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Research Paper

Low-Density Lipoprotein Receptor-Related Protein-1 Protects Against Hepatic Insulin Resistance and Hepatic Steatosis

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ABSTRACT

Low-density lipoprotein receptor-related protein-1 (LRP1) is a multifunctional uptake receptor for chylomicron remnants in the liver. In vascular smooth muscle cells LRP1 controls reverse cholesterol transport through platelet-derived growth factor receptor β (PDGFR-β) trafficking and tyrosine kinase activity. Here we show that LRP1 regulates hepatic energy homeostasis by integrating insulin signaling with lipid uptake and secretion. Somatic inactivation of LRP1 in the liver (hLRP1KO) predisposes to diet-induced insulin resistance with dyslipidemia and non-alcoholic hepatic steatosis. On a high-fat diet, hLRP1KO mice develop a severe Metabolic Syndrome secondary to hepatic insulin resistance, reduced expression of insulin receptors on the hepatocyte surface and decreased glucose transporter 2 (GLUT2) translocation. While LRP1 is also required for efficient cell surface insulin receptor expression in the absence of exogenous lipids, this latent state of insulin resistance is unmasked by exposure to fatty acids. This further impairs insulin receptor trafficking and results in increased hepatic lipogenesis, impaired fatty acid oxidation and reduced very low density lipoprotein (VLDL) triglyceride secretion.

1. Introduction

Low-density lipoprotein (LDL) receptor-related protein 1 (LRP1) is a multifunctional transmembrane receptor with diverse biological properties that are essential for the maintenance of normal mammalian physiology (Dieckmann et al., 2010). In the liver, LRP1 functions in concert with the LDL receptor (LDLR) in the clearance of ApoE-containing chylomicron remnants and very low density lipoprotein (VLDL) circulating in plasma (Rohlmann et al., 1998), as well as a number of other pro-atherogenic ligands (Espirito Santo et al., 2004). Hepatic LRP1 also binds hepatic lipase and lipoprotein lipase (LPL) (Verges et al., 2004), further contributing to the role of LRP1 in overall lipid homeostasis. In addition to functioning as an uptake receptor, LRP1 regulates intracellular trafficking of other cell surface receptors. In fibroblasts and smooth muscle cells, LRP1 controls platelet-derived growth factor receptor β (PDGFRβ) trafficking and tyrosine kinase activity, which in turn regulates extracellular signal regulated kinase (Erk) activation and cell proliferation. These mechanisms are fundamental to the protective role of LRP1 in atherogenesis and the protection of the vascular wall (Boucher et al., 2003; Takayama et al., 2005; Zhou et al., 2009).

The importance of LRP1 in lipid homeostasis and atherosclerosis is well established, however the role of LRP1 in glucose metabolism is just beginning to be understood. In vitro proteomic studies demonstrate that LRP1 functions as an integral component of glucose transporter storage vesicles (GSV) and LRP1 depletion in adipocytes is associated with decreased glucose transporter 4 (GLUT4) expression and decreased insulin-induced glucose uptake (Jedrychowski et al., 2010). In addition to regulating glucose uptake through GLUT4, LRP1 itself can be regulated by insulin signaling. In both adipocytes and hepatocytes, insulin stimulates a rapid translocation of LRP1 to the cell surface, leading to increased uptake of postprandial lipoproteins (Descamps et al., 1993; Laatsch et al., 2009). Moreover, a central role for LRP1 in overall energy metabolism is further suggested by a mouse model of induced LRP1 deficiency in the

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adult forebrain. This is associated with decreased leptin signaling, leading to accelerated weight gain and obesity, decreased energy consumption, and increased food intake (Liu et al., 2011).

Recently, the LIPGENE study has identified rs4759277 in the *Lrp1* gene as one of the top single nucleotide polymorphisms (SNPs) associated with fasting insulin levels and insulin resistance in patients with Metabolic Syndrome (Delgado-lista et al., 2014). Insulin resistance and dyslipidemia, in addition to central obesity, are key features of the Metabolic Syndrome. As a clinical entity, the prevalence of Metabolic Syndrome worldwide is increasing, largely related to rising rates of obesity and sedentary lifestyles (Grundy et al., 2005). The relationship between the hallmarks of the Metabolic Syndrome, in particular insulin resistance and dyslipidemia, is not well understood. However, it has been proposed that Metabolic Syndrome in general is mediated by an underlying insulin resistance (Grundy et al., 2004).

To explore the impact of LRP1 on insulin resistance and the Metabolic Syndrome, we generated a novel genetic model of diet-induced hepatic insulin resistance based on hepatic LRP1 deficiency. We found that liver-specific LRP1 knockout (hLRP1KO) mice when maintained on a standard chow diet exhibit mild dyslipidemia and impaired insulin signaling, however, when challenged with a high-fat diet (HFD) they rapidly develop obesity, marked insulin resistance, hyperglycemia, and hepatic steatosis. Collectively, these data suggest that hepatic LRP1 is essential for modulating hepatic insulin action and highlight a pivotal mechanism in the development of Metabolic Syndrome.

2. Materials and Methods

2.1. Materials

Human insulin was purchased from Humalog. The anti-phospho-insulin receptor (pIR), anti-IR, anti-protein kinase B (AKT), anti-pAKT, anti-β-actin, anti-glycogen synthase kinase (GSK) 3β, anti-pGSK3β and anti-calnexin antibodies were purchased from Cell Signaling. Anti-GLUT2 and anti-GLUT4 antibodies were purchased from Millipore. Anti-apolipoprotein B (apoB) and anti-apoE antibodies were purchased from Calbiochem. Generation of antibodies to LDLR, LRP1 and apoAI have been described previously (Ishibashi et al., 1993; Rohlmann et al., 1998). Peroxidase-labeled anti-rabbit or mouse IgG was from GH Healthcare and Peroxidase-labeled anti-goat IgG was from Santa Cruz. ECL system was from Thermo Scientific.

2.2. Mouse Experiments

Lrp1$^{flox/flox}$ mice were generated as previously described (Rohlmann et al., 1998; Rohlmann et al., 1996), and maintained on a mixed C57BL/6x 129SvJ strain background. Albumin-Cre mice on a congenic C57BL/6 background were from Jackson Laboratory (Stock number 003574). *Lrp1*$^{flox/flox}$ mice were crossed with albumin-Cre; *Lrp1*$^{flox/flox}$ mice to generate hLRP1KO and hLRP1$^{+/+}$ littermates (WT) by brother-sister mating. Animals were housed in specific pathogen-free facilities with 12-hour light/12-hour dark cycle. Animals were fed either a normal chow diet (Teklad 18%, Harlan) or a HFD containing 60% fat (D12492, Research Diets) ad libitum with free access to water. All experimental protocols were approved by the Institutional Animal Care and Use Committees of the University of Texas Southwestern Medical Center. Unless specifically mentioned, all experiments were performed on WT and hLRP1KO mice fed with chow or HFD for 16 weeks.

2.3. Glucose and Insulin Tolerance Tests

Glucose, insulin and pyruvate tolerance tests were performed 16 weeks after chow diet or HFD feeding; glucose tolerance test (1 g glucose/kg of body weight, after overnight fasting), insulin tolerance test (1 unit human regular insulin/kg body weight, after 6 h of fasting), and pyruvate tolerance test (1 g pyruvate/kg of body weight, after overnight fasting). Blood samples were obtained by tail bleeding and analyzed for glucose content (Contour brand glucometer) immediately before and at time points of 0, 30, 60, 90 and 120 min after an intraperitoneal injection.

2.4. Hyperinsulinemic Euglycemic Clamp

Anesthesia was accomplished by 2% isofluorane. For clamps, silicone catheters were aseptically placed in the jugular vein. Rimadyl (5 mg/kg, sc) was administered for pain control and animals were allowed to recover (4–5 days) to achieve preoperative weight prior to experiments. Clamps were performed in conscious unrestrained animals as previously described (Xia et al., 2015). Clamps were initiated by primed continuous infusion of insulin (3 mU/kg/min) and glucose was maintained constant at ~130 mg/dL during the clamped state.

2.5. In Vivo Insulin Stimulation

Following an overnight fast, mice were injected with 0.9% NaCl or 1 unit of regular human insulin/kg (Humalog) intraperitoneally. 5 min after injection of the insulin bolus, livers were rapidly removed and frozen in liquid nitrogen.

2.6. Primary Hepatocytes

Mouse primary hepatocytes were isolated from 8- to 10-week-old mice by collagenase perfusion as described previously (Wang et al., 2012). Cells were cultured in the presence of 1000 mg/L glucose and starved for overnight, following by 24-hour treatment with 0.25 mM albumin-bound Palmitate or 0.5% BSA. Insulin (100 nM/L) or 0.9% NaCl was added to the culture medium for 2 min.

2.7. Surface Protein Biotinylation

Primary mouse hepatocytes were grown in 6-well culture dishes and cell surface proteins were biotinylated as previously described (Chen et al., 2010). After insulin treatment, hepatocytes were washed with cold PBS buffer and then incubated in PBS buffer containing 1.0 mg/ml sulfo-NHS-SS-biotin (Pierce) for 30 min at 4 °C. Excess reagent was quenched by rinsing in cold PBS containing 100 mM glycine. Cell lysates were prepared in 160 μl of RIPA lysis buffer [50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 2 mM EDTA, 2 mM MgCl2, and protease inhibitor mixture (Sigma), (pH8.0)]. After 20 min incubated at 4 °C, lysates were collected and centrifuged at 14,000 × rpm for 10 min. 100 μg of total proteins were incubated with 100 μl of NeutrAvidin agarose (Pierce) at 4 °C for 1.5 h. Agarose pellets were washed three times using washing buffer [500 mM NaCl, 150 mM Tris-HCl, 0.5% Triton X-100 (pH8.0)], biotinylated surface proteins were eluted from agarose beads by boiling in 2x SDS sample loading buffer. Protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, and blotted with different antibodies.

2.8. Western Blot Analysis

Protein samples from liver tissues or hepatocytes were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad). Blots were probed separately with antibodies as indicated in figures. After incubation with horseradish-peroxidase-conjugated secondary antibodies, membranes were visualized with SuperSignal West Pico Chemiluminescence reagents on X-ray films. When comparing phosphorylated and total IR and AKT protein, the same membranes incubated with phospho-antibodies were stripped and reprobed with non-phospho-antibodies. Band intensity was quantified using scanning densitometry of the autoradiogram with NIH Image J software (http://rsb.info.nih.gov/ij/).