RNAI-BASED SCREENS REVEAL NOVEL REGULATORS OF ADIPOSE TISSUE METABOLISM

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Insulin resistance in muscle is a major syndrome in obese humans that contributes to the onset of type 2 diabetes. Adipose tissue is a major regulator of whole body metabolism through its ability to secrete peptides and control blood lipid levels, and can influence the insulin sensitivity of skeletal muscle and liver. The development of obesity coincides with substantial infiltration of macrophages into adipose tissue, which is associated with increased expression of inflammatory cytokines such as TNFα. These agents, in turn upregulate adipocyte lipolysis and downregulate insulin signaling, contributing to the metabolic complications of obesity such as impaired glucose tolerance and insulin resistance. We have developed RNA interference (RNAi) screens to identify genes in cultured adipocytes that regulate insulin signaling and key metabolic pathways. These short interfering RNA (siRNA)-based screens identified the transcriptional corepressor receptor interacting protein 140 (RIP140) (*J. Clin. Invest. 116: 125, 2006*) as a negative regulator of insulin-responsive hexose uptake and oxidative metabolism. Gene expression profiling revealed that RIP140 depletion upregulates the expression of clusters of genes in the pathways of glucose uptake, glycolysis, tricarboxylic acid cycle, fatty acid oxidation, mitochondrial biogenesis and oxidative phosphorylation. RIP140-null mice resist weight gain on a high-fat diet and display enhanced glucose tolerance. Thus, RIP140 functions in isolated adipocytes and in intact mice as both a major suppressor of oxidative metabolism and as a regulator of glucose homeostasis. Moreover, we screened cultured adipocytes with siRNA directed against over two hundred protein kinases for negative regulators of insulin-sensitive glucose transport. Among several hits in this screen, we discovered the mitogen-activated protein kinase (MAP4k4) as a protein kinase of the Ste20 family that exhibits potent inhibitory effects on insulin sensitivity and adipogenesis (*Proc. Natl. Acad. Sci. USA 103: 2087, 2006*). Further experiments indicate that Map4k4 is part of a signaling cascade initiated by TNFα that down regulates protein synthesis (but not transcription) of the adipogenic transcriptional factor PPARγ in cultured adipocytes. TNFα both increases Map4k4 acute catalytic activity and Map4k4 expression in adipocytes (*J. Biol. Chem. 282: 19302, 2007*). These data suggest that Map4k4 functions during TNFα signaling events that attenuate insulin action in adipocytes. We conclude that RIP140 and Map4k4 are novel negative regulators of PPARγ and adipose tissue oxidative metabolism, and are potential therapeutic targets for controlling metabolic disease. These data validate the power of RNAi screening for discovery of new therapeutic targets for type 2 diabetes and obesity.