Monoclonal antibody to six transmembrane epithelial antigen of prostate-4 influences insulin sensitivity by attenuating phosphorylation of P13K (P85) and Akt: Possible mitochondrial mechanism

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Abstract We examined the effects of anti-six-transmembrane epithelial antigen of the prostate-4 (STEAP4) antibodies on glucose transport in mature adipocytes and determined the mechanism of insulin resistance in obesity. Western blotting was performed to determine STEAP4 expression, to assess translocation of insulin-sensitive glucose transporter 4 (GLUT4), and to measure phosphorylation and total protein content of insulin-signaling proteins. Confocal laser microscopy and flow cytometry were used to detect intracellular reactive oxygen species (ROS) and fluctuations in mitochondrial membrane potential (ΔΨ). ATP production was measured by using a luciferase-based luminescence assay kit. After the application of anti-STEAP4 antibodies at 0.002 mg/mL, adipocytes exhibited reduced insulin-stimulated glucose transport by attenuating the phosphorylation of IRS-1, PI3K (p85), and Akt. The antibodies also potentially increase the level of ROS and decrease cellular ATP production and ΔΨ. In conclusion, (i) STEAP4 regulates the function of IRS-1, PI3K, and Akt and decreases insulin-induced GLUT4 translocation and glucose uptake; (ii) ROS-related mitochondrial dysfunction may be related to a reduced IRS-1 correlation with the PI3K signaling pathway, leading to insulin resistance. These observations highlight the potential role of STEAP4 in glucose homeostasis and possibly in the pathophysiology of type 2 diabetes related to obesity and may provide new insights into the mechanisms of insulin resistance in obesity.

Keywords STEAP4 antibody · Obesity · Insulin sensitivity · Insulin receptor substrate-1 · Phosphatidylinositol-3-kinase

Abbreviations

STEAP4 six-transmembrane epithelial antigen of the prostate 4
GLUT4 glucose transporter 4
IR insulin receptor
IRS insulin receptor substrate
PI3K phosphatidylinositol-3-kinase
ERK1/2 extracellular signal-regulated kinases 1 and 2
JNK c-Jun N-terminal kinase
PM Plasma membrane
MAPK mitogen-activated protein kinase
ROS reactive oxygen species

Introduction

The six-transmembrane epithelial antigen of the prostate 4 (STEAP4; also referred to as STAMP2 and
TIARP), a member of the six-transmembrane proteins family, has attracted considerable interest in obesity and energy metabolism research (Alessi et al. 1996; Andjelković et al. 1996; Andjelković et al. 1997; Amer et al. 2008). Biochemical and secondary structural analyses suggest that this protein could potentially function as a channel, receptor, or transporter protein; however, its true function has not yet been clarified. In STAMP2−/− mice, the visceral adipose tissue exhibits overt inflammation, thereby manifesting insulin resistance, glucose intolerance, mild hyperglycemia, dyslipidemia, and fatty liver disease (Andjelković et al. 1996). Our previous data showed that the human STEAP4 gene is highly expressed in omental adipose tissue and participates in obesity development (Boura-Halfon and Zick 2008). Recently, we also found that over-expression of the STEAP4 gene promoted insulin-stimulated glucose uptake in mature human adipocytes (Challita-Eid et al. 2007). Taken together, the data indicate that this gene may influence insulin sensitivity; however, the mechanisms underlying insulin sensitivity are only partially understood. Therefore, additional research on the STEAP4 function is necessary and will aid in our efforts to study the STEAP4 mechanism and how it influences insulin sensitivity.

Monoclonal antibodies have been used widely in clinical and experimental studies (Chen et al. 2010; Corvera and Czech 1998; Dani et al. 2010a). In addition, immunohistochemical analysis has confirmed that STEAP4 was associated with adipocyte plasma membranes (Boura-Halfon and Zick 2008), and therefore, provides a basis for our decision to use antibodies against human adipocytes. In recent studies, the generated monoclonal antibodies had bound to the extracellular domains of human STEAP4, suggesting that these antibodies may function to promote or inhibit STEAP4 function (Dani et al. 2010b). In this study, we examined the effects of anti-STEAP4 antibodies on glucose transport in mature adipocytes and determined the molecular events underlying these effects. The results suggested that upon anti-STEAP4 antibody exposure at 0.002 mg/mL, adipocytes reduced insulin-stimulated glucose transport (Ducluzeau et al. 2002) by attenuating phosphorylation of the insulin receptor substrate (IRS)-1, phosphatidylinositol-3-kinase (PI3K) p85, and Akt. Meanwhile, it also increases the intracellular reactive oxygen species (ROS), while reducing cellular ATP production and mitochondrial ΔΨ. Thus, the mechanism by which STEAP4 affects insulin sensitivity may lie in the PI3K-protein kinase B/Akt (PKB) pathway, and the ROS-related mitochondrial dysfunction may be related to a reduced IRS-1 correlation with the PI3K signaling pathway, leading to insulin resistance.

Materials and methods

Antibodies

Mouse monoclonal antibodies against STEAP4 were purchased from Lifespan Biosciences (LifeSpan Biosciences, Seattle, WA, USA). Primary polyclonal glucose transporter 4 (GLUT4) antibodies and horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (1:500; Santa Cruz, CA, USA). Anti-phospho-insulin receptor (IR) beta (Tyr1146), anti-IR beta, phosphatidylinositol-3-kinase [(PI3k) p85α/γ (Y463)], and anti-IRS-1 were purchased from Cell Signaling Technology (Danvers, MA, USA). The phospho-specific polyclonal antibody against IRS-1 (Tyr612) was obtained from Biosource (1:500; Camarillo, CA, USA). Antibodies against Akt, ERK1/2, and the phosphorylated forms of these proteins were obtained from Bioworld (1:500; Georgia, USA). Antibodies against p38, JNK, and the phosphorylated forms of these proteins were also obtained from Bioworld (1:500; Georgia, USA).

Cell culture and treatment

Human pre-adipocytes (Sciencell Research Laboratories, San Diego, CA, USA) were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 50 μg/mL streptomycin at 37°C in 5% CO2. To induce differentiation, confluent human pre-adipocytes (D0) were cultured in serum-free DMEM that contained 50 nM insulin, 100 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 100 nM TNF-α. The medium was changed every 2 days for the first 4 days. Thereafter, the medium was replaced with serum-free DMEM that contained 50 nM insulin and 100 nM dexamethasone, and the medium was changed every 2 days until lipid droplets had accumulated (days 14–17). Fat accumulation was assessed by staining formalin-fixed cells with Oil Red O.

Immunoblotting

Human pre-adipocytes were induced to differentiate as described above. On day 15, cells were exposed to antibodies against STEAP4 for 24 h, then serum-starved for 3 h, and finally incubated with or without 100 nM insulin. Total proteins and phosphorylated proteins were extracted as described previously (Garcia-Hernandez Mde et al. 2007). Plasma membrane (PM) proteins were extracted using Eukaryotic Membrane Protein Extraction Reagent (Merck KGaA, Darmstadt, Germany). Protein