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## Regulation of Insulin Signaling via Myosin Motor Protein and Actin Cytoskeletal Network

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**Abstract** Impairment of systemic insulin action causes insufficient glucose uptake to cells and compensatory hyper-secretion of insulin from pancreatic  $\beta$ -cells. This insulin-resistant state promotes development of type 2 diabetes, obesity, hypertension, dyslipidemia, and atherosclerosis. Numerous attempts have been made to clarify this pathogenesis and molecular mechanisms of insulin resistance. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is one of adipocytokines and a major factor causing insulin resistance. This cytokine attenuates insulin signaling by phosphorylation of insulin receptor substrate-1 (IRS-1) Ser residues. However little is known about the precise molecular mechanisms. Recently we reported that I $\kappa$ B kinase- $\beta$  (IKK- $\beta$ ) interacted with IRS-1, and phosphorylated IRS-1 Ser<sup>307</sup> through molecular motor and actin-cytoskeleton. In this review, we overview this unique molecular mechanism and propose a new model that molecular motor and cytoskeletal network organize signaling cross-talk.

*Key words:* NEMO/IKK- $\gamma$ , Myo1c, IRS-1, TNF- $\alpha$ , insulin resistance

### Introduction

Systemic glucose homeostasis is strictly regulated by insulin and other counter-regulatory hormones. Insulin resistance, a condition in which the cells become resistant to the effects of insulin, cause type 2 diabetes, obesity, hypertension, dyslipidemia, and atherosclerosis.<sup>1)</sup> Although the precise mechanism has been unknown, recent studies are constructing rough outline of pathogenesis of insulin resistance. This brief review will focus on recent molecular studies that have clarified the cellular and molecular mechanisms of insulin resistance.

### IKK complex and insulin resistance induced by TNF- $\alpha$

Many factors including TNF- $\alpha$ , IL-6 and

free fatty acid have been reported to induce insulin resistance.<sup>2)</sup> The machinery mediating insulin resistance induced by TNF- $\alpha$  has been studied intensively because of its principal impact on the development of type 2 diabetes.<sup>3)</sup> TNF- $\alpha$  is an inflammatory adipocytokine which is secreted from adipocytes. A TNF- $\alpha$  signal results in the phosphorylation of Ser<sup>307</sup> of insulin receptor substrate 1 (IRS-1), in turn attenuating the metabolic insulin signal.<sup>4)</sup> Many serine kinases such as IKKs, JNK, glycogen synthase kinase 3, and mammalian target of rapamycin have been reported to phosphorylate serine residues of IRS-1. Among those serine kinases that potentially attenuate insulin signaling, we showed prime contribution of IKK- $\beta$  on the development of insulin resistance.<sup>5)</sup>

The IKK complex consists of two catalytic subunits, IKK- $\alpha$  and IKK- $\beta$ , and one scaffold

subunit designated nuclear factor  $\kappa$ B essential modulator (NEMO)/IKK- $\gamma$ .<sup>6)</sup> Gao et al. reported that IRS-1 was direct substrate of IKK- $\beta$  and was phosphorylated at serine residues including Ser.<sup>307-4)</sup>

### NEMO/IKK- $\gamma$ co-localizes with IRS-1

To determine the interaction between IKKs and IRS-1, we tried to visualize IKKs and IRS-1 in culture adipocytes. First, we examined the intracellular localization of NEMO as a representative isoform of IKKs. NEMO exists in a fine punctate or granular appearance throughout the cytoplasm under basal and TNF- $\alpha$ -treated conditions. In contrast, addition of insulin to culture adipocytes yields rapid translocation of NEMO to the cell periphery, especially in the membrane ruffles. This translocation is similar to that seen in other cell types.<sup>7)</sup> Interestingly, treatment with the actin depolymerizer latrunculin B inhibited that translocation, whereas the microtubule disrupter nocodazole did not. These data suggest that insulin stimulates accumulation of NEMO at the membrane ruffles through the cortical actin network. To determine the hypothesis that NEMO may interact with IRS-1, we next investigated the localization of NEMO and IRS-1. In the basal state, IRS-1 was present in the cytoplasm, whereas with insulin stimulation, IRS-1 and NEMO co-localized to discrete foci in the cytoplasm as well as membrane ruffles. This observation indicates that interaction of IKKs with IRS-1 was facilitated by insulin treatment and this IRS-1-IKKs complex formation was organized at membrane ruffles.

### Motor protein Myo1c conveys IKKs

A proper localization of IKKs must be essentially important for IRS-1 down regulation. To gain further understanding the mechanism of intracellular translocation of IKKs, we screened proteins which interact with NEMO by insulin stimulation using mass spectrometry. With in-gel digestion followed by peptide mass fingerprinting, we identified two candidate proteins, Myo1c and actin, showing increased binding to NEMO in the presence of insulin. Myo1c is a motor

protein that is classified as an unconventional myosin I. This class of myosins is widely distributed, having been identified in organisms from yeast to human.<sup>8)</sup>

We hypothesized that the IKK complex containing NEMO is transported from the cytosol to membrane ruffles by Myo1c. To examine this possibility, we conducted experiments using the dominant inhibitory cargo domain of Myo1c. Co-expression of Myo1c WT and eGFP-NEMO enhanced NEMO translocation to the membrane ruffles with insulin stimulation. Interestingly, Myo1c WT also accumulated in the membrane ruffles, resulting in the extensive formation of membrane ruffles. In contrast, cells expressing Myo1c cargo domain and NEMO showed marked inhibition of insulin-stimulated NEMO translocation. Similar inhibition of NEMO translocation was observed in Myo1c knockdown cells using adenovirus encoding short hairpin RNA. These data indicate the scaffold protein NEMO to be transported to the membrane ruffles by the motor protein Myo1c. We also confirmed the direct interaction of NEMO with Myo1c by pull-down assay using GST fused and His tag fused protein. This result indicates that NEMO works as a molecular receptor on the IKKs-Myo1c complex formation.

Based on the data presented above, we proposed a simple model whereby Myo1c and its motor receptor NEMO cooperatively facilitate IKK-IRS-1 complex formation, as illustrated in Fig. 1. NEMO is well known as a scaffold protein of the IKK complex. Recent studies suggest that some scaffold proteins serve as links between molecular motors and intracellular vesicles, thereby functioning as vesicle binding proteins. Our data suggest that the scaffold protein NEMO links motor and signaling molecules as cargos. It is noteworthy that Myo1c organizes the signaling complex and serves as a platform for the two distinct signals to interact (i.e., the insulin signal and the TNF- $\alpha$  signal mediating insulin resistance).

### Conclusion

Our recent studies have revealed important new insights into the molecular mechanism of

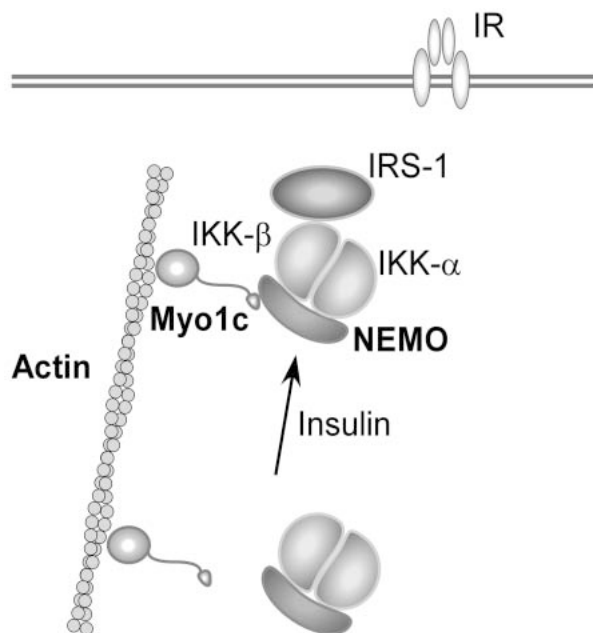


Fig. 1 Schematic model of Myo1c-mediated IRS-1-IKKs complex formation. IRS-1 is a substrate of serine/threonine kinase IKK- $\beta$ . Phosphorylation of IRS-1 serine residues attenuates insulin signaling. Insulin stimulation promotes binding of IKKs with myosin motor Myo1c. In this interaction, NEMO works as a molecular receptor which links IKKs with Myo1c. Myo1c conveys IKKs from cytoplasm to membrane ruffles. This translocation facilitates IKKs-IRS-1 complex formation and mediates down regulation of insulin signaling.

TNF- $\alpha$  induced insulin resistance. In the process of IRS-1 Ser<sup>307</sup> phosphorylation mediated by IKK- $\beta$ , the machinery composed of motor protein and actin cytoskeletal network is essential for the IRS-1-IKK complex formation. In this context, it is possible that NEMO is a molecular receptor linking motor (Myo1c) and cargo (IKK- $\alpha$  and - $\beta$ ). These results suggest a novel mechanism that molecular motor mediates signaling cross-talks. Our proposal may be applicable to other complex signal transduction.

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