Insulin-like growth factor I (IGF-I) plays an important role in cell survival, proliferation, and differentiation. Diverse kinases, including AKT/protein kinase B, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK), can be activated by IGF-I. Here, we show that the receptor-interacting protein (RIP), a key mediator of tumor necrosis factor-induced NF-κB and JNK activation, plays a key role in IGF-I receptor signaling. IGF-I induced a robust JNK activation in wild type but not RIP null (RIP−/−) mouse embryonic fibroblast cells. Reconstitution of RIP expression in the RIP−/− cells restored the induction of JNK by IGF-I, suggesting that RIP is essential in IGF-I-induced JNK activation. Reconstitution experiments with different RIP mutants further revealed that the death domain and the kinase activity of RIP are not required for IGF-I-induced JNK activation. Interestingly, the AKT and ERK activation by IGF-I was normal in RIP−/− cells. The phosphatidylinositol 3-kinase inhibitor, wortmannin, did not affect IGF-I-induced JNK activation. Additionally, binding of IGF-I to the IGF-IR also induces the transient activation of c-Jun N-terminal kinase (JNK) (17). IGF-I-induced JNK activation could be anti-apoptotic (18, 19). The mechanism of the IGF-IR-mediated activation of JNK has not been well elucidated. Because the C terminus of the IGF-IR, IRS-1, and IRS-2, which are required for AKT activation, are unnecessary for JNK activation, it appears that signals that activate JNK are mediated by the IGF-IR. The death domain kinase receptor-interacting protein (RIP) plays a central role in tumor necrosis factor (TNF)-induced nuclear factor-κB (NF-κB) activation, which contributes signif-

As a potent mitogen, insulin-like growth factor I (IGF-I) plays an important role in cell proliferation, differentiation, and survival (1–3). Accumulating evidence also suggests that IGF-I contributes to carcinogenesis. Epidemiological studies have shown consistently that elevated circulating IGF-I is associated with increased risk for several common cancers (4–7). In many cell and tumor systems, IGF-I is involved in cell transformation and maintenance of the malignant phenotype. The combination of mitogenic and anti-apoptotic properties of IGF-I has a profound impact on tumor growth, rendering the IGF-I system a good target for potential adjunct therapy to standard chemotherapy (8–10). The anti-apoptosis of IGF-I may involve activation of AKT and the regulation of the ratio of Bcl2 and Bax that leads to blockade of the initiation of the apoptotic pathway (11, 12).

The binding of IGF-I to its cell membrane IGF-I receptor (IGF-IR) is regulated by a group of six specific IGF-I binding proteins (IGFBP1–6), which belong to the IGF family (13–15). The IGF-IR is a heterotetramer of two identical α- and β-subunits, IGF-IRα and IGF-IRβ, respectively, that are generated by proteolysis and glycosylation of the αβ precursor encoded by a single gene. Binding of IGF-I to the IGF-IR activates the tyrosine kinase of the receptor, which in turn triggers a cascade of interactions among a number of molecules involved in signal transduction (15). Distinct signal transduction pathways have been identified for the IGF-IR, and it appears that there is a considerable overlap in the pathways used for the receptor functions (14, 15). One pathway activated by the IGF-IR is that through the insulin receptor substrate (IRS)-1 or IRS-2, leading to the activation of phosphoinositol (PI) 3-kinase and AKT, which promote suppression of apoptosis via phosphorylation of downstream factors such as caspase-9, Bad, GSK3-β, and the transcription factors FKHR1 and CREB (16). The next pathway activates the extracellular signal-regulated kinase (ERK) through the Ras/Raf/MAP or Ras/Rsk-1/MAP cascade (14, 15). Additionally, binding of IGF-I to the IGF-IR also induces the transient activation of c-Jun N-terminal kinase (JNK) (17). IGF-I-induced JNK activation could be anti-apoptotic (18, 19). The mechanism of the IGF-IR-mediated activation of JNK has not been well elucidated. Because the C terminus of the IGF-IR, IRS-1, and IRS-2, which are required for AKT activation, are unnecessary for JNK activation, it appears that signals that activate JNK are mediated by the IGF-IR and are distinct to those leading to PI 3-kinase and AKT activation (18).
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To cell survival (20–22). RIP also contributes to TNF-induced JNK, ERK, and p38 MAP kinases activation (23, 24). Recent studies in different cell models have revealed that RIP is also critical in NF-κB activation by other agents, including TNF-related apoptosis-inducing ligand, DNA damaging agents, and double-stranded RNA (25–28). In addition, RIP is crucial for toll-like receptor (TLR)4- and TLR6-mediated AKT activation (29). Further, it is reported that RIP interacts with the epidermal growth factor receptor, contributing to epidermal growth factor–induced NF-κB activation (30).

In this study, we address whether RIP is involved in IGF-IR signaling. With RIP+/− mouse embryonic fibroblast (MEF) cells, we demonstrated that RIP is required for IGF-1-induced JNK activation. The results indicate that the death domain kinase RIP, a key factor in TNF signaling, also plays a pivotal role in the IGF-IR–mediated activation of JNK.

**EXPERIMENTAL PROCEDURES**

**Reagents and Plasmids—**Recombinant human IGF-I and wortmannin were purchased from Calbiochem. TNF and interleukin (IL)-1 were from R&D Systems (Minneapolis, MN). Anti-RIP, c-Myc, and JNK1 antibodies were purchased from BD Pharmingen. Anti-IGF-IRβ, Xpress, and -HA antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-AKT, -ERK, -phospho-AKT, and -phospho-ERK antibodies were from Upstate Biotechnology (Chicago, IL). Anti-phospho-c-Jun was purchased from Cell Signaling (Beverly, MA). The mammalian expression plasmids for RIP, RIP-(324–671), CrmA, and HA-JNK1 are confirmed by DNA sequencing.

Cell Culture and Transfection—MEF and HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. The medium was then replaced with serum-free Dulbecco’s modified Eagle’s medium. After continuing the culture for 4 h, the cells were treated with IGF-I. Cells were collected and counted at 1, 2, or 3 days post-treatment. The experiments were repeated at least three times, and data shown (mean ± S.D.) are representative of three independent experiments.

**RESULTS**

**Impaired JNK Activation by IGF-I in RIP+/− Cells—**RIP plays a pivotal role in activation of NF-κB, JNK, and p38 induced by diverse stimuli (23–28, 33). Using a BLAST search for RIP homolog(s), we found a significantly high homology between the RIP and the IGF-I receptor β subunit (IGF-IRβ) (data not shown). This prompted us to address whether RIP is involved in IGF-I-induced signaling. Based on the fact that RIP is involved in JNK activation by different stimulations, we explored the possible role of RIP in IGF-I–induced JNK activation with RIP+/− MEF cells as a cell model. JNK activity was measured by an in vitro kinase assay with glutathione–S-transferase-c-Jun (1–79 amino acids) as the substrate. In WT MEF cells IGF-I treatment caused a robust activation of JNK, which started at 10 min, peaked at 20–40 min, and lasted up to 60 min post-treatment (Fig. 1A). However, there was no detectable JNK induction by IGF-I in RIP−/− cells (Fig. 1B, top panel). RIP−/− cells were confirmed by Western blot analysis to be null of RIP and defective to TNF-induced JNK activation by an in vitro kinase assay (Fig. 1B and data not shown). Because comparable expression levels of JNK1 and IGF-IR were detected in both RIP−/− and WT cells (Fig. 1B, second and fourth panels), it is unlikely that the decrease of JNK activation in RIP−/− cells resulted from the altered expression of JNK or the IGF-IR.

Deficient JNK activation by IGF-I in RIP−/− cells was further confirmed with an anti-phospho-JNK antibody. In this
experiment, JNK activation by IGF-I was detected in a dose-dependent manner in WT cells (Fig. 1C, upper panel). The induction of c-Jun, the direct downstream target of JNK, was also impaired in RIP−/− cells (Fig. 1C, middle panel). These results suggest that RIP is required for transducing the IGF-I-induced signal to activate JNK. Defective JNK activation in RIP−/− cells was specific to IGF-I treatment because RIP−/− cells responded to IL-1 and ultraviolet treatment as efficiently as the WT cells did in terms of JNK activation (Fig. 1D and data not shown).

IGF-I–Induced Normal AKT and ERK Activation in RIP−/− Cells—Upon activation by IGF-I, the IGF-IR transduces signals to activate ERK and AKT through Ras/Raf and PI 3-kinase, respectively. Activation of AKT or ERK is associated with the phosphorylation of AKT or ERK proteins. Because RIP is also involved in AKT and ERK activation under certain situations (23, 24, 28), we addressed whether RIP is also involved in mediating IGF-I–induced AKT and ERK activation. RIP−/− and WT cells were treated with IGF-I and collected at different time points post-treatment, and the activated forms of AKT and ERK were detected with anti-phospho-AKT and anti-phospho-ERK antibodies, respectively. As shown in Fig. 2, IGF-I–induced AKT phosphorylation was comparable in the RIP−/− and WT cells. A similar result was obtained regarding the IGF-I–induced ERK activation. These results suggested that RIP is unnecessary for AKT and ERK activation pathways.

Although RIP is a key factor for TNF-induced NF-κB activation, and IGF-I was found to activate NF-κB activity in some cell types such as multiple myeloma cells (34), we did not detect NF-κB activation by IGF-I in MEF cells, either by Western blot for IκBα degradation or NF-κB-reporter assay for NF-κB activity (Fig. 2 and data not shown). Whether RIP is involved in IGF-IR–mediated activation of NF-κB remains to be determined in other cell models.

Restoration of IGF-I–Induced JNK Activation in RIP–Reconstituted Cells—To rule out the possibility that some other flaws in the signaling pathway of IGF-I–induced JNK activation are present in RIP−/− cells, we tested whether IGF-I–induced JNK activation could be restored when RIP is reconstituted in those cells. To examine the reconstitution of IGF-I–induced JNK activation, the expression vector for myc-RIP was co-transfected with HA-JNK1 into RIP−/− cells. Following treatment with IGF-I, the transfected HA-JNK1 was immunoprecipitated for in vitro JNK activity assay. As shown in Fig. 3, RIP expression restored JNK activation in response to IGF-I treatment in RIP−/− cells (Lane 4). This result indicated that defective IGF-I–induced JNK activation in RIP−/− cells is caused by the absence of RIP.

IGF-I–Induced JNK Activation Does Not Require the Death Domain and Kinase Activity of RIP—RIP possesses three functional domains: kinase domain (1–300 amino acids), intermediate domain (300–582 amino acids), and death domain (583–653 amino acids). The death domain is essential in TNF...
receptor signaling as it is a protein-protein interacting motif that is used to recruit RIP to the tumor necrosis factor receptor signaling complex. The intermediate domain is crucial for NF-κB activation. The biological relevance of the kinase domain is not well elucidated, although it may be involved in TNF-induced ERK activation and necrotic cell death (23, 35). To determine the domain prerequisite for IGF-I-induced JNK activation, the expression vectors for either myc-RIP (K45A), a kinase dead RIP mutant, or myc-RIP-(1–558), a death domain deleted RIP mutant, was co-transfected with HA-JNK1 into RIP+/− cells. WT RIP was used as a positive control. After treatment with IGF-I, HA-JNK1 was immunoprecipitated, and JNK activity was measured by in vitro kinase assay. As shown in Fig. 3, both RIP (K45A) and RIP-(1–558) expression restored the JNK activation in response to IGF-I treatment in RIP+/− cells as efficiently as WT RIP did. The expression levels of RIP (K45A), RIP-(1–558), and RIP WT were comparable (Fig. 3, bottom panel). These results indicated that both the kinase and death domains of RIP are dispensable for IGF-I-induced JNK activation in MEF cells.

**IGF-I-induced JNK Activation Is Not Mediated by PI 3-Kinase**—It was reported that the IGF-I-induced JNK activation pathway is distinct to that of the PI 3-kinase-mediated AKT activation (18). The results indicating that RIP is unnecessary for IGF-I-induced ERK and AKT activation supported the notion that JNK activation does not involve PI 3-kinase (Fig. 2). To further test this hypothesis, WT cells were pretreated with the PI 3-kinase inhibitor wortmannin followed by IGF-I treatment. The cell extracts were subjected to in vitro JNK assay and Western blot analysis for phospho-AKT. TNF treatment was included as a control. When treated with a moderate concentration of wortmannin (100 nM), which specifically and efficiently suppressed PI 3-kinase-mediated AKT activation by IGF-I, the IGF-I-induced JNK activity was barely affected (Fig. 4A). As expected, pre-incubation of wortmannin did not interfere with TNF-induced JNK activation (Fig. 4A). This result supported the conclusion that IGF-I-induced JNK activation does not involve the PI 3-kinase pathway.

When cells were treated with a high concentration of wortmannin (10 μM), the IGF-I-induced JNK activation was completely blocked, whereas TNF-induced activation was not affected (Fig. 4B). Because high concentrations of wortmannin inhibit other kinases in addition to the PI 3-kinase, the blockage of JNK activation under this condition likely resulted from the inhibition of an unidentified kinase by wortmannin. However, as the TNF-induced JNK activation was not affected under the same condition, the mechanism of IGF-I-induced JNK activation might be different from that of TNF, although both IGF-I and TNF-induced signaling to JNK activation require RIP.

**RIP Interacts with the IGF-IR**—Because our data suggested that RIP is essential in IGF-I-induced JNK activation, RIP might also interact with the IGF-IR. To test this possibility, we investigated whether RIP interacts with the IGF-IR by ectopic expression of RIP and the IGF-IRβ in HEK293 cells. The Xpress-tagged IGF-IRβ was immunoprecipitated with an anti-Xpress antibody, and the immunoprecipitants were analyzed by Western blot with an anti-myc antibody. As shown in Fig. 5A, myc-tagged RIP was specifically co-precipitated with the Xpress-tagged IGF-IRβ when RIP and the IGF-IRβ were co-expressed. There was no detectable precipitation of RIP when it was expressed alone, although the expression level was similar to that of RIP and IGF-IRβ co-expression (Fig. 5A). Conversely, the IGF-IRβ was specifically co-immuno-
precipitated with myc-RIP when the two proteins were co-expressed (Fig. 5B). These results suggested a specific binding between RIP and the IGF-IRβ.

With ectopic expression and co-immunoprecipitation, we mapped the IGF-IRβ binding region in RIP. Whereas the death domain (RIP-(559–671)) was unable to bind IGF-IRβ, RIP-(324–671) as well as RIP-(1–558) retained the binding activity (Fig. 5C). Therefore, the minimal IGF-IRβ binding region is mapped to be between 324–558 amino acids, locating in the intermediate domain of RIP. It is noteworthy that the binding of RIP-(324–671) and RIP-(1–558) to IGF-IRβ was greatly reduced compared with that of WT RIP. It is possible that the sequence flanking this minimal binding region may help to stabilize the association between RIP and IGF-IRβ. Nevertheless, these results are consistent with the RIP reconstitution experiment, showing that the death domain and kinase activity of RIP are unnecessary for IGF-I-induced JNK activation (Fig. 3).

**RIP Is Part of the IGF-IR Signaling Complex**—To test whether endogenous RIP is recruited to the IGF-IR complex during IGF-I signaling, we performed co-immunoprecipitation experiments with cell extracts derived from WT cells with or without IGF-I treatment. In these experiments, the endogenous IGF-IRβ was immunoprecipitated with an anti-IGF-IRβ antibody, and the immunoprecipitants were analyzed with an anti-RIP antibody. The co-precipitated IGF-IRβ protein was detected by Western blot analysis with an anti-Xpress antibody. The expression of Xpress-IGF-IRβ (middle) and myc-RIP proteins (bottom) was detected by Western blot analysis with anti-Xpress and anti-myc, respectively. 5, endogenous c-Myc. D, 2 × 10^5 of WT cells were serum-starved for 4 h followed by treatment with IGF-I (100 ng/ml) for 10 or 20 min or remained untreated. Immunoprecipitation experiments were performed with anti-IGF-IRβ, and the co-precipitated RIP protein was detected by Western blot analysis. Detection of JNK1 was performed as a control.
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association of RIP and IGF-IRβ induced by IGF-I is specific (data not shown). These results suggested that IGF-I induces the recruitment of RIP to the IGF-IR. Interestingly, only a trace amount of RIP was detected after 20 min of treatment (Lane 6), suggesting that RIP was released rapidly from the IGF-IR signaling complex after being recruited there. This release may enable a downstream signaling that activates JNK, which is not recruited to IGF-IR (Fig. 5D).

RIP Is Required for IGF-I-induced Cell Proliferation—It was suggested that JNK contributes to IGF-I-induced anti-apoptosis (18). However, IGF-I had no detectable effect on serum withdrawal-induced death in MEF cells (data not shown). We then compared IGF-1-induced cell proliferation in WT and RIP−/− cells in a serum-free medium. RIP-reconstituted RIP−/− cells (RIP−/−/RIP) were also included (32). After exposure to IGF-I for 1, 2 and 3 days, cell numbers were counted. IGF-I stimulated WT cell proliferation. However, although RIP−/− cells retain some proliferating capacity, IGF-I exposure did not trigger cell proliferation in RIP−/− cells. Importantly, in RIP−/−/RIP cells, the response in IGF-I-induced proliferation was restored, suggesting that the insufficient response to IGF-I-induced proliferation in RIP−/− cells was caused by RIP deficiency (Fig. 6). Similar results were obtained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (26). These results suggested that the insufficient response to IGF-I-induced proliferation in RIP−/− cells, these results suggest that the RIP-mediated JNK pathway may contribute to IGF-I-induced proliferation in MEF cells. This notion is supported by the observation that RIP inhibition SP600125 suppressed IGF-I-induced proliferation in WT cells (data not shown).

DISCUSSION

Although tremendous effort has been made to investigate the biological functions of IGF-I and its receptor, IGF-IR, the molecular mechanism of IGF-IR signaling to JNK activation still is not well elucidated. In this study we found that RIP, a critical effector of TNF receptor signaling, plays an important role in IGF-IR-mediated JNK activation. The kinase activity and death domain of RIP are not required for IGF-I-induced JNK activation. In addition, we also demonstrated that RIP is recruited into the IGF-IR signaling complex when the IGF-IR is activated by the ligation of IGF-I. Therefore, RIP is a critical effector in IGF-IR signaling.

RIP is an essential effector for NF-κB activation induced by the TNF family cytokines (22, 33, 36). Previous studies with overexpression of the dominant negative mutant RIP have shown that RIP is involved in TNF-induced JNK activation (20). Although the study using RIP knock-out MEF cells showed that RIP had little effect on TNF-induced JNK activation (33), we found that the TNF-induced JNK activation was significantly decreased in RIP−/− cells (23). We also have found that RIP is required for TNF-related apoptosis-inducing ligand-induced JNK activation (25). These studies suggested that, in addition to NF-κB activation, RIP also plays a role in JNK activation that is induced by the TNF family of cytokines. In this study, we show evidence indicating that RIP is essential in IGF-1-induced JNK activation. We found that IGF-I-induced JNK activation in RIP−/− cells was impaired but could be restored once RIP expression was ectopically reconstituted. Additionally, RIP was recruited into the IGF-IR complex as early as 10 min, suggesting that RIP is an early signaling adapter for the IGF-IR. It seems that the interaction between RIP and the IGF-IRβ is direct, as it is easily detected when both were overexpressed in the cells.

It appears that JNK activation by IGF-I is a direct event rather than a secondary one that takes place through activation of the TNF receptor signaling system. First, IGF-I-induced JNK activation starts as early as 10 min, and it is unlikely through the induction of auto- or paracrine signaling of TNF. Second, in IGF-I-treated fibroblast cells there is undetectable NF-κB activation, which is normally detected after activation of the tumor necrosis factor receptor in these cells. Third, the death domain of RIP, which is essential for recruitment of RIP to the TNF receptor, is not required for the association between RIP and the IGF-IRβ or for IGF-I-induced JNK activation. Lastly, IGF-
but not TNF-induced JNK activation could be suppressed by high concentrations of wortmannin. Therefore, the participation of RIP in IGF-1 signaling to JNK activation is likely authentic in the cells. Because the kinase activity of RIP is dispensable for IGF-1-induced JNK activation, it is highly likely that RIP functions as an adaptor to mediate signals to downstream kinases that activate JNK. Based on the differentiated sensitivities of IGF-1- and TNF-induced JNK activation pathways to high concentrations of wortmannin, we speculate that the mechanism of IGF-I-induced JNK activation might be distinct to that of TNF. High doses of wortmannin have been suggested to inhibit PI 4-kinase, ataxia-telangiectasia mutation, ataxia-telangiectasia mutation and Rad3-related kinase, and myosin light chain kinase (37–39). However, all these kinases except ataxia-telangiectasia mutation have not been implicated in JNK activation. It will be interesting to determine whether these kinases, particularly ataxia-telangiectasia mutation, are involved in IGF-I-induced JNK activation. Whether the kinases involved in TNF-induced JNK activation such as MEKK1, MEKK3, ASK1, JNKK1 and JNKK2 are also involved in IGF-I-induced JNK activation remains to be determined. Therefore, further studies are needed to identify the JNK-activating kinase involved in IGF-1 receptor signaling.

When the IGF-IR is activated, RIP is rapidly recruited to and then released from the IGF-IR complex. This suggested that RIP is activated rapidly by the IGF-IR signaling complex and then released to transduce the JNK activation signal to the downstream factors. This is analogous to that of TNF receptor signaling in that a dynamic change of the signaling complex is critical for activation of distinct pathways (40). The results of this study showed that although RIP is recruited to the IGF-IR signaling complex, it only specifically functions in signaling to induce JNK activation, whereas RIP is unnecessary for IGF-I-induced AKT and ERK activation. Together, with the observation that IGF-I-induced JNK activation does not involve the PI 3-kinase pathway, these results are consistent with other studies that demonstrated that the pathways leading to the IGF-IR-mediated activation of JNK, ERK, and AKT are distinct (18).

The involvement of JNK activation in controlling cell death has not been well understood. Depending on cell contents and the nature of different stimuli, JNK can be pro-apoptotic, anti-apoptotic, or have no effect on killing cells (20, 41–43). It is also assumed that JNK activation can mediate necrotic cell death (44). Although it is well known that IGF-I is a potent suppressor of apoptosis through AKT-dependent and -independent pathways (18, 19), suppression of JNK with ducmolar blocked IGF-I-induced anti-apoptosis, suggesting that JNK plays an important role in AKT-independent apoptosis suppression (18, 19). In our MEF cell system, IGF-I showed no significant effect on serum-deprivation-induced apoptosis, and the role of IGF-I-induced JNK in controlling apoptosis remains to be addressed in other cell systems. Instead, we found that IGF-I-induced cell proliferation in RIP−/− cells is dramatically impaired. Because IGF-I-induced-JNK activation is abolished in RIP−/− cells whereas other pathways such as AKT and ERK remain normal, it is plausible that JNK is involved in IGF-I-induced proliferation in MEF cells.

As both IGF-I and TNF are involved in the regulation of cell proliferation, survival, and apoptosis, the sharing of RIP as a common signaling mediator suggested that there might be some cross-talk between the IGF-IR and tumor necrosis factor receptor signal transduction pathways. Indeed, in astroglial cells, treatment of IGF-I inhibited TNF-induced phosphorylation of IkBα and nuclear translocation of NF-κB (45). Also, IGF-I was found to enhance TNF-induced NF-κB and JNK activation in bovine aortic endothelial cells (46). In HT29-D4 epithelial adenocarcinoma colic cells, IGF-I down-regulated TNF-induced NF-κB activation and up-regulated IL-8 gene expression (47). In addition, IGF-I was reported to negatively regulate TNF- and TNF-related apoptosis-inducing ligand-induced apoptosis in human adipocytes (48). Conversely, TNF was found to inhibit insulin action though phosphorylation of IRS-1 by JNK (49). Whether RIP is involved in the crosstalk between IGF-I and TNF signaling needs to be addressed in future studies.

Although RIP was originally characterized in TNF signaling, recent studies in different cell models have revealed that RIP is also critical in NF-κB activation by TNF-related apoptosis-inducing ligand, DNA damaging agents, and double-stranded RNA (25–28, 50), and AKT activation mediated by TLR4 and TLR6 (29). Physical interaction of RIP and the epidermal growth factor receptor has been reported, and the role of RIP in epidermal growth factor-induced NF-κB activation has been proposed (30). The results of this study add IGF-I signaling to the list of RIP-involved pathways, suggesting that RIP functions as a signal transduction integrator relaying a broad spectrum of signals to downstream survival pathways (51). Therefore, RIP could be a molecular target for modulating survival signaling, which may be implicated in cancer therapy.

Taken together, the results presented herein demonstrate that RIP plays a critical role in IGF-IR-mediated JNK activation. Because IGF-I is involved in tumorigenesis, and JNK activation contributes to the IGF-I-induced proliferation and suppression of apoptosis, further studies on the mechanism of RIP-mediated JNK activation during IGF-IR signaling would aid in designing new strategies for cancer therapy and prevention.

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