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Suppression of the mTOR-Raptor Signaling Pathway by the Inhibitor of Heat Shock Protein 90 Geldanamycin

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Heat shock protein 90 (Hsp90) was co-immunoprecipitated with raptor, the binding partner of the mammalian target of rapamycin (mTOR) from HEK293 cells. Hsp90 was detected in the anti-raptor antibody immunoprecipitates prepared from the cell extract by immunoblot analysis using the anti-Hsp90 antibody, and the association of these two proteins was confirmed by immunoprecipitation from the cells co-expressing Hsp90 and raptor as epitope-tagged molecules. Geldanamycin, a potent inhibitor of Hsp90, disrupted the *in vivo* binding of Hsp90 to raptor without affecting the association of raptor and mTOR, and suppressed the phosphorylation by mTOR of the downstream translational regulators p70 S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). The protein kinase activity of S6K as well as the phosphorylation of the substrate, 40S ribosomal protein S6, were lowered in the geldanamycin-treated cells. These results indicate that Hsp90 is involved in the regulation of protein translation by facilitating the phosphorylation reaction of 4E-BP1 and S6K catalyzed by the mTOR/raptor complex through the association with raptor, and that the mTOR signaling pathway is a novel target of geldanamycin.

Key words: geldanamycin, Hsp90, mTOR, protein phosphorylation, raptor.

Abbreviations: 4E-BP1, eIF4E-binding protein 1; eIF, eukaryotic initiation factor; Hsp90, heat shock protein 90; mTOR, mammalian target of rapamycin; S6K, p70 S6 kinase.

Protein synthesis is essential for cell growth and proliferation, and the translational regulators p70 S6 kinase (S6K) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) play critical roles in this process (*1*, *2*). The activity of these regulators is dependent on phosphorylation. S6K is activated by phosphorylation and recognizes 40S ribosomal protein S6 (S6 protein) for the translation of mRNAs encoding a polypyrimidine tract at their 5′-untranslated region. 4E-BP1 binds to eIF4E (a 7-methylguanosine mRNA cap binding protein) and prevents the formation of the active translational complex with eIF4G, and phosphorylation of 4E-BP1 results in the dissociation and disinhibition of eIF4E.

It is known that protein synthesis is under the control of growth factors and nutrients (*3*, *4*). In the former control mechanism, signals are mediated by phosphatidylinositol 3-kinase and protein kinases such as PDK1 and PKB, and PDK1 phosphorylates S6K in response to insulin and mitogens (*5*, *6*). On the other hand, amino acid sufficiency is linked to these translational regulators by a giant protein-serine/threonine kinase, TOR (the target of rapamycin). TOR proteins, TOR1 and TOR2, were first discovered in *Saccharomyces cerevisiae* as the products of genes whose mutation confers resistance to growth inhibition by the macrolide antibiotic rapamycin, and later the mammalian ortholog mTOR was identified (*7*). In mammalian cells, S6K and 4E-BP1 are phosphorylated by mTOR. Amino acid deprivation induces dephosphorylation of these translational regulators through inactivation of mTOR (*8*, *9*), and rapamycin in complex with FK506-binding protein FKBP12 interacts with mTOR to block phosphorylation of these translational regulators by mTOR (*10*, *11*). Thus, S6K is directly regulated by PDK1 and mTOR in the downstream pathways of growth factors and nutrients, respectively, and the modification by mTOR is suggested to be a priming signal for S6K (*12*). 4E-BP1 is, in contrast, phosphorylated by mTOR, but is not a substrate of S6K; however, cell stimulation with insulin or mitogens induces phosphorylation of this initiation factor-binding protein, presumably by mTOR (*13*). It seems, therefore, that mTOR could be involved in the growth factor signaling pathway.

Recent studies have established that mTOR functions in a complex with other proteins such as raptor (*14*, *15*) and mLST8 (also known as GβL) (*16*, *17*), and that raptor binds predominantly to the amino-terminal half of mTOR, whereas mLST8 appears to bind to the mTOR catalytic

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domain independently of the association with raptor (*14*, *17*). Raptor, a 150-kDa polypeptide, serves as a scaffold for the apposition of mTOR with its substrates S6K and 4E-BP1, and its association with mTOR is indispensable for mTOR signaling *in vivo* (*14*, *15*). This scaffold protein does not alter the catalytic activity of mTOR, but the association of raptor with mTOR increases mTOR phosphorylation of S6K and is required absolutely for the mTOR-catalyzed phosphorylation of 4E-BP1 (*14*, *15*, *18*). Importantly, the association of mTOR with raptor is sensitive to rapamycin (*14*, *15*, *19*).

In this study, we screened cell extracts for proteins binding to raptor and found that heat shock protein 90 (Hsp90) is co-immunoprecipitated with raptor. Hsp90 is a cellular chaperone required for the ATP-dependent refolding of denatured or unfolded proteins and for the conformational maturation of key proteins involved in cell proliferation such as DNA-binding proteins, for example, steroid receptors and p53, as well as protein kinases including Raf, Src, MAP kinase, PDK1, and PKB (*20–23*). It has a unique pocket in the amino-terminal region that binds ATP with a low affinity and possesses weak ATPase activity. Geldanamycin, a naturally occurring ansamycin antibiotic, disrupts the association of Hsp90 with client proteins by specifically occupying the pocket of the chaperone protein and shows anti-tumor properties (*24*, *25*). Therefore, geldanamycin was employed to investigate the role of Hsp90 in the mTOR-signaling pathway.

MATERIALS AND METHODS

Cell Culture—HEK293 cells were maintained in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL) at 37°C in a 5% CO_2 incubator. For starvation of serum, cells at subconfluence were cultured in Dulbecco's modified Eagle's medium in the absence of FBS for 16 h. Cells were then incubated in the medium containing geldanamycin (Sigma) or rapamycin (Calbiochem) for 8 h. Where indicated, the cells cultured with FBS were transfected with the expression vectors of FLAG-tagged raptor (*15*) and myc-tagged Hsp90α by lipofection using Lipofectamine (Life Technologies) according to the manufacturer's protocol, and further incubated for 48 h. The myc-tagged Hsp90α expression vector was constructed by introducing the cDNA of Hsp90α, provided by Image Consortium, into pcDNA3 (Invitrogen) with the myc-epitope sequence at the 5′ end.

Antibodies—The antibodies against the FLAG epitope and S6 protein were purchased from Sigma and Cell Signaling, respectively. Normal rabbit globulin and the antibodies against S6K (monoclonal), 4E-BP1, and Hsp90 were obtained from Santa Cruz Biotechnology. Phospho-specific antibodies against S6K (Thr412), S6 protein (Ser235/236), 4E-BP1 (Thr37/46, Ser65, and Thr70), and mTOR (Ser2481 and Ser2448) were obtained from Cell Signaling. Antibodies against S6K (polyclonal) (*8*), mTOR (*26*), raptor (*15*), and mLST8 (*19*) were prepared as described.

Identification of Raptor-Binding Proteins—HEK293 cells (approximately 4×10^7 cells) transfected with the FLAG-raptor vector were disrupted by sonication in ice-cold Buffer A (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 20 mM NaF, 1 mM EDTA, 6 mM EGTA, 20 mM β-glycerophosphate, 0.5 mM dithiothreitol, 50 μM 4-amidinophenylmethanesulfonylfluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin). The following procedures were carried out at 0–4°C. The homogenate was centrifuged at 10,000 × *g* for 20 min, and the supernatant was incubated with 50 μl of Protein G-Sepharose (Amersham Biosciences) coupled with the anti-FLAG antibody for 2 h. After washing with Buffer A containing 500 mM NaCl, the bound proteins were eluted from the resin with Buffer A containing 100 μg/ml of FLAG peptide (Sigma) and applied to SDS-PAGE with the molecular mass markers (Protein Ladder 10 kDa, Gibco BRL) including proteins of 80, 90, 100, 110, 120, and 200 kDa. The proteins separated were visualized by silver staining or reverse staining, and the proteins recovered with FLAG-tagged raptor were cut out from the reverse-stained gel, digested with *Acromobacter* Protease I, and subjected to the mass spectrometric analysis for protein identification by the peptide mass fingerprinting and tandem mass spectrometry (MS/MS) ion search using Mascot as described previously (*15*, *19*).

Immunoprecipitation—Immunoprecipitation was carried out essentially as described (*11*). Briefly, the cells were lysed in ice-cold Buffer B (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.3% CHAPS, 20 mM NaF, 1 mM EDTA, 6 mM EGTA, 20 mM β-glycerophosphate, 1 mM dithiothreitol, 50 μM 4-amidinophenylmethanesulfonylfluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin). The extract was incubated with each antibody and 15 μl of Protein G-Sepharose, and the immunocomplex was washed twice with Buffer B and then used for immunoblotting. The immunocomplex was further washed twice with Buffer C (20 mM MOPS, pH 7.2, 10 mM β-glycerophosphate) when employed for the S6K kinase assay.

Immunoblotting and Kinase Assay—The cell extract and immunocomplex were subjected to SDS-PAGE. The proteins were transferred onto a polyvinylidene difluoride membrane, immunoblotted with the appropriate antibody, and visualized by the enhanced chemiluminescence method. When the same sample was analyzed with different antibodies, the membrane was stripped and employed for the subsequent immunoblotting. The phosphorylation of 4E-BP1 was estimated by the mobility shift on immunoblotting as described (*27*). The S6K activity was assayed by monitoring the incorporation of radioactivity from [γ-^{32}P]ATP into the 40S ribosomal subunit by S6K immunoprecipitated with the polyclonal anti-S6K antibody as described previously (*11*). After the kinase reaction, the reaction mixture was separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. It was then analyzed by autoradiography using X-ray film or bioimaging analyzer BAS2500 (Fujix). Then, the membrane was immunoblotted with the anti-S6K monoclonal antibody and visualized as described above.

RESULTS

Identification of Hsp90 as Raptor-Interacting Protein—The cell extract of HEK293 cells expressing FLAG-tagged raptor was incubated with the anti-FLAG antibody coupled to Protein G-Sepharose, and the epitope-tagged protein was separated from the immunocomplex by competition with the FLAG peptide and applied to SDS-PAGE to be visualized by silver staining (Fig. 1A). A protein with an approximate molecular mass of 290 kDa was recovered

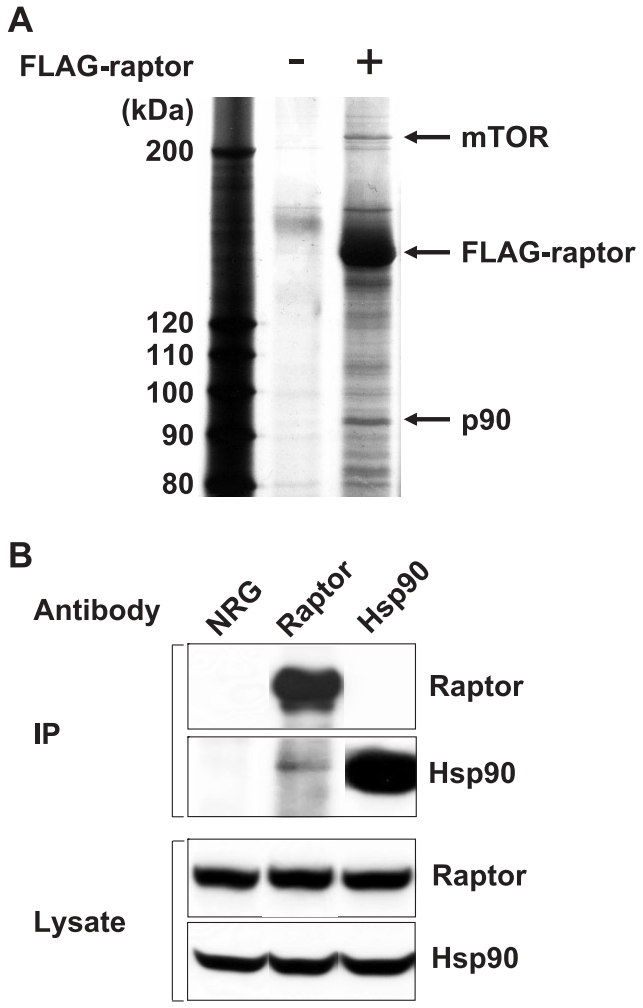

Fig. 1. **Association of Hsp90 with raptor.** (A) Isolation of raptor-interacting proteins. Extracts from cells expressing FLAG-tagged raptor and control cells were incubated with Protein G-Sepharose coupled with the anti-FLAG antibody. The proteins eluted from the resin with FLAG peptide were applied to SDS-PAGE on 6% gel and visualized by silver staining. FLAG-tagged raptor, mTOR, and a 90-kDa protein (p90) are indicated by arrows. The positions of the molecular mass markers are shown in kDa. (B) Interaction of raptor and Hsp90 in the cells. Cell lysates were prepared from untransfected cells, and immunoprecipitation and immunoblotting were carried out using anti-raptor and anti-Hsp90 antibodies as indicated with normal rabbit globulin (NRG) as a control. The results are representative of three reproducible experiments.

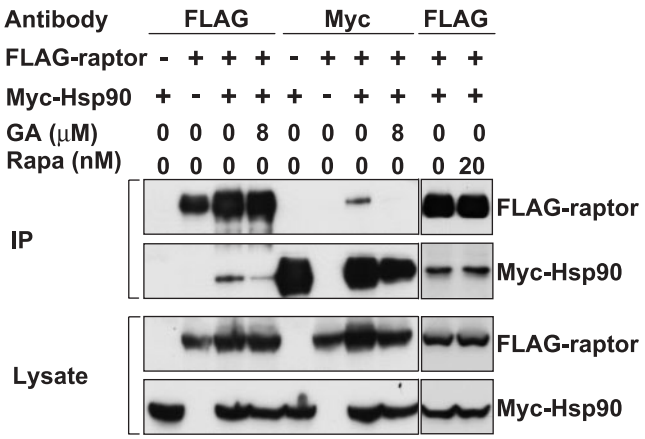

Fig. 2. **Effects of geldanamycin on the association between raptor and Hsp90.** Cells were transfected with cDNA encoding FLAG-tagged raptor and/or myc-tagged Hsp90, incubated in the presence or absence of geldanamycin (GA) and rapamycin (Rapa), and immunoprecipitation and immunoblotting were carried out using anti-FLAG and anti-myc antibodies. The results are representative of three reproducible experiments.

with FLAG-tagged raptor, and it was revealed to be mTOR expressed endogenously in the cells by immunoblotting using the specific antibody against mTOR as well as by the peptide mass fingerprinting analysis (data not shown). Thus, raptor was isolated with its associated protein by this immunoaffinity purification procedure. Several binding protein bands were detected on the gel along with mTOR, and a major protein among them with an apparent molecular mass of 90 kDa was subjected to in-gel digestion followed by mass spectrometric analysis for protein identification. The peptide mass fingerprinting and tandem mass spectrometry ion search using Mascot revealed that the 90-kDa protein is a mixture of Hsp90α and Hsp90β (SWISS-PROT accession nos. P07900 and P08238, respectively). Four peptide fragments common to both proteins, one fragment specific to Hsp90α and three fragments specific to Hsp90β were identified: IRYESLTDPSK (β: amino acids 53–63), ADLINNLGTIAK (α: amino acids 100–111, β: amino acids 95–106), YIDQEELNK (α: amino acids 283–291, β: amino acids 275–283), PIWTRNPDDITNEEYGEFYK (α: amino acids 294–313), PIWTRNPDDITQEEYGEFYK (β: amino acids 286–305), SLTNDWEDHLAVK (α: amino acids 314–326, β: amino acids 306–318), CLELFSELAEDK (β: amino acids 411–422), EGLELPEDEEEK (α: amino acids 546–557, β: amino acids 538–549).

Association of Hsp90 and Raptor—The interaction of Hsp90 with raptor was examined by co-immunoprecipitation from the cell extract of HEK293 cells (Fig. 1B). As expected, Hsp90 was detected in the anti-raptor antibody immunoprecipitates by immunoblot analysis using the anti-Hsp90 antibody. The heat shock protein was not found when normal rabbit globulin was employed for immunoprecipitation. Thus, Hsp90 was immunoprecipitated specifically with raptor under the conditions employed. Raptor was, however, not detected in the anti-Hsp90 antibody immunoprecipitates, presumably because Hsp90 is more abundant than raptor in HEK293 cells. The interaction of Hsp90 with raptor was further analyzed in HEK293 cells by co-expressing these two proteins as FLAG- and myc-tagged constructs, respectively (Fig. 2). Immunoblot analysis showed that myc-tagged Hsp90α was detected in the anti-FLAG antibody immunoprecipitates, as in the case of the endogenously expressed proteins, and FLAG-tagged raptor was co-immunoprecipitated with the anti-myc antibody. Although we were unable to show the binding of the endogenously expressed raptor to Hsp90, it is reasonable to assume that Hsp90 is associated with raptor in the cells. Therefore, the interaction between Hsp90 and raptor was analyzed using the transfected cells. The binding of the two proteins was disrupted by geldanamycin, a potent inhibitor of Hsp90 (*24*, *25*), whereas rapamycin, which breaks the

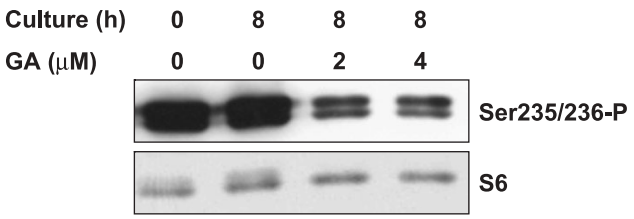

Fig. 3. **Suppression of the phosphorylation of S6 protein by geldanamycin.** Cells cultured in serum-free medium were treated with geldanamycin (GA) at the indicated concentrations, and the lysates were subjected to immunoblotting with the antibodies indicated for the analysis of S6 protein on 10% SDS-PAGE gels. The results are representative of three reproducible experiments.

mTOR-raptor complex as a specific inhibitor of mTOR (*19*), did not change the association of Hsp90 with raptor. In the cells treated with rapamycin, the phosphorylation of S6 protein was abolished (data not shown) as described previously (*2*), indicating that the association of Hsp90 with raptor is independent of the binding of mTOR to the scaffold protein.

Inhibition of mTOR Pathway by Geldanamycin—mTOR links amino acid sufficiency to the regulation of the translational regulators S6K and 4E-BP1, and raptor is essential for their mTOR-catalyzed phosphorylation (*14*, *15*, *18*). Thus, the effect of geldanamycin was examined on the regulation of S6K and 4E-BP1 and on S6 protein, a specific substrate of the former enzyme in serum-starved HEK293 cells. In S6 protein, multiple residues Ser235, Ser236, Ser240, Ser244, and Ser247 have been identified as *in vivo* phosphorylation sites, and this protein appears as a doublet of highly and less phosphorylated bands on SDS-PAGE (*28*, *29*). S6 protein was also detected as a doublet in the control cells by immunoblot analysis using the anti-S6 protein antibody and the phospho-specific antibody for Ser235/236 of the protein (Fig. 3). As these two bands were detected by the phospho-specific antibody, their different mobilities on the gel probably result from phosphorylation at site(s) distinct from Ser235/236. The upper faint band detected by the anti-S6 protein antibody was not observed in the geldanamycin-treated cells, although the amounts of the protein did not change considerably (Fig. 3, lower panel). The phosphorylation of S6 protein at Ser235/236, which is catalyzed by S6K (*2*), was heavily reduced in the geldanamycin-treated cells (Fig. 3, upper panel). The low-mobility band was still found in the phospho-specific antibody blot, presumably because the phospho-specific antibody is more sensitive than the anti-S6 protein antibody.

Next, the effect of geldanamycin on S6K was studied by analyzing the *in vitro* kinase activity of S6K against S6 protein (Fig. 4A) and the phosphorylation at Thr412 (Fig. 4B), which is catalyzed by mTOR and is critical for the *in vitro* S6K activity (*2*), using 12% and 6% of SDS-PAGE gels, respectively. The amounts of S6K immunoprecipitated from the cells did not change significantly as revealed by immunoblotting after separation on 12% gel (Fig. 4A, lower panel), but the catalytic activity of the enzyme was reduced by the geldanamycin treatment when measured *in vitro* using S6 protein as a substrate (Fig. 4A, upper panel). S6K isolated from the control cells was separated on 6% gel into several bands that were

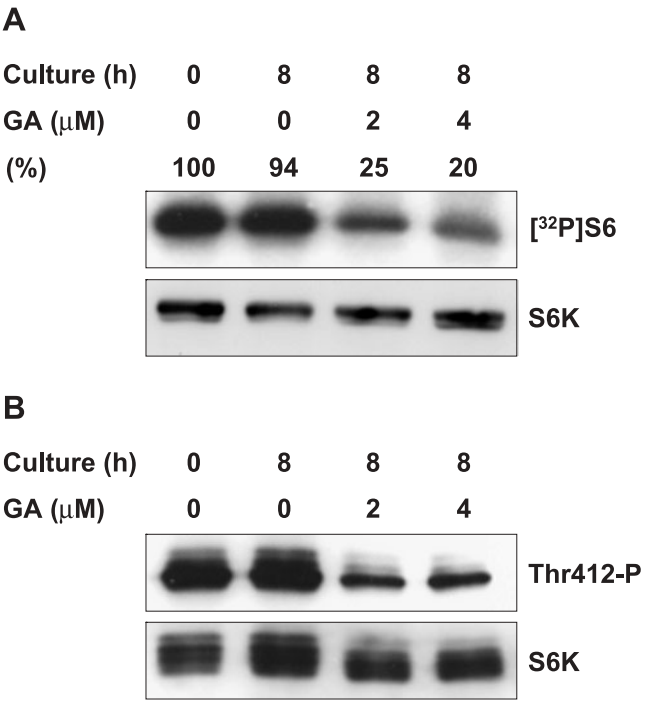

Fig. 4. **Suppression of the S6K activity by geldanamycin.** Cells cultured in serum-free medium were treated with geldanamycin (GA) at the indicated concentrations. (A) Assay of S6K. S6K immunoprecipitated from the lysates was incubated in the presence of S6 protein and [γ-^{32}P]ATP. The phosphorylated S6 protein was separated by SDS-PAGE on 12% gel and transferred onto polyvinylidene difluoride membrane. The membrane was analyzed by autoradiography (upper panel), then immunoblotted with the anti-S6K antibody (lower panel). ^{32}P radioactivity incorporated into S6 protein was quantified by BAS2500, and the PSL values relative to that of the control cells as 100% are shown on the top of the panel. (B) Phosphorylation of S6K. S6K immunoprecipitated from lysates was subjected to SDS-PAGE on 6% gel followed by immunoblotting using the indicated antibodies. The results are representative of three reproducible experiments.

detectable by immunoblotting, and the geldanamycin treatment reduced the amounts of the low-mobility bands (Fig. 4B, lower panel). The amounts of the low-mobility bands detected by the antibody specific to S6K phosphorylated at Thr412 were consistently reduced, and those of the highest-mobility band were also lowered in the geldanamycin-treated cells (Fig. 4B, upper panel). Thus, geldanamycin suppressed the mTOR-catalyzed phosphorylation of S6K at Thr412 to attenuate the catalytic activity of S6K.

The phosphorylation of 4E-BP1, another downstream target of mTOR, was also analyzed by using the phospho-specific antibodies at Thr37/46, Ser65, and Thr70 of the protein as well as the antibody against the translational regulator protein (Fig. 5). Phosphorylation of 4E-BP1 at Thr37 and Thr46 is relatively insensitive to serum deprivation, and phosphorylation of these residues is required for the subsequent phosphorylation at Thr70 and then at Ser65 (*30*). 4E-BP1 thus appears as three distinct bands, designated as α, β, and γ, according to the order of the mobility on SDS-PAGE, that roughly correspond to the protein phosphorylated at Thr37/46 alone, further phosphorylated at Thr70 and then at

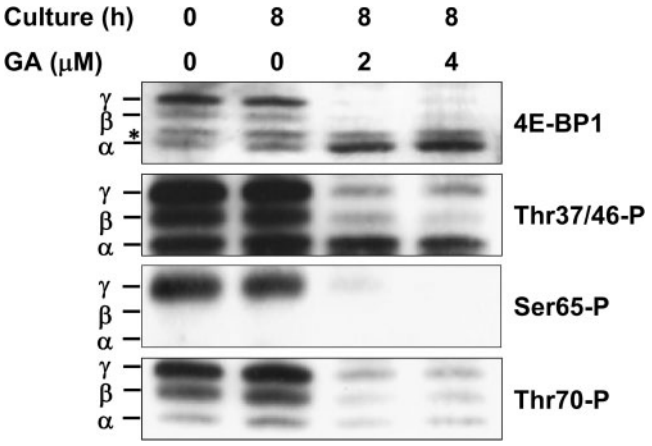

Fig. 5. **Suppression of the phosphorylation of 4E-BP1 by geldanamycin.** Cells cultured in serum-free medium were treated with geldanamycin (GA) at the indicated concentrations, and the lysates were subjected to immunoblotting with the antibodies indicated for the analysis of 4E-BP1 on 12% SDS-PAGE gels. The positions of 4E-BP1 in different phosphorylation states and a protein non-specifically recognized by the anti–4E-BP1 antibody are indicated by α, β, γ and the asterisk (*), respectively. The results are representative of three reproducible experiments.

Ser65, respectively (*31*). Immunoblot analysis using the anti-4EBP1 antibody revealed that low mobility bands of β and γ were almost eliminated, and the protein appeared as a single protein of band α with the highest mobility in the cells treated with geldanamycin (Fig. 5, top panel). The studies using the phospho-specific antibodies at Ser65 and Thr70 confirmed that phosphorylation at Ser65, which appeared at the position of γ, was abolished and that phosphorylation at Thr70, mostly found at the positions of β and γ, was heavily reduced in the geldanamycin-treated cells. The bands phosphorylated at Thr37/46 at the position of β and γ also considerably decreased, but the phosphorylated band was still detected at the position of α in the treated cells. Therefore, the geldanamycin treatment significantly reduced the mTOR-catalyzed phosphorylation of S6K and 4E-BP1, although it did not completely eliminate the phosphorylation of these proteins in the cells.

Effect of Geldanamycin on mTOR—As phosphorylation of S6K and 4E-BP1 was attenuated by geldanamycin, the effect of the Hsp90 inhibitor was examined on mTOR (Fig. 6). The amounts of mTOR did not change, but autophosphorylation at Ser2481 (*32*) as well as phosphorylation by other kinase(s) at Ser2448 (*33*, *34*) were reduced in the geldanamycin-treated cells. In contrast, geldanamycin did not change the association of mTOR with raptor or that with another binding protein, mLST8. These results indicate that geldanamycin induces the attenuation of the protein kinase activity of mTOR and suppresses the phosphorylation of mTOR catalyzed by other enzyme(s) in the cells.

DISCUSSION

In this study, Hsp90 was revealed to be a novel binding protein of raptor, which is responsible for chaperoning various proteins in cells such as transcription factors and protein kinases (*20*). Raptor is associated with mTOR

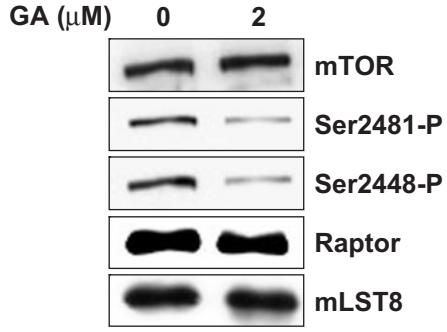

Fig. 6. **Effects of geldanamycin on mTOR and its association with the binding proteins.** Cells cultured in serum-free medium were treated with geldanamycin (GA), and mTOR immunoprecipitated from the extracts was subjected to SDS-PAGE on 10% gel, followed by immunoblotting with the indicated antibodies. The results are representative of three reproducible experiments.

as a partner protein, and thus Hsp90 seems to interact with the mTOR-raptor complex through the scaffold protein. Geldanamycin, an inhibitor specific to Hsp90, disrupted the association of Hsp90 with raptor, and suppressed the protein kinase activity of mTOR as judged by the phosphorylation of its downstream proteins S6K and 4E-BP1 as well as the mTOR autophosphorylation activity on Ser2481. On the other hand, rapamycin, which breaks the association between mTOR and raptor and thereby prevents mTOR-catalyzed phosphorylation of the target proteins, did not affect the binding of Hsp90 with raptor. Therefore, geldanamycin appears to attenuate the mTOR protein kinase activity in a manner distinct from that of rapamycin. It is plausible that Hsp90 facilitates the phosphorylation catalyzed by the mTOR/raptor complex through the association with raptor. We could not, however, exclude the possibility that geldanamycin affects the mTOR protein kinase activity, and further studies are required to reveal the action mechanism of geldanamycin.

S6K is not only regulated by mTOR sensing amino acid sufficiency but is also located in the signaling pathway of growth factors (*2*). The latter pathway includes PDK1 and PKB, two protein kinases that are client proteins of Hsp90 (*21*, *23*). Therefore, geldanamycin could suppress S6K activity by preventing the association of Hsp90 with PDK1, which can phosphorylate S6K. It is, however, unlikely that geldanamycin attenuates S6K by inhibiting the growth factor pathway, because the effect of this inhibitor was observed in serum-starved cells in this study. Recently, a new mTOR complex, TORC2, was identified as a rapamycin-insensitive complex (*35*, *36*), which may be responsible for the phosphorylation of the carboxyl-terminal end of PKB (*37*). On the other hand, mTOR is suggested to be phosphorylated at Ser2448 by PKB, which is located in the signaling pathway of growth factors (*33*, *34*). Phosphorylation at Ser2448 was, however, reduced in the cells treated with geldanamycin. Thus, the dephosphorylation of Ser2448 is enhanced or the kinase reaction is attenuated by geldanamycin in the cells cultured in the absence of growth factors. In the latter case, a protein kinase distinct from PKB may recognize this Ser residue of mTOR under the control of nutritional conditions. Very recently, S6K has been reported to

phosphorylate Ser2448 (*38*, *39*). As geldanamycin induces inhibition of the S6K activity as reported in this study, it is reasonable to assume that S6K is a Ser2448 kinase. Alternatively, it remains possible that there is a protein kinase other than PKB and S6K that phosphorylates and activates mTOR in a geldanamycin-sensitive manner. It is necessary to examine in detail the protein kinases responsible for the phosphorylation of Ser2448 of mTOR. In addition, it is interesting to know whether Hsp90 is involved in the regulation of phosphatidylinositol kinase-related kinase family members other than mTOR, such as ATM, ATR, and DNA-dependent kinase.

Rapamycin, famous as an anti-cancer agent as well as an immunosuppressant (*40*, *41*), binds to mTOR and induces dissociation of the mTOR-raptor complex when coupled with FKBP12 (*19*). In contrast, geldanamycin, which has anti-tumor properties (*24*, *25*), suppressed the mTOR activity, although it did not affect the association of raptor with mTOR. Both rapamycin and geldanamycin, thus, target the mTOR/raptor complex, but their mechanisms of action as anti-cancer agents are different. The combination these compounds may show a synergistic effect on cancer cells. It is important to study the signaling mechanisms of the mTOR pathway in detail for the development of anti-cancer agents.

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