SMURF1 Plays a Role in EGF-Induced Breast Cancer Cell Migration and Invasion

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Epidermal growth factor (EGF) is a well-known growth factor that induces cancer cell migration and invasion. Previous studies have shown that SMAD ubiquitination regulatory factor 1 (SMURF1), an E3 ubiquitin ligase, regulates cell motility by inducing RhoA degradation. Therefore, we examined the role of SMURF1 in EGF-induced cell migration and invasion using MDA-MB-231 cells, a human breast cancer cell line. EGF increased SMURF1 expression at both the mRNA and protein levels. All ErbB family members were expressed in MDA-MB-231 cells and receptor tyrosine kinase inhibitors specific for the EGF receptor (EGFR) or ErbB2 blocked the EGF-mediated induction of SMURF1 expression. Within the signaling pathways examined, ERK1/2 and protein kinase C activity were required for EGF-induced SMURF1 expression. The overexpression of constitutively active MEK1 increased the SMURF1 to levels similar to those induced by EGF. SMURF1 induction by EGF treatment or by the overexpression of MEK1 or SMURF1 resulted in enhanced cell migration and invasion, whereas SMURF1 knockdown suppressed EGF- or MEK1-induced cell migration and invasion. EGF treatment or SMURF1 overexpression decreased the endogenous RhoA protein levels. The overexpression of constitutively active RhoA prevented EGF- or SMURF1-induced cell migration and invasion. These results suggest that EGF-induced SMURF1 plays a role in breast cancer cell migration and invasion through the downregulation of RhoA.

INTRODUCTION

Breast cancer is one of the most commonly identified cancers and accounts for approximately 23% of all female cancers worldwide (Jemal et al., 2008). Breast cancer can metastasize to the liver, lungs, brain, bone, or muscle. The bones are the most frequent sites for the metastasis of breast cancer; in fact, most breast cancer patients die with bone metastasis (Solomayer et al., 2000). Many cancer cell lines are used to study breast cancer metastasis, and MDA-MB-231, an aggressive breast cancer cell line, is known to have more migratory capacity to the bone compared with other noninvasive breast cancer cell lines (Nannuru and Singh, 2010).

Cancer metastasis occurs when tumor cells penetrate the blood and lymph vessels and are transferred to distant organs as a result of tumor cell migration and invasion. Cell migration and invasion is initiated through the activation of specific signaling pathways that control the cytoskeletal dynamics and the cell-matrix or cell-cell junction complexes (Fidler, 2003; MacDonald et al., 2002). The stimulation of cancer cells with various growth factors, such as hepatocyte growth factor, fibroblast growth factor, transforming growth factor beta, and epidermal growth factor (EGF), induces changes in cell-cell junction-related molecules and ultimately increases cell migration (Barr et al., 2008; Miettinen et al., 1994; Muller et al., 2002; Valles et al., 1990).

EGF not only increases cell proliferation but also promotes cancer cell migration and invasion (Barr et al., 2008; Citri and Yarden, 2006; De Luca et al., 2008; Price et al., 1996; Shibata et al., 1996). EGF and its receptors are frequently overexpressed in breast cancers with a higher incidence of distant metastases (Eccles, 2001; De Luca et al., 2008; Giltnane et al., 2009). Inhibitors of epidermal growth factor receptor (EGFR) signaling have been found to reduce the metastasis to the brain and bone in mouse breast cancer model systems, which suggests that EGF/EGFR signaling is involved in the metastasis of breast cancers (Du et al., 2010; Gril et al., 2008; Molli et al., 2008; Nie et al., 2012).

SMAD ubiquitination regulatory factor 1 (SMURF1) was first identified as an E3 ligase specific for SMAD1 and SMAD5 (Zhu et al., 1999). In addition to the SMAD proteins, SMURF1 targets RhoA and thereby regulates cell polarity and protrusion (Ozdamar et al., 2005; Sahai et al., 2007; Wang et al., 2003). SMURF1 is involved in the transforming growth factor beta-induced epithelial-to-mesenchymal transition (Ozdamar et al., 2005; Townsend et al., 2008). It has also been demonstrated that SMURF1 gene amplification promotes invasiveness in pancreatic cancer (Kwei et al., 2011; Suzuki et al., 2008). The upregulation of SMURF1 enhances breast cancer cell migration and invasion *in vitro* and bone metastasis *in vivo* (Fukunaga et al., 2008). The results from these studies suggest that SMURF1 plays a role in cancer cell migration and invasion. Therefore, in this study, we investigated whether SMURF1 is involved in EGF-induced cell migration and invasion in breast cancer cells.

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MATERIALS AND METHODS

Reagents and antibodies

Recombinant human EGF was purchased from Sigma-Aldrich (USA). Anti-SMURF1 antibody was obtained from Invitrogen (USA), and p-ERK1/2 and ERK1/2 antibodies were obtained from Cell Signaling Technology (USA). RhoA antibody was purchased from Santa Cruz (USA). The following kinase inhibitors were obtained from Calbiochem (USA) and used in this study: EGFR and ErbB2 receptor tyrosine kinases (AG1478, AG825), mitogen-activated protein kinases (MAPKs; U0126, SB203580, SP600125), and protein kinase C (PKC; calphostin C). Matrigel and type I collagen were purchased from BD Bioscience (USA).

Constitutively active (*ca*) RhoA plasmid was kindly provided by Prof. Hyun-Man Kim from Seoul National University (Yang and Kim, 2012). The construction of *ca*MEK1 and SMURF1 expression vectors has been previously described (Jin et al., 2004; Jun et al., 2010). Transient transfection was performed using the Lipofectamine reagent (Invitrogen).

SMURF1 small interfering RNA (siRNA) and control siRNA (ON-TARGETplus non-targeting siRNA D-001810-02-20) were purchased from Dharmacon (USA). The SMURF1 siRNA used in this study is a mixture of four siRNAs targeting four independent SMURF1 mRNA sequences. The sequences of each SMURF1 siRNA are as follows: J-007191-09 5'-GCACUAU GAUCUAUAUGUU-3', J-007191-10 5'-GGAGGAGACCUGC GGGUUU-3', J-007191-11 5'-GAUCGACAUUCCACCAUAU-3' and J-007191-12 5'-AAGAAUACGUCCGGUUGUA-3'. The transient transfection of siRNA was performed using Dharmafect (Dharmacon) according to the manufacturer's instructions.

Cell culture

MDA-MB-231 cells, a human breast cancer cell line, were grown in Dulbecco's modified Eagle's medium (DMEM, Hyclone; USA) supplemented with 10% fetal bovine serum (BioWhittaker; USA), 100 U/ml penicillin, and 100 µg/ml streptomycin.

Migration and invasion assay

The MDA-MB-231 cells were seeded on the upper chamber of a 24-well transwell culture plate (Corning, USA; 8-µm pore size). For the migration assay, 1×10^4 cells were seeded on a type I collagen-coated transwell plate, whereas 2×10^5 cells were seeded on a matrigel-coated transwell plate for the invasion assay. After a 24-h incubation, the cells were serum-starved for an additional 24 h. The upper chambers were then filled with serum-free DMEM, and the lower chambers were filled with serum-free DMEM containing either vehicle or 10 ng/ml EGF. After a 48-h incubation, the remaining cells on the top surface of the upper chamber were removed with a cotton swab, and the cells that migrated or invaded to the undersurface were stained with H&E. The migrated or invaded cells were counted using the Image J program.

When indicated, the transient transfection of the MDA-MB-231 cells with expression plasmids or siRNAs was performed 24 h before the cells were seeded in the transwell plates.

Western blot analysis

At the end of the culture period, the cells were washed with phosphate-buffered saline and scraped into lysis buffer containing 10 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM EDTA (pH 8.0), 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM sodium fluoride, 0.2 mM sodium orthovanadate, 1 mM PMSF, 1 µg/ml aprotinin, 1 µM leupeptin, and 1 µM pepstatin.

Fig. 1. EGF induces SMURF1 expression in MDA-MB-231 breast cancer cells. MDA-MB-231 cells were incubated in the presence of EGF (10 ng/ml) for the indicated periods, and the expression level of SMURF1 was evaluated by real-time PCR (A) and Western blot analysis (B). The data are presented as the means $\pm$ SD. * $p < 0.05$ compared to the 0 h time point.

The protein samples were subjected to SDS-PAGE and subsequently transferred onto a PVDF membrane. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween20, incubated with the indicated primary antibody overnight, and incubated with a HRP-conjugated secondary antibody for 2 h. The immune complexes were then visualized with the Suprex reagent (Dyne-Bio, Korea), and the luminescence was detected with a LAS1000 instrument (Fuji Photo Film; Japan).

Reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA was extracted with the easy-BLUE™ RNA extraction reagents (iNtRON Biotechnology, Korea). The complementary DNA was synthesized from 2.5 µg of total RNA using the AccuPower™ RT PreMix (Bioneer, Korea) and subsequently subjected to PCR amplification with *StarTaq*™ polymerase (iNtRON Biotechnology) for the semi-quantitative RT-PCR. The PCR products were electrophoresed on a 1.2% agarose gel, stained with ethidium bromide, and visualized under UV light. The primer sequences were as follows: EGFR (f) 5'-GAG AGG AGA ACT GCC AGA A-3', (r) 5'-GTA GCA TTT ATG GAG AGT G-3'; ErbB2 (f) 5'-CCA GGA CCT GCT GAA CTG GT-3', (r) 5'-TGT ACG AGC CGC ACA TCC-3'; ErbB3 (f) 5'-GGT GCT GGG CTT GCT TTT-3', (r) 5'-CGT GGC TGG AGT TGG TGT TA-3'; ErbB4 (f) 5'-TGT GAG AAG ATG GAA GAT GGC-3', (r) 5'-GTT GTG GTA AAG TGG AAT GGC-3'; and GAPDH (f) 5'-TCA CCA TCT TCC AGG AGG G-3', (r) 5'-CTG CTT ACC ACC TTC TTG A-3'.

The quantitative RT-PCR of SMURF1 was performed using SYBR premix EX Taq (TaKaRa, Japan) in an AB 7500 Fast Real-Time system (Applied Biosystems; Foster City, CA, USA). The primer sequences were as follows: *SMURF1* (f) 5'-GTC CAG AAG CTG AAA GTC CTC AGA-3', (r) 5'-CAC GGA ATT

Fig. 2. SMURF1 knockdown blocks EGF induction of cell migration and invasion. MDA-MB-231 cells were transfected with control siRNA (*si control*) or SMURF1 siRNA (*si SMURF1*) and seeded in the upper compartment of transwell plates coated with type I collagen (A) or matrigel (B). After 24-h serum-starvation, the cells were incubated in the presence or absence of EGF for 48 h. The cells that migrated (A) or invaded (B) to the undersurface of the transwell were observed through H&E staining under a microscope (100 $\times$, upper panels). The number of cells was counted, and the data are presented relative to the quantity of migrated or invaded cells in the vehicle-treated *si control* group. * $p < 0.05$ compared to the vehicle-treated *si control* cells; # $p < 0.05$ for the indicated pairs.

TCA CCA TCA GCC-3' and GAPDH (f) 5'-CCA TCT TCC AGG AGC GAG ATC-3', (r) 5'-GCC TTC TCC ATG GTG GTG AA-3'.

Statistical analysis

The statistical significance was characterized by Student's *t*-test. A *p* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

EGF induces SMURF1 expression in MDA-MB-231 cells

It is well known that EGF promotes cancer cell migration and invasion (Barr et al., 2008; De Luca et al., 2008; Price et al., 1996). Additionally, SMURF1 regulates cell protrusion and motility (Ozdamar et al., 2005; Wang et al., 2003). However, the connection between SMURF1 and EGF in cancer cell migration and invasion remains unclear. Therefore, we first examined whether EGF regulates SMURF1 expression in breast cancer cells.

The MDA-MB-231 cells were serum-starved and incubated for up to 48 h in the presence of 10 ng/ml EGF. The quantitative RT-PCR results showed that EGF significantly enhanced the SMURF1 mRNA levels (Fig. 1A). The increase in SMURF1 mRNA expression was obvious as early as 6 h after treatment, and the maximum level of SMURF1 mRNA was observed after a 48-h incubation with EGF. The Western blot analysis demonstrated that a significant increase in the SMURF1 protein level started to appear after 24 h of treatment with EGF, and the SMURF1 protein level was further increased in the samples incubated for 48 h (Fig. 1B). In addition to MDA-MB-231 cells, EGF-induced SMURF1 expression was observed in other breast cancer cell lines, such as MCF-7 and MDA-MB-468 (Supplementary Figs. 1 and 2). These results suggest that EGF enhances SMURF1 expression in breast cancer cells.

SMURF1 is involved in the EGF induction of breast cancer cell migration and invasion

To examine the possible role of SMURF1 in EGF-induced cell migration and invasion, we induced SMURF1 knockdown using siRNA and observed the effects on cell migration and invasion. In the presence of non-targeting control siRNA, EGF significantly increased the number of migrated and invaded cells (Fig. 2). SMURF1 knockdown suppressed the basal cell migration and invasion. In the presence of EGF treatment, SMURF1 knockdown reduced the number of migrated and invaded cells to those of the levels observed with the vehicle-treated control siRNA cells. The silencing efficiency of SMURF1 siRNA was observed by Western blot analysis (Fig. 3A, right panel). The basal and EGF-induced SMURF1 protein levels were reduced in the SMURF1 siRNA-transfected cells compared to those of the control siRNA-transfected cells. The results of the present study are consistent with those of a previous study that showed that the knockdown of SMURF1 in MDA-MB-231 cells decreases cell migration (Fukunaga et al., 2008). These results suggest that the EGF induction of SMURF1 expression contributes to EGF-induced cancer cell migration and invasion.

caRhoA blocks the stimulatory effect of EGF and SMURF1 overexpression on cell migration and invasion

It was previously demonstrated that SMURF1 induces RhoA ubiquitination and that SMURF1 regulates tumor cell plasticity and motility through localized RhoA degradation in lamellipodia and filopodia (Sahai et al., 2007; Wang et al., 2003). However, in podocytes, SMURF1 acts as a global suppressor of RhoA signaling deep in the cell and in the cell periphery, and the overall suppression of both the RhoA activity and the level of the RhoA impairs cell motility (Asanuma et al., 2006).

Rho GTPases, such as RhoA, Rac1 and Cdc42, are key players in the regulation of cell migration and invasion (Raftopoulos and Hall, 2004). The activity of RhoA is suppressed in the leading edge during cell migration because RhoA activity reduces lamellipodia formation; however, at the rear side, RhoA

Fig. 3. Overexpression of constitutively active RhoA blocks EGF- and SMURF1-induced cell migration and invasion. MDA-MB-231 cells were transfected with the indicated expression plasmids (pcDNA, SMURF1, *caRhoA*) or siRNAs (*si control*, *si SMURF1*) and incubated for 24 h. The cells were then incubated for an additional 48 h in the presence or absence of EGF. (A) The levels of Smurf1 and RhoA proteins were evaluated by Western blot analysis. EGF decreased the RhoA protein levels, and SMURF1 is involved in the EGF-induced RhoA reduction. (B) Transwell migration and invasion assays were performed. The migrated and invaded cells were stained with H&E and observed under a microscope (100 $\times$). The number of migrated and invaded cells was presented relative to those found in the vehicle-treated pcDNA-transfected cells. * $p < 0.05$ compared to the vehicle-treated pcDNA-transfected cells; # $p < 0.05$ for the indicated pairs.

activity is involved in the promotion of actin-myosin contraction (Ridley et al., 2003; Worthylake and Burridge, 2003). Although SMURF1-induced localized RhoA degradation is known to increase cell motility in breast cancer cells (Sahai et al., 2007), increased activity and/or expression level of RhoA has also been reported to enhance cell migration and invasion in gastric cancer, colorectal cancer, and pancreatic cancer cells and trophoblasts (Cardoso et al., 2013; Han et al., 2010; Kusama et al., 2006). Therefore, it remains unclear whether the EGF induction of SMURF1 expression reduces the global expression level of RhoA and whether the rescue of RhoA activity blocks the EGF-induced migration and invasion in breast cancer cells.

We examined whether EGF reduces the RhoA protein level in MDA-MB-231 cells. The treatment of the cells with EGF for 48 h downregulated the total cellular RhoA protein level and upregulated the SMURF1 level compared with the vehicle-treated control cells (Fig. 3A, left panel). The overexpression of SMURF1 in MDA-MB-231 cells as a positive control decreased the RhoA protein to a level similar to that observed in the EGF-treated cells. When the cells overexpressing SMURF1 were treated with EGF, the SMURF1 level was much higher than that obtained with EGF or SMURF1 alone, and the RhoA level was further decreased (Fig. 3A, left panel). In SMURF1-silenced cells, the cellular RhoA protein level was higher than that obtained in the control siRNA-transfected cells (Fig. 3A, right panel). Furthermore, SMURF1 knockdown partially rescued the RhoA protein level in the presence of EGF treatment. These results suggest that SMURF1 induction is involved in the EGF downregulation of RhoA in MDA-MB-231 cells. These results are contradictory to those of a previous report that revealed that EGF increases RhoA expression levels in a time-dependent

manner in trophoblast cells (Han et al., 2010). The reason for this discrepancy is not clear, but a previous report found that EGF increases lamellipodia and reduces stress fiber formation in breast cancer cells (Molli et al., 2008); therefore, it is suggested that the nature of the EGF regulation of the RhoA level depends on the cell type.

We then investigated whether the rescue of the RhoA level blocked the EGF induction of cell migration and invasion. The MDA-MB-231 cells were transiently transfected with *caRhoA*-expressing plasmids, and the effect on cell migration and invasion was observed in the presence or absence of EGF treatment or SMURF1 overexpression. The overexpression of SMURF1 increased the quantity of migrated and invaded cells to levels similar to those obtained with the EGF-treated cells (Fig. 3B), further confirming the enhancing role of SMURF1 in the regulation of migration and invasion in breast cancer cells. When the level of RhoA increased, both migration and invasion were downregulated in the vehicle-treated cells (Fig. 3B). Furthermore, the overexpression of *caRhoA* prevented EGF and SMURF1 overexpression from stimulating cell migration and invasion (Fig. 3B). A previous study also reported similar results: the overexpression of SMURF1 in epicardial cells increased cellular invasion, and the overexpression of *caRhoA* abolished the transforming growth factor β 2-induced invasion (Sanchez and Barnett, 2012). In addition, the silencing of EGFR has been shown to reduce lamellipodia and filopodia in MDA-MB-231-BR cells, which are a brain-seeking derivative of MDA-MB-231 cells (Nie et al., 2012). Taken together, the present results further suggest that a global reduction in RhoA expression by EGF-induced SMURF1 contributes to the EGF induction of migration and invasion in breast cancer cells.

Fig. 4. ERK1/2 and PKC activation is required for the EGF induction of SMURF1 expression. (A) The expression of the ErbB family members in MDA-MB-231 cells was examined by conventional RT-PCR. All of the examined members were expressed, but the level of ErbB2 was the highest. (B, C) The SMURF1 expression levels were examined in MDA-MB-231 cells treated with EGF for 48 h in the presence or absence of the indicated kinase inhibitor. Both EGFR and ErbB2 activation is involved in the EGF induction of SMURF1 (B). Among the downstream signaling pathways examined, ERK1/2 and PKC activation plays a role in SMURF1 induction (C). AG1478, EGFR receptor tyrosine kinase inhibitor; AG825, ErbB2 receptor tyrosine kinase inhibitor; SB (SB203580), p38 MAPK inhibitor; SP (SP600125), JNK inhibitor; U (U0126), ERK1/2 inhibitor; C (calphostin C), PKC inhibitor. With the exception of calphostin C (1 μ M), all of the inhibitors were used at a concentration of 10 μ M. * p < 0.05 compared to the vehicle control; # p < 0.05 compared to EGF alone.

EGF induction of SMURF1 requires PKC and ERK1/2 activation

For many years, ErbB-related signaling has been found to play an important role in breast cancer cell migration and invasion and is one of the major targets in breast cancer therapy (Hynes and Lane, 2005; Lo et al., 2006). The EGF receptors belong to the ErbB family of transmembrane receptor tyrosine kinases. The ErbB family includes EGFR/ErbB1/HER1, ErbB2/HER2/Neu, ErbB3/HER3, and ErbB4/HER4 (Klapper et al., 2000; Olayioye et al., 2000). Upon ligand binding, EGFR homodimerizes or heterodimerizes with all members of the ErbB family and activates downstream effectors, including the Ras/MAPK and the phospholipase C γ 1 (PLC γ 1)/PKC pathways.

To investigate the signaling pathways involved in the EGF induction of SMURF1, we first examined the expression of the ErbB family members in MDA-MB-231 cells. All of the family members were detected by conventional RT-PCR, but ErbB2 was the most highly expressed member in MDA-MB-231 cells (Fig. 4A). Because a previous report demonstrated that synergistic EGFR/ErbB2 and ErbB2/ErbB3 heterodimer signaling

leads to breast cancer cell migration (Balz et al., 2012), we determined the effect of receptor tyrosine kinase inhibitors specific for EGFR (AG1478) or ErbB2 (AG825) on the EGF-induced SMURF1 expression. Real-time PCR and Western blot analyses demonstrated that both AG1478 and AG825 blocked the EGF induction of SMURF1 (Fig. 4B). These results suggest that both EGFR and ErbB2 are involved in the EGF-induced expression of SMURF1 in breast cancer cells.

To further delineate the signaling pathways involved in EGF-induced SMURF1 expression, MDA-MB-231 cells were treated with EGF in the presence or absence of inhibitors for the MAPKs or PKC. Real-time PCR and Western blot analyses showed that U0126 (an ERK1/2 inhibitor) and calphostin C (a PKC inhibitor) decreased both the basal and the EGF-induced SMURF1 expression (Fig. 4C). However, the addition of SB203580 (a p38 MAPK inhibitor) or SP600125 (a JNK inhibitor) did not affect the expression levels of SMURF1. These results suggest that the ERK1/2 and PKC signaling pathways but not the JNK and p38 MAPK pathways are involved in the EGF induction of SMURF1 expression.

Fig. 5. ERK1/2 activation by caMEK1 increases cell migration and invasion in a SMURF1-dependent manner. (A) MDA-MB-231 cells were transfected with constitutively active MEK1 (caMEK1) or treated with EGF for 48 h, and the expression levels of SMURF1 were determined by real-time PCR and Western blot analyses. The overexpression of caMEK1 alone is sufficient to induce SMURF1 expression to a level similar to that obtained with EGF treatment. * $p < 0.05$ compared to the pcDNA-transfected cells. (B) MDA-MB-231 cells were transfected with the indicated expression plasmid and siRNAs, and cell migration and invasion assays were performed using the transwell system. * $p < 0.05$ compared to the si control-transfected control cells; # $p < 0.05$ for the indicated pairs.

We previously showed that ERK1/2 activity is involved in tumor necrosis factor α -induced SMURF1 expression in C2C12 murine premyoblast cells (Lee et al., 2013). To further examine the role of ERK1/2 in SMURF1 induction in breast cancer cells, MDA-MB-231 cells were transfected with caMEK1, and the level of SMURF1 expression was observed by real-time PCR and Western blot analyses. Similarly to the previous study, the SMURF1 mRNA and protein expression levels were upregulated by caMEK1 overexpression (Fig. 5A).

We then examined whether the activation of ERK1/2 by caMEK1 overexpression increased cell migration and invasion. Transwell cell migration and invasion assays showed that caMEK1 overexpression increased cell migration and invasion and that this stimulatory effect of caMEK1 was blocked by the knockdown of SMURF1 (Fig. 5B). These results suggest that SMURF1 is involved in the MEK1/ERK activation-induced increase in breast cancer cell migration and invasion.

Previous reports have demonstrated that the induction of breast cancer cell migration by EGF is initiated by the heterodimerization of EGFR/ErbB2 and the activation of downstream signaling, such as PLC γ 1, which results in actin reorganization (Brandt et al., 1999; Dittmar et al., 2002). It has also been reported that ERK1/2 activity is required for the basal and EGF-induced migration of intestinal epithelial cells and that the overexpression of caMEK1 increases lamellipodia formation (Vaidya et al., 2005). The transactivation of EGFR is also required for angiotensin II-induced smooth muscle cell migration (Yang et al., 2005). In the present study, we demonstrated that PKC and

ERK1/2 activity is required for the EGF induction of SMURF1 expression. Interestingly, the overexpression of caMEK1 alone increased SMURF1 expression to a level similar to that obtained with EGF treatment. However, it remains unclear whether PKC and ERK1/2 regulate SMURF1 expression in an independent- or interdependent-manner, and this thus necessitates further study.

In this study, we demonstrated that EGF induces SMURF1 expression in human breast cancer cells and that EGFR- and ErbB2-induced PKC and ERK1/2 play a role in the upregulation of SMURF1 expression. The levels of RhoA protein were downregulated by both EGF treatment and SMURF1 overexpression. Both the knockdown of SMURF1 and the overexpression of caRhoA blocked the EGF-induced cell migration and invasion. These results indicate that EGF-induced SMURF1 plays a role in breast cancer cell migration and invasion through the downregulation of RhoA.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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