

# ErbB2 Is Necessary for Induction of Carcinoma Cell Invasion by ErbB Family Receptor Tyrosine Kinases

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**Abstract.** The epidermal growth factor (EGF) family of tyrosine kinase receptors (ErbB1, -2, -3, and -4) and their ligands are involved in cell differentiation, proliferation, migration, and carcinogenesis. However, it has proven difficult to link a given ErbB receptor to a specific biological process since most cells express multiple ErbB members that heterodimerize, leading to receptor cross-activation. In this study, we utilize carcinoma cells depleted of ErbB2, but not other ErbB receptor members, to specifically examine the role of ErbB2 in carcinoma cell migration and invasion. Cells stimulated with EGF-related peptides show increased invasion of the extracellular matrix, whereas cells devoid of functional ErbB2 receptors do not. ErbB2 facilitates cell in-

vasion through extracellular regulated kinase (ERK) activation and coupling of the adaptor proteins, p130CAS and c-CrkII, which regulate the actin-myosin cytoskeleton of migratory cells. Overexpression of ErbB2 in cells devoid of other ErbB receptor members is sufficient to promote ERK activation and CAS/Crk coupling, leading to cell migration. Thus, ErbB2 serves as a critical component that couples ErbB receptor tyrosine kinases to the migration/invasion machinery of carcinoma cells.

**Key words:** epidermal growth factor • ERK • cell migration • adaptor proteins • signal transduction

## Introduction

The ErbB family of receptors (ErbB1, -2, -3, and -4) consists of a cysteine-rich extracellular domain, a single transmembrane spanning region, and a cytoplasmic tail containing tyrosine kinase activity and several tyrosine residues that are phosphorylated upon ligand binding (for reviews see Hynes and Stern, 1994; Alroy and Yarden, 1997; Riese and Stern, 1998). This family of receptors and their ligands have been implicated in the genesis of a number of human carcinomas (Slamon et al., 1989; Dougall et al., 1994; Hynes and Stern, 1994; Gilbertson et al., 1998). ErbB2 (HER2/neu) is most notable since amplification of the ErbB2 gene occurs in a variety of tumors, including 20–30% of breast cancer patients (Hynes and Stern, 1994). In fact, ErbB2 amplification is used as an independent prognostic indicator of patient survival and is correlated with a number of adverse prognostic factors in breast cancer, including increased occurrence of metastasis and micrometastatic bone marrow disease (Slamon et al., 1987; Pantel et al., 1993).

The ligands for ErbB receptors can be divided into three separate groups of peptides that activate distinct sets of in-

dividual receptors (Beerli and Hynes, 1996; Tzahar et al., 1996; Pinkas-Kramarski et al., 1998; Riese and Stern, 1998). The first group comprises epidermal growth factor (EGF)<sup>1</sup>, transforming growth factor alpha (TGF- $\alpha$ ), and amphiregulin, which bind to ErbB1, but not other ErbB receptor members. The neuregulins (NRG, also known as neu differentiation factor, NDF, and heregulin), which specifically bind to ErbB3 and ErbB4, represent the second group. The third group consists of  $\beta$ -cellulin (BTC), heparin-binding EGF (HB-EGF), and epiregulin, which bind to ErbB1 and ErbB4. Importantly, ErbB2 is an orphan receptor with no characterized ligand, but can be activated by homodimerization or in trans by heterodimerization with another ErbB family receptor (Graus-Porta et al., 1995, 1997; Karunakaran et al., 1996). In fact, ErbB2 has recently been shown to be the preferred heterodimerization partner of all activated ErbB family members (Graus-Porta et al., 1997). Moreover, there are specific

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<sup>1</sup>Abbreviations used in this paper:  $\beta$ -gal,  $\beta$ -galactosidase; BTC,  $\beta$ -cellulin; CAS, crk-associated substrate; ECM, extracellular matrix; ERK, extracellular regulated kinase; FBM, fibroblast basal media; MAP, mitogen-activated protein; MBP, myelin basic protein; MEK, MAP kinase kinase; NDF, neu differentiation factor; p130CAS, Crk-associated substrate; SH2, src-homology 2 domain.

heterodimer combinations formed between ErbB2 and ErbB receptors in response to EGF family peptides (Tzahar et al., 1996; Pinkas-Kramarski et al., 1998). For example, NDF promotes ErbB3 and ErbB4 heterodimerization with ErbB2, but not with ErbB1. BTC promotes ErbB4 as well as ErbB1 dimers with ErbB2, but not with ErbB3. EGF induces primarily ErbB1/ErbB2 heterodimers (Riese and Stern, 1998).

ErbB2 dimerization with ErbB family members and its transactivation may allow for the recruitment of distinct effector molecules leading to diversification of signal transduction responses. For example, ErbB2, but not other ErbB members, couple to CHK (Csk-homologous kinase; Zrihan-Licht et al., 1997). On the other hand, the protooncogene Cbl forms a complex only with ErbB1 (Levkowitz et al., 1996). The specific interaction of these effector proteins with ErbB receptors likely result from differences in tyrosine residues phosphorylated in trans by distinct ErbB receptor pairs (Olayioye et al., 1998). An important component of ErbB2-containing heterodimers is sustained activation of various biochemical signals, including the mitogen-activated protein (MAP) kinases, ERK1 and ERK2 (extracellular regulated kinase; Graus-Porta et al., 1995, 1997; Karunakaran et al., 1996; Pinkas-Kramarski et al., 1998). This signaling event may contribute to the increased metastatic potential associated with ErbB2-containing tumors, as ERK can regulate both actin-myosin motor activity and proliferation of migratory cells (Klemke et al., 1997; Nguyen et al., 1999).

Recent evidence indicates that ErbB2 amplification in cells leads to kinase activation and cell proliferation (Di Fiore et al., 1987b; Tzahar et al., 1996; Pinkas-Kramarski et al., 1998; Riese and Stern, 1998). These findings suggest that aberrant activation of biochemical signals as the result of ErbB2 overexpression contributes to the development of metastatic breast cancer. However, the molecular signaling mechanisms responsible for ErbB2-induced malignancy are poorly understood. In fact, it has been difficult to determine the specific role of ErbB2 signals in a given biological response as this receptor is widely expressed and most always in the context of other ErbB members. In this report, we used cells functionally devoid of ErbB2 to directly study its role in cell migration and invasion. We provide evidence that ErbB2 activation by ErbB family receptors and their ligands is a fundamental event necessary for carcinoma cell invasion of the extracellular matrix (ECM). Furthermore, we show that cell invasion induced by ErbB2 is mediated by ERK activation and the coupling of the adaptor proteins p130CAS (Crk-associated substrate) and c-CrkII.

## Materials and Methods

### Antibodies and Reagents

Rabbit antibodies to the ErbB1, -2, -3, -4, p130CAS, and ERK2 were from Santa Cruz Biotechnology, Inc. Phospho-ERK-specific antibody was from Promega. Monoclonal antiphosphotyrosine antibody 4G10, rabbit anti-MEK, and rat tail collagen I were from Upstate Biotechnology, Inc. Anti-Crk and CAS antibodies were from Transduction Laboratories, Inc. Functional blocking mAbs to the  $\beta 1$  integrin subunit (P4C10) and  $\alpha \nu \beta 5$  (P1F6) have been described (Leavesley et al., 1992). NDF was from NeoMarkers. EGF was from Genzyme Corp. BTC was from Sigma Chemical

Co. Insulin was from Boehringer Mannheim Corp. PD98059 (2-[2'-amino-3'-methoxyphenyl]-oxanaphthalen-4-one) was from Calbiochem-Novabiochem Corp.

### Expression Vectors and Constructs

The pEBC expression plasmid containing glutathione S transferase (gst)-tagged or untagged wild-type CAS, or CAS with an in-frame deletion of its substrate domain (CAS-SD, aa 213-514) has been described previously (Mayer et al., 1995). pUCCAGGS vector containing myc-tagged wild-type or src-homology 2 domain (SH2) mutated c-CrkII (arginine 38 to valine), and vectors encoding full-length human ErbB receptors and mutationally activated MEK has been described (Matsuda et al., 1993; Graus-Porta et al., 1995; Klemke et al., 1997). In some cases, ErbB receptor cDNAs were subcloned into pcDNA3 using standard molecular cloning protocols.

### Haptotaxis Migration Assays

The breast adenocarcinoma cell lines MCF7, T47D, and MDA-MB-435, and derivatives without cell surface ErbB2 receptors have been described (Graus-Porta et al., 1995; Karunakaran et al., 1996; Pinkas-Kramarski et al., 1998). Cell adhesion and migration experiments were performed as described previously (Klemke et al., 1997, 1998). In brief, migration experiments were performed using modified Boyden chambers (tissue culture treated, 6.5-mm diam, 10- $\mu$ m thickness, 8- $\mu$ m pores, from either Transwell, Costar Corp., or the QCM migration kit from Chemicon International, Inc.). The polycarbonate membranes were coated on the underside of the membrane with 10  $\mu$ g/ml vitronectin or collagen type I in PBS for 2 h at 37°C, rinsed once with PBS, and were then placed into the lower chamber containing migration buffer (fibroblast basal medium; FBM with 0.5% BSA; Clonetics). Serum-starved cells were removed from culture dishes with HBSS containing 5 mM EDTA and 25 mM HEPES, pH 7.2, and 0.01% trypsin, washed twice with migration buffer, and then suspended in FBM 0.5% BSA (10<sup>6</sup> cells/ml). 100,000–200,000 cells were then added to the top of each migration chamber and allowed to migrate to the underside of the top chamber for 2 h in the presence or absence of various concentrations of either insulin, EGF, NDF, or BTC which had been added to the lower chamber. In some cases, cells were allowed to migrate in the presence or absence of function blocking antiintegrin antibodies (25  $\mu$ g/ml). The nonmigratory cells on the upper membrane surface were removed with a cotton swab and the migratory cells attached to the bottom surface of the membrane stained with 0.1% crystal violet in 0.1 M borate, pH 9.0, and 2% ethanol for 20 min at room temperature. The number of migratory cells per membrane were either counted (with an inverted microscope using a 20 $\times$  objective) or the stain eluted with 10% acetic acid and the absorbance determined at 600 nm, and migration enumerated from a standard curve. Each determination represents the average of three individual experiments and error bars represent the SEM. All values have had background subtracted, which represents cell migration on membranes coated on the bottom with BSA (1%). In control experiments, cell migration on BSA was <0.01% of the total cell population.

### Time-lapse Cell Migration and Invasion Assays

MCF7 or MCF7-5R cells devoid of cell surface ErbB2 receptors were seeded onto glass coverslips coated with 3.5  $\mu$ g/ml collagen type I and cultured overnight in DME containing 10% FBS. Cells were then serum-starved and coverslips placed into Attofluor cell chambers (Molecular Probes, Inc.), overlaid with light mineral oil, and placed into a stage heater (20/20 Technologies) on a Zeiss Axiovert 100TV microscope equipped with a BioRad 1024 confocal microscope. Cells were examined for morphological changes and cell movement in the presence or absence of NDF (50 ng/ml) by collecting time-lapse images every 60 s using a Zeiss 32 $\times$  Achromat lens and LaserSharp software (BioRad).

Cell invasion experiments were performed with 8- $\mu$ m porous invasion chambers coated with Matrigel (Becton Dickinson) according to the manufacturer's recommendation with minor modifications. Serum-starved cells (75,000 in 100  $\mu$ l of FBM/0.5% BSA) were placed into the upper chamber. The lower chamber contained 500  $\mu$ l of FBM/0.5% BSA with or without either 50 ng/ml EGF, NDF, BTC, or 10% FBS. Cells were allowed to invade through the Matrigel membrane for 24 h. Noninvasive cells were removed from the upper membrane and the invasive cells on the underneath stained and counted with an inverted microscope using the 10 $\times$  objective as described for the above migration experiments.

## Determination of ERK Kinase Activity in Migratory Cells

Cells were exposed to NDF (50 ng/ml) for various times and were then examined for kinase activity using either an immunocomplex kinase assay and myelin basic protein (MBP) as a substrate, as previously described (Klemke et al., 1997), or by immunoblotting with an antibody to the phosphorylated activated form of this enzyme according to the manufacturer's recommendation (Promega). Blots were stripped and reprobed with an antibody to ERK2 to confirm that equal amounts of enzyme was present. In some cases, cells were pretreated with PD98059 (50  $\mu$ M) for 60 min before being stimulated with NDF (50 ng/ml). In other experiments, cells were treated with NDF for 30 min before the addition of PD98059. Cells were then examined for both kinase activity and cell migration as described above.  $^{32}$ P incorporation into MBP was measured by excising protein bands from the gel and scintillation counting. Fold increase in ERK kinase activity represents changes in  $^{32}$ P incorporation into MBP induced by NDF, relative to basal kinase activity present in serum-starved non-treated cells.

## Transfection of MCF7 and CHO Cells, and Determination of CAS/Crk Complexes and ErbB Protein Expression in Migratory Cells

Transient transfection of MCF7 and MCF7-5R cells and determination of cell migration were performed with Transwell migration chambers as previously described (Klemke et al., 1997, 1998). In brief, MCF7 cells ( $1.5 \times 10^6$  cells/10-cm plate) were cotransfected with lipofectamine (40  $\mu$ l/10-cm plate; GibcoBRL) and 7  $\mu$ g of the expression vector containing the cDNA encoding wild-type Crk or Crk with a mutated SH2 domain (Crk-SH2) or wild-type CAS, or CAS without its substrate domain (CAS-SD), along with 2  $\mu$ g of a reporter construct encoding  $\beta$ -galactosidase (pCMV- $\beta$ -gal). CAS-SD and Crk-SH2 have been described and serve as dominant negative proteins that prevent the assembly of CAS/Crk complexes in cells (Klemke et al., 1998). In some cases, cells were transfected with mutationally activated MEK (Klemke et al., 1997) as described above. Mock cells were transfected with the appropriate amount of the empty expression vectors. CHO cells were transfected with 3  $\mu$ g of the appropriate vector using 50  $\mu$ l of effectine (Qiagen). In some cases, cells were transfected as described above with vectors encoding human wild-type ErbB1, -2, -3, or -4 and the reporter construct, and examined for expression of ErbB proteins as previously described (Graus-Porta et al., 1996). Cells were allowed to incorporate the cDNA constructs for 16 h, washed, and were then incubated for 40 h, which provides optimal transient expression. Cells were then prepared for migration as outlined above or analyzed for expression of specific proteins and CAS/Crk complexes as previously described (Klemke et al., 1997, 1998). Cells cotransfected with  $\beta$ -gal were developed using X-gal as a substrate according to the manufacturer's recommendation (Promega).

Importantly, controls for transfection efficiency and cell adhesion to ECM proteins were performed as previously described (Klemke et al., 1998). In brief, an aliquot of cells from the migration experiments above were allowed to attach to culture dishes coated with purified ECM proteins. The dishes were washed and adherent cells transfected with the  $\beta$ -gal reporter gene were detected using X-gal as a substrate according to the manufacturer's recommendation (Promega). In typical transfection experiments, we obtain 50–70% efficiency of MCF7 and 60–70% of CHO cells as determined by counting the number of  $\beta$ -gal positive cells relative to the total number of cells attached per field (200 $\times$ ). It is important to note that in an individual experiment, transfection efficiency varies <10%. The efficiency and adhesion control assures that changes observed in cell migration is not simply the result of differences in transfection efficiency, expression of the  $\beta$ -gal reporter gene, or differences in the ability of transfected cells to attach to the ECM (Klemke et al., 1997, 1998).

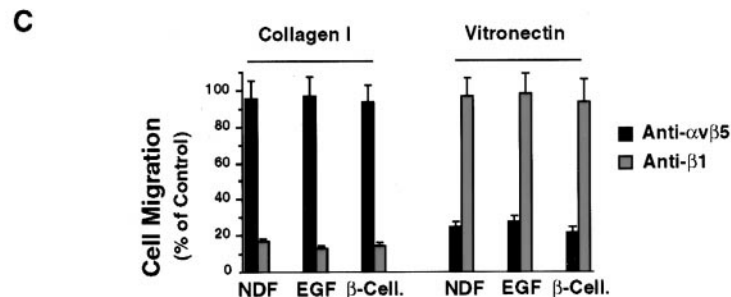
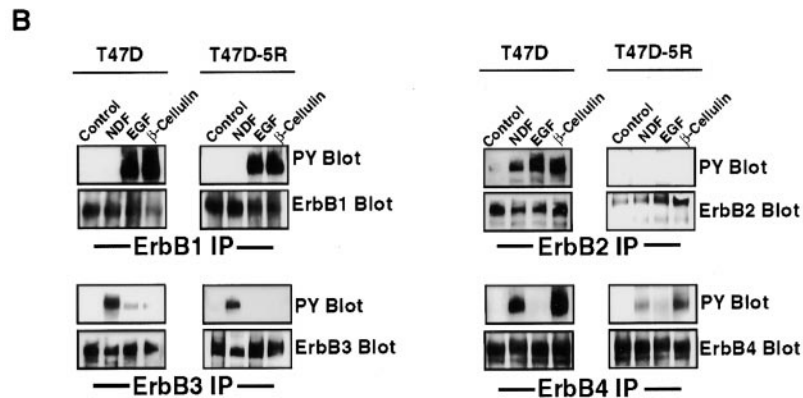
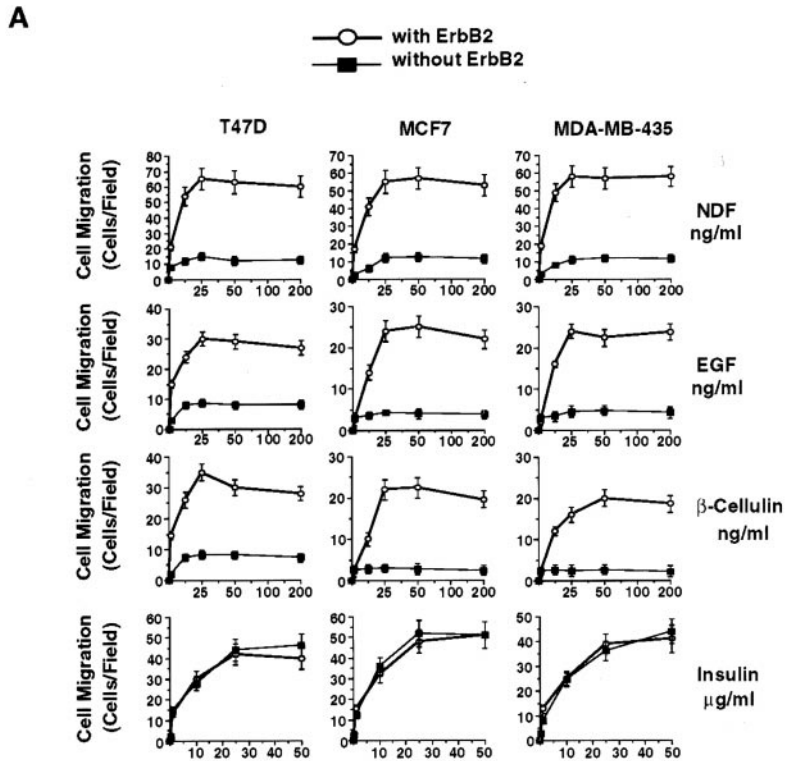
## Results

### ErbB2 Activation Is Necessary for Breast Carcinoma Cell Migration Induced by EGF Family Peptides

To determine if ErbB2 plays a direct role in cell migration on ECM proteins, we used a novel cell model system that employs mammary adenocarcinoma cells (MCF7, T47D,

and MDA-MB-435) devoid of cell surface ErbB2 receptors (referred to as 5R). These cells were obtained by retroviral infection with a vector that directs the expression of an ErbB2-specific single-chain antibody genetically engineered to prevent ErbB2 transit through the ER. This results in loss of cell surface expression and functional inactivation of the receptor (Beerli et al., 1994; Graus-Porta et al., 1995). Importantly, since ErbB1, -3, and -4 receptor levels, as well as basal cell proliferation rate, remain unchanged in these cells; this allowed us to examine the specific role of ErbB2 in cell migration (Graus-Porta et al., 1995, 1997). To investigate whether ErbB2 was necessary for haptotaxis cell migration, cells with and without ErbB2 receptors were serum-starved and were then examined for their ability to migrate on the ECM proteins collagen type I and vitronectin in the presence or absence of EGF, NDF, or BTC. MCF7-5R, T47D-5R, and MDA-MB-435-5R cells showed a low level of basal migration on collagen and vitronectin that is comparable to control cells expressing endogenous levels of ErbB2 receptors, indicating that loss of ErbB2 function did not impact the general adhesive or basal migratory properties of these cells (data not shown). However, exposure of wild-type cells to NDF promoted ErbB2 tyrosine kinase activation (Fig. 1 B), resulting in a significant (greater than tenfold) increase in cell migration on collagen (Fig. 1 A) and vitronectin (data not shown). As expected, NDF-induced cell migration was associated with strong activation of ErbB3 and ErbB4, but not ErbB1 receptors (Fig. 1 B). Time-lapse microscopy revealed that five minutes after exposure to NDF, cells showed increased membrane spreading and surface ruffling (Fig. 2). By 30 min, cells showed reduced cell-cell contacts leading to disruption of the epithelial cell colonies and random cell movement that was clearly visible by 60–90 min (Fig. 2). In contrast, serum-starved control cells not exposed to NDF did not migrate and showed little membrane ruffling. Exposure of wild-type cells to EGF and BTC also induced cell migration, although not as strongly as NDF. This event was also associated with ErbB2 activation (Fig. 1, A and B). As expected, exposure of cells to EGF specifically activated ErbB1, whereas BTC promoted only ErbB1 and ErbB4 activation. Significantly, cells devoid of functional ErbB2 receptors failed to migrate in response to EGF, NDF, or BTC (Fig. 1 A). These cells not only showed decreased cell spreading and ruffling in response to NDF, but retained their cell-cell contacts and remained in discrete epithelial colonies. Similar findings were obtained with cells exposed to EGF and BTC (data not shown). As expected, reexpression of ErbB2 in these cells restored their ability to migrate in response to EGF-like peptides (data not shown).

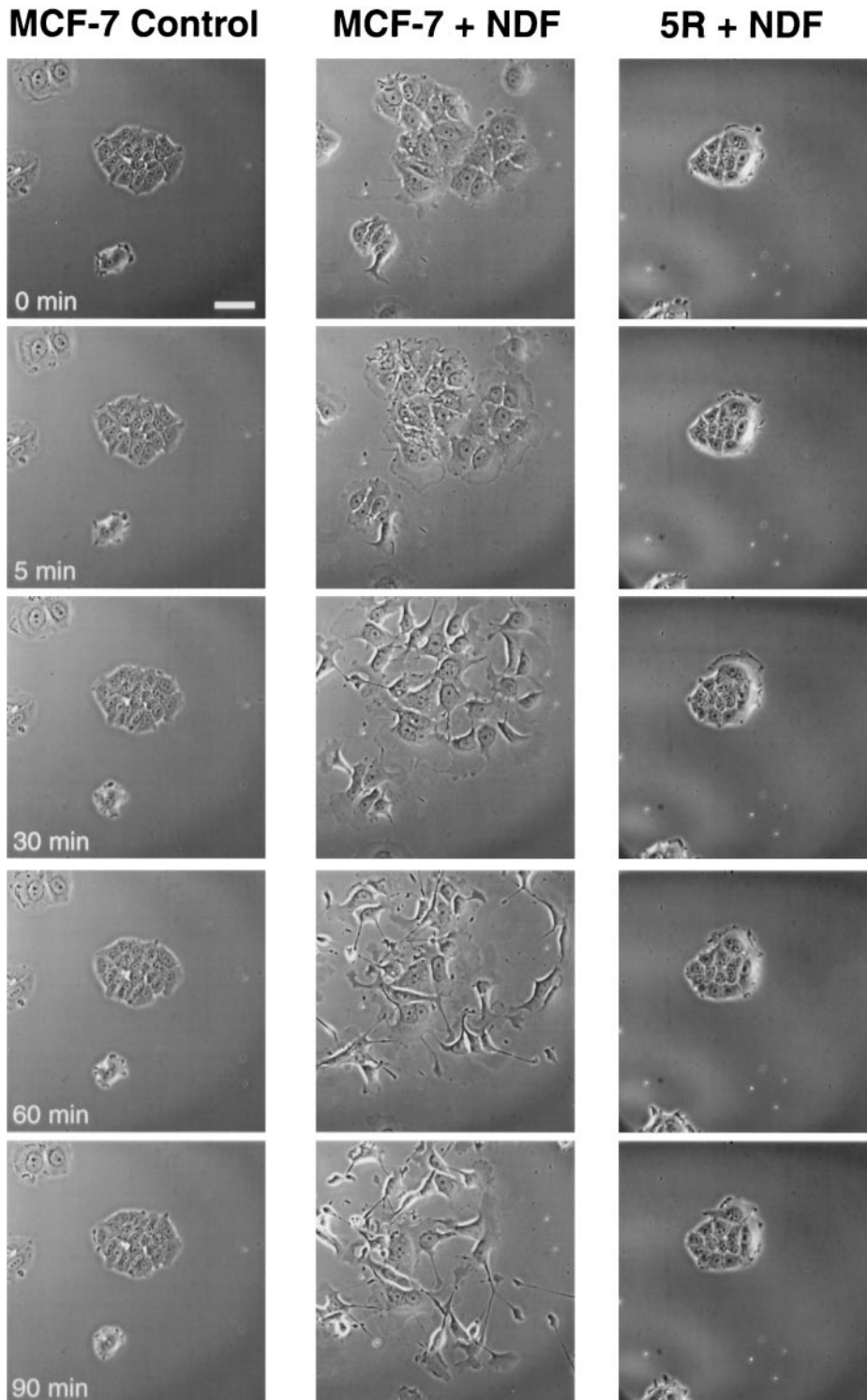
Interestingly, whereas the loss of ErbB2 function dramatically impaired the process of cell movement, it did not abolish ErbB receptor activation in response to EGF family peptides. In this case, EGF and BTC-induced ErbB1 activation was reduced by  $\sim$ 30–40%, whereas NDF-induced ErbB3 and -4 activation was decreased by  $\sim$ 50% (Fig. 1 B). Thus, the loss of cell migration in these cells did not result from a complete loss of ErbB receptor activity, but most likely resulted from the ability of ErbB receptor members to transactivate ErbB2 and engage its downstream signals that specifically impact the migration ma-



**Figure 1.** Carcinoma cell migration induced by EGF family peptides requires functional ErbB2 receptors. **A**, Mammary adenocarcinoma cell lines (MCF7, T47D, and MDA-MB-435) with or without cell surface ErbB2 receptors were serum-starved and allowed to migrate on collagen-coated Transwell membranes for 2 h in the presence of various concentrations of NDF, EGF, BTC, or insulin as described in Materials and Methods. Each point represents the mean  $\pm$  SEM of at least three independent experiments. The background migration in cells with or without ErbB2 receptors was the same and has been subtracted. **B**, ErbB family receptors immunoprecipitated from T47D cells with or without cell surface ErbB2 (5R) after exposure to 50 ng/ml of NDF, EGF, BTC, or buffer only (control) for 10 min. Immunoprecipitates (IP) were examined by immunoblotting for the presence of ErbB receptors, as well as changes in phosphotyrosine (PY) as described in Materials and Methods. **C**, T47D cells were allowed to migrate on collagen I or vitronectin-coated Transwell membranes for 2 h in the presence of 50 ng/ml NDF, EGF, BTC ( $\beta$ -cell.) with or without 25  $\mu$ g/ml of function blocking antibodies to  $\beta$ 1 (P4C10) or  $\alpha$ v $\beta$ 5 (P1F6) integrins. Data are expressed as percent of ligand-induced migration in the absence of antibodies and represents the mean  $\pm$  SEM of at least three independent experiments.

chinery of cells. Importantly, the loss of cell surface ErbB2 expression did not impair the general migration machinery of these cells since insulin induced significant migration on collagen (Fig. 1 A) and vitronectin (data not shown), which was comparable to wild-type cells with ErbB2 at the plasma membrane. Cell migration induced by EGF family peptides and insulin (data not shown) were mediated by

integrin adhesion receptors, as function blocking antibodies directed to  $\alpha$ v $\beta$ 5 and  $\beta$ 1 integrins prevented T47D cell migration on vitronectin and collagen substrates, respectively (Fig. 1 C). Together, these findings indicate that cell surface expression and activation of ErbB2 is necessary for induction of carcinoma cell migration on the ECM in response to EGF-related ligands.

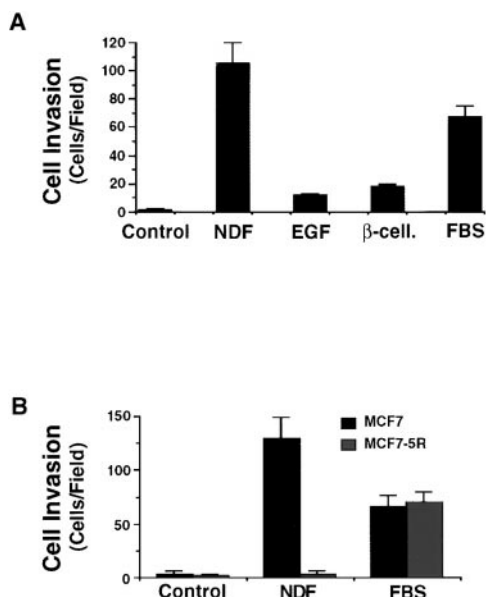


*Figure 2.* Induction of carcinoma cell migration by NDF is associated with loss of cell-cell contacts, epithelial cell scattering, and random migration. Serum-starved MCF7 cells with or without cell surface ErbB2 receptors (5R) were cultured on collagen-coated coverslips before being stimulated with NDF (50 ng/ml) or buffer only (control) and examined by time-lapse phase microscopy for changes in cell morphology and migration at the indicated times. Bar, 50  $\mu$ m.

### ***ErbB2 Activation Is Necessary for Breast Carcinoma Cell Invasion of the ECM***

Cell metastasis not only requires cell movement, but also the ability to degrade the basement membrane and invade the ECM. To investigate whether ErbB2 played a role in this event, MCF7 cells were examined for their ability to invade the basement membrane-like material Matrigel. Exposure of cells to NDF significantly increased their abil-

ity to invade this substrate (Fig. 3 A). Cells exposed to EGF and BTC also showed a slight increase in invasion (Fig. 3 A). Importantly, cells devoid of cell surface ErbB2 receptors failed to invade the basement membrane in response to NDF (Fig. 3 B), EGF, or BTC (data not shown). However, MCF7-5R cells were capable of invasion in response to FBS, indicating that the loss of ErbB2 function in these cells did not impair their general invasive ability



**Figure 3.** Functional ErbB2 receptors are required for induction of carcinoma cell invasion of the ECM in response to EGF family peptides. **A**, MCF7 cells were serum-starved and allowed to invade Transwell membranes coated with the reconstituted basement membrane material Matrigel for 24 h in the presence or absence of 50 ng/ml of NDF, EGF, BTC ( $\beta$ -cell.), or 10% FBS as described in Materials and Methods. Each bar represents the mean  $\pm$  SEM of at least three independent experiments. **B**, MCF7 cells with and without cell surface ErbB2 receptors (MCF7-5R) were serum-starved, then examined for their ability to invade the reconstituted basement membrane as described above in the presence or absence of 50 ng/ml of NDF or 10% FBS. Each bar represents the mean  $\pm$  SEM of at least three independent experiments.

(Fig. 3 B). Similar findings were obtained with T47D and MDA-MB-435 cells (data not shown). Therefore, ErbB2 activation is required for induction of carcinoma cell migration, as well as invasion of the ECM in response to EGF family peptides.

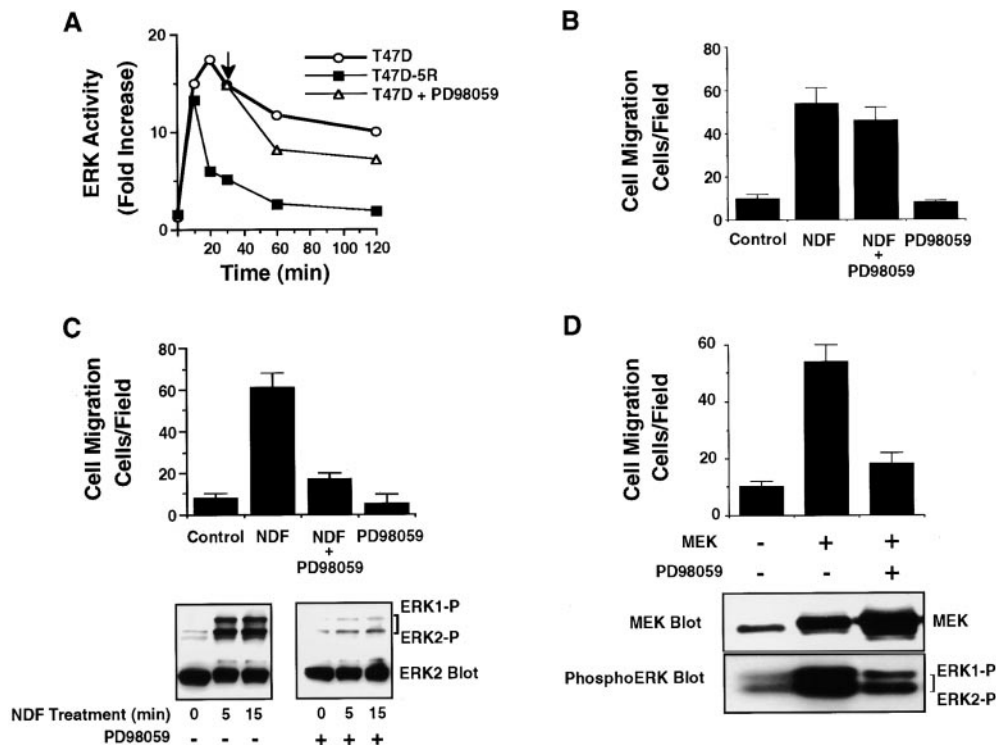
### ***ERK Activation Is Necessary for ErbB2-mediated Carcinoma Cell Migration***

Exposure of cells to EGF-related peptides results in sustained ERK activation ( $>2$  h), while cells devoid of functional ErbB2 receptors show only an early transient peak of kinase activity that returns to basal levels by 30–40 min (Fig. 4 A; also see Graus-Porta et al., 1995, 1997; Pinkas-Kramarski et al., 1998). Together with our findings that ErbB2 is required for cell migration, suggests that sustained ERK activation is important for cell movement. To examine this possibility, wild-type cells were stimulated with NDF for 30 min and then treated with PD98059, which prevents ERK phosphorylation and activation by MAP kinase kinase (MEK; Dudley et al., 1995). These cells were then examined for their ability to migrate and for ERK activity using an *in vitro* kinase assay. As shown in Fig. 4, A and B, exposure of cells to the MEK inhibitor had little effect on NDF-induced cell migration ( $<20\%$ ) and sustained ERK activity ( $<30\%$ ). However, exposure of cells to the MEK inhibitor before NDF stimulation

blocked ERK activation and migration induced by this peptide, indicating that ERK activity was necessary for the migratory response (Fig. 4 C). Similar findings were obtained with cells exposed to EGF and BTC (data not shown). Importantly, expression of mutationally activated MEK in cells without functional ErbB2 was sufficient to promote cell migration and sustained ERK activation (Fig. 4 D). Therefore, the loss of cell surface expression of ErbB2 did impact the general ability of these cells to migrate in response to ERK activation. Together, these findings suggest that sustained ERK activity is important for ErbB2-mediated cell migration in response to EGF family peptides.

### ***ErbB2-dependent Migration Requires Coupling of the Adaptor Protein Crk to p130CAS***

Ligation of growth factor and integrin receptors promotes tyrosine phosphorylation of the substrate domain of CAS and its subsequent association with the SH2 domain of Crk (Feller et al., 1994; Matsuda and Kurata, 1996; Ojaniemi and Vuori, 1997; Casamassima and Rozengurt, 1998; Klemke et al., 1998). Assembly of a CAS/Crk complex represents a second signaling pathway distinct from ERK that can contribute to the invasive behavior of some cells through its ability to regulate the small GTPase, Rac (Kiyokawa et al., 1998; Klemke et al., 1998). To investigate the role of ErbB2 in assembly of CAS/Crk complexes, cells were either held in suspension or allowed to attach to a collagen substrate in the presence or absence of NDF. These cells were then examined for changes in CAS tyrosine phosphorylation and the formation of CAS/Crk complexes. Cells in suspension showed little CAS phosphorylation and Crk binding, which was not altered by the presence of NDF (Fig. 5, A and B). However, cells attached to collagen in the presence of NDF showed significantly increased CAS tyrosine phosphorylation and Crk-binding, compared with cells attached to this substrate without NDF (Fig. 5, A and B). The amount of detergent-soluble CAS immunoprecipitated from cells attached to collagen was reduced, compared with nonadherent cells (Fig. 5 B). This may result from the ability of CAS to associated with the Triton X-100-insoluble cytoskeleton upon cell adhesion to the ECM (Polte and Hanks, 1997). In any case, NDF clearly enhanced CAS phosphorylation and formation of CAS/Crk complexes in MCF7, but not MCF7-5R cells attached to collagen (Fig. 5, A and B). In fact, cells devoid of ErbB2 activity showed only basal CAS/Crk binding upon adhesion to collagen, and NDF did not alter this response or the solubility of these proteins (Fig. 5 B). Thus, it appears that ErbB2 can cooperate with integrin adhesion receptors to potentiate the formation of CAS/Crk complexes in migratory cells. To determine if assembly of this adaptor protein complex was necessary for ErbB2-dependent migration, cells were transfected with CAS without its substrate domain (CAS-SD) or Crk with a mutated SH2 domain (Crk-SH2). These mutant proteins have been shown to interfere with formation of CAS/Crk complexes and prevent downstream signals in cells (Klemke et al., 1998). Expression of either CAS-SD or Crk-SH2 in cells blocked migration induced by NDF (Fig. 5 C), EGF, and BTC (data not shown). Importantly, ex-



**Figure 4.** ErbB2-mediated cell migration requires ERK activity. **A**, Kinase activity of ERK2 immunoprecipitated from T47D cells with or without cell surface ErbB2 receptors (T47D-5R) after treatment with 50 ng/ml NDF for the indicated times as described in Materials and Methods. In some cases, PD98059 (50  $\mu$ M) was added to T47D cells 30 min after exposure to NDF to block ERK activation by MEK. Arrow indicates time of exposure of cells to PD98059. **B**, T47D cells pretreated for 30 min with or without NDF (50 ng/ml) and then allowed to migrate on collagen-coated Transwell membranes for 2 h in the presence or absence of PD98059 (50  $\mu$ M). Each bar represents the mean  $\pm$  SEM of at least three independent experiments. **C**, T47D cells pretreated for 1 h with or without PD98059 (50  $\mu$ M)

and were then allowed to migrate on collagen-coated Transwell membranes in the presence or absence of NDF for 2 h. Each bar represents the mean  $\pm$  SEM of at least three independent experiments. An aliquot of cells treated as for the migration experiment above was lysed in detergent then analyzed for changes in ERK activity by immunoblotting with antibodies to the phosphorylated activated form of ERK1 and ERK2. Immunoblots were stripped and re probed with antibodies to ERK2 to confirm equal amounts of ERK protein were present in these lysates. **D**, MCF7-5R cells were transiently transfected with a  $\beta$ -gal reporter construct, together with either the empty vector or the vector containing mutationally activated MEK. Cells were allowed to migrate on collagen-coated Transwell membranes in the presence or absence of PD98059 for 3 h and were then stained with X-gal reagent to detect transfected cells. Transfection efficiency, as well as cell adhesion to collagen, was monitored to assure that equal numbers of cells were transfected and loaded into migration chambers as described in Materials and Methods. Each bar represents the mean  $\pm$  SEM of at least three independent experiments. An aliquot of cells treated as for the migration experiment above was lysed in detergent and analyzed for MEK expression or changes in ERK activity by immunoblotting with antibodies to MEK or the phosphorylated activated forms of ERK1 and ERK2, respectively.

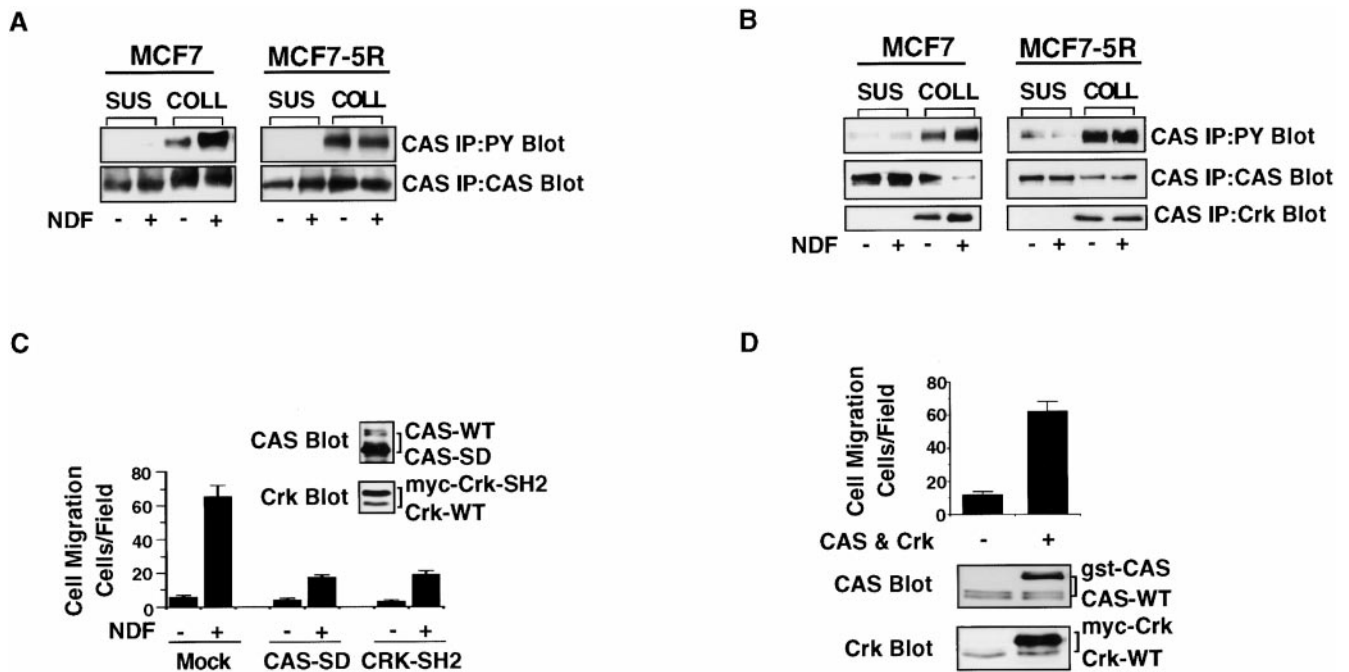
pression of wild-type CAS and Crk in cells without functional ErbB2 promoted cell migration (Fig. 5 D) and CAS/Crk complexes (data not shown). Therefore, the loss of cell surface expression of ErbB2 did not impair, nonspecifically, the ability of these cells to migrate in response to CAS/Crk coupling and its downstream signals. Together, these findings indicate that ErbB2 activation potentiates the assembly of a CAS/Crk signaling complex, and this event is necessary for cell migration mediated by ErbB family tyrosine kinases.

### **Overexpression of ErbB2 Is Sufficient to Induce Carcinoma Cell Migration, as well as CAS/Crk Coupling and ERK Activation**

In some carcinomas, ErbB2 protein can be overexpressed as much as 100-fold as the result of gene amplification and/or transcriptional alterations (Hynes and Stern, 1994; Riese and Stern, 1998). This may lead to ErbB2 tyrosine kinase activation in the absence of ligand and induction of cell migration and invasion. To determine whether overexpression of ErbB2 was sufficient to induce cell migra-

tion/invasion, cells were transiently transfected with a vector encoding wild-type human ErbB2 and examined for changes in cell migration on collagen, as well as invasion of a reconstituted basement membrane (Matrigel). As shown in Fig. 6 A, cells overexpressing ErbB2 showed increased receptor activation and a three- to fourfold increase in both migration and invasion, compared with mock cells transfected with the empty vector. Importantly, ErbB2-induced cell migration/invasion was associated with ERK activation and increased assembly of CAS/Crk complexes (Fig. 6, B and C). Treatment of these cells with the MEK inhibitor or transfection with CAS-SD blocked ErbB2-mediated cell migration and invasion of the basement membrane (Fig. 6 A). These findings indicate that overexpression of ErbB2 was sufficient to induce cell migration and invasion and this process required ERK activity and CAS/Crk coupling.

To directly investigate the role of ErbB2 in cell migration independent of other ErbB members, we used CHO cells that express a moderate level of endogenous ErbB2, but no ErbB1, -3, or -4 (Karunagaran et al., 1996). This allowed us to examine whether ErbB2 directly couples to



**Figure 5.** Coupling of the adaptor proteins CAS and Crk are necessary for ErbB2-mediated cell migration. **A**, CAS immunoprecipitated from MCF7 cells with and without cell surface ErbB2 receptors (MCF7-5R) and either held in suspension (SUS) or allowed to attach to collagen-coated (COLL) dishes for 15 min in the presence or absence of NDF (50 ng/ml), and then lysed in boiling 1% SDS to completely solubilize cellular proteins. The proteins were diluted in buffer and CAS immunoprecipitated and examined for tyrosine phosphorylation by immunoblotting with anti-phosphotyrosine antibodies as described in Materials and Methods. **B**, CAS immunoprecipitated from cells treated, as described in **A**, and examined for CAS tyrosine phosphorylation, as well as Crk binding by immunoblotting as described in Materials and Methods. 1% Triton X-100 was used to solubilize cellular proteins in these experiments in place of SDS. The milder lysis conditions allows for the retention CAS/Crk complexes. However, some of the CAS protein is not completely solubilized in adherent cells, most likely because it strongly associates with the detergent-insoluble cytoskeleton in cells attached to ECM proteins (Polte and Hanks, 1997). **C**, MCF7 cells were transiently transfected with a  $\beta$ -gal reporter construct, together with either the empty vector or the vector containing CAS with the Crk binding sites deleted (CAS-SD) or Crk with its CAS binding domain mutated (Crk-SH2). Cells were allowed to migrate on collagen-coated Transwell membranes in the presence or absence of NDF (50 ng/ml) for 3 h and were then stained with X-gal reagent to detect transfected cells. Transfection efficiency, as well as cell adhesion to collagen, was monitored to assure that equal numbers of cells were transfected and loaded into migration chambers as described in Materials and Methods. Each bar represents the mean  $\pm$  SEM of at least three independent experiments. An aliquot of cells treated as for the migration experiment above was lysed in detergent then analyzed for CAS and Crk expression by immunoblotting with antibodies to CAS and Crk. Note that the Crk-SH2 is myc-tagged and shows reduced mobility, compared with the endogenous wild-type Crk (Crk-WT) protein as the result of the molecular tag. CAS-SD shows faster mobility, compared with endogenous CAS (CAS-WT) since its substrate domain has been truncated. **D**, MCF7-5R cells without cell surface ErbB2 receptors were transiently transfected with a  $\beta$ -gal reporter construct, together with either the empty vector or the vector containing CAS and Crk, and then allowed to migrate as described in **C**. An aliquot of cells treated as for the migration experiment above was lysed in detergent and then analyzed for CAS and Crk expression by immunoblotting with antibodies to CAS and Crk. Note that Crk and CAS are myc- and gst-tagged, respectively, and show reduced mobility, compared with endogenous wild-type (WT) forms of these proteins.

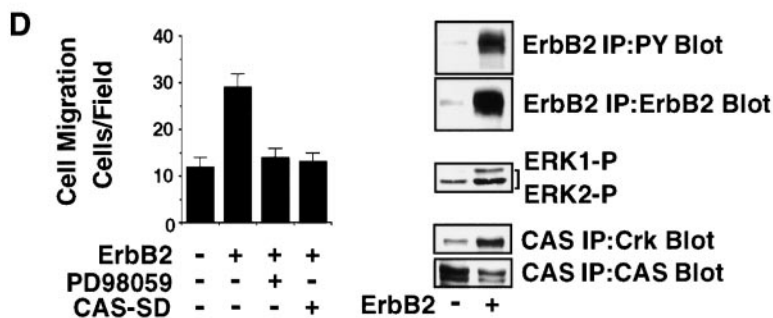
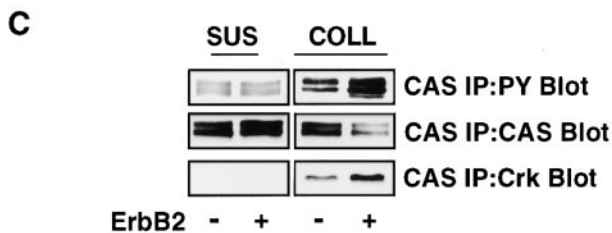
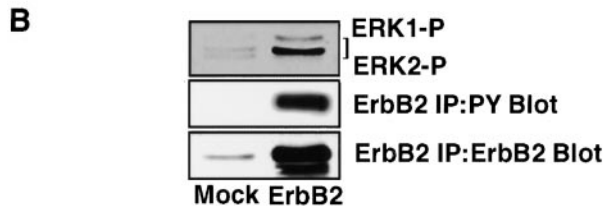
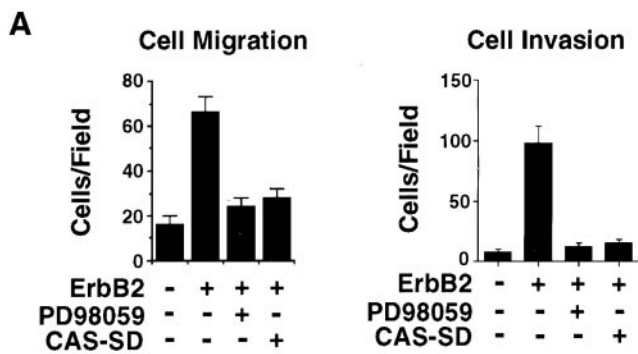
the migration machinery of cells. As shown in Fig. 6 D, cells overexpressing ErbB2 showed a 2.5-fold increase in cell migration, compared with mock-transfected control cells. This was associated with a significant increase in ERK activity and assembly of CAS/Crk complexes (Fig. 6 D). Importantly, the MEK inhibitor or CAS-SD blocked ErbB2-induced cell migration, indicating that ERK activity and CAS/Crk coupling were necessary for this response (Fig. 6 D). These findings indicate that ErbB2 can directly couple to the migration machinery of cells by activating ERK and assembling CAS/Crk complexes.

#### Overexpression of ErbB Family Receptors Induce Cell Migration that Depends on ErbB2 Activation

Amplification of ErbB family members can lead to ty-

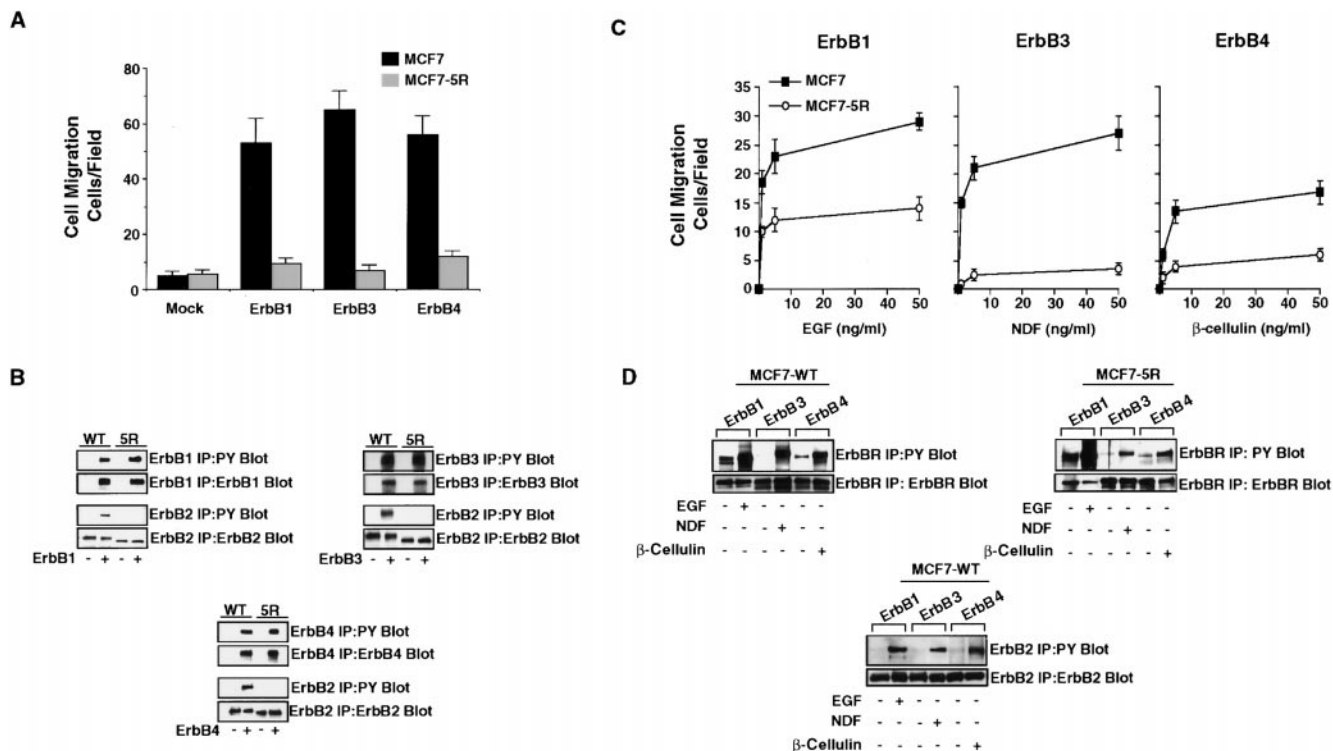
rosine kinase activation and development of various cancers (Di Fiore et al., 1987a,b; Friess et al., 1995; Gilbertson et al., 1998; Li et al., 1999). Therefore, we investigated whether overexpression of ErbB1, -3 and -4 receptors could also potentiate cell migration and whether ErbB2 played a role in this event. MCF7 and MCF7-5R cells were transfected with either ErbB1, -3, or -4, and then examined for their ability to migrate and for changes in ErbB receptor activation, including endogenous ErbB2. Whereas overexpression of ErbB1, -3, or -4 was sufficient to induce ErbB2 activation and MCF7 cell migration, it failed to stimulate these events in MCF7-5R cells devoid of cell surface ErbB2 receptors (Fig. 7, A and B). Interestingly, ErbB1, -3, and -4 showed significant tyrosine phosphorylation that was similar in cells with or without surface ErbB2 receptors (Fig. 7 B). These findings indicate that overexpression





of ErbB receptor tyrosine kinases in carcinoma cells is sufficient for their activation in the absence of functional ErbB2 or exogenous ligand (Fig. 7 B). However, it appears that the basal activation of ErbB1, -3, and -4 under these conditions does not support efficient cell migration on the ECM in the absence of ErbB2. Furthermore, ligand-induced migration of cells overexpressing ErbB family receptors also required ErbB2. In fact, ErbB2-deficient cells overexpressing ErbB3 and -4 showed little capacity to migrate in response to EGF ligands, compared with cells with functional ErbB2 receptors (Fig. 7, C and D). However, while the loss of ErbB2 activity in these cells clearly impaired their ability to migrate, it did not prevent receptor activation in response to their ligands (Fig. 7 D). Interestingly, ErbB2-deficient cells overexpressing ErbB1 showed a moderate level of ligand-induced migration, although it

was not as prominent as cells with functional ErbB2 receptors (Fig. 7 C). These findings suggest that cells with high levels of ErbB1 receptor activity can engage the migration machinery of cells in the absence of ErbB2 activation. In contrast, overexpression and strong activation of ErbB3 and -4 in cells does not appear to fully compensate for the loss of ErbB2 activity necessary for cell migration. The activation of ErbB receptors in the absence of ErbB2 likely results from their ability to form functional homodimers, as well as heterodimers with endogenous ErbB members present in these cells (Tzahar et al., 1996, Li et al., 1999). Alternatively, competition for phosphatases or secretion of EGF-like peptides could lead to ErbB receptor activity and migration. In any case, these findings provide additional evidence that ErbB2 is critical for cell migration mediated by ErbB family receptors.



**Figure 7.** ErbB2 activation is necessary for cell migration induced by overexpression of ErbB family receptors. **A**, Serum-starved MCF7 cells with or without cell surface ErbB2 receptors (MCF7-5R) were transiently transfected with either the empty vector (Mock) or the vector (pcDNA3) containing either ErbB1, ErbB3, or ErbB4 along with the  $\beta$ -gal reporter gene. Cells were allowed to migrate on collagen-coated Transwell membranes for 3 h and then stained with X-gal to identify transfected migratory cells. Transfection efficiency, as well as cell adhesion to collagen, was monitored to assure that equal numbers of transfected cells were loaded into the migration chamber as described in Materials and Methods. **B**, Immunoprecipitation of ErbB1, -3, or -4 from cells treated as for the migration experiments above and examined for expression, as well as changes in tyrosine phosphorylation by immunoblotting as described in Materials and Methods. Note that endogenous ErbB2 was also immunoprecipitated from these cells and examined for changes in tyrosine phosphorylation. In these experiments, ErbB1, -3, and -4 were overexpressed above endogenous proteins by  $\sim$ 56-, 30-, and 48-fold, respectively, as determined by immunoblotting with ErbB antibodies and ECL. MCF7 cells express moderate levels of endogenous ErbB3, -4, and a low level of ErbB1 receptors, which are not visible at the short exposure time (5 s) used to visualize the overexpressed proteins in these experiments. **C**, Cells transfected as described in **A** were allowed to migrate for 3 h in the presence of various concentrations of EGF, NDF, or BTC as described above. The basal migration of these cells in the absence of EGF-like peptides has been subtracted as background. **D**, Immunoprecipitation of ErbB1, -3, or -4 from cells treated as for the migration experiments in **C**. ErbB receptor (ErbBR) expression, as well as changes in tyrosine phosphorylation, were determined in cells exposed to the indicated ligand (50 ng/ml for 10 min) by immunoblotting as described in Materials and Methods. Note that endogenous ErbB2 was also immunoprecipitated from these cells and examined for changes in tyrosine phosphorylation. Each bar or data point represents the mean  $\pm$  SEM of at least three independent experiments.

## Discussion

### Evidence that ErbB2 Activation Is Critical for Induction of Cell Migration and Invasion of the ECM

Cell migration is an underlying component of development, angiogenesis, wound healing, and tumor cell metastasis. While ErbB family receptors and their ligands are involved in the regulation of these biological processes, it has been difficult to link specific ErbB receptor activation events to a given biological response since most cells express multiple ErbB members (Hynes and Stern, 1994; Riese and Stern, 1998). Even more complexity is introduced by the fact that these receptors undergo multiple heterodimerization and transactivation events upon ligand binding or overexpression (Tzahar et al., 1996). It appears then that the potential of an ErbB receptor to regulate a given biological process is dependent on the type of ligand

present, as well as the complement of ErbB receptors expressed by a given cell type. The ability for receptor cross-talk is important as it diversifies downstream signals and the potential to control numerous biological processes. In this study, we used carcinoma cells depleted of cell surface ErbB2 receptors to study its specific role in cell invasion of the ECM. We provide several lines of evidence that ErbB2 serves as a central signaling component required for cell migration and invasion induced by EGF family receptors. First, breast carcinoma cell lines exposed to several EGF-related peptides were induced to migrate and invade the ECM, whereas cells devoid of functional ErbB2 receptors did not. Second, only cells expressing functional ErbB2 receptors potentiated ERK activity and CAS/Crk coupling, two signaling pathways associated with induction of cell movement (Klemke et al., 1997, 1998). Third, overexpression of ErbB2 in cells was sufficient to induce cell invasion

and facilitate ERK activity and CAS/Crk coupling. Finally, overexpression of ErbB1, -3, and -4 induced cell migration that was mediated by ErbB2 activation. Interestingly, it is known that ErbB1 facilitates cell migration through critical tyrosine residues in its cytoplasmic tail (Chen et al., 1994). Recent evidence indicates that this region of ErbB1 may be dispensable for its signaling properties since it can associate with and signal through the ErbB2 receptor (Wong et al., 1999). Furthermore, mice deficient in ErbB2 display almost identical developmental abnormalities as NDF, and ErbB4 deficient animals (Gassmann et al., 1995; Lee et al., 1995; Riethmacher et al., 1997). Together, these findings suggest that ErbB2 serves as an integral partner of the ErbB receptor signaling network and the physiological processes mediated by this family of receptor tyrosine kinases.

### ***Mechanisms of ErbB2-dependent Cell Invasion***

One of the most significant effects associated with ErbB2 activation is enhanced and prolonged signaling events, including sustained activation of ERK (Graus-Porta et al., 1995; Karunakaran et al., 1996; Pinkas-Kramarski et al., 1998). We show here that an important consequence of this event is to facilitate cell invasion. ERK can phosphorylate myosin light chain kinase, leading to increased myosin phosphorylation and motor activity during cell migration (Klemke et al., 1997). This may be due to the ability of ErbB2 to enhance binding affinities for EGF peptides, leading to an increase in the half-life of receptor-ligand complexes and downstream signals that impact ERK activity and myosin function (Karunakaran et al., 1996). However, ErbB2 alone can stimulate sustained ERK activation independent of other ErbB members, indicating that it directly couples to this signaling pathway (Fig. 4 D; and Ben-Levy et al., 1994). The ability of ErbB2 to facilitate sustained ERK signaling may be responsible for the highly proliferative, as well as malignant, nature of tumors that arise from aberrant ErbB2 receptor activation. In breast cancer patients, ERK activity is sustained five- to tenfold over benign conditions and is localized to metastatic cells within the lymph nodes (Sivaraman et al., 1997).

The inability of ErbB2-deficient cells to move in response to EGF family peptides does not appear to result from a complete loss of ErbB receptor activity. Therefore, it seems that the role of ErbB2 in migration is to diversify receptor transphosphorylation events and to provide unique downstream effector molecules that specifically link ErbB receptors to the migration and invasion machinery of cells. Our findings, that overexpression of ErbB2 in cells devoid of other ErbB members is sufficient to induce cell migration, support this notion. Furthermore, ErbB2-deficient cells overexpressing ErbB1, -3, or -4 show strong receptor activation, but impaired migration in response to EGF-like peptides. Recent evidence also indicates that overexpression of ErbB2 in NIH 3T3 fibroblast cells promotes cell migration (Verbeek et al., 1998). However, it is not clear whether ErbB1 present in these cells contributes to the migration response.

ErbB receptor heterodimerization with ErbB2 was recently shown to cause differential patterns of tyrosine phosphorylation of ErbB2 (Olayioye et al., 1998). These

phosphotyrosine residues may serve as SH2 docking sites for unique effectors, such as CHK (CSK-homologous kinase), Grb2, c-src, Shc, and the p85 subunit of PI3 kinase (Alroy and Yarden, 1997). CHK is of particular interest since this kinase specifically binds to the activated ErbB2 receptor and regulates c-src activation (Zrihan-Licht et al., 1997). c-Src is known to facilitate CAS/Crk coupling, as well as ERK activation in cells (Vuori et al., 1996). Furthermore, mammary carcinomas that result from ErbB2 amplification show significantly elevated c-src activity (Ottenhoff-Kalff et al., 1992; Luttrell et al., 1994; Muthuswamy et al., 1994). It seems possible, then, that c-src could play a pivotal role in coupling ErbB2 to the migration machinery of cells by mediating ERK activation and CAS/Crk coupling.

ErbB2 may also serve to target ErbB receptor/signaling complexes to a specific site(s) in cells. Both CAS/Crk and ErbB2 localize to membrane ruffles of migratory cells (De Potter and Quatacker, 1993; Klemke et al., 1998; Wang et al., 1999). Assembly of a CAS/Crk complex is associated with Rac activation and formation of membrane ruffles (Kiyokawa et al., 1998; Klemke et al., 1998). This, and the fact that ErbB2 activation facilitates down-regulation of cell-cell contacts resulting in an epithelial-mesenchymal transition, may explain the increased metastatic properties associated with carcinoma cells with amplified ErbB2. Rac activation and loss of cell-cell contacts enhances cell invasion and metastasis of breast carcinoma cells (Keely et al., 1997). Interestingly, ErbB2 can associate with integrin adhesion receptors (Falcioni et al., 1997), and integrin ligation induces ErbB1 activation (Moro et al., 1998). Therefore, ErbB2 may play a role in regulation of integrin adhesiveness and/or signaling during cell migration on the ECM. Our findings, that ErbB2 activation can potentiate CAS/Crk coupling during cell adhesion to the ECM, suggests that integrin and ErbB2 receptors cooperate to regulate cell migration.

### ***Biological Importance of ErbB2-mediated Cell Migration/Invasion***

Our findings that ErbB2 is necessary for the process of cell migration has implications for a number of biological, as well as certain pathological, processes associated with ErbB family receptors and their ligands. For example, ErbB receptors have been implicated in the development of a number of human cancers (Slamon et al., 1989; Dougall et al., 1994; Hynes and Stern, 1994; Gilbertson et al., 1998). In fact, many of these tumors were found to overexpress one or more of the ErbB receptors that were associated with increased occurrence of visceral metastasis and micrometastatic bone marrow disease (Pantel et al., 1993). Our findings raise the possibility that the increased invasive potential and poor prognosis associated with these carcinomas may be directly related to their ability to couple to and transactivate ErbB2 and the migration machinery. Since ErbB2 is the preferred partner recruited by all activated ErbB receptors, it seems reasonable that an ErbB2 homo- or heterodimer is responsible for these migration/metastasis events (Graus-Porta et al., 1997). This could occur as a result of ErbB receptor amplification, which is known to facilitate increased molecular interac-

tions among receptor members, leading to their dimerization and activation in the absence of ligand (Stern et al., 1988). Our findings, that overexpression of ErbB receptors in carcinoma cells is sufficient to promote ErbB2 activation in the absence of exogenous ligand, support this notion (Fig. 7). ErbB2 containing heterodimers would also be expected to occur in carcinoma cells exposed to EGF family peptides that are common in the extracellular environment of many tissues, including mammary glands (Yang et al., 1995; Jones et al., 1996). Alternatively, some carcinoma cells are known to secrete EGF-related peptides, including NDF and TGF- $\alpha$ , suggesting that ErbB2 activation may occur by an autocrine mechanism (Bacus et al., 1993). Thus, the poor prognosis associated with cancers that arise from altered ErbB receptor members could be due, in part, to enhanced migration/invasion as the result of ErbB2 activation via an autocrine and/or paracrine mechanism.

Mice lacking ErbB2 die in utero due to defects in neural and cardiac development (Lee et al., 1995). The neural defects seen in these animals may result from a deficiency in neural crest cell proliferation and/or migration (Lee et al., 1995; Britsch et al., 1998). Remarkably, mice deficient in ErbB4 or NDF show an almost identical phenotype to that of ErbB2 deficient animals (Gassmann et al., 1995; Meyer and Birchmeier, 1995). These findings are consistent with the idea that ErbB2 activation is a central event in the signal transduction mechanism of ErbB family receptors and their biological responses, including cell migration. ErbB receptors and EGF family peptides are also expressed during normal development of the mammary gland (Yang et al., 1995; Jones et al., 1996). NDF has been shown to promote in vitro cell migration and morphogenic changes that resemble the development of tubular and alveolar structures present in the mammary gland (Chausovsky et al., 1998; Niemann et al., 1998). Moreover, these events are associated with both ErbB2 and ErbB3 activation, suggesting that an ErbB2/3 heterodimer is important for this process.

There may also be instances in which cells lacking ErbB2 can migrate in response to EGF family peptides (Rio et al., 1997). Fibroblast cells overexpressing ErbB1 or -4 alone can be stimulated to migrate with EGF and HB-EGF, respectively (Elenius et al., 1997). We found that activation of ErbB1 in carcinoma cells overexpressing this receptor was sufficient to induce a moderate level of migration in the absence of ErbB2 (Fig. 7). Thus, it is possible that some cells compensate for the lack of ErbB2 signals through receptor transactivation events associated with receptor amplification and dimerization. This, in turn, could upregulate and/or provide unique signals for cell migration.

In summary, our findings demonstrate that ErbB2 plays a central role in promoting breast carcinoma cell invasion of the ECM. ErbB2 serves as a fundamental signaling component that links ErbB family receptor tyrosine kinases to the migration/invasion machinery of carcinoma cells by facilitating ERK activation and CAS/Crk coupling. These findings contribute to the understanding of how ErbB family receptors and their ligands regulate signal transduction events associated with development, wound repair, angiogenesis, and tumor cell dissemination.

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