Overexpression of ErbB2 receptor inhibits IGF-I-induced Shc–MAPK signaling pathway in breast cancer cells☆,☆☆

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Abstract

Overexpression of the ErbB2 receptor in one-third of human breast cancers contributes to the transformation of epithelial cells and predicts poor prognosis for breast cancer patients. We report that the overexpression of ErbB2 inhibits IGF-I-induced MAPK signaling. IGF-I-induced MAPK phosphorylation and MAPK kinase activity are reduced in ErbB2 overexpressing MCF-7/HER2-18 cells relative to control MCF-7/neo cells. In SKBR3/IGF-IR cells, reduction of ErbB2 by antisense methodology restores the IGF-I-induced MAPK activation. The inhibition of IGF-I-induced MAP kinase activation in ErbB2 overexpressing breast cancer cells is correlated with decreased IGF-I-induced Shc tyrosine-phosphorylation, leading to a decreased association of Grb2 with Shc and decreased Raf phosphorylation. However, IGF-I-induced tyrosine-phosphorylation of IGF-I receptor and IRS-I and AKT phosphorylation were unaffected by ErbB2 overexpression. Consistent with these results, we observed that the proportion of IGF-I-stimulated proliferation blocked by the MAPK inhibitor PD98059 fell from 82.6% in MCF-7/neo cells to 41.2% in MCF-7/HER2-18 cells. These data provide evidence for interplay between the IGF-IR and ErbB2 signaling pathways. They are consistent with the view that the IGF-IR mediated attenuation of trastuzumab-induced growth inhibition we recently described is dependent on IGF-I-induced PI3K signaling rather than IGF-I-induced MAPK signaling.

Keywords: ErbB2; Overexpression; IGF-I receptor; MAPK; Shc; Grb2

ErbB2 is expressed in a variety of tissues where it plays fundamental roles in development, proliferation, and differentiation [1,2]. Although no direct ligand has been found for ErbB2, it can be activated by its overexpression or transactivated by various ligands of EGF family [3]. The activation of ErbB2 by the formation of heterodimers with other ErbB receptors is well described [3–5] and involves prolongation of the signaling by ErbB2-containing heterodimers [6–9]. Ras/MAPK and PI3K/Akt are two downstream pathways of ErbB2, which link ErbB2 to its biological functions [3].

The insulin-like growth factor-I receptor (IGF-IR) is a membrane-bound heterotetramer with ligand-induced tyrosine kinase activity [10]. Phosphorylation of the IGF-IR results in the tyrosine phosphorylation of insulin receptor substrate (IRS) proteins and Shc, which in turn activate the survival phosphatidylinositol 3 kinase (PI3K) and the mitogenic Ras/MAPK pathways [11–13]. IRS/PI3K and Shc/Ras/MAPK pathways represent two distinct pathways for IGF-I response in many cell types. There is evidence that mitogenic response to IGF-I is thought to be related to the tyrosine-phosphorylation of Shc [11,13]. Phosphorylated Shc is then associated with Grb2–mSOS complex to activate p21/Ras, which leads to mitogenesis by activation of the Ras/Raf/MAPK pathway [14–16]. Although IRS-I also recruits Grb2 [17], the Shc–Grb2 pathway seems to be predominant activator of p21/Ras in IGF-IR signaling,

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☆☆ *Abbreviations:* IGF-I, insulin-like growth factor I; IGFBP-3, IGF binding protein-3; IRS-1, insulin receptor substrates 1; MBP, myelin basic protein; SFM, serum free medium, RTK, protein tyrosine kinase.

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because blocking antibodies raised against Shc effectively block the mitogenic response to IGFs [13,18,19].

There are three major MAPK pathways: the extracellular-signal regulated kinases (ERK1/ERK2), the c-jun N-terminal kinases (JNK), and p38 kinase [20]. Activation of the Ras/MAP kinase (ERK1/ERK2) pathway is important in RTK-induced signal transduction, including ErbB2 and IGF-IR-mediated signaling [21,22]. Activation of this pathway by appropriate ligands involves sequential participation of adaptor protein Shc and Grb2, Ras-GTP, Raf, MEK1/MEK2, and ERK1/ERK2 [22,23]. The downstream effectors of ERKs are nuclear transcription factors such as myc, c-fos, and c-jun, which trigger cell proliferation via direct impact on gene expression [24].

Overexpression of ErbB2 has been found in one-third of breast cancer patients and correlates with a higher relapse rate and poor clinical prognosis [2,25,26]. A recent study showed that ErbB2-overexpressing breast cancer cells display an increased requirement for the PI3K signaling pathway [27]. Furthermore, ErbB2-induced MAPK activity is not stimulated by serum and does not lead to cell proliferation in ErbB2 overexpressing breast cancer cells [28,29]. In the present study, we investigated the effect of overexpression of ErbB2 on IGF-I-induced MAPK activation in MCF-7/HER2-18 cells and SKBR3/IGF-IR cells [30]. We recently showed using these models that IGF-IR signaling can attenuate trastuzumab-induced growth inhibition [30].

Materials and methods

Materials. All culture materials were from Invitrogen (Carlsbad, CA). [γ-^{32}P]ATP was from Amersham–Pharmacia Biotech (Piscataway, NJ). IGF binding protein-3 (IGFBP-3) and IGF-I were from Protigen Corporation (Mountain View, CA). PD98059, transferrin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and myelin basic protein (MBP) were from Sigma (St. Louis, MO). Lipofectin was from Invitrogen (Carlsbad, CA). Protein A–agarose, anti-ErbB2, anti-IGF-IRβ, anti-ERK2, anti-c-fos, anti-jun, and anti-α-tubulin were purchased form Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Shc (monoclonal) and anti-Grb2 were from Transduction Laboratories (Lexington, KY). Anti-Shc (polyclonal), anti-IRS-1, anti-phospho-Raf-1, and anti-phospho-tyrosine specific antibodies were from Upstate Biotechnology (Lake Placid, NY). Anti-phospho-MAPK, anti-total MAPK, anti-phospho-AKT, and anti-AKT were from New England BioLabs (Mississauga, Ontario).

Cell lines. MCF-7/neo and MCF-7/HER2-18 cells [31] were provided by Dr. Alaoui-Jamali (McGill University). SKBR3/IGF-IR are human breast cancer cells transfected with pcDNA3.1(+)/IGF-IR and the characterization of these cell lines has been described in our previous paper [30].

IGF-I treatment. MCF-7/neo and MCF-7/HER2-18 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 700 μg/ml G418 at 37 °C and 5% CO_2. SKBR3/IGF-IR cells were cultured in McCoy'5A medium supplemented with 10% fetal bovine serum (FBS) and 800 μg/ml G418 at 37 °C and 5% CO_2. For studies assessing the effects of exposure to IGF-I and IGFBP-3 on IGF-I receptor and its downstream molecule activation in MCF-7/neo, MCF-7/HER2-18, and SKBR3/IGF-I R cells, 70–80% confluent cultures were washed twice with ice-cold PBS solution and then cultured in SFM for 24 h. During the last 10 min of culture, the cells were either treated with vehicle, 100 ng/ml IGF-I alone or with 1 μg/ml IGFBP-3 at 37 °C. Monolayers were quickly washed twice with ice-cold PBS solution and lysed with 0.4 ml lysis buffer (10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium vanadate, 0.2 mM phenylmethylsulfonyl fluoride, 0.5% NP-40, and 0.2 U/ml aprotinin). For studies assessing the effect of IGF-I on regulating its downstream targets, quiescent MCF-7/neo and MCF-7/HER2-18 cells were stimulated by 40 ng/ml IGF-I for 1, 4, 8, 16, or 24 h. After treatment, cells were lysed in RIPA buffer (0.1 mM dibasic sodium phosphate, 1.7 mM monobasic sodium phosphate, 150 mM NaCl, 1% NP-49, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.2 mM sodium vanadate, 0.2 mM phenylmethylsulfonyl fluoride, and 0.2 U/ml aprotinin).

MAKP kinase activity assay. Cells were similarly treated and lysed in the buffer which contained 20 mM Hepes (pH 7.5), 10 mM EGTA, 40 mM β-glycerophosphate, 1% Nonidet P-40, 2.5 mM Na_3VO_4, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 μg aprotinin per ml, and 20 μg/ml leupeptin per ml. Clarified protein lysates (200 μg) were subjected to immunoprecipitation in the lysis buffer at 4 °C overnight in the presence of anti-ERK2 antibody and protein A–agarose beads. Phosphorylation of myelin basic protein (MBP) was measured by incubating the beads with 40 μl kinase buffer which contained 12.5 mM Mops (morpholinepropanesulfonic acid), 12.5 mM β-glycerophosphate, 7.5 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM sodium fluoride, 0.5 mM Na_3VO_4, 10 μg MBP, 10 μCi [γ-^{32}P]ATP, and 20 mM cold ATP for 30 min at 30 °C. The reaction was stopped by boiling the samples in SDS buffer for 5 min. The samples were analyzed by 12% SDS–PAGE and gel was dried and subjected to autoradiography.

Oligodeoxynucleotide treatment. Antisense (5′-CTCCATGGTGCT CAC-3′) and sense (5′-GTGAGCACCATGGAG-3′) phosphorothioate ODNs targeting the 5′ region of the erbB-2 mRNA molecule were obtained from AlphaDNA (Montreal, QC). The lyophilized ODNs were reconstituted in sterile distilled water to 1 mM, filter-sterilized, and stored in aliquots at −20 °C as stock solutions. For subsequent experiments, the stock solutions of ODNs were diluted to give final concentration of 1 μM. Diluted ODNs were mixed with 2 μg/ml Lipofectin, and SKBR3/IGF-IR cells were exposed to the mixture for 3 h, after which the mixture-containing medium was replaced with the culture medium.

Western blots. Clarified protein lysates from each experimental condition (20–60 μg) were electrophoretically resolved on denaturing SDS–polyacrylamide gel (8–12%), transferred to nitrocellulose membranes, and probed with the following primary antibodies: anti-c-jun, anti-c-fos, anti-α-tubulin, anti-ErbB2, anti-phospho-MAPK, anti-MAPK, anti-phospoAKT, and anti-AKT; proteins were revealed by using horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies.

Immunoprecipitation. Clarified protein lysates (200–300 μg/ml) were precleaned with 25 μl protein A–agarose and then precipitated with 2 μg anti-IGF-IRβ, anti-IRS-1, anti-Shc or anti-Grb2 antibody and 25 μl protein A–agarose overnight at 4 °C. The next day, beads were collected by centrifugation and washed with lysis buffer. The samples were denatured with 25 μl of 2 × SDS–PAGE sample buffer and subjected to SDS–PAGE on a 12% gel. After separated proteins were transferred to membranes, the membranes were probed with an anti-phospho-tyrosine specific antibody, anti-Grb2 antibody, or anti-Shc antibody, followed by peroxidase-conjugated appropriate secondary antibody and visualization by ECL detection system.

Cell synchronization. To make MCF-7/neo and MCF-7/HER2-18 cells quiescent, they were seeded at a density of $1 \times 10^4/cm^2$ in the presence of serum. After 24 h the cells were washed with PBS solution and incubated in serum-free medium (SFM) (RPMI 1640 supplemented with 50 μg/ml transferrin). The cells became quiescent after 48 in SFM and medium was renewed every 24 h.

Cell growth assay. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was employed to test cell growth. We plated 10^5 MCF-7/neo and MCF-7/HER2-18 cells in 6-well plates in medium containing 10% FBS. After cells were pretreated with vehicle or with 75 μm PD98059 in serum-free condition for 3 h, cells were then incubated with or without IGF-I (40 ng/ml) for additional 72 h. The MTT assay was done in triplicate: MTT was added to a final concentration of 5 mg/ml, the reaction mixture was incubated for 3 h at 37 °C, and the absorbency was measured at 570 nm.

Results and discussion

IGF-I-induced MAPK phosphorylation and MAP kinase activity are inhibited by overexpression of ErbB2 in breast cancer cells

MCF-7/HER2-18 cells are MCF-7 human breast cancer cells engineered to overexpress ErbB2 [31]. After both MCF-7/HER2-18 and control MCF-7/neo cells were starved for 24 h in serum-free condition, the two cell lines were incubated with 100 ng/ml IGF-I for 10 min. Compared to MCF-7/neo, reduced phosphorylated ERK-1 and ERK-2 in response to IGF-I stimulation were observed in MCF-7/HER2-18 cells (Fig. 1A). MAP kinase activities induced by IGF-I stimulation were also dramatically decreased in MCF-7/HER2-18 cells (Fig. 1B).

To further study the inhibition of MAPK activation seen in ErbB2 overexpression, we used SKBR3/IGF-IR as another cell model. SKBR3/IGF-IR cells are ErbB2 overexpressing human breast cancer cells engineered to overexpress IGF-I receptor [30]. After ErbB2 antisense oligos were transfected to the SKBR3/IGF-IR cells, the ErbB2 protein level was reduced (Fig. 1C). IGF-I-induced MAPK phosphorylation was increased in SKBR3/IGF-IR cells transfected with ErbB2 antisense oligos but not in the cells transfected with ErbB2 sense oligos. The levels of MAPK protein were not affected by any of these treatments or transfections.

IGF-I-induced phosphorylation of Shc, Grb2, and Raf is also inhibited by ErbB2 overexpression in breast cancer cells

To investigate the involvement of the immediate upstream event of MAPK activation, the IGF-I-induced Shc/Grb2/Raf/MAPK pathway was examined. Interestingly, a higher baseline level of phosphorylated Shc was observed in serum-starved MCF-7/HER2-18 cells than in serum-starved control MCF-7/neo cells. However, significant increases in phosphorylation level of Shc by IGF-I stimulation were only observed in MCF-7/neo cells, and IGFBP-3 specifically blocked this action of IGF-I. In MCF-7/HER2-18 cells, IGF-I failed to increase Shc phosphorylation under the same condition (Fig. 2A, upper panel). Similar results were found in terms of Grb2 (Fig. 2A lower panel).

We next examined the activation of Raf-1 by the detection of its phosphorylation. Compared to control MCF-7/neo cells, decreased phosphorylation of Raf by IGF-I stimulation was observed in MCF-7/HER2-18 cells (Fig. 2B). This was confirmed by downregulation of ErbB2 by ErbB2 antisense in SKBR3/IGF-IR cells: reducing ErbB2 expression resulted in increases in IGF-I-induced Raf phosphorylation in this cell line (Fig. 2C).

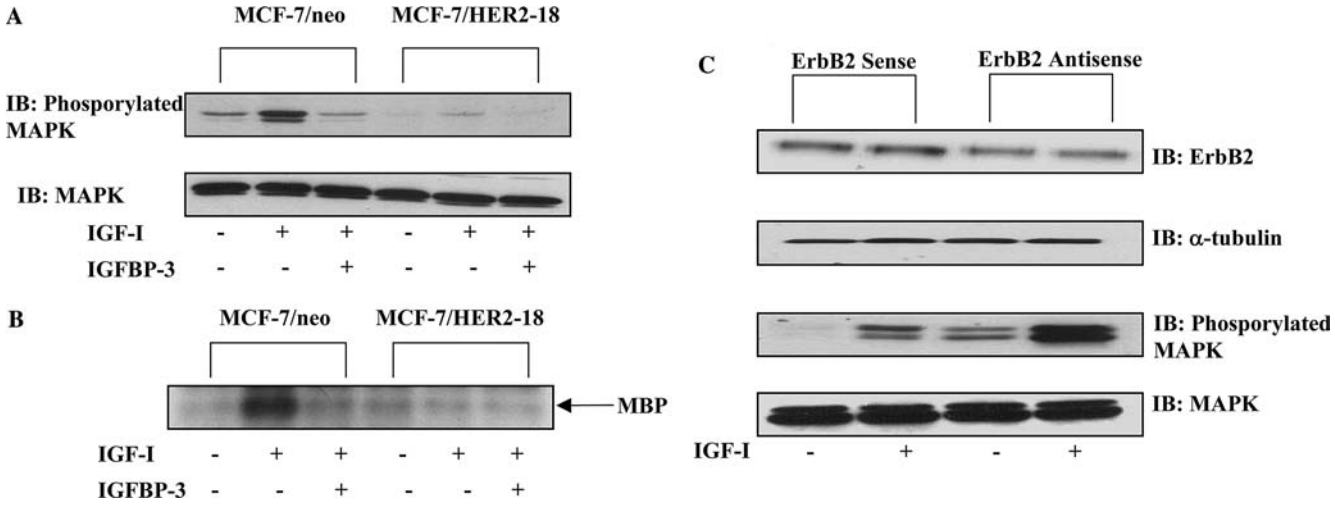

Fig. 1. IGF-I-induced MAPK phosphorylation and MAPK activity are inhibited by overexpression of ErbB2 in breast cancer cells. (A) Serum-starved MCF-7/HER2-18 and MCF-7/neo cells were stimulated with 100 ng/ml IGF-I or IGF-I plus IGFBP-3 for 10 min. MAPK phosphorylation was detected by using specific anti-phosphMAPK antibody. (B) MAPK activity was determined by immunoprecipitation with ERK2 and followed by analyzing the incorporation of ^{32}P into MBP in MCF-7/HER2-18 and MCF-7/neo cells. (C) SKBR3/IGF-IR cells were transfected with sense or antisense ErbB2 and starved in serum-free condition for 24 h. Cells were stimulated with IGF-I (100 ng/ml) for 10 min. ErbB2, α-tubulin, phospho-MAPK, and total MAPK expression were determined by Western blot.

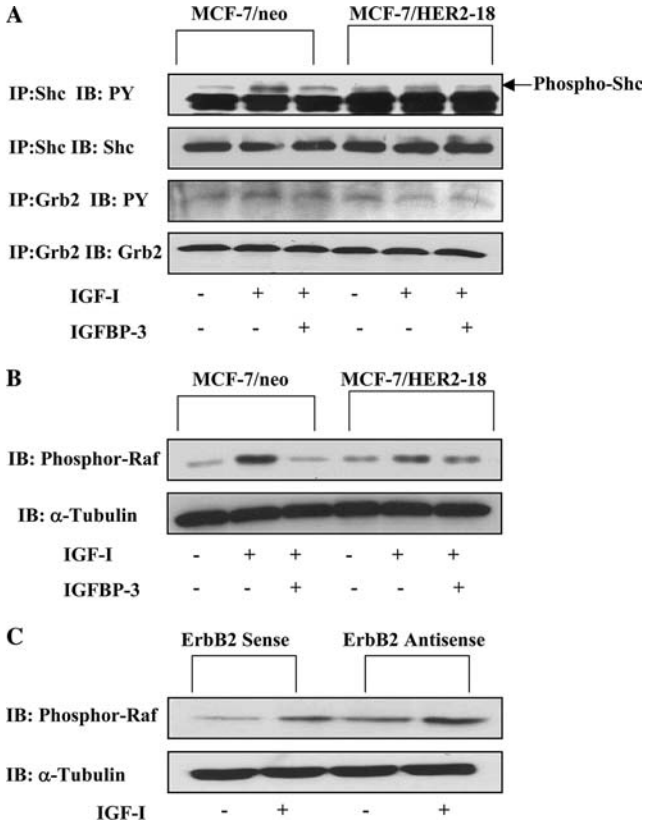

Fig. 2. IGF-I-induced phosphorylation of Shc, Grb2, and Raf is inhibited by ErbB2 overexpression in breast cancer cells. Serum-starved MCF-7/HER2-18 and MCF-7/neo cells were stimulated with 100 ng/ml IGF-I for 10 min. (A) Cell extracts were immunoprecipitated with a polyclonal anti-Shc antibody or a polyclonal anti-Grb2 followed by immunblotting with specific anti-phospho-tyrosine antibody. The same blots were reprobed with monoclonal anti-Shc or anti-Grb2. (B) Phospho-Raf and α-tubulin were determined in MCF-7/HER2-18 and MCF-7/neo cells by Western blot. (C) SKBR3/IGF-IR cells were transfected with sense or antisense ErbB2 and starved in serum-free condition for 24 h. Cells were stimulated with IGF-I (100 ng/ml) for 10 min. Phospho-Raf and α-tubulin were determined by Western blot.

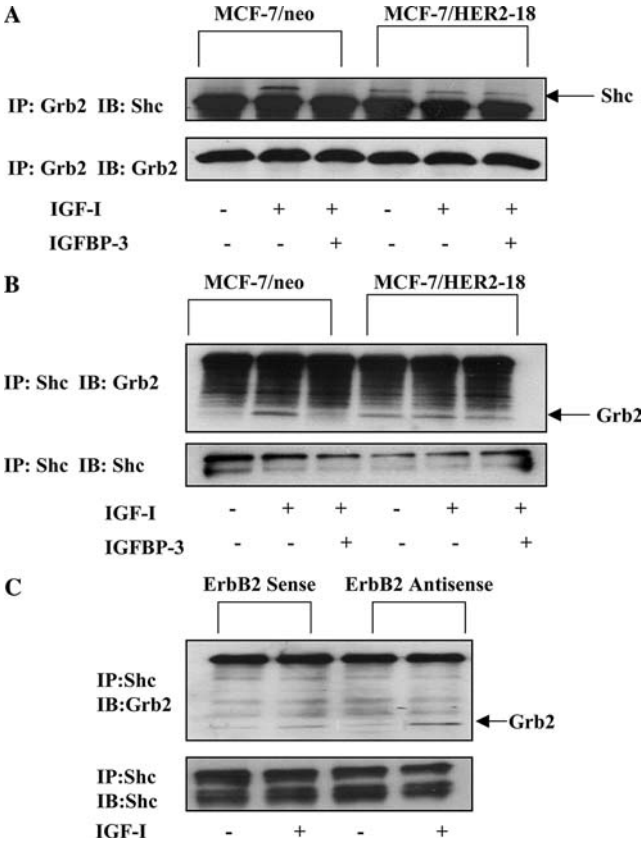

Fig. 3. Inhibition of Shc phosphorylation by overexpression of ErbB2 leads to decreased association of Grb2 with Shc. (A) Serum-starved MCF-7/HER2-18 and MCF-7/neo cells were stimulated with 100 ng/ml IGF-I for 10 min. Cell extracts were immunoprecipitated with a polyclonal anti-Grb2 followed by immunblotting with monoclonal anti-Shc. (B) Same cell extracts were immunoprecipitated with a polyclonal anti-Shc antibody followed by immunblotting with monoclonal anti-Grb2 in MCF-7/HER2-18 and MCF-7/neo cells. (C) SKBR3/IGF-IR cells were transfected with sense or antisense ErbB2 and starved in serum-free condition for 24 h. Cells were stimulated with IGF-I (100 ng/ml) for 10 min. Cell extracts were immunoprecipitated with a polyclonal anti-Shc antibody followed by immunblotting with monoclonal anti-Grb2.

Inhibition of IGF-I-induced Shc phosphorylation by ErbB2 overexpression leads to decreased association of Grb2 with Shc in human breast cancer cells

Following growth factor stimulation, tyrosine-phophorylated Shc is associated with Grb2/Sos complex and activates p21/ Ras, then leading to activation of Raf and MAPK [22,23]. Therefore, we also analyzed the effect of IGF-I stimulation on the Shc and Grb2 association in MCF-7/neo and MCF-7/HER2-18. Anti-Grb2 immunoprecipitates were analyzed by immunoblotting with anti-Shc antibody. Consistent with Shc phosphorylation results, increasing ErbB2 expression in MCF-7/HER2-18 cells resulted in increased baseline of Shc–Grb2 association in serum-starved conditions. Consistently, IGF-I-induced Shc–Grb2 association and IGFBP-3 completely blocked this effect in MCF-7/neo cells, but failed to increase Shc–Grb2 association in MCF-7/HER2-18 cells (Fig. 3A). These data were further confirmed by immunoblotting with anti-Grb2 after anti-Shc immunoprecipitation (Fig. 3B).

To investigate if increased IGF-I-induced MAPK activation by ErbB2 antisense is through modulation of Shc–Grb2 association, anti-Shc immunoprecipitates were analyzed by immunoblotting with anti-Grb2 in SKBR/IGF-IR cells. As shown in Fig. 3C, the baseline Shc–Grb2 association was significantly decreased by downregulation of ErbB2 expression in serum-starved SKBR3/IGF-IR cells which were transfected with ErbB2 antisense oligos. Increased Shc–Grb2 association on IGF-I stimulation was observed in the cells transfected with ErbB2 antisense oligos, but not in the cells transfected with ErbB2 sense oligos. These data were confirmed by immunoblotting with anti-Shc after anti-Grb2 immunoprecipitation (data not shown).

Overexpression of ErbB2 does not affect IGF-I-induced IGF-I receptor, IRS-1, and PKB/AKT phosphorylation

We next studied the effects of ErbB2 overexpression on the phosphorylation of IGF-I receptor and its direct downstream substrate IRS-1 by IGF-I stimulation. In contrast to effects of ErbB2 overexpression on IGF-I-induced MAPK activation, overexpression of ErbB2 in MCF-7 cell changed neither IGF-I-induced phosphorylation of IGF-I receptor nor phosphorylation of IRS-1 (Figs. 4A and B). IGF-I-induced AKT phosphorylation, a component in the PI3K pathway (Fig. 4C), also remains the same in MCF-7/HER2-18 and MCF-7 neo cells. Reduction of ErbB2 expression in ErbB2 overexpressing SKBR3/IGF-IR cells by using ErbB2 antisense did not have any effect on IGF-I-induced activation of IGF-IR, IRS-1, and AKT (data not shown).

IGF-I-induced c-jun and c-fos expression, the downstream targets of MAPK pathway, is inhibited in ErbB2 overexpressing MCF-7 cells

To determine if the inhibition of IGF-I-induced MAPK activity in ErbB2 overexpressing cell line also has an effect on its nuclear targets, c-jun and c-fos protein expression was analyzed. To make MCF-7/HER2-18 and MCF-7/neo cells quiescent, they were cultured in SFM supplemented with 50 μg/ml transferrin for 48 h. Fig. 5 shows that IGF-I stimulation increased in c-jun and c-fos protein level in a time-dependent fashion in the control MCF-7/neo-22 cell line. However, overexpressing ErbB2 in this cell line resulted in a significantly reduced IGF-I-induced protein expression of c-jun and c-fos. These results provide evidence that overexpression of ErbB2 antagonizes IGF-I-induced activation of MAPK pathway.

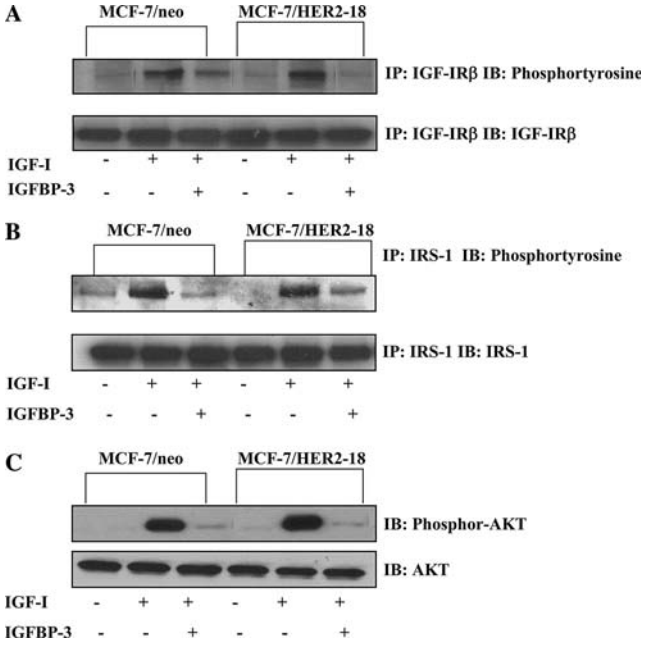

Fig. 4. Overexpression of ErbB2 does not affect IGF-I-induced IGF-I receptor, IRS-1, or PKB/AKT phosphorylation. Serum-starved MCF-7/HER2-18 and MCF-7/neo cells were stimulated with 100 ng/ml IGF-I for 10 min. (A) IGF-I receptor phosphorylation of MCF-7/neo cells and MCF-7/HER2-18 cells in the presence or absence of IGF-I and IGFBP-3. (B) IRS-1 phosphorylation of MCF-7/HER2-18 and MCF-7/neo cells in the presence or absence of IGF-I and IGFBP-3. (C) PKB/AKT phosphorylation of MCF-7/neo cells and MCF-7/HER2-18 cells in the presence or absence of IGF-I.

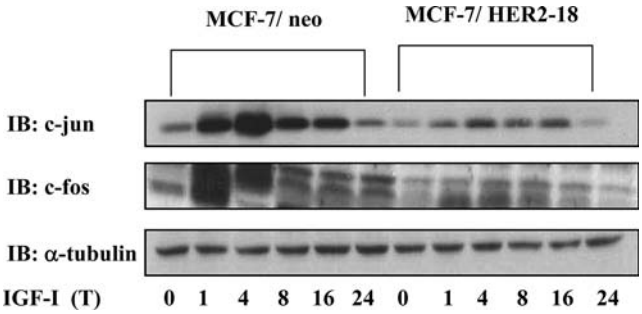

Fig. 5. Overexpression of ErbB2 inhibits IGF-I-induced changes downstream of MAPK pathway. IGF-I-induced c-jun and c-fos expression is inhibited in ErbB2 overexpressing MCF-7 cells. When 50% confluent, MCF-7/HER2-18 and MCF-7/neo cells were synchronized in SFM for 48 h. IGF-I (40 ng/ml) was added in different time periods as indicated in the figure. Cell lysates were collected and c-jun, c-fos, and α-tubulin were determined by Western blot.

Overexpression of ErbB2 decreases IGF-I-stimulated MAPK pathway-dependent cell growth in MCF-7 cells

Fig. 6 shows that the MAPK inhibitor PD98059 at 75 μm abolishes 82.6% of IGF-I-stimulated growth of MCF-7/neocells, but only 41.2% of IGF-I-stimulated growth of MCF-7/HER2-18 cells. This is consistent with the effects that we described of overexpression of ErbB2 on IGF-I-stimulated MAPK activity.

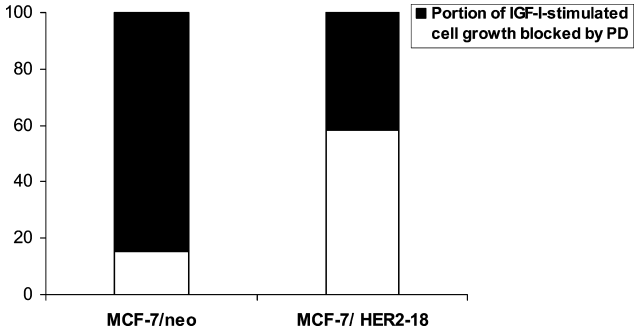

Fig. 6. Overexpression of ErbB2 decreases IGF-I-stimulated MAPK pathway-dependent cell growth in MCF-7 cells. 1×10^5 MCF-7/neo and MCF-7/HER2-18 cells were plated in 6-well plates for 24 h attachment. After cells were pretreated with vehicle or with 75 μm PD98059 in serum-free condition for 3 h, cells were then incubated with or without IGF-I (40 ng/ml) for further 72 h. MTT was used to examine cell proliferation. Experiments were repeated in triplicate.

Overexpression of ErbB2 has received attention because it indicates poor prognosis in the breast cancer patients and predicts response to trastuzumab treatment [2,9,25]. Recent studies have shown that ErbB2-overexpressing cancer cells have the distinct mitogenic signal transduction pathway relative to cancer cells without ErbB2-overexpression [27–29]. Our data provide the first evidence that overexpression of ErbB2 interferes with IGF-I-induced Shc–MAPK mitogenic signaling pathway in breast cancer cells, indicating that ErbB2-overexpression not only influences its own downstream signaling pathway, but also can interfere with the actions of other growth factors. This is consistent with the view that cellular control system consists of interacting signaling network rather than independent linear signaling pathways [32,33].

Although both IRS-1 and Shc act as docking molecules linking the IGF-IR to downstream signaling pathways, tyrosine phosphorylation sites in IRS-1 provide binding motifs for several distinct SH2 domain-containing proteins including the 85 kDa regulatory subunit of PI3K, the tyrosine-specific phosphatase Syp, and the Grb2 [34]; while tyrosine-phosphorylated Shc proteins upon IGF-IR activation appear to bind only to the SH2 domain of Grb2 [35,36]. The Shc proteins mainly interact with the Tyr-950 residue of the IGF-IR, whereas the interaction between the IGF-IR and IRS-1 requires at least two major determinants on the receptor: the Tyr-950 residue and the major tyrosine autophosphorylation sites of the receptor [37]. Phosphorylated IRS-1 and Shc upon IGF-IR activation further competing for a limited cellular pool of Grb2 has been observed by expression of IRS-1 or Shc cDNA [38]. Our results show that overexpression of ErbB2 increases the baseline level of Shc phosphorylation and association of Shc with Grb2, but reduces IGF-I-induced Shc phosphorylation, IGF-I-induced Shc association with Grb2 and also reduces IGF-I-induced Ras/Raf/MAPK signaling. It is possible that induction of Shc phosphorylation and its association with Grb2 by ErbB2 overexpression may result in less free Shc and Grb2 available upon IGF-IR activation in these cells. In contrast, neither baseline IRS-I phosphorylation nor IGF-I-induced IRS-1 phosphorylation is influenced by levels of ErbB2 expression.

Recently, we established that IGF-I receptor signaling can attenuate trastuzumab (a specific antibody for blocking ErB2 mediated signaling)-induced growth inhibition in ErbB2 overexpressing breast cancer cells, mainly through activation of PI3K pathway [39]. In this study, we demonstrate an additional unexpected interaction between IGF-I signaling and ErbB2 overexpression: the attenuation of IGF-I-induced MAPK signaling in the presence of ErbB2 overexpression.

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