effector - glycogen synthase kinase (and thereby the glycogenic axis of insulin signalling). PEDF turned off both the molecular switches of GLUT4 translocation: IRS-Akt/PKB-AS160 mediated and IR-pCbl-dependent GLUT4 translocation (the molecular axis of glucose uptake). These findings implicate a direct effect of PEDF on multiple insulin-dependent molecular mechanisms of glucose homeostasis in skeletal muscle cells, thereby enabling it to contribute to peripheral insulin resistance at the cellular level.

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1. Introduction

Insulin resistance is the reduced capacity of endogenous insulin to elicit its response of glucose uptake and metabolism in target tissues such as liver, fat and skeletal muscle (Moller and Flier, 1991). It is also a ubiquitous correlate of obesity and a central component of metabolic syndrome (DeFronzo and Ferrannini, 2001). Skeletal muscle is the predominant insulin-responsive peripheral site of glucose metabolism and plays a crucial role in maintaining systemic glucose homeostasis through interactive cross-talk with hepatic and adipose tissues (DeFronzo and Tripathy, 2009). Skeletal muscle insulin resistance impacts whole body glucose homeostasis and is a consequence of impaired signalling events - defective post-receptor mechanisms that mediate various events of glucose metabolism and precedes the clinical diagnosis of metabolic diseases (Vaag et al., 1992).

The signalling pathways involved in insulin-dependent glucose metabolism are well characterised in skeletal muscle and can be dissected into two molecular axes: one governing glycogenesis and the other governing GLUT4 translocation (Carnagarin et al., 2015a, b). The stimulation of glycogen synthesis from glucose involves phosphatidylinositol-3-kinase (PI3K) activation through association with insulin receptor substrate 1 (IRS1), tyrosine phosphorylated by the activated insulin receptor (IR) (White et al., 1998). IRS1 is the predominant metabolic signal adaptor molecule involved in muscle (Yamauchi et al., 1996). PI3K catalyses the formation of phosphatidylinositol-3,4,5-trisphosphate which leads to the activation of Akt (v-akt murine thymoma viral oncogene homolog, also known as PKB or RAC kinase), through sequential phosphorylation on Thr-308 and Ser-473 by PDK1 and PDK2 (mammalian target of rapamycin complex 2 (MTORC2) in complex with Rictor and Sin),

Abbreviations: AS-160, akt substrate-160; Cbl, casitas B-lineage lymphoma; GSK, glycogen synthase kinase; IR, insulin receptor; IRS, insulin receptor substrate; PEDF, pigment epithelium-derived factor; PKB, protein kinase B.

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respectively (Alessi and Cohen, 1998). Akt in turn phosphorylates and inhibits glycogen synthase kinase (GSK), the most important kinase regulating the activity of glycogen synthase through phosphorylation-inhibition (Skurat and Roach, 1996).

The other rate-limiting step determining insulin resistance is glucose transport, mediated by increased translocation of GLUT4 glucose transporter levels (the predominant GLUT isoform in skeletal muscle). Two independent signalling pathways - tyrosine phosphorylation of Cbl (casitas b-lineage lymphoma) directly by the activated IR (Ribon et al., 1998), along with Akt-dependent phosphorylation-inactivation of AS160 (Akt substrate 160), have evolved as the predominant pathways of insulin-dependent glucose uptake mediated by GLUTs (Saltiel and Kahn, 2001). Thereby, defective glycogen synthesis or GLUT4 translocation play a dominant role in insulin resistance. A schematic overview of insulin-dependent molecular events involved in glucose homeostasis of skeletal muscle is depicted in Fig. 1.

PEDF is associated with insulin resistance in major metabolic disorders, endocrine, cardiovascular, hepatic and inflammatory disorders (Carnagarin et al., 2015a, b). PEDF is a multifunctional serpin with therapeutic potential due to its antiangiogenic property (Dawson et al., 1999; Tombran-Tink et al., 1991). PEDF levels are elevated in metabolic disorders and in insulin-resistant states in humans such as type 2 diabetes mellitus (Nakamura et al., 2010), obesity (Crowe et al., 2009), metabolic syndrome (Chen et al., 2010; Yamagishi et al., 2006), polycystic ovarian syndrome (PCOS) (Yang et al., 2011) and hepatic disorders (Yamagishi et al., 2010). Clinically, PEDF is positively correlated with several metabolic risk factors such a high body mass index (BMI), waist circumference, elevated triglycerides, glucose, and insulin, and is negatively correlated with circulating high density lipoprotein levels (Sabater et al., 2010). PEDF is an independent determinant of insulin resistance in patients with essential hypertension (Nakamura et al., 2010) and is associated with cardiovascular mortality and morbidity in humans (Rychli et al., 2009).

In the context of PEDF-mediated insulin resistance, the role of PEDF is evolving and is identified as a result of its lipolytic and inflammatory effects. While studies have shown that PEDF impairs insulin-stimulated glucose uptake and disposal in skeletal muscle and reduced the whole-body insulin sensitivity as measured by hyperinsulinemic-euglycemic clamps (Crowe et al.), investigations into molecular signalling mechanisms responsible for these metabolic responses of skeletal muscle are yet be studied. There is no evidence to date suggesting a direct role of PEDF in cellular insulin resistance at the molecular level. The present study examines the effect of PEDF on insulin signal transduction in skeletal muscle cells and demonstrates that PEDF can cause cellular insulin resistance across species. PEDF is shown to exert inhibitory effect right from IR autophosphorylation to the effector response of GSK3β phosphorylation and GLUT4 translocation in an AKT/PKB-dependent and -independent manner. These data are the first characterisation of PEDF-induced insulin resistance at the cellular level.

2. Methods

2.1. Materials

Recombinant human PEDF and PEDF polyclonal rabbit antibody were purchased from MD Bioproducts (Bethesda, MD, USA) and PEDF was dissolved in water to a 0.5 mg/ml stock solution. Fetal calf serum was purchased from Gibco (Fort Worth, TX, USA), human recombinant insulin, Dulbecco's Modified Eagle's Medium, penicillin/streptomycin and anti-GLUT4 antibody were obtained from Sigma–Aldrich (St Louis, MO, USA). The universal DNA ladder kit was purchased from KAPA Biosystems (Wilmington, MA, USA) and PCR master mix, Alexa Fluor® 594 F(ab')2 goat anti-rabbit IgG, Pierce 660 nm protein assay from Thermo Fisher Scientific (Waltham, Massachusetts, USA). PEDF siRNA (m), siRNA dilution buffer, control siRNA-A and siRNA-B and all RT-PCR primers and antibodies: IRS-1, pIRS-1(tyr 632) and insulin Rβ, p-insulin Rβ (tyr 1162/1163) and pCBL (Tyr 700)-R were purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA). PNPLA2 from EMD Millipore Corporation (Temecula, CA, USA). Pan-Akt, phospho-Akt(Ser473), phospho-Akt(Thr308), phospho-Gsk-3β(Ser9) and LY294002 (PI3K inhibitor) and secondary anti-rabbit IgG, HRP-linked antibody and phospho-AS160 (Thr642) from Cell Signaling

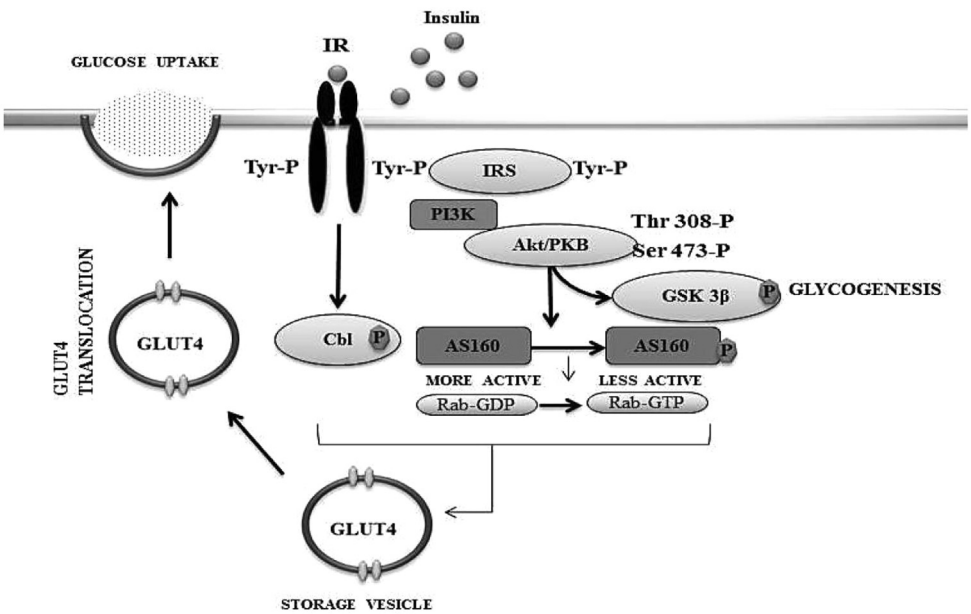

Fig. 1. Schematic overview of the insulin mediated molecular events involved in glucose homeostasis. Insulin binds and activates IR which tyrosine-phosphorylates IRS to initiate the dual critical axes of glycogenesis – IR/IRS/Akt/GSK3β and GLUT4 translocation-IR/IRS/Akt/As160/GLUT4 and IR/Cbl/GLUT4.

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