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Biochemistry

Ligand-specific activation of HER4/p180^{erbB4}, a fourth member of the epidermal growth factor receptor family

(receptor tyrosine kinase/*ERBB4* gene product)

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ABSTRACT **This report describes the isolation and recombinant expression of a cDNA clone encoding HER4, the fourth member of the human epidermal growth factor receptor (EGFR) family. The *HER4/erbB4* gene encodes a 180-kDa transmembrane tyrosine kinase (HER4/p180^{erbB4}) whose extracellular domain is most similar to the orphan receptor HER3/p160^{erbB3}, whereas its cytoplasmic kinase domain exhibits 79% and 77% identity with EGFR and HER2/p185^{erbB2}, respectively. *HER4* is most predominantly expressed in several breast carcinoma cell lines, and in normal skeletal muscle, heart, pituitary, brain, and cerebellum. In addition, we describe the partial purification of a heparin-binding HER4-stimulatory factor from HepG2 cells. This protein was found to specifically stimulate the intrinsic tyrosine kinase activity of HER4/p180^{erbB4} while having no direct effect on the phosphorylation of EGFR, HER2, or HER3. Furthermore, this heparin-binding protein induces phenotypic differentiation, and tyrosine phosphorylation, of a human mammary tumor cell line that overexpresses both HER4 and HER2. These findings suggest that this ligand–receptor interaction may play a role in the growth and differentiation of some normal and transformed cells.**

Transmembrane receptors that contain a cytoplasmic tyrosine kinase domain are of particular interest to developmental and tumor biologists, since their activation often initiates a cascade of events leading to cell growth and differentiation (1). Included in this group are the receptors for polypeptide growth factors such as epidermal growth factor (EGF), insulin, platelet-derived growth factor, fibroblast growth factor, and neurotrophins. Recently, the ligands for several "orphan" receptors have been identified, including those for c-kit (steel factor), met (hepatocyte growth factor), trk (neurotrophins), and HER2 (heregulin) (1–3). However, numerous receptor tyrosine kinases (RTKs) have been isolated, including eph, eck, elk, ret, and HER3 (1, 4), for which a ligand has not yet been identified.

The EGF receptor (EGFR) family is one group of RTKs that is frequently overexpressed in a variety of aggressive epithelial carcinomas and comprises three members: EGFR, HER2/p185^{erbB2}, and HER3/p160^{erbB3} (4–6). Increased expression of EGFR has been associated with more aggressive cancers of the breast, bladder, lung, and stomach (7); amplification and overexpression of HER2 have been reported for breast and ovarian carcinoma, where high levels of HER2 directly correlate with a poor clinical prognosis (7, 8); and HER3 expression is amplified in a variety of human adenocarcinomas (9). While several structurally related polypeptides have been identified that specifically bind to the EGFR, including EGF, transforming growth factor α (TGF-α), amphiregulin, heparin-binding EGF, and vaccinia virus growth factor (for review see refs. 7 and 10), none of these ligands has been shown to interact with HER2 or HER3. However, several groups have recently reported the identification of candidate ligands for HER2 (2, 3, 11–13). Elucidation of the primary structure of one of these molecules, heregulin/Neu differentiation factor (NDF), reveals it to be an additional member of the EGF family (2, 3). Due to the biological importance of this extended family of ligands and receptors, we have continued our search for additional homologues. This report describes the cloning and expression of a fourth member of the EGFR family, HER4.*

MATERIALS AND METHODS

Molecular Cloning. Several pools of degenerate oligonucleotides were synthesized on the basis of conserved sequences from EGFR family members. Total genomic DNA was isolated from murine K1735 melanoma cells and used as a template with these oligonucleotide primers in a 40-cycle polymerase chain reaction (PCR) amplification (4). Using the degenerate oligonucleotides H4TVWELM and H4VYMIIL, we identified one clone (MER4-85) that contained a 144-nt insert corresponding to murine *erbB4*. This ^{32}P-labeled insert was used to isolate a 17-kb fragment from a murine T-cell genomic library (Stratagene) that was found to contain two exons of the murine *erbB4* gene. A specific oligonucleotide (4M3070) was synthesized on the basis of the DNA sequence of an *erbB4* exon and used in a PCR protocol with a degenerate 5′-oligonucleotide (H4PIKWMA) on a template of single-stranded MDA-MB-453 cDNA. This reaction generated a 260-nt fragment (pMDAPIK) corresponding to human *HER4*. cDNA libraries were constructed in λ ZAP II (Stratagene) from oligo(dT)- and specific-primed MDA-MB-453 and human heart RNA (4, 10). *HER4*-specific clones were isolated by probing the libraries with the ^{32}P-labeled insert from pMDAPIK. To complete the cloning of the 5′ portion of *HER4*, we used a PCR strategy to allow for rapid amplification of cDNA ends (4). All cDNA clones and several PCR-generated clones were sequenced on both strands, using T7 polymerase with oligonucleotide primers.

The oligonucleotide mixtures (including their degeneracy, corresponding amino acid residues, and orientation) which were used for PCR are as follows:

H4TVWELM 5′-ACNGTNTGGGARYTNAYHAC-3′
(256-fold, TVWELMT, sense)

H4VYMIIL 5′-ACAYTTNARDATDATCATRTANAC-3′
(576-fold, VYMIILK, antisense)

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Abbreviations: EGF, epidermal growth factor; EGFR, EGF receptor; RTK, receptor tyrosine kinase; mAb, monoclonal antibody; TGF-α, transforming growth factor α.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L07868).

H4PIKWMA 5′-GACGAATTCCNATHAARTGGATGGC (48-fold, PIKWMA, sense)

4M3070 5′-CTGCTGTCAGCATCGATCAT-3′ (antisense).

Degenerate residues are: D = A, G, or T; H = A, C, or T; N = A, C, G, or T; R = A or G; and Y = C or T.

Assays of Tyrosine Kinase Stimulatory Activity. A panel of four recombinant cell lines, each of which expresses only a single member of the human EGFR family, was generated. The complete 4-kb *HER4* coding sequence was reconstructed and inserted into a glutamine synthetase expression vector, pEE14 (14). The resulting construct (pEE14HER4) was transfected into CHO-KI cells, and a stable cell line (CHO/HER4 #3) expressing high levels of recombinant human $p180^{erbB4}$ was selected by immunoblotting solubilized cell extracts with a sheep polyclonal antipeptide antibody against HER2 residues 929–947 (Cambridge Research Biochemicals, Valley Stream, NY). CHO/HER2 cells (clone 1-2500) were selected to express high levels of recombinant human $p185^{erbB2}$ by dihydrofolate reductase-induced gene amplification in *dhfr*-deficient CHO cells. NRHER5 cells (15) expressing $\approx 10^6$ human EGFRs per cell were obtained from Hsing-Jien Kung (Case Western Reserve University, Cleveland). 293/HER3 cells were selected to express high levels of human $p160^{erbB3}$ (16).

Cells were allowed to attach in six-well tissue culture plates (Falcon) at 37°C for 18–24 hr and then incubated in serum-free medium for at least 1 hr prior to addition of the indicated amounts of ligand preparations. After treatment with ligand for 10 min at room temperature, cells were solubilized (17) and the cleared supernatants were allowed to react with 1 μg of murine monoclonal antibody (mAb) to phosphotyrosine (PY20, ICN) for CHO/HER4 and 293/HER3 cells, or 1 μg of murine mAb to HER2 (Neu-Ab3, Oncogene Sciences, Manhasset, NY) for CHO/HER2 cells, or 1 μg of mouse mAb R1 to the human EGFR (Amersham) for NRHER5 cells. Anti-mouse IgG-agarose (for PY20 and Neu-Ab3 mAbs) or staphylococcal protein A-Sepharose (for EGFR-R1 mAb) was used to precipitate the immune complexes. Proteins were separated by SDS/7% PAGE and analyzed by immunoblotting with PY20 mAb essentially as described (12), except phosphoproteins were detected with ^{125}I-labeled goat anti-mouse IgG $F(ab')_2$ and exposure on a Molecular Dynamics phosphorimager.

Cell Differentiation Assay. MDA-MB-453 cells (ATCC) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum and 1× essential amino acids. Cells (7500 per well) were allowed to adhere to 96-well plates for 24 hr. Samples were diluted in the above medium and added to the cell monolayer in 50 μl final volume, and the incubation was continued for an additional 3 days. Cells were then examined by inverted light microscopy for morphologic changes.

Protein Purification. HepG2 cells (ATCC) were cultured in DMEM containing 10% fetal bovine serum. Ten liters of serum-free conditioned medium (HepG2-CM) was cleared by centrifugation, concentrated 16-fold by using an Amicon ultrafiltration unit (10,000 molecular weight cutoff), and precipitated with 2 M ammonium sulfate. The supernatant was dialyzed against phosphate-buffered saline (PBS) and passed through a DEAE-Sepharose (Pharmacia) column. The flow-through fraction was then applied onto a 4-ml heparin-acrylic (Bio-Rad) column. The MDA-MB-453 cell differentiation assay was used throughout the purification procedure to monitor the column fractions. Active fractions, which eluted from the heparin column between 0.4 and 0.8 M NaCl, were precipitated with 2.0 M ammonium sulfate, and the resulting supernatant was loaded onto a phenyl-5PW column (8 × 75 mm, Waters). Bound proteins were eluted by using a 2.0–0.0 M ammonium sulfate gradient in 0.1 M Na_2HPO_4, pH 7.4. Dialyzed fractions were assayed for tyrosine phosphorylation of MDA-MB-453 cells, essentially as described (2).

RESULTS

Isolation of cDNA Clones Encoding a Fourth Member of the Human EGFR Gene Family. EGFR and the related proteins HER2, HER3, and Xmrk exhibit extensive amino acid homology in their tyrosine kinase domains (4–6, 18). In addition, there is strict conservation of the exon–intron boundaries within the genomic regions that encode these catalytic domains (ref. 5 and unpublished observations). Degenerate oligonucleotide primers were designed on the basis of conserved amino acids encoded by a single exon or by adjacent exons from the kinase domains of these four proteins. These primers were used in a PCR protocol to isolate genomic fragments corresponding to murine EGFR, *erbB2* and *erbB3*. In addition, a highly related DNA fragment (designated MER4) was identified as distinct from these other genes. A similar strategy was used to obtain a cDNA clone corresponding to the human homologue of MER4 from the breast cancer cell line MDA-MB-453. Using this fragment as a probe, we found several breast cancer cell lines and human heart to be an abundant source of the EGFR-related transcript. cDNA libraries were constructed by using RNA from human heart and MDA-MB-453 cells, and overlapping clones spanning the complete open reading frame of *HER4/erbB4* were isolated.

The assembled *HER4* cDNA sequence contains a single open reading frame that encodes 1308 amino acids. The coding region is flanked by a 33-nt 5′-untranslated region and a 1517-nt 3′-untranslated region ending with a poly(A) tail. A 25-amino acid hydrophobic signal sequence follows a consensus initiating methionine at position 34. Removal of this signal sequence would give a mature protein beginning at Gln-26 followed by 1283 amino acids with a calculated M_r of 144,260 (Fig. 1).

Structural Analysis of HER4. HER4 has all the structural features of the EGFR family of RTKs (19). Excluding the N-terminal signal sequence, the HER4 protein contains a single hydrophobic stretch of 26 amino acids that is characteristic of a transmembrane region and bisects the protein into a 625-residue extracellular ligand binding domain and a 633-residue C-terminal cytoplasmic domain. The ligand binding domain can be further divided into four subdomains (I–IV), including two cysteine-rich regions (II, residues 186–334; and IV, residues 496–633) and two flanking domains (I, residues 29–185; and III, residues 335–495). This organization is similar to that of EGFR, where domains I and III have been implicated to define specificity for ligand binding (ref. 20; Fig. 1). The extracellular domain of HER4 is most similar to that of HER3: domains II–IV of HER4 share 56–67% identity to the respective domains of HER3, while the same regions of EGFR and HER2 exhibit 43–51% and 34–46% identity to HER4, respectively (Fig. 1). In contrast, the four extracellular subdomains of EGFR and HER2 share 39–50% identity. HER4 also conserves all 50 cysteines present in the extracellular portion of EGFR, HER2, and HER3, except that the HER2 protein lacks the fourth cysteine in domain IV (4–6). There are 11 potential N-linked glycosylation sites in HER4, conserving 4 of 12 potential sites in EGFR, 3 of 8 sites in HER2, and 4 of 10 sites in HER3.

Following the transmembrane domain of HER4 is a cytoplasmic juxtamembrane region of 37 amino acids. This region shares the highest degree of homology with EGFR (73% amino acid identity) and contains one potential protein kinase C phosphorylation site, Ser-679, that is not likely to be used due to its close proximity to the transmembrane domain. Notably, HER4 lacks a site analogous to Thr-654 in the

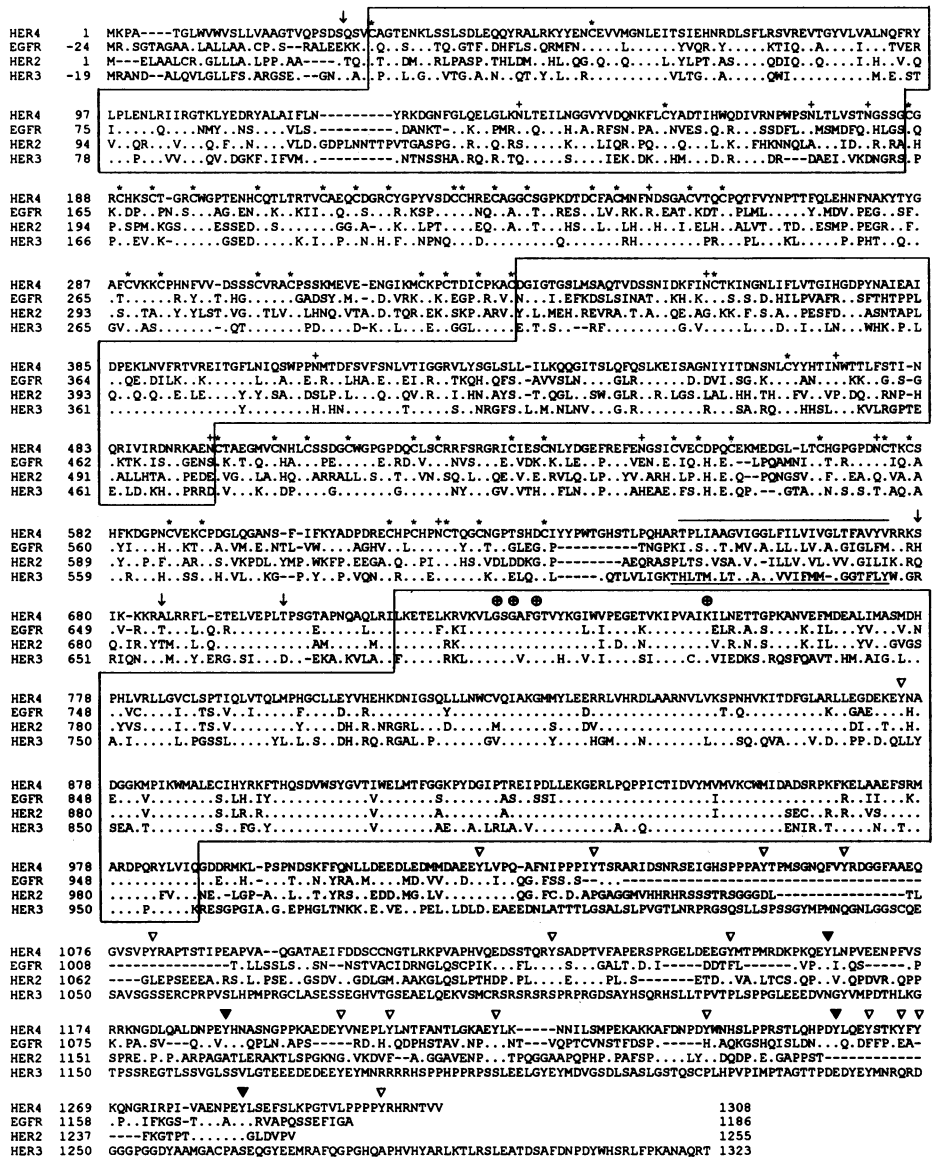

FIG. 1. Deduced amino acid sequence of human HER4 and alignment with other human EGFR family members. Sequences are displayed in the single-letter code and are numbered at left. Identical residues are denoted with dots, gaps (introduced for optimal alignment) are represented by hyphens, cysteine residues are marked with asterisks, and N-linked glycosylation sites are denoted with plus signs. Potential sites of phosphorylation by protein kinase C (Ser-679) or mitogen-activated protein kinase (Thr-699) are shown with arrows. An additional arrow at Ala-685 marks the location of the major site of protein kinase C-induced phosphorylation of EGFR. The predicted ATP-binding site is shown with four circled crosses, C-terminal tyrosines are denoted with open triangles, and tyrosines in HER4 that are conserved with the four major autophosphorylation sites in the EGFR are indicated with filled triangles. The predicted extracellular domain extends from the boundary of the signal sequence marked by an arrow at position 25 to the hydrophobic transmembrane domain which is overlined from position 650 to position 675. Various subdomains are labeled on the right: I, II, III, and IV = extracellular subdomains with II and IV being cysteine-rich; TM = transmembrane domain; and TK = tyrosine kinase domain. Domains I, III, and TK are boxed.

EGFR, which is its major site of protein kinase C-induced phosphorylation. Phosphorylation at this residue in the EGFR appears to block ligand-induced internalization and plays an important role in its transmembrane signaling (21). HER4 also conserves Thr-699 with Thr-669 in EGFR, which is its major EGF-stimulated mitogen-activated protein kinase phosphorylation site (22).

The remaining cytoplasmic portion of HER4 consists of three domains: a 276-amino acid tyrosine kinase domain, an acidic helical structure of 38 amino acids that is homologous to a domain required for ligand-induced internalization of the EGFR (23) and contains a single conserved tyrosine residue, and a 282-amino acid region containing 18 tyrosine residues characteristic of the autophosphorylation domains of other EGFR-related proteins (Fig. 1). The 276-amino acid domain conserves all the diagnostic structural motifs of a tyrosine kinase (19) and is most related to the catalytic domains of EGFR (79% identity) and HER2 (77% identity), and to a lesser degree, HER3 (63% identity). In this same region, EGFR and HER2 share 83% identity. The C-terminal 282 amino acids of HER4 has limited homology with HER2 (27%) and EGFR (19%). However, the C-terminal domain of each receptor is proline-rich and conserves stretches of 2–7 amino acids that are generally centered on a tyrosine residue. These residues include the major tyrosine autophosphorylation sites of EGFR at residues 1068, 1086, 1148, and 1173 (Fig. 1, filled triangles; ref. 24).

The HER4 sequence was unique compared (October 1992) with sequences in the available DNA sequence data bases, but a search of the protein data bases revealed a stretch of 60/64 amino acid identity with HER2 and a 54/54 amino acid identity with an entry termed tyro-2. This entry was deduced from the nucleotide sequence of a small PCR-generated fragment of a rat protein-tyrosine kinase gene (25).

Several related cDNAs were also discovered from the MDA-MB-453 library that were identical to the consensus HER4 sequence but diverged at the 5′ or 3′ ends. These cDNA variants comprised two forms: one encodes a transmembrane HER4 with the deletion of its C-terminal autophosphorylation domain and the second encodes only a portion of the HER4 cytoplasmic domain.

Tissue Distribution of HER4 Transcripts. Northern blots of poly(A)$^+$ mRNA from human tissues were hybridized with an antisense RNA probe to the 3′ end of *HER4*. An ≈6-kb mRNA transcript was most abundant in the heart and skeletal muscle (Fig. 2), while an mRNA of greater than 15 kb was detected in the brain, with lower levels in heart, skeletal muscle, kidney, and pancreas. To confirm and extend the *HER4* expression profile, we performed quantitative reverse transcriptase PCR, using primers from sequences in the

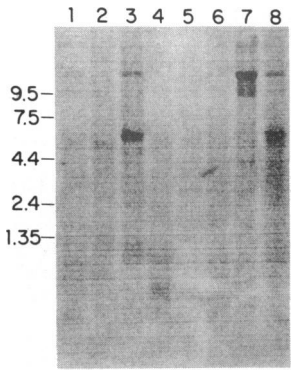

FIG. 2. Northern blot analysis of mRNA from human tissues hybridized to a 3′ *HER4*-specific [α-^{32}P]UTP-labeled antisense RNA probe. RNA size markers (in kb) are shown on the left. Lanes 1–8 represent 2 μg of poly(A)$^+$ mRNA from pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, and heart, respectively.

HER4 kinase domain. As observed by Northern analysis, brain, heart, and kidney express the highest levels of *HER4* transcripts, in addition to parathyroid, cerebellum, pituitary, spleen, testis, and breast. Lower levels were found in thymus, lung, salivary gland, and pancreas, and low or undetectable expression was found in liver, prostate, ovary, adrenal, colon, duodenum, epidermis, and bone marrow. Various human cell lines were also examined by PCR analysis, revealing the highest expression of *HER4* RNA in four mammary adenocarcinoma cell lines (T-47D, MDA-MB-453, BT-474, and H3396) and in neuroblastoma (SK-N-MC) and pancreatic carcinoma (Hs766T) cell lines. Intermediate expression was detected in three additional mammary carcinoma cell lines (MCF-7, MDA-MB-330, and MDA-MB-361). Low or undetectable expression was found in other cell lines derived from carcinomas of the breast (MDB-MB-231, MDA-MB-157, MDA-MB-468, and SK-BR-3), kidney (Caki-1, Caki-2, and G-401), liver (SK-HEP-1 and HepG2), pancreas (PANC-1, AsPC-1, and Capan-1), colon (HT-29), cervix (CaSki), vulva (A-431), ovary (PA-1 and Caov-3), melanoma (SK-MEL-28), or in a variety of leukemic cell lines.

Recombinant Expression of the HER4 Kinase. To determine the binding characteristics of HER4, we stably overexpressed its complete coding sequence in CHO-KI cells. These cells lack any detectable EGFR, HER2, or HER3 by immunoblot, tyrosine phosphorylation, and immunoprecipitation analysis of ^{35}S-labeled proteins (data not shown). The HER4 protein was detected by immunoblot analysis on solubilized cells or membrane preparations by using an antiserum generated to a 19-amino acid region of the HER2 kinase domain, which coincidentally is identical to the HER4 sequence (residues 927–945). The recombinant HER4 migrated with an apparent M_r of 180,000, slightly faster than HER2, while the parental CHO cells showed no cross-reactive bands (data not shown). These cells were then used to assess ligand-specific binding and autophosphorylation of the HER4 tyrosine kinase. EGF, TGF-α, and amphiregulin are three related ligands whose growth-regulatory signals are mediated in part by their interaction with EGFR (7, 10, 17). All three ligands stimulated tyrosine phosphorylation of EGFR (for EGF, see Fig. 3*C*, lane 2), but not of HER4, HER2, or HER3 (Fig. 3 *A*, *B*, and *D*, lanes 2 and 3). On the basis of the structural homologies between EGFR family members and EGFR, we presumed these orphan receptors may bind yet-unidentified ligands.

Identification and Partial Purification of a Ligand That Specifically Activates the HER4 Kinase. In an effort to identify ligands specific for HER2, HER3, or HER4, we took advantage of the receptor expression profile of MDA-MB-453 cells. This cell line expresses HER2 and HER3 but contains no detectable EGFR (4, 26). In addition, *HER4* cDNA was first isolated from this cell line. Serum-free conditioned media from numerous human cancer cells were screened for growth-regulatory activity on MDA-MB-453 cells, and HepG2 human hepatocarcinoma cells were found to secrete

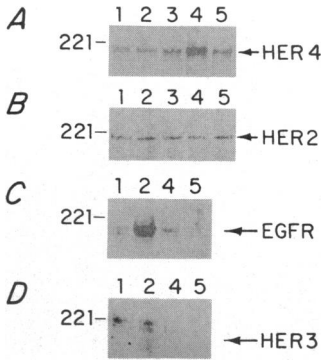

FIG. 3. Specific activation of HER4 tyrosine kinase by a breast cancer differentiation factor. Four recombinant cell lines were developed that each overexpress a single member of the EGFR family of tyrosine kinase receptors (EGFR, HER2, HER3, and HER4). These cells were then stimulated with various ligand preparations and assayed for receptor tyrosine phosphorylation. The cell lines include the following: *A*, CHO/HER4 #3 cells; *B*, CHO/HER2 cells; *C*, NRHER5 cells; and *D*, 293/HER3 cells. Lane 1, buffer control; lane 2, EGF at 100 ng/ml; lane 3, amphiregulin at 200 ng/ml; lane 4, 10 μl of phenyl-5PW column fraction 17; lane 5, 10 μl of phenyl-5PW column fraction 14. The sizes (in kDa) of the prestained molecular weight markers are indicated on the left of each gel. The phosphorylated receptor in each series migrates just below the 221-kDa marker.

a factor(s) that induced a morphologic differentiation in these breast cancer cells (Fig. 4). Whereas untreated MDA-MB-453 cells are moderately adherent, with a rounded morphology (Fig. 4*A*), the addition of semipurified material induced the cells to display a flat morphology with larger nuclei and increased cytoplasm (Fig. 4 *B* and *C*) and had minimal effects on cell growth. This differentiation factor(s) binds to heparin (data not shown) and elutes from a phenyl-5PW column at 1.0 M ammonium sulfate (fractions 16–18, Fig. 4*D*). Active fractions were also found to stimulate tyrosine phosphorylation of a 185-kDa protein in MDA-MB-453 cells (Fig. 4*E*); fraction 16 induced a 4.5-fold increase compared with the unstimulated control (Fig. 4*F*). This activity was then tested against a panel of cell lines that each overexpress a single member of the EGFR-family. Fraction 17 induced a significant and specific activation of the 180-kDa HER4 kinase (Fig. 3*A*, lane 4) without directly affecting the phosphorylation of HER2, EGFR, or HER3 (Fig. 3 *B–D*, lane 4). An adjacent fraction from the phenyl-5PW column (fraction 14) was used as a control and had no effect on the phosphorylation of any of these receptors (Fig. 3, lane 5).

DISCUSSION

This report describes the cloning and expression of HER4, an additional member of the EGFR family whose extracellular domain is most similar to HER3, whereas its cytoplasmic kinase domain is strikingly related to EGFR and HER2. We also describe the partial purification of a heparin-binding factor that specifically stimulates the intrinsic tyrosine kinase activity of this receptor while having no direct effect on EGFR, HER2, or HER3. In contrast, several ligands that bind the EGFR fail to interact with HER4. Owing to ubiquitous expression of EGFR, the presence of numerous EGFR ligands, and the recognition that crosstalk exists between related RTKs, we elected to set up assay systems that would permit us to look at specific interactions with each of these receptors in absence of crosstalk. For screening potential ligands, we have generated recombinant cell lines that each express only one member of the EGFR family. The heparin-binding factor described in this report activates only the cell line containing HER4 and not the parental CHO cells or the

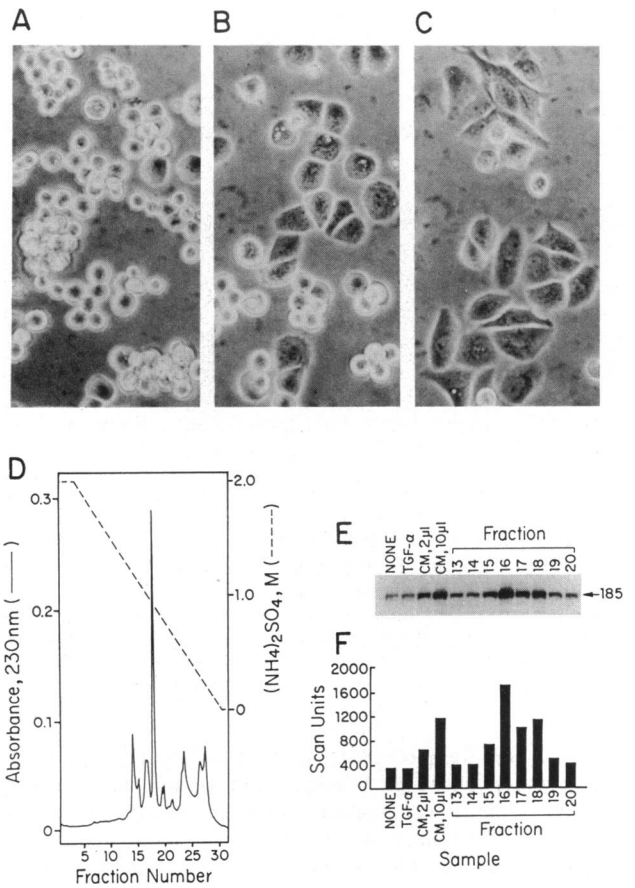

FIG. 4. Biological and biochemical properties of the MDA-MB-453-cell differentiation activity. (*A–C*) Induction of MDA-MB-453 differentiation. Protein in conditioned medium from HepG2 cells was cleared by precipitation with ammonium sulfate and the supernatant was dialyzed prior to being added to cells in the following amounts: *A*, control; *B*, 80 ng per well; *C*, 2.0 μg per well. (*D*) Phenyl-5PW column elution profile. (*E*) Phosphotyrosine immunoblot analysis of MDA-MB-453 cells stimulated with the following ligand preparations: None, media control; TGF-α, 50 ng/ml; CM, 16-fold-concentrated HepG2 conditioned medium tested at 2 μl and 10 μl per well; fraction, phenyl-5PW column fractions 13–20 (10 μl per well). (*F*) Densitometry analysis of the phosphorylated bands shown in *E*.

CHO cells expressing HER2, strongly suggesting that this is a direct ligand–receptor interaction. Further confirmation will require crosslinking and binding studies with homogeneous purified material.

Recently, several groups have reported the identification of specific ligands for HER2 (2, 3, 11–13). Some of these ligands, such as gp30 (11), interact with both EGFR and HER2, while others are reported to bind specifically to HER2 (2, 3). In contrast to these molecules, the cell differentiating activity described in this report shows no direct activation of HER2 and appears to transduce its signal by interaction with the highly related receptor HER4.

The identification of this member of the EGFR family and the availability of a ligand that specifically activates it will expedite the characterization of the biological function of HER4. Since EGFR and HER2 have been shown to act synergistically (27–29) it is conceivable that HER4 may also interact with other EGFR family members either by heterodimer formation or receptor crosstalk. Understanding the role of HER4 and its ligand in the process of growth and differentiation as well as in neoplasia might contribute to the development of new anticancer therapies.

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