MAPK Scaffold IQGAP1 Binds the EGF Receptor and Modulates Its Activation*

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Cellular responses produced by EGF are mediated through the receptor (EGFR) and by various enzymes and scaffolds. Recent studies document IQGAP1 as a scaffold for the MAPK cascade, binding directly to B-Raf, MEK, and ERK and regulating their activation in response to EGF. We previously showed that EGF is unable to activate B-Raf in cells lacking IQGAP1. However, the mechanism by which IQGAP1 links B-Raf to EGFR was unknown. Here we report that endogenous EGFR and IQGAP1 co-localize and co-immunoprecipitate in cells. EGFR has no effect on the association, but Ca²⁺ attenuates binding. In vitro analysis demonstrated a direct association mediated through the IQ and kinase domains of IQGAP1 and EGFR, respectively. Calmodulin disrupts this interaction. Using a mass spectrometry-based assay, we show that EGF induces phosphorylation of IQGAP1 Ser¹⁴⁴³, a residue known to be phosphorylated by PKC. This phosphorylation is eliminated by pharmacological inhibition of either EGFR or PKC and transfection with an IQGAP1 isoform. In IQGAP1-null cells, EGF-stimulated tyrosine phosphorylation of EGFR is severely attenuated. Normal levels of autophosphorylation are restored by reconstituting wild type IQGAP1 and enhanced by an IQGAP1 S¹⁴⁴³D mutant. Collectively, these data demonstrate a functional interaction between IQGAP1 and EGFR and suggest that IQGAP1 modulates EGFR activation.

The epidermal growth factor receptor (EGFR; also known as ErbB1 or HER1) is a member of the ErbB family of receptor tyrosine kinases (reviewed in Refs. 1 and 2). These receptors mediate signaling pathways that govern cell growth and differentiation. Overactivation of EGFR contributes to several human cancers (3). The EGFR comprises an extracellular ligand binding domain, a single transmembrane domain, and an intracellular region with intrinsic tyrosine kinase activity. Binding of EGF to the extracellular domain causes receptor homo- or heterodimerization. This leads to phosphorylation of distinct tyrosine residues within the C-terminal tail, which then serve as docking sites for Src homology domain-containing effectors. Stimulation of EGFR kinase activity triggers signaling through several downstream pathways. Binding of activated EGFR to Grb2 induces the Ras-Raf-MAPK cascade (4), whereas its association with phospholipase Cγ leads to inositol trisphosphate formation and Ca²⁺ mobilization (5). The EGFR signaling network is highly complex and relies heavily on the formation of multiprotein complexes.

IQGAP1 is a ubiquitously expressed 190-kDa protein (reviewed in Ref. 6). It contains several protein interaction domains, including an IQ region (containing four tandem IQ motifs), a calponin homology domain, and a WW domain. IQGAP1 participates in protein-protein interactions and integrates diverse signaling pathways (6). Proteins that bind IQGAP1 include calmodulin, Cdc42, Rac1, actin, β-catenin, Rap1, and cadherin (reviewed in Refs. 6–9). Importantly, IQGAP1 regulates the function of its binding partners. For example, IQGAP1 inhibits cell-cell adhesion mediated by the E-cadherin-β-catenin complex (10, 11) and increases β-catenin-mediated transcription (12). IQGAP1 therefore plays a role in multiple cellular activities, ranging from cell-cell adhesion and transcriptional regulation to cell migration, proliferation, and neurite outgrowth (6). Furthermore, accruing evidence strongly implicates IQGAP1 and its interaction with several binding proteins in tumorigenesis (13, 14) and microbial pathogenesis (15).

In recent years, multifunctional adaptor proteins known as scaffold proteins have come to be appreciated as important mediators of many signaling pathways (16, 17). One of the best characterized scaffolds in the MAPK cascade is KSR (kinase suppressor of Ras). KSR binds to C-Raf, MEK1/2, and ERK1/2, and optimal KSR expression is required for maximal MAPK activation (18, 19). We have documented that IQGAP1 is a scaffold for MAPK signaling (20–22). IQGAP1 binds directly to B-Raf (20), MEK1/2 (22), and ERK1/2 (21) and modulates MAPK activity in response to EGF (20–22), CD44 (23), and N-cadherin (24). Altering the intracellular concentration of IQGAP1 attenuates EGF-dependent MEK and ERK activation (21, 22). Several stimuli activate MAPK signaling (25). Neither EGF, PDGF, nor VEGF is able to significantly activate B-Raf in IQGAP1-deficient cells (20). Reconstitution of IQGAP1-null
cells with IQGAP1 restores the ability of EGF to increase B-Raf kinase activity, suggesting that IQGAP1 is necessary for activation of B-Raf by EGF (20). However, the mechanism by which IQGAP1 links B-Raf and the MAPK cascade to EGFR has so far remained unknown.

Here, we present evidence that IQGAP1 and EGFR associate both in vitro and in intact cells. EGF-Induced activation of EGFR catalyzes phosphorylation of IQGAP1 at Ser1443. Importantly, IQGAP1 phosphorylation on Ser1443 enhances EGF-stimulated EGFR tyrosine phosphorylation, suggesting that phosphorylation of Ser1443 enables IQGAP1 to modulate EGFR activation through a positive feedback mechanism. Collectively, these data establish IQGAP1 as a molecular scaffold that modulates EGFR activation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cell lysis reagents, including CellLytic M, Protease Inhibitor Mixture, and Phosphatase Inhibitor Cocktails 1 and 2, were from Sigma-Aldrich. NuPAGE gel electrophoresis reagents were from Invitrogen. Tissue culture reagents and fetal bovine serum (FBS) were obtained from Invitrogen. FuGENE HD transfection reagent was obtained from Roche Applied Science. Lipofectamine RNAiMAX transfection reagent was obtained from Invitrogen. Gene-specific oligonucleotide siRNA sequences were obtained from Sigma-Aldrich, and control scrambled siRNA was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Goat anti-IQGAP1 (N-17) polyclonal antibody, anti-EGFR (3H2094), and anti-E-cadherin (G-10) monoclonal antibodies (mAbs), goat anti-TIM23 monoclonal antibody, and anti-Myc 9E10-agarose conjugate were from Santa Cruz Biotechnology, Inc. Anti-EGFR polyclonal antibody, EGFR cytoplasmic domain rabbit mAb, phospho-EGFR (Tyr1068) mAb, and anti-phosphotyrosine mAb (P-Tyr-100) were obtained from Cell Signaling Technology. Anti-phosphotyrosine mAb (PT-66) was from Sigma-Aldrich. The anti-IQGAP1 polyclonal antibody has been characterized previously (26). Secondary antibodies used for ECL detection were from Amersham Biosciences, and those for immunofluorescent microscopy were from Invitrogen. Immobilized Protein A/G-agarose was from Thermo Scientific. Recombinant human EGF was from R&D Systems. The ATP-competitive dual EGFR/ErB-2 inhibitor 4557W (4-(4-benzyloxyanilino)-6,7-dimethoxyquinoxaline) was from Calbiochem. The ATP-competitive PKC inhibitor GF109203X (bisindolylmaleimide I hydrochloride) and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich. All other reagents were of standard analytical grade.

**Plasmid Construction and Expression**—Myc-tagged constructs comprising full-length IQGAP1 (amino acids 2–1657), IQGAP1-N (amino acids 2–863), and IQGAP1-C (amino acids 864–1657) in the pcDNA3 vector have been described previously (26). IQGAP1-IQ (amino acids 717–916) and IQGAP1ΔIQ (full length minus amino acids 746–860) have been described previously (27). IQGAP1-SAA (serines 1441 and 1443 mutated to alanine) and IQGAP1-SED (serines 1441 and 1443 mutated to glutamic acid and aspartic acid, respectively), were generated as Myc-tagged constructs and have been described previously (28). Glutathione S-transferase-tagged full-length IQGAP1 fusion protein (GST-IQGAP1) has been described previously (26). GST-IQGAP1 was expressed in Escherichia coli and isolated by glutathione-Sepharose (26). The EGFR kinase domain (EGFR-KD) (containing amino acids 695–1020) was cloned and expressed at GlaxoSmithKline as a His-TEV tag fusion in Baculovirus-infected SF9 cells and purified by nickel-Sepharose chromatography. Following cleavage of the His-TEV tag, the protein was reapplied to nickel-Sepharose resin, and the untagged protein was collected in the unbound fraction. The unbound fraction was further purified with Superdex 5-75 chromatography. The EGFR intracellular domain (EGFR-ICD) (containing amino acids 671–1210 with a threonine 790 to methionine mutation) was cloned and expressed at GlaxoSmithKline as a His tag fusion in baculovirus-infected SF9 cells and purified by nickel-Sepharose chromatography. The purity of all fusion proteins as evaluated by Coomassie staining of SDS-polyacrylamide gels was >90%.

**Cell Culture and Transfection**—Mouse embryonic fibroblast (MEF) cells were isolated from day 14 embryos of IQGAP1−/− mice and normal litter mate controls and immortalized as described previously (20). HE-1 human cervical adenocarcinoma and A431 human skin epidermoid carcinoma cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) FBS, in the absence of antibiotics, and grown at 37 °C and 5% CO2. Where indicated, FBS was omitted (serum starvation). The cells were transfected with expression plasmids using FuGENE HD reagent according to the manufacturer’s instructions. The following oligonucleotide duplex siRNA sequences were used for targeted gene knockdown: PKCa, 5′-CAGAAGACUGUAUGCAUdTdT and 5′-AUUGCAUCAGUCUUCUGdTdT; PKCe, 5′-GUUGAUUGUCUGUUCGAUdTdT and 5′-AUACUGAACAGAC-AUAACdTdT; PKCγ, 5′-GUAUCUACAGGGAUUUGA-dTdT and 5′-UCAAUUCCUGAUGACdTdT. siRNAs were reverse-transfected into HeLa cells at 10 nM for 48–72 h according to the manufacturer’s instructions.

**Immunoprecipitation**—A431 cells were grown in 10-cm dishes to ~75% confluence, and the medium was replaced with serum-free DMEM for 16 h. Cells were left untreated or were stimulated with 100 ng/ml EGF in serum-free DMEM for 10 min. The cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed directly in ice-cold Celllytic buffer containing protease and phosphatase inhibitor mixtures, and scraped. Where indicated, the lysis buffer was supplemented with either 1 mM CaCl2 or 1 mM EGTA. The cell lysates were disrupted by sonication with three brief pulses using a Microson Ultrasonic Cell Disruptor (Misonix) with the microprobe tip set at 20 watts output power, followed by centrifugation at 10,000 × g for 10 min to remove the debris. Clarified lysates were frozen at −80 °C and recentrifuged to clarify following thaw. Approximately 10 mg of protein lysate was used for each pull-down experiment. Anti-IQGAP1 goat polyclonal antibody or anti-TIM23 goat polyclonal antibody used as an irrelevant immunoprecipitation control was preadsorbed to Protein A/G-agarose beads for 1 h at 4 °C and washed with lysis buffer. Immobilized antibodies were mixed with equal amounts of protein lysate and incubated at rotation for 4 h at 4 °C. Complexes were sedimented by centrifugation, washed three times.
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times with lysis buffer, and heated for 10 min at 70 °C in gel loading buffer. The samples were resolved by SDS-PAGE and transferred to PVDF, and the blots were probed with rabbit anti-EGFR, rabbit polyclonal anti-IQGAP1, or anti-phosphotyrosine monoclonal antibodies, as indicated in the relevant figure legends. Antigen-antibody complexes were visualized with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies and developed by ECL Plus.

**Immunocytochemistry**—Cells were plated onto poly-d-lysine-coated coverslips (BD Biocoat) in 24-well plates. Following serum starvation overnight, cells were stimulated with 100 ng/ml EGF for 5 min. Cells were fixed in 4% paraformaldehyde, PBS for 15 min at 22 °C and permeabilized in ice-cold methanol for 10 min at −20 °C. Cells were blocked with 10% non-immune goat serum in PBS (Zymed Laboratories Inc.) for 1 h at 22 °C. Primary antibodies were diluted 1:100–1:1000 into antibody diluent solution (Zymed Laboratories Inc.) and incubated at 4 °C overnight. Cells were washed with PBS and then incubated with the appropriate secondary antibody (Alexa Fluor 488 or 594-conjugated anti-mouse or anti-rabbit antibodies, Invitrogen), diluted 1:1000 in antibody diluent solution, for 1 h at 22 °C in the dark. Coverslips were washed with PBS and mounted on glass slides with Prolong Gold antifade reagent (Invitrogen). Confocal laser microscopy was performed on a Zeiss LSM510 laser-scanning microscope (Carl Zeiss, Thornwood, NY) with a ×40 oil objective, and images were analyzed using the LSM image browser.

**In Vitro Binding Assays**—EGFR-KD (4 µg) was incubated with 4 µg of GST-IQGAP1 on glutathione-Sepharose beads for 3 h at 4 °C in Buffer A (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 1% Triton X-100) containing 1 mM EGTA or 1 mM CaCl₂. Immobilized GST alone was used as control. Where indicated, GST fusion proteins were preincubated with 4 µg of calmodulin or 4 µg of BSA in the presence of 1 mM Ca²⁺ or 1 mM EGTA for 1 h at 4 °C before adding EGFR-KD. After sedimentation by centrifugation, samples were washed five times in Buffer A and resolved by SDS-PAGE. Following SDS-PAGE, the gel was cut in half; the top portion (containing IQGAP1) was stained with Coomassie Blue, and the bottom half was transferred to PVDF membrane and processed for Western blotting using an EGF receptor mAb that recognizes the EGFR cytoplasmic domain. Immobilized anti-glycogen phosphorylase monoclonal antibody was used as an irrelevant control for immunoprecipitation. Samples were washed five times in buffer A, and complexes were resolved by SDS-PAGE and processed by autoradiography.

**Phosphorylation of IQGAP1 in Intact Cells**—HeLa cells were grown in 10-cm dishes to ~75% confluence, at which time the medium was replaced with serum-free DMEM. After 16 h, the cells were treated with serum-free DMEM (vehicle) or stimulated with 100 ng/ml EGF in serum-free DMEM for 2, 5, 10, 20, or 60 min or alternatively with varying concentrations of EGF for 5 min. For pathway inhibition studies, cells were preincubated with 1 µM bisindolylmaleimide, 1 µM 4557W, or DMSO (vehicle) for 1 h. Cells were stimulated with either 100 ng/ml EGF for 5 min or 1 µM PMA for 20 min. Cells were washed twice with ice-cold PBS and lysed directly with Celllytic buffer containing protease and phosphatase inhibitor mixtures. Following clarification, endogenous IQGAP1 was immunoprecipitated using a rabbit anti-IQGAP1 polyclonal antibody immobilized to Protein A/G-agarose for 4 h at 4 °C. Samples were washed three times with lysis buffer, resolved by SDS-PAGE, and stained with SimplyBlue Safestain (Invitrogen). The IQGAP1 bands were excised and digested in situ with trypsin as described previously (29).

**Phosphorylation Site Analysis of IQGAP1**—All peptide sequencing and phosphorylation analysis was done by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Lyophilized tryptic digests were resuspended in 0.1% formic acid, 0.02% TFA and injected onto an Agilent 1100 NanoLC interfaced to an Agilent LC/MSD XCT Ultra ion trap mass spectrometer via an Agilent Chip cube. The Chip contains a 40-nl enrichment column and a 75 µm × 43-mm analytical column, both packed with Zorbax 300SB-C18 5-µm material. Peptides were eluted from the Chip into the mass spectrometer with an acetonitrile/water/formic acid gradient. The mass spectrometer was operated in a data-dependent mode for peptide sequencing (30).

**Quantification of IQGAP1 Ser1443 phosphorylation** was done by targeting the peptides SVKEDSNLTLQEK (3H2094) that recognizes amino acids 985–996. Western detection was done by a horseradish peroxidase-conjugated secondary antibody and ECL.

**TNT Product Production and Binding Analysis**—[³⁵S]Methionine-labeled TNT products were produced with the TNT quick coupled transcription/translation system (Promega, Madison, WI) according to the manufacturer’s instructions. Briefly, 2 µg of IQGAP1 plasmid (IQGAP1, IQGAP1-N, IQGAP1-C, IQGAP1-IQ, and IQGAP1ΔIQ) was incubated with 40 µl of TNT Quick Master Mix (Promega) and 2 µCi (1 Ci = 37 GBq) of [³⁵S]methionine at 30 °C for 90 min. The products were identified by SDS-PAGE and autoradiography. All of the products migrated to the expected position on SDS-PAGE. 10 µl of [³⁵S]methionine-labeled IQGAP1 constructs were incubated for 3 h at 4 °C with 2 µg of EGFR-ICD in 1 ml of buffer A containing 1 mM phenylmethylsulfonyl fluoride, protease inhibitor mixture, and 1 mM EGTA. Complexes were captured using 10 µl of Protein A/G-agarose-immobilized rabbit
ever, because the relative ionization efficiency of each peptide does not change under constant experimental conditions, it is possible to accurately measure changes in the apparent stoichiometry.

Activation of EGF—IQGAP1 null (IQGAP1−/−) MEFs were seeded in 6-well plates, allowed to grow for 8 h, and then transfected with either wild type IQGAP1 cDNA or IQGAP1 Ser1443 mutant cDNAs. Sixteen hours after transfection, the medium was replaced with serum-free DMEM. After 6 h, the cells were incubated with serum-free DMEM (vehicle) or stimulated with 100 ng/ml EGF (+) for 10 min. Equal amounts of protein lysates were immunoprecipitated with anti-IQGAP1 antibody (top) and probed with anti-EGFR antibody (bottom). 10% of the immunoprecipitate was processed in parallel and probed with anti-IQGAP1 antibody (bottom). Aliquots of lysate from each sample not subjected to pull-down were also processed by Western blotting. C, A431 cells were incubated with vehicle (−) or 100 ng/ml EGF (+) and processed as described in A, except that cells were lysed in buffer containing 1 mM EGTA or 1 mM CaCl2. Equal amounts of protein lysate were immunoprecipitated with anti-IQGAP1 antibody. Immunoprecipitates (IQGAP1) and unprocessed lysates were probed with anti-EGFR antibody (top) and anti-IQGAP1 antibody (bottom). D, identical aliquots were resolved separately by SDS-PAGE, transferred to PVDF, and probed with anti-phosphotyrosine antibody (pTyr). All data shown above are representative of three independent experiments.

RESULTS

IQGAP1 and EGFR Interact in Cells—IQGAP1 is a scaffold for EGF-dependent activation of the MAPK cascade and acts upstream of B-Raf (20, 31). Given this, we hypothesized that IQGAP1 might interact directly with the EGFR. Several complementary strategies were used to test this postulate. In the first approach, COS-7 cells were transiently transfected with Myc-tagged IQGAP1. Probing the lysates with anti-Myc antibody reveals expression of transfected IQGAP1 (Fig. 1A). Transfection of IQGAP1 does not alter the levels of endogenous EGFR. Immunoprecipitation with anti-Myc antibodies shows that EGFR is co-enriched with IQGAP1 (Fig. 1A). Minimal EGFR is present in immunoprecipitates from vector-transfected cells. These data indicate that EGFR associates with IQGAP1.

To ascertain whether the endogenous proteins interact in a normal cell milieu, we immunoprecipitated endogenous IQGAP1 from A431 cell lysates. We chose A431 cells because they express abundant amounts of both EGFR and IQGAP1. EGFR co-immunoprecipitated with IQGAP1 (Fig. 1B) and the binding is specific because neither IQGAP1 nor EGFR is observed in samples immunoprecipitated with irrelevant antibody (Fig. 1B, ctrl). Analysis by mass spectrometry independently confirmed that the EGFR protein is present in the IQGAP1 immune complexes (data not shown).

Because EGF promotes the interaction of EGFR with several proteins, we examined the effect of EGF on the interaction of EGFR with IQGAP1. We found that EGF bound IQGAP1 in immunoprecipitates from both serum-starved and EGF-treated cells (Fig. 1, B and C). These data indicate that the association of EGFR with IQGAP1 is constitutive and not modulated by EGF.

Ca2+ Modulates the Interaction between IQGAP1 and EGFR—Ca2+ regulates the interaction of IQGAP1 with several binding
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partners (26, 29, 32, 33). To evaluate the effect of Ca\(^{2+}\) on the association of IQGAP1 with EGFR, A431 cells were lysed in the presence of Ca\(^{2+}\) or EGTA. EGFR co-immunoprecipitated with IQGAP1 in the presence of EGTA, but the interaction was completely abrogated by Ca\(^{2+}\) (Fig. 1C). Ca\(^{2+}\) does not alter the amount of IQGAP1 in the immunoprecipitates.

In order to ascertain the activation status of the EGFR in these experiments, an identical aliquot of the immunoprecipitates was probed with anti-phosphotyrosine antibody. Because the anti-phosphotyrosine blot is unable to distinguish between IQGAP1 and EGFR and the two proteins migrate to similar positions on SDS-PAGE, we maximized the resolution of the two proteins using a 4–12% gradient gel with MOPS running buffer. Under these conditions, IQGAP1 and EGFR could be completely resolved, and we were able to be quite certain that the protein observed in the Tyr(P) blots was EGFR and not IQGAP1. As expected, EGFR is activated via abundant tyrosine phosphorylation following treatment of the cells with EGF (as observed by the whole cell lysate lanes in Fig. 1D). Notably, there is no signal from tyrosine phosphorylation at the position where IQGAP1 is expected to migrate in either the whole cell lysate or the IQGAP1 immunoprecipitation.

In the presence of Ca\(^{2+}\), EGFR does not bind IQGAP1 regardless of the state of receptor activation (Fig. 1, C and D, Ca\(^{2+}\) +). By contrast, when Ca\(^{2+}\) is removed, IQGAP1 binds both quiescent and activated EGFR (Fig. 1, C and D, EGTA +). Thus, IQGAP1 constitutively associates with EGFR in cells, in an EGF-independent but Ca\(^{2+}\)-regulated manner.

**IQGAP1 and EGFR Colocalize in Cells**—To further characterize the interaction between IQGAP1 and EGFR, we examined the localization of the endogenous proteins in cells by immunocytochemistry. Consistent with previous reports in other cell lines (11, 34, 35), IQGAP1 is diffusely distributed throughout the cytoplasm of serum-starved A431 cells, with prominent accumulation at cell-cell junctions (Fig. 2A, top, red). Total EGFR is also found primarily at cell-cell junctions (Fig. 2A, top, green). The merged and colocalization images (Fig. 2A, top) show that IQGAP1 and EGFR colocalize at cell-cell junctions under conditions of serum starvation. EGF induces translocation of both IQGAP1 (Fig. 2A, bottom, red) and EGFR (Fig. 2A, bottom, green) to the periphery of cells located at the outer edge, where the proteins colocalize in areas of ruffling consistent with lamellipodial structures (Fig. 2A, bottom).

Comparing images taken in the x-z orientation confirms that IQGAP1 is localized within the same 2-μm z slice as EGFR, both prior to and following EGF stimulation (Fig. 2B). Furthermore, the EGF-induced changes we observed in IQGAP1 and EGFR localization are not the result of any loss of adherens junction integrity. Immunostaining for IQGAP1 and E-cadherin confirms that cell-cell junctions are intact and that IQGAP1 localizes to membrane ruffles in response to EGF treatment (Fig. 2C).

To evaluate whether the observed translocation and colocalization of IQGAP1 and EGFR is due to activation of the receptor, the cells were stained with an EGFR antibody specific for phosphorylated tyrosine 1068. As anticipated, negligible phospho-EGFR staining was observed in the absence of EGF (Fig. 2D, top, green), demonstrating that serum-starved A431 cells stained for EGFR (green) and IQGAP1 (red) after incubation with vehicle (top) or EGF (bottom). B, x-z plane comparison of serum-starved A431 cells stained for EGFR (green) and IQGAP1 (red) after incubation with vehicle (top) or EGF (bottom). IQGAP1 and EGFR localize to membrane ruffles in response to EGF treatment (Fig. 2C).

**FIGURE 2.** EGFR colocalizes with IQGAP1 in cells. A431 cells were plated on poly-\(\alpha\)-lysine coverslips, serum-starved, and treated with (+) or without (−) 100 ng/ml EGF for 5 min and then fixed and processed as described under “Experimental Procedures.” Cells were double-stained with rabbit anti-IQGAP1 antibody/Alexa Fluor 594-conjugated secondary antibody and mouse anti-EGFR/Alexa Fluor 488-conjugated secondary antibody (A and B), rabbit anti-IQGAP1 antibody/Alexa Fluor 594-conjugated secondary antibody and mouse anti-E-cadherin/Alexa Fluor 488-conjugated secondary antibody (C), or rabbit anti-IQGAP1 antibody/Alexa Fluor 594-conjugated secondary antibody and mouse anti-phospho-EGFR (Tyr1068) (pT) antibody/Alexa Fluor 488-conjugated secondary antibody (D). Slides were analyzed by Zeiss LSM510 confocal laser microscopy. A, serum-starved A431 cells stained for EGFR (green) and IQGAP1 (red) after incubation with vehicle (top) or EGF (bottom). B, x-z plane comparison of serum-starved A431 cells stained for EGFR (green) and IQGAP1 (red) after incubation with vehicle (top) or EGF (bottom). IQGAP1 and EGFR localize to membrane ruffles in response to EGF treatment (Fig. 2C).
were largely quiescent. EGF stimulated intense phosphotyrosine staining of EGFR, indicating extensive receptor activation (Fig. 2D, bottom, green). EGFR induces translocation of IQGAP1 to the peripheral membrane, where it co-localizes with activated EGFR (Fig. 2D, bottom, Merge and Colocalization). Collectively, these findings corroborate the immunoprecipitation data and further support our observations that IQGAP1 interacts constitutively with both the quiescent and activated EGFR in cells. Furthermore, these data reveal that the cellular localization of IQGAP1 is substantially altered by EGF.

**IQGAP1 Binds EGFR Directly**—To determine whether the association between IQGAP1 and EGFR is direct, we examined the binding of IQGAP1 and EGFR using purified proteins. GST-IQGAP1 was incubated with purified EGFR-KD (see “Experimental Procedures” for details) and isolated using glutathione-Sepharose. Coomassie staining showed equal amounts of GST-IQGAP1 were pulled down. After proteins were resolved by SDS-PAGE, the gel was cut into two pieces. The piece containing EGFR-KD was transferred to PVDF and probed with anti-EGFR kinase domain antibody (top). The piece containing IQGAP1 was stained with Coomassie Blue (bottom). C, the experiment described in B was carried out in the presence of EGTA. All of the data in this figure are representative of three independent experiments.

Evidence from several groups has shown that Ca\(^{2+}\)/calmodulin attenuates IQGAP1 interactions with a number of binding partners (8, 26, 33, 36). To test this effect with EGFR, we performed the *in vitro* binding assay described above in the presence or absence of calmodulin. We found that Ca\(^{2+}\)/calmodulin completely abrogated the binding of EGFR to IQGAP1 (Fig. 3B). The effect is specific for calmodulin because BSA does not alter the association of IQGAP1 with EGFR. Coomassie staining showed that equal amounts of GST-IQGAP1 were pulled down (Fig. 3B, bottom). Unexpectedly, binding of EGFR to IQGAP1 was also disrupted by Ca\(^{2+}\)-free (apo)calmodulin (Fig. 3C). Possible reasons for these observations are addressed under “Discussion.”

These data reveal that IQGAP1 binds directly to the EGFR and that the intracellular kinase domain of EGFR alone, in the absence of the juxtamembrane region and the C-terminal cytoplasmic docking sites, is sufficient to accommodate this binding. These data also show that calmodulin completely disrupts this interaction.

**Identification of the EGFR Binding Domain on IQGAP**—To further understand the interaction, the specific region of IQGAP1 that binds EGFR was investigated using a series of IQGAP1 mutants. Selected constructs of IQGAP1 (depicted in Fig. 4A) were labeled with [\(^{35}\)S]methionine in a reticulocyte lysate and incubated with purified EGFR intracellular domain (EGFR-ICD; see “Experimental Procedures” for details). We chose this EGFR construct because it allowed us to use a commercially available capture antibody that recognizes an epitope outside the IQGAP1 binding region within the kinase domain. EGFR-ICD/IQGAP1 complexes were isolated using immobilized anti-EGFR-ICD antibody, resolved by SDS-PAGE, and identified by autoradiography. Analogous to the capture of EGFR by immobilized full-length GST–IQGAP1, this reciprocal experiment confirmed that [\(^{35}\)S]methionine-labeled full-length IQGAP1 bound to immobilized EGFR-ICD (Fig. 4B, top). Specificity of binding is validated by the presence of minimal IQGAP1 bound to immobilized control antibody.

Experiments with the N- and C-terminal halves of IQGAP1 revealed that only the N-terminal half (amino acid residues 2–863) bound to the EGFR (Fig. 4B, middle). No interaction between the C-terminal half of IQGAP1 and EGFR was detected (Fig. 4B, bottom). The amount of EGFR-ICD in each experiment was essentially identical (data not shown). To further narrow the binding site, we used an IQGAP1 construct

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**FIGURE 3. Calmodulin disrupts the binding of IQGAP1 to EGFR in vitro.** A, GST–IQGAP1 (IQGAP1) or GST alone bound to glutathione-Sepharose was incubated with equal amounts of purified EGFR-KD in the presence of Ca\(^{2+}\) or EGTA. The complexes were isolated as described under “Experimental Procedures” and probed with antibody to EGFR amino acids 985–996. Input is purified EGFR. B, GST–IQGAP1 or GST alone bound to glutathione-Sepharose was incubated with equal amounts of purified EGFR-KD in the presence of Ca\(^{2+}\)/calmodulin (CaM) or BSA. The complexes were isolated and washed. Input, EGFR-KD not subjected to pull-down. After proteins were resolved by SDS-PAGE, the gel was cut into two pieces. The piece containing EGFR-KD was transferred to PVDF and probed with anti-EGFR kinase domain antibody (top). The piece containing IQGAP1 was stained with Coomassie Blue (bottom). C, the experiment described in B was carried out in the presence of EGTA. All of the data in this figure are representative of three independent experiments.

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FIGURE 4. Regions of IQGAP1 necessary for EGFR binding. A, schematic representation of IQGAP1 constructs. Full-length IQGAP1 and deletion mutants of IQGAP1 are depicted. The identified protein interaction motifs and the specific amino acid residues in each construct are indicated. IQGAP1ΔIQ lacks amino acids 746–860. CHD, calponin homology domain; WW, polypolpeptide binding domain; IQ, four tandem calmodulin binding motifs; GRD, Ras-GAP-related domain; IQGAP1-N, N-terminal half of IQGAP1; IQGAP1-C, C-terminal half of IQGAP1. (Metionine-labeled IQGAP1, IQGAP1-N, and IQGAP1-C (B) and IQGAP1ΔIQ and IQGAP1-IQ (C) produced with the TNT Quick Coupled Transcription/Translation system were incubated with purified EGFR intracellular domain. Complexes were isolated by immunoprecipitation with either anti-EGFR cytoplasmic domain antibody or bated with purified EGFR intracellular domain. Complexes were resolved by SDS-PAGE. Gels were dried and processed by autoradiography.) An aliquot of [35S]methionine-labeled TNT product (equivalent to 10% of the amount that was subjected to pull-down) was resolved by SDS-PAGE, dried, and processed by autoradiography (input). Data are representative of three independent experiments.

lacking the IQ domain (IQGAP1ΔIQ). The IQ domain is essential for calmodulin binding (36), which we have shown above abrogates the interaction of IQGAP1 with EGFR. Deletion of the IQ domain completely eliminated IQGAP1 binding to EGFR (Fig. 4C, top). Finally, a polypeptide encompassing only the functional IQ domain of IQGAP1 (IQGAP1-IQ) binds to EGFR (Fig. 4C, bottom). Collectively, these data reveal that the IQ domain of IQGAP1 is both necessary and sufficient for binding EGFR.

EGFR Activation Promotes IQGAP1 Ser1443 Phosphorylation in Cells—Activation of the EGFR triggers several downstream signaling pathways, including those mediated by phospholipase Cγ and its effector, PKC. We and others have previously shown that IQGAP1 is phosphorylated in cells in a PKCε-dependent manner at Ser1443 in response to phorbol ester treatment (28, 37). It seems reasonable, therefore, to hypothesize that IQGAP1 can be phosphorylated on Ser1443 in response to EGF-stimulated activation of EGFR. To test this postulate, we treated serum-starved HeLa cells with EGF, immunoprecipitated IQGAP1, and, after SDS-PAGE purification, performed phosphorylation analysis using mass spectrometry. Using data-dependent LC-MS/MS, we found that IQGAP1 was minimally phosphorylated in serum-starved control cells but was reproducibly phosphorylated on Ser1443 in cells treated with EGF (data not shown).

To fully characterize EGF-induced phosphorylation on Ser1443, we needed a sensitive, phosphosite-specific assay that would be both quantitative and reproducible. Since our initial identification of Ser1443 phosphorylation (28), we have made several unsuccessful attempts to produce phosphoepitope-specific antibodies against the site. To overcome this difficulty, we developed a mass spectrometry-based assay to quantify Ser1443 phosphorylation in cells (Fig. 5).

IQGAP1 is readily immunopurified from cells using polyclonal antibodies directed against the whole protein, isolating both phosphorylated and non-phosphorylated forms. Enzymatic digestion of the purified IQGAP1 with trypsin releases peptides containing the Ser1443 epitope (28). To measure the abundance of both phosphorylated and nonphosphorylated epitopes, we developed a targeted, full-time LC-MS/MS experiment that rapidly cycles between the four Ser1443 epitopes produced by trypsin digestion of IQGAP1 (see Fig. 5 and “Experimental Procedures” for details), selectively sequencing each one in turn. The rapidity of the MS method permits the abundance of each epitope to be measured in real time as the peptides elute from the LC. The assay is selective and sensitive and able to precisely quantify changes in Ser1443 phosphorylation with a detection limit of ∼1% apparent stoichiometry. Using this assay, we found that stimulation of IQGAP1 Ser1443 phosphorylation was dose-dependent and saturable (Fig. 6A). The calculated EC50 for the biological effect was determined to be 40 ng/ml (∼0.7 nM) EGF.

The MS-based phosphoassay allowed us to perform a detailed study of Ser1443 phosphorylation dynamics in cells (Fig. 6, B and C). In serum-starved HeLa cells (t = 0 min), a very low level of Ser1443 phosphorylation was observed (apparent stoichiometry, 1.5 ± 0.6% (n = 3)). EGF induced phosphorylation within 2 min and reached a maximum at 5 min (Fig. 6C). Quantification revealed a 5.3 ± 0.7-fold (n = 3) increase in Ser1443 phosphorylation at 5 min relative to unstimulated cells (Fig. 6B). Phosphorylation was transient and returned essentially to basal levels within 60 min of the EGF addition. Western blotting of the whole cell lysates indicated that the kinetic profile of IQGAP1 Ser1443 phosphorylation is indistinguishable from the activation kinetics of the EGFR itself (Fig. 6D), as measured by tyrosine phosphorylation (Fig. 6D, middle). Similar to IQGAP1 phosphorylation, receptor activation is shown to peak 5 min post-EGF addition. The higher molecular weight smears are indicative of EGFR-ubiquitin conjugates because the receptor is tagged for endosomal processing (38). IQGAP1 protein levels did not change significantly during the course of the experiment (Fig. 6D, bottom). These data demonstrate that ligand-induced activation of the EGFR results in rapid, transient phosphorylation of IQGAP1 at Ser1443. This finding was observed in multiple EGF-responsive cell lines in addition to HeLa, including A431 and MCF-7 cells (data not shown).

EGFR Mediates Phosphorylation of IQGAP1 through PKC Signaling—To confirm that EGFR activation-dependent phosphorylation of Ser1443 was via PKC, HeLa cells were preincubated with either the PKC inhibitor GF109203X or the EGFR inhibitor 4557W prior to stimulation with EGF (Fig. 7A). Inhibition of either EGFR or PKC kinase activity abrogated EGF-stimulated phosphorylation of Ser1443, demonstrating that IQGAP1 phosphorylation is mediated via activation of PKC downstream of EGFR. As expected (39), direct activation of
PKC (PMA; Fig. 7B) enhanced Ser1443 phosphorylation more than EGF (7.7 ± 1.2-fold, n = 3), and the PKC inhibitor, bisindolylmaleimide, prevented PMA from inducing IQGAP1 phosphorylation at Ser1443.

EGFR triggers phospholipase C to generate both diacylglycerol and inositol 1,4,5-trisphosphate-mediated calcium fluxes, resulting in the potential activation of multiple PKC isoforms (40, 41). Previous studies have shown that Ser1443 is contained within a PKC/consensus site and that PKC is able to catalyze IQGAP1 phosphorylation at Ser1443.

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IQGAP1 Enhances EGF-stimulated Receptor Activation—The finding that IQGAP1 binds to the EGFR and is phosphorylated in response to EGF stimulation prompted us to examine the effect of IQGAP1 on EGFR activation. Potential interference from endogenous IQGAP1 was eliminated by using cells
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Figure 6. IQGAP1 is phosphorylated at Ser\(^{1443}\) in response to EGF activation. Serum-starved HeLa cells were treated with either vehicle or EGF at the indicated concentrations and times. IQGAP1 was immunoprecipitated and isolated by SDS-PAGE, digested with trypsin, and analyzed by LC-MS/MS using the assay described above. Ser\(^{1443}\) phosphorylation data are expressed as apparent stoichiometry (described under "Experimental Procedures") and represent the means ± S.E. (n = 3 independent experiments). *, a significant difference of p < 0.01 compared with vehicle. A, effect of increasing EGF concentration on Ser\(^{1443}\) phosphorylation. Responses were quantified by fitting a four-parameter Hill equation to individual experiments and calculating the EC\(_{50}\) value. B, dynamics of EGF-induced IQGAP1 Ser\(^{1443}\) phosphorylation. Serum-starved HeLa cells were treated with either vehicle (0 min) or 100 ng/ml EGF for 2, 5, 10, 20, or 60 min. C, reconstructed ion chromatograms from one channel of the LC-MS/MS assay showing the dynamics of EGF-dependent phosphorylation of Ser\(^{1443}\). Each trace shows the production of the \(y_5\) ion produced by fragmentation of the Ser\(^{1443}\) phosphopeptide SkpSWEDSN-LTLQEK (M\(^+\), m/z 618.3). D, dynamics of EGF-induced activation of EGFR. Additional aliquots of lysate from each sample described in B were probed with anti-EGFR and anti-phosphotyrosine (pY) as a measure of activated EGFR and anti-IQGAP1 antibodies. The data are representative of three independent experiments, each performed in triplicate.

Figure 7. Specificity of IQGAP1 Ser\(^{1443}\) phosphorylation to EGF pathway activation. Serum-starved HeLa cells were pretreated either with vehicle, 1 μM bisindolylmaleimide (Bis), or 1 μM 4557W for 60 min, followed by activation with 100 ng/ml EGF for 5 min (A) or 1 μM PMA for 20 min (B) where indicated. The data are expressed as apparent stoichiometry (described under "Experimental Procedures") and represent the means ± S.E. (n = 3 independent experiments). *, a significant difference of p < 0.01 compared with either EGFr- or PMA-treated samples.

from IQGAP1-null mice (20). Cells were transfected with Myc-tagged wild type or mutant IQGAP1, which was expressed at levels similar to those of endogenous IQGAP1 in control MEFs (data not shown).

In MEF cells without IQGAP1 (vector), EGF stimulated tyrosine phosphorylation of the EGFR 3-fold over serum-starved cells (3.3 ± 0.5, n = 3) (Fig. 9). Reconstitution with wild-type IQGAP1 further increased the EGF-stimulated phosphorylation to 6-fold over serum-starved (6.1 ± 0.9-fold, n = 3) or a 2-fold augmentation relative to the vector.

To ascertain whether phosphorylation of Ser\(^{1443}\) played any role in this phenomenon, we transfected the null cells with either IQGAP1-SED, a phosphomimetic construct that represents fully phosphorylated Ser\(^{1443}\) (28), or IQGAP1-SAA, a construct that cannot be phosphorylated on Ser\(^{1443}\), and treated the cells with EGF (Fig. 9A). IQGAP1-SED markedly increased EGF-stimulated tyrosine phosphorylation of EGFR relative to serum-starved (12.8-fold, n = 3). In relative terms, this is a 2-fold (2.1-fold) increase over wild type IQGAP1 (p < 0.01) and nearly 4-fold (3.9-fold) over cells lacking IQGAP1 (p < 0.005) (Fig. 9B). IQGAP1-SAA on the other hand, showed a small increase in receptor activation when compared with cells lacking IQGAP1 (1.4-fold, n = 3), but this was significantly (p < 0.05) less than that produced by wild type IQGAP1. It is possible that this low level of activation relative to the IQGAP1 null cells is from the influence of as yet unidentified phosphorylation at an additional site.

Although there is a decrease in receptor levels as expected after EGF treatment, the level is not influenced by the presence of either IQGAP1 or the particular IQGAP1 construct present. The unchanged levels of receptor confirm that the increase in EGFR phosphorylation observed in the presence of the phosphomimetic IQGAP1-SED construct is not influenced by any altered degradation of the receptor. Blots probed for IQGAP1 indicate similar transfection levels among the constructs. GAPDH levels confirmed equivalent protein loading in all cases (Fig. 9A). These data reveal that reconstitution of IQGAP1 into IQGAP1-null MEFs augments EGF-mediated activation of the EGFR and that this effect is mediated at least in part through phosphorylation of IQGAP1 at Ser\(^{1443}\). This suggests that in cells, EGF activation of IQGAP1 Ser\(^{1443}\) downstream of PKC positively feeds back to allow maximal tyrosine phosphorylation (i.e. activation) of the EGFR.

Discussion

When EGF binds to its receptor, it triggers a network of signaling pathways, culminating in responses ranging from cell division to death and motility to adhesion (1). Numerous adaptors and enzymes are recruited to these pathways and participate in signal transmission and regulating the cell’s response to EGF activation. Prior work from our laboratory has implicated IQGAP1 as a scaffold in EGF signaling (20–22). IQGAP1 binds directly to B-Raf, MEK, and ERK and is required for maximal activation of the MAPK cascade by EGF. Previously, we made the intriguing observation that IQGAP1 is necessary for EGF to stimulate B-Raf activity in cells (20). How this occurs is not known. Published reports have failed to identify an association between IQGAP1 and H-Ras (32, 43), the protein directly upstream of B-Raf. Any associations with other upstream molecules via which IQGAP1 might link EGF to B-Raf are also unknown. In this study, we propose a link between EGF, IQGAP1, and B-Raf signaling by demonstrating a functional interaction between EGFR and IQGAP1. Unexpectedly, we found that IQGAP1 is necessary for EGF to fully activate EGFR.
Analysis by both immunoprecipitation and immunocytochemistry reveals that IQGAP1 associates constitutively with EGFR. These data are consistent with recent observations that IQGAP1 can associate with EGFR from cells transfected with lipid raft-tethered RasV12 (44). Additionally, a proteomic screen identified IQGAP1 as being recruited to the activated Grb2-EGFR complex (45). Although these observations suggest an interaction between the two proteins, the possibility of an indirect association via a complex cannot be excluded. We show here for the first time with purified proteins that IQGAP1 and EGFR interact directly.

Evidence from several other groups suggests that IQGAP1 participates in multiple signaling cascades mediated by a heterogeneous group of receptors (6). For example, IQGAP1 interacts with the VEGF receptor (VEGFR2) (46), the hyaluronan receptor CD44 (23), the AMPA receptor subunit GluR4 (47), and FGFR1 (fibroblast growth factor receptor 1) (48). However, in contrast to VEGFR2 or FGFR2, where association with IQGAP1 seems to require activation of the receptors, we have shown here that the interaction between IQGAP1 and EGFR appears to be ligand-independent.

Although the interaction between IQGAP1 and EGFR in cells appears to be independent of receptor activation, it is dependent on Ca²⁺. However, the disruption of the EGFR-IQGAP1 complex by Ca²⁺ in cells, but not in vitro, suggests that calmodulin plays a potential role in this interaction. Prior studies have shown that calmodulin reduces the association between IQGAP1 and all binding partners that have been examined. The N-terminal half of IQGAP1 contains four IQ motifs, which bind calmodulin (36), and Ca²⁺ augments this interaction (26, 29, 33). Because the IQ domains are necessary for EGFR to bind IQGAP1, it is reasonable to postulate that the presence of Ca²⁺/calmodulin in the IQ domains blocks access to EGFR binding. In contrast to most other IQGAP1 binding partners, EGFR also binds calmodulin directly (49), and Ca²⁺ is required for calmodulin to bind to the EGFR at the juxtagener.
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brane region of the receptor (residues 645–660) (49). Thus, in intact cells, EGFR binding to Ca\(^{2+}/\)calmodulin may also abrogate its interaction with IQGAP1.

Whereas apocalmodulin does not bind EGFR, it does bind IQGAP1. Nevertheless, there are several reasons why apocalmodulin does not block the interaction between IQGAP1 and EGFR in cells, including the low affinity of apocalmodulin for IQGAP1. Apocalmodulin has an \( \sim 2.5 \)-fold lower affinity for IQGAP1 than Ca\(^{2+}/\)calmodulin (29), rendering it more susceptible to competition. For example, \( \beta \)-catenin can partially displace apocalmodulin, but not Ca\(^{2+}/\)calmodulin, from IQGAP1 (12).

EGF, although not altering the association of EGFR with IQGAP1, elicits two important functional sequelae. It stimulates phosphorylation of IQGAP1 on Ser\(^{1443} \), and this phosphorylation is necessary for EGF to maximally promote EGFR autophosphorylation. Using siRNA, we targeted the TPA-responsive PKC isoforms expressed in HeLa cells (42) and identified PKCa as the isoform responsible for IQGAP1 phosphorylation in cells in response to EGFR. Although phorbol esters have been shown to promote IQGAP1 phosphorylation at Ser\(^{1443} \), this work is the first conclusive evidence for \textit{in vivo} phosphorylation of IQGAP1 in response to a physiological stimulus.

We did not find any evidence for IQGAP1 phosphorylation on tyrosine residues, either by mass spectrometry or Western blot analysis. Using unbiased mass spectrometry techniques that detect phosphorylation on serine, threonine, and tyrosine equally, we found that only Ser\(^{1443} \) was activated in response to EGF (data not shown). By Western blot, phosphotyrosine immunoreactivity is only detected when EGFR protein can also be detected (see Fig. 1, C and D). When IQGAP1 is immunoprecipitated in the presence of Ca\(^{2+} \), no phosphotyrosine signal is detected (EGFR does not co-purify).

Limited data exist on the functional consequences of IQGAP1 phosphorylation. Neurite outgrowth in N1E-115 cells is significantly enhanced by expression of a Ser\(^{1443} \) phosphomimetic (28). PKCe-mediated phosphorylation of IQGAP1 participates in wound closure of bronchial epithelial cells (50), and NIH3T3 cells can be transformed by stable expression of IQGAP1 Ser\(^{1443} \) phosphomimetic (51). The molecular mechanisms underlying these findings are unknown, although Ser\(^{1443} \) phosphorylation on IQGAP1 was reported to affect binding to Cdc42 (37). Using transfected full-length IQGAP1 phosphomimetic and phosphodefective mutants in IQGAP1-null MEFs, we found that neither subcellular localization nor the extent of self-association differed significantly between the phosphomutants and wild-type IQGAP1 (data not shown). Additionally, \textit{in vitro} binding assays did not reveal differences in the ability of the phosphomutants to bind either EGFR or calmodulin (data not shown).

The kinetics of PKCa-mediated Ser\(^{1443} \) phosphorylation in response to EGF mirror the activation kinetics of EGFR. In a recent proteomics study, the temporal dynamics of the HeLa phosphoproteome in response to EGF was statistically grouped into six clusters, including one deemed signal initiators (52). Based on our data, Ser\(^{1443} \) phosphorylation kinetics match those in the signal initiator cluster, which is enriched with proteins involved in membrane-proximal signaling events (42, 52).

Previous studies have established that, following EGF stimulation, PKCa is activated and translocates from the nucleus to the membrane, where it phosphorylates EGFR at Thr\(^{654} \) leading to receptor internalization and down-regulation of EGFR signaling (53–56). In light of this, it is significant that PKCa also phosphorylates IQGAP1 and that phospho-IQGAP1 modulates EGFR autophosphorylation during signal initiation.

Our data show that in the absence of IQGAP1, EGF induces minimal tyrosine phosphorylation of EGFR and that reconstitution of IQGAP1-null cells with IQGAP1 significantly augments this phosphorylation. Our findings are consistent with the recent report that reducing IQGAP1 expression impaired phosphorylation of EGFR resulting from Ras activation at lipid rafts (44). Although it is as yet unclear how IQGAP1 facilitates EGFR activation, the site on EGFR where IQGAP1 binds may be relevant to our findings. Because IQGAP1 binds directly to the EGFR kinase domain, it is possible that this interaction alters the catalytic activity of the kinase, thus facilitating trans-autophosphorylation of tyrosine residues in the C-terminal tail (57). Another possibility is that the EGFR is phosphorylated by a non-receptor tyrosine kinase. c-Src, which catalyzes tyrosine phosphorylation of several sites within the EGFR (58–61), is a potential candidate. Consistent with this hypothesis is the observation that c-Src acts as an adaptor to bridge IQGAP1 to VEGFR-2 (62).

Replacing WT IQGAP1 with the phosphomimetic IQGAP1-SED construct significantly enhances the effect of IQGAP1 on EGF-stimulated phosphorylation of EGFR. This observation suggests that phosphorylation of IQGAP1 on Ser\(^{1443} \) induced by EGF produces a positive feedback loop that stimulates EGFR activity. The molecular mechanism underlying this effect is not known. Regardless of the mechanism, our data indicate that IQGAP1 is required for EGF to maximally stimulate tyrosine phosphorylation of the EGFR.

Collectively, the data presented here enhance our comprehension of EGF signaling and provide additional insight into the established MAPK scaffold function of IQGAP1. We show that IQGAP1 serves as both a target and an effector of EGFR signaling, and regulation of IQGAP1 through phosphorylation at Ser\(^{1443} \) is likely to play an important role in this function. Moreover, the direct interaction of IQGAP1 with multiple components of the MAPK cascade, namely EGFR, B-Raf, MEK, and ERK, firmly establishes IQGAP1 as an important scaffold in the EGF-stimulated MAPK cascade.

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