IRAK-1 Phosphorylates IRS-1 on Ser24: Crosstalk Relevant to the Pathophysiology of CardioMetabolic Syndrome

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Chronic inflammation contributes to insulin resistance in diabetes and obesity. Mouse Pelle-like kinase (mPLK, homolog of human IL-1 receptor-associated kinase-1 (IRAK-1)) participates in inflammatory signaling. We evaluated IRS-1 as a novel substrate for mPLK that may contribute to linking inflammation with insulin resistance. Wildtype mPLK, but not a kinase-inactive mutant (mPLK-KD), directly phosphorylated full-length IRS-1 in vitro. This in vitro phosphorylation was increased when mPLK was immunoprecipitated from tumor necrosis factor (TNF)-α-treated cells. In NIH-3T3IR cells, wild-type mPLK (but not mPLK-KD) co-immunoprecipitated with IRS-1. This association was increased by treatment of cells with TNF-α. Using mass spectrometry, we identified Ser24 in the pleckstrin homology (PH) domain of IRS-1 as a specific phosphorylation site for mPLK. IRS-1 mutants S24D or S24E (mimicking phosphorylation at Ser24) had impaired ability to associate with insulin receptors resulting in diminished tyrosine phosphorylation of IRS-1 and impaired ability of IRS-1 to bind and activate PI-3 kinase in response to insulin. IRS-1-S24D also had an impaired ability to mediate insulin-stimulated translocation of GLUT4 in rat adipose cells. Importantly, endogenous mPLK/IRAK was activated in response to TNF-α or interleukin1 treatment of primary adipose cells. In addition, using a phospho-specific antibody against IRS-1 phosphorylated at Ser24, we found that interleukin-1 or TNF-α treatment of Fao cells stimulated increased phosphorylation of endogenous IRS-1 at Ser24. We conclude that IRS-1 is a novel physiological substrate for mPLK. TNF-α-regulated phosphorylation at Ser24 in the pleckstrin homology domain of IRS-1 by mPLK/IRAK represents an additional mechanism for cross-talk between inflammatory signaling and insulin signaling that may contribute to metabolic insulin resistance.

Interleukin 1 receptor-associated kinase 1 (IRAK-1) mediates pro-inflammatory signaling via IL-1 receptor/Toll-like receptors, which may contribute to insulin resistance. Here, we tested this hypothesis in vivo using male irak1 null (k/o) mice to investigate the metabolic role of IRAK-1. C57BL/6 wild-type (WT) and k/o mice had comparable body weights on low-fat and high-fat diets (LFD and HFD, respectively). After 12 weeks on LFD (but not HFD), k/o mice (versus WT) had substantially improved glucose tolerance (assessed by the intraperitoneal glucose tolerance test (IPGTT)). As assessed with the hyperinsulinemic euglycemic glucose clamp technique, insulin sensitivity was 30% higher in the Irak1 k/o mice on chow diet, but the Irak1 deletion did not affect IPGTT outcomes in mice on HFD, suggesting that the deletion did not overcome the impact of obesity on glucose tolerance. Moreover, insulin-stimulated glucose-disposal rates were higher in the k/o mice, but we detected no significant difference in hepatic glucose production rates (insulin infusion). Positron emission/computed tomography scans indicated higher insulin-stimulated glucose uptake in muscle, but not liver, in Irak1 k/o mice in vivo. Moreover, insulin-stimulated phosphorylation of Akt was higher in muscle, but not in liver, from Irak1 k/o mice ex vivo. In conclusion, Irak1 deletion improved muscle insulin sensitivity, with the effect being most apparent in LFD mice.

To investigate relevance of IRAK-1/IRS-1 interactions to human pathophysiology we examined human skeletal muscle (SkM) from NGT (n=10), IGT (n=6), and T2D (n=12) (1st cohort). Fasting protein expression of IRAK-1 and phospho-IRAK-1 (p-IRAK-1, activity proxy) and IRS-1 (pS24) did not differ significantly across groups. Insulin infusion during hyperinsulinemic glucose clamp (HGC), caused acute reductions in IRS-1 (pS24) in NGT that was diminished in IGT, and absent in T2D (p < 0.05, vs. NGT). p-IRAK-1 was unaltered during HGC in 1st cohort.
insulin-induced reduction of IRS-1 (pS^{24}) in SkM was also found in women with PCOS (2\textsuperscript{nd} cohort, n=12). Insulin sensitivity improved after pioglitazone treatment (pio, 6 mo). This was associated with reduction in IRS-1 (pS^{24}) (both fasting and HGC (acute insulin stimulation); (vs. pio pre-treatment). In a 3\textsuperscript{rd} cohort, non-diabetic (n=13) and T2D subjects (n=13), associations between IRAK-1 phosphorylation in the fasting state and measures of insulin sensitivity were noted: HOMA-IR (r=0.57, p=0.01; consistent with HGC data from PCOS pre- and post-pio, and trend in 1st cohort). In sum: 1) p-IRAK-1 in SkM (activity surrogate), predicts insulin resistance (IR) in human subjects. 2) Ability of acute insulin stimulation to reduce IRS-1 (pS^{24}) is absent in T2D and improved after pio intervention in PCOS. 3) Changes in IRAK-1 phosphorylation of IRS-1 (S^{24}) represents cross-talk between immune signaling and insulin signaling that may cause IR in human SkM. Our findings suggest that IRS-1 (pS^{24}) and p-IRAK-1 are biomarkers of IR that may be molecular targets for therapy of IR in human metabolic diseases.