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Regulation of EGF-induced ERK/MAPK Activation and EGFR Internalization by G Protein-coupled Receptor Kinase 2

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Abstract G protein-coupled receptor kinases (GRKs) mediate agonist-induced phosphorylation and desensitization of various G protein-coupled receptors (GPCRs). We investigate the role of GRK2 on epidermal growth factor (EGF) receptor signaling, including EGF-induced extracellular signal-regulated kinase and mitogen-activated protein kinase (ERK/MAPK) activation and EGFR internalization. Immunoprecipitation and immunofluorescence experiments show that EGF stimulates GRK2 binding to EGFR complex and GRK2 translocating from cytoplasm to the plasma membrane in human embryonic kidney 293 cells. Western blotting assay shows that EGF-induced ERK/MAPK phosphorylation increases 1.9-fold, 1.1-fold and 1.5-fold (P<0.05) at time point 30, 60 and 120 min, respectively when the cells were transfected with GRK2, suggesting the regulatory role of GRK2 on EGF-induced ERK/MAPK activation. Flow cytometry experiments show that GRK2 overexpression has no effect on EGF-induced EGFR internalization, however, it increases agonist-induced G protein-coupled δ opioid receptor internalization by approximately 40% (P<0.01). Overall, these data suggest that GRK2 has a regulatory role in EGF-induced ERK/MAPK activation, and that the mechanisms underlying the modulatory role of GRK2 in EGFR and GPCR signaling pathways are somewhat different at least in receptor internalization.

Key words G protein-coupled receptor kinase; receptor tyrosine kinase; epidermal growth factor receptor; ERK/MAPK; internalization

G protein-coupled receptors (GPCRs) constitute a superfamily of plasma membrane receptors. Members of this family include receptors for many hormones, neurotransmitters, chemokines and calcium ion, as well as sensory receptors for various odors, and bitter and sweet tastes, so GPCRs play important roles in a variety of cellular functions [1]. Repeated agonist stimulation triggers a negative feedback regulatory mechanism that attenuates GPCR-mediated signal transduction (desensitization). The initial event of GPCR desensitization is the phosphorylation of GPCR catalyzed by G protein-coupled receptor kinases (GRKs). GRKs are a family of Ser/Thr kinases and can phosphorylate agonist-activated GPCRs and initiate their desensitization and subsequent down-regulation. Thus GRKs are a key modulator of GPCR signaling [2].

Receptor tyrosine kinases (RTKs) constitute another family of plasma membrane receptors. RTKs are primary mediators of physiological cell responses, such as cell proliferation, differentiation, motility and survival [3]. Epidermal growth factor receptor (EGFR) belongs to the RTK family. Binding of EGFR with its ligand induces dimerization of EGFR, resulting in autophosphorylation of their cytoplasmic domains, thus recruiting the Src homology 2 and phosphotyrosine binding domain-containing proteins, which subsequently activates multiple signaling cascades and ultimately induces altered gene expression in the nucleus [4]. Disregulation of EGFR by overexpression,

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mutation or continuous activation of its intrinsic tyrosine kinase is frequently linked to hyperproliferative diseases such as cancer [5]. Thus EGFR signaling must be under stringent control. Previous studies have demonstrated that EGFR signaling is modulated by tyrosine dephosphorylation [6], receptor internalization and degradation [7,8].

Previous studies have shown that overexpression of GRK2, a member of GRK family, could attenuate phosphoinositide hydrolysis, cell chemotaxis and proliferation evoked via platelet-derived growth factor receptor β (PDGFRβ) [9,10], thus expanding our understanding of the roles GRKs may play. A recent study has shown that EGF stimulation induces GRK2-EGFR complex formation via Gβγ- and Src-dependent mechanisms [11]. But the modulatory role of GRKs on RTK signaling pathways is limited to signals mediated by PDGFRβ to date, moreover, the mechanisms underlying the modulatory role of GRK2 remains unknown.

In the present study, we investigated the regulatory role of GRK2 on EGF-induced extracellular signal-regulated kinase and mitogen-activated protein kinase (ERK/MAPK) activation and EGFR internalization.

Materials and Methods

Materials

Human EGF and [D-Pen^2,D-Pen^5]enkephalin (DPDPE) were obtained from Sigma Chemical Co. (St. Louis, USA). Modified Eagle's medium (MEM) and fetal bovine serum (FBS) were purchased from Life Technologies Incorporated (Grand Island, USA). Protein A-Sepharose was obtained from Amersham Pharmacia Biotech (Piscataway, USA). Rabbit anti-phospho and total ERK1/2 were supplied by New England Biolabs (Beverley, USA). Mouse monoclonal antibody against GRK2 was kindly provided by Dr. Martin OPPERMANN (Georg-August University, Göttingen, Germany). Mouse monoclonal antibody against DYKDDDDK octapeptide (FLAG) epitope and mouse monoclonal antibody 12CA5 recognizing influenza hemagglutinin (HA) epitope were supplied by Roche Molecular Biochemicals (Indianapolis, USA). Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG was purchased from Jackson Immunoresearch (West Grove, USA).

Cell culture and plasmid transfection

Human embryonic kidney 293 (HEK293) cells were obtained from American Type Culture Collection (Manassas, USA). HEK293 cells cultured in MEM containing 10% FBS were seeded in 35 mm or 60 mm tissue culture dishes at 0.2–1×10^6 cells/dish 20 h before transfection. Plasmids encoding bovine GRK2, GRK2-GFP (green fluorescence protein), human FLAG-tagged EGFR and mouse HA-tagged δ opioid receptor (DOR, one kind of GPCR) were prepared as described previously. Plasmids (1–3 μg each) were transfected into the HEK293 cells using the calcium phosphate/DNA co-precipitation method as described previously. Experiments were performed 44–48 h after transfection and the cells were maintained overnight in FBS-free medium before the experiments.

Co-immunoprecipitation and Western blotting

HEK293 cells were incubated at 37 °C in the presence or absence of 100 ng/ml EGF for 5 min, then the cells were washed twice with ice-cold phosphate buffered saline and lysed in 800 μl ice-cold NP-40 solubilization buffer (250 mM NaCl, 50 mM HEPES, 0.5% NP-40, 10% glycerol, 2 mM EDTA, pH 8.0, 1 mM Na_3VO_4, 10 μg/ml aprotinin, 10 μg/ml benzamidine and 0.2 mM PMSF) as described previously [12] for 1.5 h. The lysate was centrifuged, and the supernatant was incubated with 1 μg of anti-FLAG antibody and 15 μl of 50% slurry of protein A-Sepharose beads at 4 °C for 16 h. The beads were subsequently washed three times with NP-40 solubilization buffer. The proteins bound to the beads were eluted using the SDS-PAGE sample buffer and separated by SDS-PAGE. The presence of EGFR and GRK2 in the immunocomplexes was detected in the subsequent Western blotting with antibody specifically against FLAG epitopes and GRK2 respectively. The immunoblots were visualized using an enhanced chemiluminescence (ECL) kit (Amersham Biosciences) following the manufacturer's suggested protocol. An aliquot (2.5%) of the cell lysate was analyzed by Western blotting to quantify the expression level of the protein studied.

Laser confocal fluorescence microscopy

HEK293 cells were transfected with plasmid encoding GRK2-GFP. For real-time fluorescence analysis of GRK2-GFP in living cells, the fluorescence was observed under a microscope equipped with a temperature controller at 37 °C. The EGF was applied directly over the selected cells. The image scanned before EGF application represented GRK2-GFP distribution in cells. After EGF treatment, the same cells were scanned again in a time series. Scanning images were recorded with a TCS NT laser confocal microscope (Leica Microsystems, Bensheim, Germany).

Quantitation of receptor internalization by fluorescence flow cytometry assay

Receptor internalization was quantitated using fluorescence flow cytometry assay. Briefly, stably transfected HEK293 cells were chilled on ice after agonist stimulation and the surface receptors were labeled with corresponding antibody for 1 h at 4 °C. After sufficient washing, the cells were incubated with FITC-conjugated goat anti-mouse antibody for 1 h at 4 °C. The cells were then collected and fixed and the surface receptor staining intensity was analyzed using FACScalibur flow cytometry (Becton Dickenson, Mountain View, USA). Basal cell fluorescence intensity was determined with cells stained with the secondary antibody alone.

Statistical analysis

Data were analyzed using Student's *t*-test for comparison of independent means with pooled estimates of common variances.

Results

EGF stimulates GRK2-EGFR complex formation in HEK293 cells

HEK293 cells expressing FLAG-tagged EGFR and GRK2 or GRK2 alone were incubated in the presence or absence of 100 ng/ml EGF, then FLAG-EGFR was immunoprecipitated with specific anti-FLAG antibody. As shown in **Fig. 1(A)**, there was little GRK2 in the EGFR immunoprecipitation complex before EGF stimulation. After EGF stimulation there was a large amount of GRK2 detected in the EGFR immunoprecipitaion complex using GRK-specific antibody. In the cells expressing GRK2 alone there was no GRK2 detected in the EGFR immunoprecipitation complex upon EGF stimulation [**Fig. 1(A)**, upper panel]. This result indicates that the detected GRK2 in the EGFR immunoprecipitation complex was specific. Direct Western blot analysis of the total cell lysate detecting

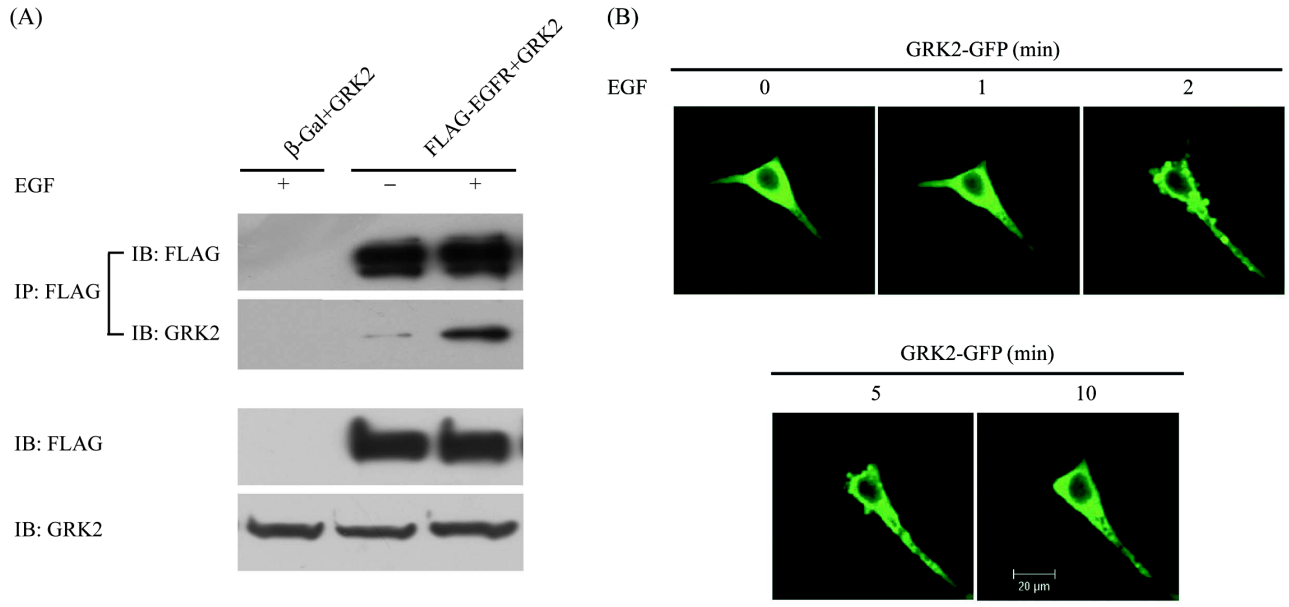

Fig. 1 EGF stimulates GRK2-EGFR complex formation in HEK293 cells

(A) Human embryonic kidney (HEK) 293 cells were transfected with G protein-coupled receptor kinases 2 (GRK2) alone or DYKDDDDK octapeptide (FLAG)-tagged epidermal growth factor receptor (FLAG-EGFR) and GRK2 encoding plasmids. The cells were incubated in the presence or absence of 100 ng/ml EGF for 5 min, and then cell lysate was prepared. Immunoprecipitation of the receptor complex was carried out using anti-FLAG antibody. FLAG-EGFR immunoprecipitation complex and the cell lysates were resolved by 8% SDS-PAGE, and electrotransferred onto nitrocellulose membranes. The upper and the lower parts of the membrane were probed with antibodies against FLAG and GRK2 respectively. One representative result of three independent experiments is shown. (B) HEK293 cells were transfected with GRK2-GFP (green fluorescence protein). The cells were challenged with 100 ng/ml EGF at 37 °C and real-time fluorescence images of the living cells before and after EGF exposure were recorded under a laser confocal microscope. Pictures shown are corresponding GRK2-GFP fluorescence images taken at 0, 1, 3, 5 and 10 min of EGF exposure. IB, immunoblot; IP, immunoprecipitation.

FLAG-tagged EGFR and GRK2 expression [**Fig. 1(A)**, lower panel] was shown to ensure similar expression levels. These results are in accordance with our previous study [11], and clearly demonstrate that EGF stimulates GRK2-EGFR complex formation in HEK293 cells overexpressing GRK2 and EGFR.

To further demonstrate that EGF stimulates GRK2-EGFR complex formation, we observed the subcellular redistribution of GRK upon EGF stimulation in HEK293 cells transiently expressing GRK2-GFP using a laser confocal microscope. As shown in **Fig. 1(B)**, the green fluorescence representing GRK2 mainly resided in the cytoplasm before EGF stimulation. The real-time recording of GRK2-GFP fluorescence images in living cells showed that after EGF stimulation the GRK2-GFP fluorescence increased quickly on the membrane. At the same time GRK2-GFP fluorescence decreased in the cytoplasm, and this redistribution was accompanied by changes in membrane shape [**Fig. 1(B)**, 3 min and 5 min]. The redistribution of GRK2-GFP was restored to the basal state at 10 min of EGF stimulation [**Fig. 1(B)**, 10 min].

These results demonstrated that EGF stimulation could induce translocation of GRK2 from cytoplasm to the plasma membrane and form a complex with EGFR on the membrane in HEK293 cells overexpressing GRK2 and EGFR.

Overexpression of GRK2 enhances EGF-stimulated ERK/MAPK activation

EGFR activation leads to activation of the ERK/MAPK pathway. To demonstrate whether GRK-EGFR complex formation upon EGF stimulation leads to modulation of EGFR signaling, we observed the effect of GRK2 on EGF-stimulated ERK/MAPK phosphorylation. HEK293 cells overexpressing GRK2 or β-Gal were incubated in the presence or absence of 10 ng/ml EGF for a period ranging from 0 min to 120 min. Phospho-ERK/MAPK and total ERK/MAPK were probed employing phospho-specific and total ERK/MAPK antibody. Western blotting analysis showed that phospho-ERK/MAPK was detected at 2 min of EGF stimulation and reached its maximum at 5 min, then it gradually decreased. Total ERK/MAPK did not show detectable change before or after EGF stimulation. Phospho-ERK/MAPK in GRK2 transfected cells was significantly increased compared with β-Gal transfected controls, although the time course of phospho-ERK/MAPK was similar [**Fig. 2(A)**].

The resulting phospho-ERK/MAPK levels from four independent sets of experiments were quantified normalizing with total ERK as a loading control. The mean values are presented graphically in **Fig. 1(B)**. Phospho-

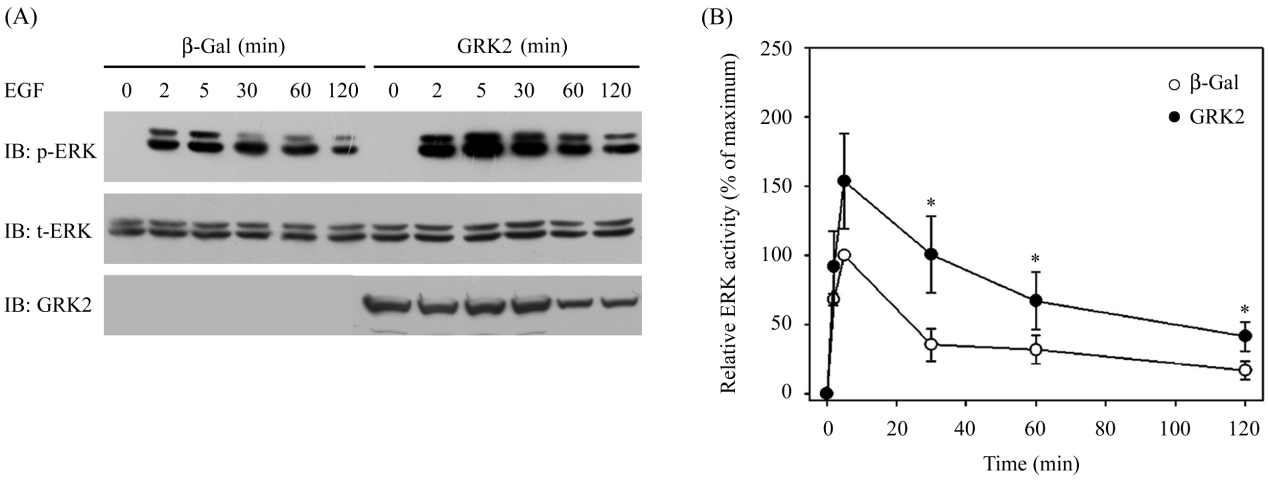

Fig. 2 Overexpression of GRK2 enhances EGF-stimulated ERK/MAPK activation in HEK293 cells

(A) Human embryonic kidney (HEK) 293 cells were transfected with β-Gal or G protein-coupled receptor kinase 2 (GRK2) encoding plasmids. The cells were incubated in the presence or absence of 10 ng/ml epidermal growth factor (EGF) for indicated time (min) and the cells were then lysed. Cell lysates were subjected to 10% SDS-PAGE and electrotransferred onto nitrocellulose membranes. The membranes were probed with anti-phospho-extracellular signal-regulated kinase (ERK)1/2, then reprobed with anti-total-ERK1/2 after stripping. The GRK2 expression was detected with anti-GRK2 antibody. Shown was one experiment representative of four independent experiments performed. (B) Phospho-ERK1/2 signals were quantified normalizing with total-ERK1/2 and represented as a percentage of maximal ERK activity in the graphical plot. Data were represented as mean±SE (n=4). *P<0.05 compared with β-Gal. IB, immunoblot; MAPK, mitogen-activated protein kinase; p-ERK, phospho-ERK; t-ERK, total-ERK.

ERK/MAPK was enhanced 1.9-fold, 1.1-fold and 1.5-fold respectively (P<0.05) in GRK2 transfected cells compared with β-Gal transfected controls at time point 30 min, 60 min and 120 min [**Fig. 2(B)**].

The effect of GRK2 overexpression on EGF-induced EGFR internalization and DPDPE-induced DOR internalization

Agonist-induced EGFR internalization is a critical regulatory mechanism in EGFR signaling. EGFR internalization induces receptor down-regulation by decreasing the amount of EGFR present on the plasma membrane. To determine whether the modulatory role of GRK2 on EGFR signaling is through affecting EGFR internalization, the following experiments were carried out. First we constructed a stable HEK293 cell line expressing FLAG-tagged EGFR, then we used flow cytometry to quantitatively determine the characteristics of EGF-induced EGFR internalization and the effect of GRK2 overexpression on the receptor internalization. Cell surface EGFR declined gradually after EGF stimulation. At 30 min of EGF stimulation there was about 70% of EGFR left on the cell surface compared with the cells left untreated, indicating that about 30% of cell surface EGFR was internalized into cytoplasm (data not shown). This data was in accordance with a previous study [13], suggesting EGFR was sequestered from plasma membrane in response to EGF stimulation. Then, overexpression of GRK2 on EGFR and G protein-coupled DOR internalization was examined. **Fig. 3(A)** shows representative results from a set of experiments. Stimulation of cells with indicated agonist led to the reduction of both the percentage of the positive cells and the mean fluorescence density. Quantitatively, overexpression of GRK2 in HEK293 cells stably expressing FLAG-EGFR did not have any significant effect on EGF-induced EGFR internalization (P=0.96) compared with control cells overexpressing β-Gal [**Fig. 3(B)**]. But in an HEK293 cell line stably expressing DOR, DOR internalization induced by its agonist DPDPE was enhanced by approximately 40% (P<0.01) when the cells were transfected with GRK2 compared with control cells transfected with β-Gal [**Fig. 3(B)**]. To exclude the possible effect of high density of plasma membrane EGFR on EGFR internalization and the possible saturability of the EGFR internalization pathway [14], we measured the internalization of endogenously expressed EGFR in

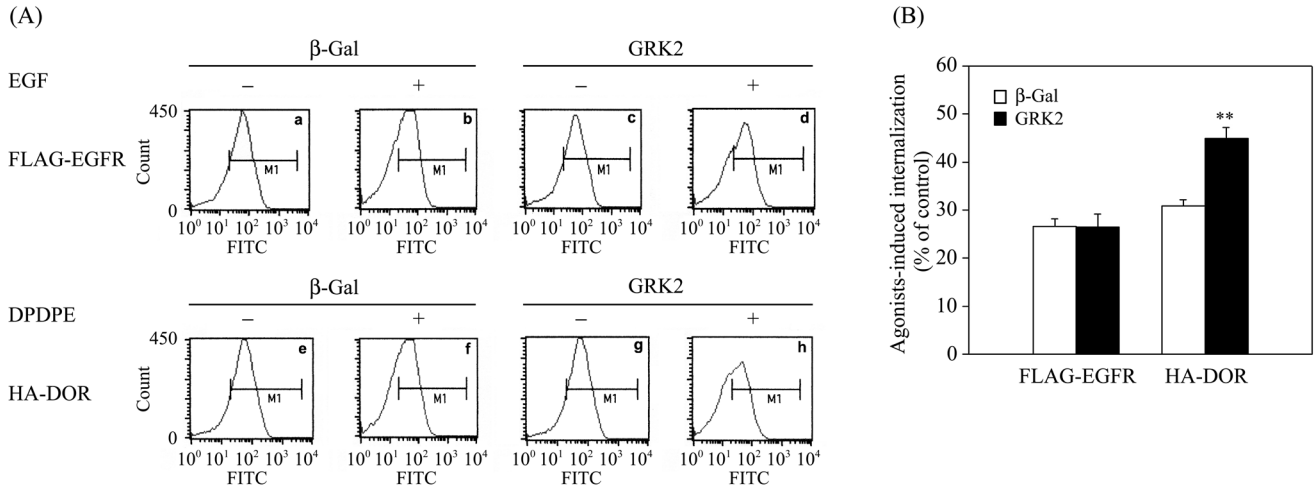

Fig. 3 Effect of GRK2 overexpression on agonist-stimulated internalization of EGFR and DOR

(A) The human embryonic kidney 293 (HEK293) cells stably expressing DYKDDDDK octapeptide (FLAG)-tagged epidermal growth factor receptor (FLAG-EGFR) (a–d) or hemagglutinin-tagged δ opioid receptor (HA-DOR) (e–h) were transiently transfected with β-Gal (a, b, e, f) as control or G protein-coupled receptor kinase 2 (GRK2) (c, d, g, h) cDNAs. The transfected cells were either left unstimulated (a, c, e, g) or stimulated with 100 ng/ml EGF (b, d) for EGFR or 1 μM [D-Pen^2,D-Pen^5]enkephalin (DPDPE) for HA-DOR (f, h) for 30 min as indicated. The cell surface receptors were stained with anti-FLAG antibody for FLAG-EGFR or anti-HA antibody for HA-DOR then fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody and analyzed by flow cytometry. Data are presented as FITC/Counts plots. The representative result from one experiment is shown. (B) The effect of GRK2 on receptor internalization as indicated. The opened bars represent β-Gal overexpression control, the closed bars represent GRK2 overexpression. The data are presented as a percentage of reduction of cell surface fluorescence, which represents the internalization of the indicated receptors. The percentage of receptor internalization is presented as mean±SE (n=3). **P<0.01 compared with β-Gal.

HEK293 cells and HeLa cells. GRK2 overexpression had no significant effect on EGF-induced EGFR internalisation either, although the internalization of endogenously expressed EGFR upon EGF stimulation was more rapid and to a greater degree (data not shown).

Discussion

Previous studies have shown that GRK2 plays a role in the negative regulation of signaling pathways mediated by PDGFRβ, besides its classical role in phosphorylating and desensitizing agonist-activated GPCRs. In the current study we investigated the role of GRK2 in the EGFR signaling pathway, including ERK/MAPK activation induced by EGFR activation and the role of GRK2 in EGF-induced EGFR internalization, as well as the possible mechanisms underlying it. Our results have shown that overexpression of GRK2 enhances ERK/MAPK activation induced by EGF stimulation. EGF stimulation induces GRK2 translocation from cytoplasm to the plasma membrane and GRK-EGFR complex formation. But overexpression of GRK2 had no significant effect on EGF-induced EGFR internalization; however, agonist-induced DOR internalization increased significantly.

Our present and previous studies have shown that EGF stimulates GRK2-EGFR complex formation. There are several lines of evidence to support this. First, co-immunoprecipitation experiment showed that there was a large amount of GRK2 in EGFR immunoprecipitation complex upon EGF stimulation. Second, confocal laser microscopy experiment showed that GRK2-GFP translocated from cytoplasm to plasma membrane in a real-time confocal fluorescence record for living cells. The mechanisms by which GRK activity is regulated can be divided into three categories: subcellular localization, alterations in intrinsic kinase activity and alterations in GRK expression level [15,16]. Our results showed that GRK2 translocated to plasma membrane upon EGF stimulation, indicating that upon EGFR activation GRK2 was also activated. Previous studies have demonstrated that GRK2 exhibits a primarily cytosolic distribution in unstimulated cells and appear to translocate to the plasma membrane upon GPCR activation [15,16]. Our results are in accordance with this, suggesting that GRK2 may exert its modulatory role in EGFR signaling via mechanisms similar to GPCRs.

EGFR activation leads to its dimerization and autophosphorylation of the cytoplasmic domains of EGFR. Adaptor proteins such as SHC bind to the phospho-tyrosine residues and subsequent formation of an SHC-Grb2-Sos complex and induction of Raf function, thus, the Ras/MAPK pathway was activated. This cascade couples agonist stimulation to gene transcription [4]. The current study has shown that overexpression of GRK2 enhances EGF-induced ERK/MAPK activation, suggesting that, in contrast to its negative regulation of GRK2 on PDGFRβ signaling, GRK2 may exert a positive regulation on EGF-induced ERK/MAPK phosphorylation. Phospho-ERK/MAPK can enter the nucleus and phosphorylate transcription factors such as Elk-1 [17], so enhanced ERK/MAPK activation by GRK2 overexpression may also lead to changes in gene transcription mediated by EGFR activation.

GRK-catalyzed GPCR phosphorylation leads to GPCR desensitization and subsequent internalization and degradation, thus GRK plays an important role in GPCR internalization. The present study showed that overexpression of GRK2 had no effect on EGF-induced EGFR internalization. However, overexpression of GRK2 significantly enhanced agonist-induced DOR internalization. Ligand-induced EGFR internalization requires intrinsic receptor tyrosine kinase activity [18] and specific sequences in the carboxyl-terminus of the receptor distal to the kinase domain. Some adaptors, such as the μ2 subunit of the AP2 protein recognize the endocytic signals thus involved in receptor endocytosis and recycling [19]. The GRK2 binding domain on EGFR and the possible phosphorylation sites on EGFR catalyzed by GRK2 remain elusive. We presume that EGF-induced GRK2 binding to EGFR does not affect the specific sequences involved in EGFR endocytosis due to the long distance between the binding domain or phosphorylation sites and the endocytic sequences, thus, EGF-induced EGFR internalization was unaffected by GRK2 overexpression.

Taken together, these data demonstrate in HEK293 cells overexpressing GRK2 and EGFR that GRK2 has the regulatory role in EGF-induced ERK/MAPK activation. However, EGF-induced EGFR internalization is not affected, suggesting that the mechanisms underlying the modulatory role of GRK2 in EGFR and in GPCR signaling pathways is somewhat different, at least in receptor internalization.

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References

1 Ji TH, Grossmann M, Ji I. G protein-coupled receptors. I. Diversity of receptor-ligand interactions. J Biol Chem 1998, 273: 17299–17302

2 Penela P, Ribas C, Mayor F Jr. Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases. Cell Signal 2003, 15: 973–981

3 Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000, 103: 211–225

4 Zwick E, Hackel PO, Prenzel N, Ullrich A. The EGF receptor as central transducer of heterologous signalling systems. Trends Pharmacol Sci 1999, 20: 408–412

5 Holbro T, Civenni G, Hynes NE. The ErbB receptors and their role in cancer progression. Exp Cell Res 2003, 284: 99–110

6 Ostman A, Bohmer FD. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. Trends Cell Biol 2001, 11: 258–266

7 Vieira AV, Lamaze C, Schmid SL. Control of EGF receptor signaling by clathrin-mediated endocytosis. Science 1996, 274: 2086–2089

8 Wiley HS.Trafficking of the ErbB receptors and its influence on signaling. Exp Cell Res 2003, 284: 78–88

9 Peppel K, Jacobson A, Huang X, Murray JP, Oppermann M, Freedman NJ. Overexpression of G protein-coupled receptor kinase-2 in smooth muscle cells attenuates mitogenic signaling via G protein-coupled and platelet-derived growth factor receptors. Circulation 2000, 102: 793–799

10 Peppel K, Zhang L, Huynh TT, Huang X, Jacobson A, Brian L, Exum ST *et al*. Overexpression of G protein-coupled receptor kinase-2 in smooth muscle cells reduces neointimal hyperplasia. J Mol Cell Cardiol 2002, 34: 1399–1409

11 Gao J, Li J, Chen Y, Ma L. Activation of tyrosine kinase of EGFR induces Gβγ-dependent GRK-EGFR complex formation. FEBS Lett 2005, 579: 122–126

12 Maudsley S, Pierce KL, Zamah AM, Miller WE, Ahn S, Daaka Y, Lefkowitz RJ *et al*. The β_2-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. J Biol Chem 2000, 275: 9572–9580

13 Kim J, Ahn S, Guo R, Daaka Y. Regulation of epidermal growth factor receptor internalization by G protein-coupled receptors. Biochemistry 2003, 42: 2887–2894

14 Wiley HS. Anomalous binding of epidermal growth factor to A431 cells is due to the effect of high receptor densities and a saturable endocytic system. J Cell Biol 1988, 107: 801–810

15 Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. Annu Rev Biochem 1998, 67: 653–692

16 Penn RB, Pronin AN, Benovic JL. Regulation of G protein-coupled receptor kinases. Trends Cardiovasc Med 2000, 10: 81–89

17 Papavassiliou AG. The role of regulated phosphorylation in the biological activity of transcription factors SRF and Elk-1/SAP-1. Anticancer Res 1994, 14: 1923–1926

18 Wiley HS, Herbst JJ, Walsh BJ, Lauffenburger DA, Rosenfeld MG, Gill GN. The role of tyrosine kinase activity in endocytosis, compartmentation, and down-regulation of the epidermal growth factor receptor. J Biol Chem 1991, 266: 11083–11094

19 Wiley HS. Trafficking of the ErbB receptors and its influence on signaling. Exp Cell Res 2003, 284: 78–88

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