The Small GTP-binding Protein Rac1 Induces Cardiac Myocyte Hypertrophy through the Activation of Apoptosis Signal-regulating Kinase 1 and Nuclear Factor-κB*

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The small guanine nucleotide-binding protein Rac1 has emerged as an important molecule involved in cardiac myocyte hypertrophy. Recently, we reported on apoptosis signal-regulating kinase (ASK) 1 and a transcriptional factor, nuclear factor-κB (NF-κB), as novel signaling intermediates in cardiac myocyte hypertrophy. The aim of the study presented here was to clarify the role of Rac1 in the ASK1-NF-κB signaling pathway. Infection of isolated neonatal cardiac myocytes with an adenovirus expressing a constitutively active form of Rac1 (RacV12) enhanced the expression of a NF-κB-dependent reporter gene construct and induced the degradation of IκB. Expression of a degradation-resistant mutant of IκBα inhibited the RacV12-induced hypertrophic responses, including increases in protein synthesis and atrial natriuretic factor production and the enhancement of sarcomeric organization. An immune complex kinase assay indicated that the expression of RacV12 activated ASK1. Expression of a dominant negative mutant of ASK1 eliminated the RacV12-induced NF-κB activation and the biochemical and morphological hypertrophic responses, whereas expression of a dominant negative form of Rac1 attenuated phenylephrine-induced activation of ASK1 and NF-κB and cardiac myocyte hypertrophy. These findings suggest that Rac1 induces cardiac myocyte hypertrophy mediated through ASK1 and NF-κB.

Cardiac myocyte hypertrophy is an important adaptive process in response to various extracellular stimuli such as mechanical stress, cytokines, and growth factors. The hypertrophic response is compensatory in nature, but sustained excessive workloads may lead to heart failure. Epidemiological studies suggest that cardiac hypertrophy is an independent risk factor for cardiac morbidity and results in a significant increase in the relative risk of mortality from cardiovascular diseases (1).

Analysis of the signal transduction cascade in the development of cardiac hypertrophy has used neonatal ventricular myocytes as a model system. The hypertrophic response is characterized by an increase in cell volume, the assembly of contractile proteins into reorganized sarcomeric units, and the reactivation of fetal genes such as the atrial natriuretic factor (ANF) (2). The signaling pathways, in which extracellular signals are transmitted into myocytes to induce the hypertrophic responses, are probably multifold, but the small (21-kDa) guanine nucleotide-binding proteins (G-protein) of the Ras and Rho families have been implicated in the regulation of this process (3).

The Rho family GTPases, including Rho, Rac, and Cdc42, participate in regulation of the actin cytoskeleton and various cell adhesion events (4). Rac is involved in membrane ruffling, cell motility, actin polymerization, and cadherin-mediated cell-cell adhesion in non-muscle cells. In addition to its roles in cell morphology, Rac participates in regulating transcription both through the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinase pathways (5, 6). Furthermore, Rac has been reported to activate the transcription factor, nuclear factor-κB (NF-κB) (7), whereas the generation of intracellular reactive oxygen species (ROS) production by Rac might be a trigger for NF-κB activation (7). In addition, the activated form of Rac1 can also stimulate cell cycle progression to promote DNA synthesis in fibroblasts (8).

In cardiac myocytes, Rac has been implicated in their hypertrophic response, mediating both the morphological and transcriptional changes. Previous studies have demonstrated that the hypertrophic agonists such as endothelin-1 and phenylephrine induce rapid activation of Rac1 in cardiac myocytes (9). Transfection of cardiac myocytes with a constitutively active mutant of Rac1 (RacV12) was found to increase ANF, brain natriuretic factor (BNP), and α-skeletal actin promoter activities (10), and the adenoviral gene transfer of RacV12 resulted

* The abbreviation used are: ANF, atrial natriuretic factor; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; ERK, extracellular signal-related kinase; MEKK, MAP kinase/ERK kinase kinase; MKK6, MAP kinase kinase 6; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; ASK1, apoptosis signal-regulating kinase 1; ASK1(KM), dominant negative form of ASK1 with mutant K709M; AdASK1(KM), adenoviral vector expressing ASK1(KM); AdASKA(KM), adenoviral vector expressing ASK1(KM); ASK1N, constitutively active form of ASK1; AdIκBαS23A, adenoviral vector expressing a mutated form of human IκBα (S23A/S23A/Rα); RacV12, constitutively active mutant of Rac1; AdRacV12, adenoviral vector expressing AdRacV12; RacN17, dominant negative mutant of Rac1 (S17N); AdRacN17, adenoviral vector expressing RabB17; AdLacZ, adenoviral vector expressing bacterial β-galactosidase; GPCR, G-protein coupled receptor; DMEM, Dulbecco’s modified Eagle’s medium; HA, hemagglutinin; NAC, N-acetyl cysteine; PAK, p21-activated kinase.

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in the enhancement of sarcomeric organization and increases in cell size and protein synthesis, along with increased ANF expression. Furthermore, infection with an adenovirus expressing a dominant negative mutant of Rac1 (RacN17) inhibited the phenylephrine-induced hypertrophic responses (11). Finally, overexpression of Rac1 in mouse heart resulted in the induction of a cardiomyopathic phenotype characterized by hypertrophy or dilation (12). The downstream effectors of Rac, however, have not yet been thoroughly characterized.

Recently, we identified apoptosis signal-regulating kinase 1 (ASK1) and NF-κB as novel signaling intermediates involved in cardiac myocyte hypertrophy (13, 14). ASK1 was recently identified as an ROS-sensitive MAP kinase kinase kinase that activates JNK and p38 (15), and the JNK/p38 pathways are known to play important roles in cardiac myocyte hypertrophy (16–18). We have demonstrated that the G-protein-coupled receptor (GPCR) agonists and tumor necrosis factor-α activate NF-κB in rat neonatal cardiac myocytes (13, 14). Inhibition of NF-κB activation resulted in elimination of the agonist-induced cardiac myocyte hypertrophy, suggesting that NF-κB is required for the hypertrophic growth of cardiac myocytes. Moreover, GPCR agonists activate ASK1, and overexpression of a dominant negative form of ASK1, ASK(KM), inhibits GPCR agonist-induced myocyte hypertrophy. The constitutively active form of ASK1 (ASK(ΔN)) activates NF-κB, leading to hypertrophy. We therefore hypothesized that ASK1 and NF-κB might be present downstream of Rac1 in the signal transduction pathway during cardiac myocyte hypertrophy. In this study, we demonstrated that Rac1 induces cardiac myocyte hypertrophy via the activation of NF-κB and that ASK1 is required for the activation of NF-κB and the development of cardiac myocyte hypertrophy.

**EXPERIMENTAL PROCEDURES**

Primary Culture of Neonatal Rat Ventricular Myocytes—Rat ventricular myocytes from 1- or 2-day-old Wister rats were prepared and cultured overnight in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum as described previously (19). The media were changed to serum-free DMEM supplemented with transferrin (5 μg/ml) and insulin (1 μg/ml) 24 h before adenoviral infection and/or treatments.

Recombinant Adenovirus Vectors—The replication-defective E1 and E3 adenoviral vector expressing either a mutated form of human IκBα (AdIκBα32/36A; mutant Ser52 and Ser36 to Ala), a hemagglutinin (HA)-tagged dominant negative mutant of Rac1 (AdRacN17; mutant Ser17 to Asn), or bacterial β-galactosidase (AdLacZ) was described previously (13, 14, 20). Adenoviral vectors expressing a dominant negative (AdASK(KM)); mutant Lys789 to Met) and a constitutively active (AdASK(ΔN)) form of ASK1 were also described in previous reports (21). Adenovirus AdRacV12, containing the myc-tagged constitutively active Rac1 cDNA, was constructed by means of homologous recombination in human embryonic kidney 293 cells using the adenovirus-based plasmid JM17 (22). Cardiac myocytes were infected with the recombinant adenovirus vectors at a multiplicity of infection of 10 plaque-forming units per cell for 1 h. Subsequently, the cells were cultured in serum-free DMEM for an additional 24 h prior to treatment. To confirm successful infection with adenoviruses, myocytes infected with AdLacZ were co-stained with 5-bromo-4-chloro-3-indolyl-β-D-galactoside and rhodamine-conjugated phalloidin. Myocytes infected with epitope-tagged adenoviruses were co-stained with rhodamine-conjugated phalloidin and fluorescein isothiocyanate-conjugated secondary antibodies followed by incubation with epitope-specific first antibodies (anti-HA for AdRacN17, AdASK(ΔN), and AdASK(KM)) and anti-myc for AdRacV12) (Santa Cruz Biotechnology). Adenoviruses were successfully infected to more than 95% of all myocytes.

Expression of the epitope-tagged RacN17 and RacV12 was assessed by Western blotting using 20 μg of myocardial protein lysates with HA- and myc-specific antibodies, respectively. The probed proteins were detected with secondary antibodies by an ECL Western blotting detection kit (Amersham Biosciences).

**Measurement of NF-κB Activity**—Cardiac myocytes plated on 22-mm-diameter culture dishes were exposed to 5 μg of lipofectin with the luciferase reporter construct containing consensus NF-κB binding sites (1.67 μg) according to the manufacturer’s instructions (Invitrogen) (23). After incubation for 24 h in serum-free DMEM, viral infection was initiated, and the myocytes were cultured for 24 h. Myocytes were assayed for luciferase activity using a luciferase reporter assay kit (Promega). The activity was normalized to total protein concentration and expressed as -fold induction relative to untreated control. Values represent the mean ± S.E. of data for three experiments performed in triplicate.*, p < 0.05 versus control; #, p < 0.05 versus AdRacV12. B, immunoblotting was performed with anti-IκBα antibody.

**FIG. 1.** Activation of NF-κB by RacV12. A, cardiac myocytes, transfected with x-B-luciferase construct, were infected with AdLacZ, AdRacV12, or AdIκBα32/36A alone or co-infected with AdRacV12 and AdIκBα32/36A. Adenoviral infection was performed at a multiplicity of infection of 10 viral particles per cell. Twenty-four hours after adenoviral infection, the cultures were extracted and assayed for luciferase. Luciferase activity was normalized to protein content for each sample and is expressed as -fold induction relative to untreated control. Values represent the mean ± S.E. of data for three experiments performed in triplicate.*, p < 0.05 versus control; #, p < 0.05 versus AdRacV12.
Statistical Analysis—Data are expressed as mean ± S.E. Differences between experimental groups were analyzed for statistical significance with one-way analysis of variance (ANOVA) followed by Bonferroni’s post-test. \( p \) values \( < 0.05 \) were considered to be statistically significant.

RESULTS

Activation of Rac1 Results in Stimulation of NF-κB Activity in Cardiac Myocytes—A previous study has shown that infection of cardiac myocytes with AdRacV12 induced cardiac myocyte hypertrophy (11). We examined whether NF-κB is involved as a transcription factor in RacV12-induced cardiac myocyte hypertrophy. Isolated cardiac myocytes were transiently transfected with a luciferase reporter construct containing consensus NF-κB binding sites. Infection of myocytes with AdRacV12 led to increased luciferase activity, whereas infection of a control adenoviral vector expressing bacterial β-galactosidase, AdLacZ, had no effect on NF-κB activation (Fig. 1A).

To confirm the activation of NF-κB by Rac1, we examined the degradation of IκBα by using an anti-IκBα antibody for Western blot analysis (Fig. 1B). IκBα was indeed degraded in cells infected with AdRacV12. These findings indicated that Rac1 activates NF-κB in cardiac myocytes.

NF-κB Is Involved in Activated Rac1-induced Cardiac Myocyte Hypertrophy—We also examined the role of NF-κB in...
myocyte hypertrophy induced by RacV12. The effectiveness of an adenovirus expressing a degradation-resistant form of IkBa, (AdIkBa32/36A) in blocking activation of NF-κB was assessed in a reporter gene assay. The luciferase induction mediated by overexpression of RacV12 was reduced to near control levels in the myocytes co-infected with AdIkBa32/36A (Fig. 1A). Next, we examined the effects of AdIkBa32/36A on cardiac myocyte hypertrophy induced by RacV12. At the cellular level, the hypertrophic response is characterized by an increase in protein synthesis, an induction of sarcomere organization, and an increase in the expression of embryonic genes such as ANF. As shown in Fig. 2A, infection with AdRacV12 increased [3H]leucine uptake into cardiac myocytes, but the increase was suppressed by co-infection with AdIkBa32/36A, whereas infection with AdIkBa32/36A alone had no effect on [3H]leucine uptake. We also analyzed the cytoskeletal organization in cardiac myocytes by phalloidin staining (Fig. 2B) and the expression of ANF by co-infection with AdRacV12 and AdASK(KM) (Fig. 2C). Both uninfected cells and cells infected with AdLacZ showed a reticular actin organization lacking sarcomeric formation, whereas infection with AdRacV12 significantly induced the reorganization of the actin cytoskeleton into a sarcomeric structure as was also reported previously (11, 12). Co-infection with AdIkBa32/36A led to the elimination of the enhancement of the sarcomere organization, but infection with AdIkBa32/36A alone had no effect on cell morphology. The percentage of myocytes expressing the ANF protein was increased by infection with AdRacV12 compared with uninfected cells or cells infected with AdLacZ (Fig. 2D). Inhibition of the RacV12-induced NF-κB activation eliminated the increase in ANF expression. These findings suggested that the activation of NF-κB is involved in morphological and biochemical hypertrophic responses induced by Rac1.

Activation of Rac1 Results in Stimulation of ASK1 Activity—We demonstrated previously that ASK1 was necessary for NF-κB activation and cardiac myocyte hypertrophy by GPCR agonists (14). This time, we examined whether the overexpression of RacV12 could activate ASK1 in cardiac myocytes. Cardiac myocytes were transfected with HA-tagged ASK1. Eight hours after infection with AdRacV12, the extracts were immunoprecipitated with the anti-HA antibody. An ASK1 kinase assay was then carried out using MKK6 as a substrate (Fig. 3A, top). As shown in Fig. 3A, the expression of RacV12 resulted in the activation of ASK1. To confirm the activation of ASK1 by RacV12, we estimated endogenous ASK1 activation. The immune complex kinase assay using the anti-ASK1 antibody indicated that RacV12 significantly activated endogenous ASK1 (Fig. 3A, bottom). Western blotting showed a significant increase in the expression of embryonic genes such as ANF as determined by [3H]leucine uptake (Fig. 5). Twenty-four hours after adenoviral infection, the cultures were extracted and assayed for luciferase. Luciferase activity was normalized to protein content for each sample and is expressed as fold induction relative to untreated control. Values show the mean ± S.E. of data for three experiments performed in triplicate. *p < 0.05 versus uninfected control; #p < 0.05 versus AdRacV12. B, immunoblotting was performed with anti-IκBα antibody.

Fig. 3. Activation of ASK1 by RacV12. A, cardiac myocytes transfected (top) or untransfected (bottom) with HA-ASK1 were infected with AdLacZ or AdRacV12. After 8 h of infection, cell lysates were incubated with anti-HA antibody (top) or anti-ASK1 antibody (bottom). ASK1 activity was measured by immune complex kinase assay with His-MKK6 as a substrate. B, adenoviral-mediated gene transfer expression at 8 h of infection. Western blot analysis using a myc-specific antibody demonstrated the expression of the 21-kDa epitope-tagged form of Rac1.

Fig. 4. Rac1-induced NF-κB activation is mediated through ASK1. A, cardiac myocytes, transfected with the NF-κB-luciferase construct, were infected with AdLacZ, AdRacV12, or AdASK(KM) alone or co-infected with AdRacV12 and AdASK(KM). Adenoviral infection was performed at a multiplicity of infection of 10 viral particles per cell. Twenty-four hours after adenoviral infection, the cultures were extracted and assayed for luciferase. Luciferase activity was normalized to protein content for each sample and is expressed as fold induction relative to untreated control. Values show the mean ± S.E. of data for three experiments performed in triplicate. *p < 0.05 versus AdLacZ; #p < 0.05 versus AdRacV12. B, immunoblotting was performed with anti-IκBα antibody.

ASK1 Is Required for RacV12-induced NF-κB Activation and Cardiac Myocyte Hypertrophy—To determine the involvement of ASK1 in the RacV12-induced NF-κB activation, we infected cardiac myocytes with an adenovirus expressing a dominant negative form of ASK1 (AdASK(KM)). Overexpression of ASK1(KM) completely eliminated the activation of NF-κB-dependent luciferase (Fig. 4A) and the degradation of IκBα induced by RacV12 (Fig. 4B). These findings suggested that ASK1 connects Rac1 with NF-κB.

Next, we examined the involvement of ASK1 in RacV12-induced myocyte hypertrophy. Infection of myocytes with AdASK(KM) significantly suppressed the increase in [3H]leucine uptake (Fig. 5A), the enhancement of sarcomeric reorganization, and the expression of ANF (Fig. 5B) induced by RacV12. The percentage of cells expressing ANF was similar to the basal level (8.1 ± 2.4% for the co-infection with AdRacV12 + AdASK(KM)). We thus concluded that ASK1 is involved in the RacV12-induced NF-κB activation, which leads to the induction of morphological and biochemical hypertrophic responses.

Inhibition of Rac1 Activity Results in Abrogation of ASK1 and NF-κB Activation by the Hypertrophic Agonist—Previous reports have demonstrated that expression of a dominant neg-
Rac1 and Cardiac Hypertrophy

Fig. 5. RacV12-induced myocyte hypertrophy is mediated through ASK1. Cardiac myocytes were infected with AdLacZ, AdRacV12, or AdASK(KM) alone or co-infected with AdRacV12 and AdASK(KM). Values represent the mean ± S.E. of data for three experiments performed in triplicate. A, ([3H]leucine incorporation. *, p < 0.05 versus control; #, p < 0.05 versus AdRacV12. B, Organization of sarcomere structure (top row) and ANF protein expression (bottom row).

Fig. 6. Phenylephrine-induced ASK1 activation is mediated through Rac1. A, cardiac myocytes transfected or untransfected with HA-ASK1 were infected with AdLacZ or AdRacN17. After 24 h of infection, myocytes were treated with phenylephrine. Ten minutes after the treatment, cell lysates were incubated with anti-HA (top) or anti-ASK1 (bottom) antibodies. ASK1 activity was measured by immune complex kinase assay with His-MKK6 as a substrate. B, adenovirally mediated gene transfer expression. Western blot analysis demonstrated the expression of RacN17.

which is characterized by increases in [3H]leucine uptake and ANF expression and the enhancement of sarcomeric organization. Infection with AdRacN17, on the other hand, inhibited the hypertrophic response to phenylephrine as was also previously reported (11) (Fig. 7, B–D). However, ASK1(AK)–induced increases in [3H]leucine uptake and ANF expression and the enhancement of sarcomeric organization were not affected by co-infection with AdRacN17 (Fig. 7, B–D). This suggested that ASK1 is present downstream of Rac1 during cardiac myocyte hypertrophy.

N-acetyl Cysteine, an Antioxidant, Abolished the RacV12-induced ASK1 and NF-κB Activation Leading to Hypertrophic Responses—In HeLa cells, a previous report has demonstrated that Rac1 activates NF-κB via ROS generation (7). To explore the role of ROS in Rac1-induced cardiac myocyte hypertrophy, cardiac myocytes were preincubated with an antioxidant, N-acetyl cysteine (NAC), for 1 h prior to AdRacV12 infection. Preincubation with NAC significantly suppressed the activation of NF-κB by RacV12 and also inhibited the increase in [3H]leucine incorporation (Fig. 8A), the enhancement of sarcomeric structure, and the expression of ANF (Fig. 8B). The percentages of NF-κB–positive cells were similar to control level (9.0 ± 1.6% for NAC+AdLacZ and 8.8 ± 1.0% for NAC+AdRacV12). Next, we investigated the effect of NAC on ASK1 activation by RacV12. The immune complex kinase assay revealed that RacV12-induced ASK1 activation was significantly abolished by preincubation with NAC (Fig. 8C). These findings suggested that ROS is involved in Rac1-induced ASK1 and NF-κB activation and myocyte hypertrophy.

DISCUSSION

Rac1 has been found to be involved in cardiac myocyte hypertrophy (11, 12). Expression of RacV12 in cardiac myocytes leads to the characteristic features of cardiac hypertrophy, including morphological changes, increased protein synthesis, and alteration of gene expression, which are indistinguishable from the characteristics of the GPCR agonist-stimulated hypertrophy. The results presented here suggest a role for Rac1 in the signal transduction pathway leading to cardiac hypertrophy mediated through ASK1 and NF-κB.

In HeLa cells, a transient expression of RacV12 was found to induce the activation of NF-κB (7). In the study presented here, we demonstrated that infection of cardiac myocytes with...
AdRacV12 led to increases in κB-dependent luciferase activity as well as IκBα degradation. In addition, the expression of RacN17 inhibited phenylephrine-induced NF-κB activation. These findings indicate that Rac1 functions upstream of NF-κB activation in cardiac myocytes. In agreement with the previous report (11), RacV12 induced morphological and biochemical hypertrophic responses, whereas RacN17 inhibited phenylephrine-induced cardiac myocyte hypertrophy. Inhibition of NF-κB activation by infection with AdIκBα32/36A resulted in the elimination of RacV12-induced cardiac hypertrophic responses, such as increases in protein synthesis and ANF expression and the enhancement of sarcomere organization. These results clearly indicate that Rac1 functions upstream of NF-κB activation for the development of cardiac hypertrophy.

It has been reported that Rac participates in regulating transcription both through JNK and p38 MAP kinase pathways (5, 6). ASK1 was recently identified as a MAP kinase kinase, which activates both JNK and p38 (15). We identified the involvement of ASK1 in GPCR agonist-induced cardiac myocyte hypertrophy via the activation of NF-κB (14). In the current study, we showed that overexpression of activated Rac1 induced ASK1 activation and that infection with AdRacN17 eliminated phenylephrine-induced ASK1 activation. Because wild-type MKK6 was used as substrate for ASK1 kinase assays rather than catalytically inactive MKK6, autophosphorylation of the recombinant protein might exaggerate the extent of ASK1 activation. However, the extent of ASK1 activation by Rac1 was significantly larger than that in control

**FIG. 7.** The effect of RacN17 on ligand-induced or gene transfer-mediated myocyte hypertrophy. Cardiac myocytes were infected with AdLacZ or AdASK(ΔN) alone or coinfected with AdRacN17 and AdASK(ΔN). After 24 h of infection, myocytes were treated with or without phenylephrine. Values show the mean ± S.E. of data for three experiments performed in triplicate. A, NF-κB luciferase activation and Western blot with anti IκBα antibody. B, [3H]leucine incorporation. C, organization of sarcomere structure. D, ANF protein expression.
These results indicate that Rac1 induces NF-κB mediated through ASK1 and NF-κB activity using anti-ASK1 antibody. The immune complex kinase assay was performed to measure endogenous ASK1 activation measured by immune complex kinase assay. The immune control; #, open bars 0.05 versus AdRacV12. B, organization of sarcomere structure (top row) and ANF protein expression (bottom row). C, ASK1 activation measured by immune complex kinase assay. The immune complex kinase assay was performed to measure endogenous ASK1 activity using anti-ASK1 antibody.

Fig. 8. ROS is involved in RacV12-induced myocyte hypertrophy mediated through ASK1 and NF-κB activation. Cardiac myocytes were infected with AdLacZ or AdRacV12. NAC was added to the medium 1 h prior to adenoviral infection. A, NF-κB activation (closed bars) and [3H]leucine incorporation (open bars). *, p < 0.05 versus control; #, p < 0.05 versus AdRacV12. B, organization of sarcomere structure (top row) and ANF protein expression (bottom row). C, ASK1 activation measured by immune complex kinase assay. The immune complex kinase assay was performed to measure endogenous ASK1 activity using anti-ASK1 antibody.

with AdRacN17. This is the first report to suggest that ASK may act as a Rac target. The ASK-NF-κB signaling pathway can mediate both morphological and biochemical changes induced by RacV12.

To date, several potential targets of Rac have been identified, including p21-activated kinase (PAK), phosphatidylinositol 4-phosphate 5-kinase, and MAP kinase kinase kinases such as the mixed-lineage kinases MEKK1 and MEKK4 (6, 24). PK has been reported to be a likely candidate for controlling the activation of JNK and p38 by Rac (25–28). In fibroblasts, expression of active PAK can stimulate NF-κB activation (29). Previous experiments have demonstrated that PAK was translocated from a cytoplasmic to a cytoskeletal distribution in Rac1 transgenic mouse hearts, exhibiting cardiac hypertrophy (12). These findings suggest that PAK could be a potential target of Rac1 in cardiac hypertrophy. To the best of our knowledge, however, there have been no reports indicating a positive role for PAK in cardiac hypertrophy. Although AdASK(KM) completely eliminated activated Rac1-induced cardiac hypertrophy, our results do not exclude a possible role for PAK downstream of Rac1 in cardiac hypertrophy. It should be of interest to determine the possible interplay between the ASK1 and PAK pathways as a downstream target of Rac1 in regard to the development of myocyte hypertrophy.

In addition, the Rac and the extracellular signal-related kinase (ERK) pathways appear to be closely connected in human embryonic kidney 293 cells, because RacN17 was found to inhibit Ras-mediated ERK2 activation (25). Activated Rac synergizes with an active form of Raf to increase ERK activity. In cardiac myocytes, a previous transfection study has demonstrated that transient expression of RacV12 slightly increased ANF-luciferase (9). However, the combination of RacV12 with ΔN-Raf (an activated form of Raf) synergistically stimulated a response in ANF-luciferase similar to that induced by endothelin-1, suggesting that activation of Rac1 by hypertrophic stimuli contributes to the modulation of the ERK cascade. Therefore, some of the effects of Rac1 on hypertrophy may be attributable to an input into the ERK cascade in addition to that into the ASK1 pathway, hence, however, Rac1 is a key intermediate molecule that mediates the hypertrophic signal from GPCR to ASK1, not identified yet, we strongly suggest Rac1 as the candidate molecule that mediates the hypertrophic signal from GPCR to the downstream target, ASK1. The probability of this signaling sequence was confirmed by the finding that ASK1 activation was not affected by co-infection...
system in phagocytic cells (32–35). It has recently been proposed that the oxidase is expressed in a variety of cells (36). It can therefore be hypothesized that Rac proteins may act directly via protein-protein interaction to organize the ROS generating system, NADPH oxidase, in a manner similar to what occurs in neutrophils.

These are in vitro studies and used experimental designs that require overexpression of the proteins involved. Our approach has a disadvantage in that it may influence the function/expression of other related proteins. Also, an in vitro study does not always mimic the phenomenon in vivo. Defining precise and physiological roles for Rac1 will require cardiac specific knockout mice.

In conclusion, we have demonstrated a signal transduction pathway in which the small G protein Rac1 induces cardiac myocyte hypertrophy via the activation of NF-κB, and ASK1 mediates the Rac1-induced signal transduction pathway toward NF-κB activation.

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The Small GTP-binding Protein Rac1 Induces Cardiac Myocyte Hypertrophy through the Activation of Apoptosis Signal-regulating Kinase 1 and Nuclear Factor-κB

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