IKKβ suppression of TSC1 function links the mTOR pathway with insulin resistance

DUNG-FANG LEE1,2, HSU-PING KUO1,2, CHUN-TE CHEN1,2, YONGKUN WEI1, CHAO-KAI CHOU1,2, JEN-YU HUNG1,4, CHIA-JUI YEN1,5 and MIEN-CHIE HUNG1,2,3

1Department of Molecular and Cellular Oncology, The University of Texas M.D. Anderson Cancer Center; 2The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA 3Center for Molecular Medicine and Graduate Institute of Cancer Biology, China Medical University and Hospital, Taichung, Taiwan

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Abstract. The proinflammatory cytokine TNFα is one of the factors that links obesity-derived chronic inflammation with insulin resistance. Activation of mTOR signaling pathway has been found to suppress insulin sensitivity through serine phosphorylation and the inhibition of IRS1 by mTOR and its downstream effector, S6K1. It remains elusive that whether the mTOR pathway has a role in TNFα-mediated insulin resistance. In the present study, we demonstrated that TNFα-IKKβ-mediated inactivation of TSC1 resulted in increasing phosphorylation of IRS1, and the association between IRS1 and PI3K p85. Furthermore, a higher expression of pIKKß (S181), pTSC1(S511), and pS6(S240/244) was found in livers obtained from both C57BL/6J mice on a high-fat diet and B6.V-Lepob ob-ob mice. Collectively, dysregulation of the TSC1/mTOR signaling pathway by IKKβ is a common molecular switch for both cancer pathogenesis and diet- and obesity-induced insulin resistance.

Introduction

Insulin action is essential for growth, development, and metabolism. Upon stimulation with insulin, insulin receptor (IR) stimulates its intrinsic tyrosine kinase activity and phosphorylates IR substrate 1 (IRS1). The tyrosine-phosphorylated IRS1 functions as an adaptor to activate downstream phosphoinositide 3-kinase (PI3K)/AKT signaling, which leads to enhancement of glucose uptake, synthesis of protein and glycogen, and execution of the growth-promoting and metabolic effects of insulin. In contrast with the positive effect of tyrosine phosphorylation of IRS1, serine phosphorylation actually inhibits the function of IRS1 (1). Although serine phosphorylation of IRS1 induced by insulin can function as a feedback control, other factors, such as proinflammatory cytokines, also increase phosphorylation of IRS1 and function as negative regulators. A large number of kinases function as IRS1 serine kinases and play negative regulatory roles in insulin action. These include mammalian target of rapamycin (mTOR)-mediated phosphorylation of IRS1 serine 636 (Ser636) and serine 639 (Ser639) (2), ribosomal S6 kinase 1 (S6K1)-mediated phosphorylation of IRS1 serine 307 [Ser307 (mouse serine 318)] (7). IRS1 Ser312 directly inhibits IR-induced tyrosine phosphorylation and the inhibition of IRS1 by mTOR and its downstream effector, S6K1. It remains elusive that whether the mTOR pathway has a role in TNFα-mediated insulin resistance. In the present study, we demonstrated that TNFα-IKKβ-mediated inactivation of TSC1 resulted in increasing phosphorylation of IRS1, and the association between IRS1 and PI3K p85. Furthermore, a higher expression of pIKKß (S181), pTSC1(S511), and pS6(S240/244) was found in livers obtained from both C57BL/6J mice on a high-fat diet and B6.V-Lepob ob-ob mice. Collectively, dysregulation of the TSC1/mTOR signaling pathway by IKKβ is a common molecular switch for both cancer pathogenesis and diet- and obesity-induced insulin resistance.

Correspondence to: Dr Mien-Chie Hung, The University of Texas M.D. Anderson Cancer Center, Department of Molecular and Cellular Oncology, Unit 108, 1515 Holcombe Blvd., Houston, TX 77030, USA E-mail: mhung@mdanderson.org

Present addresses: 4Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung 807, 5Department of Medicine, National Chen Kung University, Tainan 70428, Taiwan, R.O.C.

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phorylation of IRS1 and disrupts the association between IRS1 and p85 (a PI3K regulatory subunit), thereby interfering with insulin signaling. Although the involvement of IKKβ and JNK in TNFα-mediated insulin resistance is generally approved, recent studies raise interesting possibilities that other mechanisms may be involved in this inhibition. For instance, activation of the mTOR pathway suppresses insulin signaling by modulating the serine phosphorylation of IRS1, which serves as a feedback regulator of the insulin signaling pathway. mTOR and its downstream effector S6K1 suppress IRS1 activity by directly phosphorylating IRS1 at Ser636/639 and Ser307, respectively, which leads to desensitization of insulin signaling (3,10). Remarkably, S6K1-deficient mice are protected against nutritionally and genetically driven insulin resistance (11), and genetic loss of either tuberous sclerosis 1 (TSC1) or tuberous sclerosis 2 (TSC2) results in insulin resistance (11,10). 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PBS; incubated with antibodies against pIKKβ(S181), pTSC1(S511), pS6(S240/244), and pAKT(S473); incubated with goat anti-rabbit immunoglobulin G conjugated with fluorescein isothiocyanate in the dark; and examined under a fluorescent microscope (Zeiss). The nuclei of liver cells were stained with 4,6-diamidino-2-phenylindole.

**Results**

**TNFα-mediated insulin resistance via inappropriate activation of the TSC1/mTOR/S6K1 signaling cascade**

Activation of the mTOR pathway functions as a feedback regulator of insulin action by increasing phosphorylation of IRS1 at Ser307 [pIRS(S307)] and Ser636/639 [pIRS (S636/639)] by S6K1.
and mTOR (2), respectively. Also, studies showed that treatment with rapamycin prevents the development of insulin resistance by inhibiting the serine phosphorylation of IRS1 (3,10). Recently, we demonstrated that the pro-inflammatory cytokine TNFα activates the mTOR pathway and results in tumor angiogenesis (12,13). This raised the intriguing question of whether activation of the mTOR pathway contributes to TNFα-induced insulin resistance by increasing phosphorylation of IRS1 Ser307 and Ser636/639. In the present study, we found that treatment with TNFα substantially induced the expression of pIRS1(S307) and pIRS1(S636/639) in MDA-MB-453 cells within 10 to 30 min (Fig. 1A). To further address this issue, we used stable transfectants of the paired stable transfectants MDA-MB-453-TSC1(SSAA) and MDA-MB-453-TSC1(SSDD), which have constitutive suppression and activation, respectively, of the IKKβ/TSC1/mTOR pathway, to determine whether suppression of TSC1 function by IKKβ has a role in TNFα-mediated insulin resistance. In determining the phosphorylation status of S6K1 at T389 [pS6K1(T389)] and 4EBP1 at S65 [p4EBP1(S65)], which are two downstream phosphorylation sites of mTOR, we found higher expression of pS6K1(T389) and p4EBP1(S65) in 453-TSC1(SSDD) stable transfectants than in MDA-MB-453-TSC1(WT) or MDA-MB-453-TSC1(SSAA) stable transfectants (Fig. 1B), confirming our previous finding that IKKβ phosphorylation of TSC1 activates the mTOR pathway (12,13). This result prompted us to examine whether IKKβ-derived phosphorylation of TSC1 can serve as a switch for TNFα- and mTOR-induced phosphorylation of IRS1 at Ser307 and Ser636/639. Examination of the serine phosphorylation status of IRS1 [pIRS(S307) and pIRS(S636/639)] in various MDA-MB-453-TSC1 stable transfectants showed higher expression of pIRS(S307) and pIRS(S636/639) in MDA-MB-453-TSC1(SSDD) stable transfectants than in MDA-MB-453-TSC1(WT) or MDA-MB-453-TSC1(SSAA) stable transfectants (Fig. 1C), indicating that activation of mTOR signaling by IKKβ-mediated phosphorylation of TSC1 increases phosphorylation of IRS1 through up-regulation of S6K1 and mTOR activity, respectively.

IKKβ-mediated TSC1 phosphorylation impairs insulin action. Since serine phosphorylation of IRS1 has a significant role in countering insulin action (IR-induced IRS1 tyrosine phosphorylation and association with PI3K p85) (1,18,19), increased serine phosphorylation of IRS1 by IKKβ/TSC1/mTOR signaling likely inhibits insulin response. To further validate the physiological relevance of our observations described above, we studied the effect of IKKβ-mediated phosphorylation of TSC1 on insulin-induced tyrosine phosphorylation of IRS1, the association between IRS1 and PI3K p85, and the level of glucose uptake by insulin stimulation. As expected, we observed less of a response to insulin-induced tyrosine phosphorylation of IRS, less of an association between IRS1 and PI3K p85 (Fig. 2A), and a lower level of glucose uptake (Fig. 2B) in MDA-MB-453-TSC1(SSDD)
stable transfectant than in MDA-MB-453-TSC1(WT) or MDA-MB-453-TSC1(SSAA) stable transfectants. Thus, activation of the mTOR/S6K1 signaling pathway by IKKß-induced phosphorylation and inactivation of TSC1 may contribute to TNFα-mediated insulin resistance by increasing serine phosphorylation of IRS1.

Up-regulation of IKKß/TSC1/mTOR/S6K1 signaling in C57BL/6J mice on an HFD, and in ob/ob mice. Obesity is significantly associated with insulin resistance and is a state of chronic inflammation as indicated by increased plasma concentrations of TNFα in obese humans and animals (8,9). Protection against obesity-induced insulin resistance by either knockout or neutralization of TNFα by a soluble TNFα receptor provides solid evidence supporting the concept that TNFα has an essential role in obesity-mediated insulin resistance (9). To further determine whether suppression of TSC1 function by TNFα-activated IKKß is related to obesity-induced insulin resistance, we sought to determine whether the TNFα/IKKß/TSC1/mTOR/S6K1 pathway is activated in mice on an HFD and in genetically obese mice. We measured the pIKKß(S181), pTSC1(S511), and pS6(S240/244) (which is phosphorylated by S6K1 and used as an indicator of S6K1 activity) status in frozen section of livers obtained from C57BL/6J mice that were fed the HFD and genetically obese C57BL/6J ob/ob mice, in which leptin deficiency leads to hyperglycemia, hyperinsulinemia, and insulin resistance (20). We found that the expression of pIKKß(S181), pTSC1(S511), and pS6(S240/244) was significantly higher in livers obtained from 16-week-old HFD C57BL/6J mice and ob/ob mice than in those obtained from NCD C57BL/6J mice (Fig. 3). Since insulin resistance impairs the PI3K/AKT signaling pathway, we determined the pAKT(S473) status in these livers. We observed a lower expression of pAKT(S473) in the livers obtained from HFD C57BL/6J mice and C57BL/6J ob/ob mice than in those obtained from NCD C57BL/6J mice (Fig. 3). These observations indicated that up-regulation of mTOR and S6K1 activity via suppression of TSC1 by IKKß may contribute to diet- and obesity-induced insulin resistance.

Discussion

TNFα has an important role in mediating diet- and obesity-induced insulin resistance by increasing serine phosphorylation of IRS1, which inhibits insulin action. Several studies have suggested that IKKß and JNK are central coordinators in the regulation of TNFα-induced insulin resistance (16,21,22), whereas the cellular and molecular mechanisms, by which TNFα impairs IRS1 function, are not fully elucidated. The findings of the present study of diet- and obesity-induced insulin resistance in murine models suggest that suppression of TSC1 by IKKß activates mTOR and S6K1, which in turn phosphorylate IRS1 at Ser636/639 and Ser307, thereby inhibiting IRS1 function. Based on these and previous findings, herein we propose a model in which the high levels of TNFα secreted by adipocytes and infiltrating macrophages (23) decrease the insulin response of cells to insulin through IKKß-, JNK-, mTOR-, and S6K1-mediated phosphorylation and inactivation of IRS1 in obese populations (Fig. 4). This in turn prevents glucose uptake by GLUT4 and increases glucose concentrations in the blood, symptoms of diabetes. In addition to TNFα, interleukin-6 (IL-6) is a pro-inflammatory cytokine involved in obesity-derived insulin resistance via the signal transducer and activator of transcription 3/suppressor of cytokine signaling 3 (STAT3) pathway. Notably, HFD- and obesity-derived increases in IL-6 expression may also occur due to transcriptional up-regulation by TNFα/ IKKß-induced nuclear factor κB (NF-κB) activation (22). Collectively, these findings emphasize the vital role of TNFα/IKKß signaling in diet- and obesity-induced insulin resistance.

Importantly, dysregulation of TNFα contributes not only to obesity-mediated insulin resistance but also to cancer development. We recently demonstrated that the upregulation of the IKKß/TSC1/mTOR/S6K1 signaling pathway enhances angiogenesis and culminates in breast cancer development. Clinical studies further suggest that dysregulation of this pathway is associated with poor clinical outcome of breast cancer. Findings of our present study and previous work (12,13) suggest that the interaction between IKKß and TSC1 is a molecular switch for triggering both cancer and obesity-mediated type 2 diabetes and offers a rationale for the role of obesity as a risk factor for both diseases (24).

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