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Biochemistry

Molecular cloning and expression of an additional epidermal growth factor receptor-related gene

(receptor tyrosine kinase/sequence/amphiregulin/ERBB2/ERBB3)

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ABSTRACT **Epidermal growth factor (EGF), transforming growth factor α (TGF-α), and amphiregulin are structurally and functionally related growth regulatory proteins. These secreted polypeptides all bind to the 170-kDa cell-surface EGF receptor, activating its intrinsic kinase activity. However, amphiregulin exhibits different activities than EGF and TGF-α in a number of biological assays. Amphiregulin only partially competes with EGF for binding EGF receptor, and amphiregulin does not induce anchorage-independent growth of normal rat kidney cells (NRK) in the presence of TGF-β. Amphiregulin also appears to abrogate the stimulatory effect of TGF-α on the growth of several aggressive epithelial carcinomas that overexpress EGF receptor. These findings suggest that amphiregulin may interact with a separate receptor in certain cell types. Here we report the cloning of another member of the human EGF receptor (HER) family of receptor tyrosine kinases, which we have named "*HER3/ERRB3*." The cDNA was isolated from a human carcinoma cell line, and its 6-kilobase transcript was identified in various human tissues. We have generated peptide-specific antisera that recognizes the 160-kDa HER3 protein when transiently expressed in COS cells. These reagents will allow us to determine whether HER3 binds amphiregulin or other growth regulatory proteins and what role HER3 protein plays in the regulation of cell growth.**

Epidermal growth factor (EGF), transforming growth factor α (TGF-α), and amphiregulin (1–3) all bind to the 170-kDa cell-surface EGF receptor (EGF-R), resulting in activation of its intrinsic kinase activity (3–6). Amphiregulin was originally identified from phorbol ester-treated human breast carcinoma cells (MCF-7) on its ability to inhibit the growth of several carcinoma cell lines while stimulating the proliferation of normal cells (7). The secreted form of amphiregulin is a 78-amino acid glycoprotein that is proteolytically cleaved from a larger transmembrane precursor (4). Amphiregulin contains a motif that is 38% identical with EGF and conserves most residues implicated as involved in binding to the EGF-R (3, 4). In addition, mature amphiregulin contains a distinct NH_2-terminal sequence of 43 predominantly hydrophilic amino acids, not present in other EGF-like proteins. Because related ligands often bind structurally similar receptors (8–10), we speculated that EGF, TGF-α, or amphiregulin may differentially interact with a homolog of EGF-R. After failing to show any interaction between these three secreted growth factors and HER2/neu, a known EGF-R-related protein, we initiated a search for other members of this family of receptor tyrosine kinases. This report describes the cloning and expression of human EGF receptor (HER)3,* a candidate receptor for EGF-like growth factors.

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MATERIALS AND METHODS

Cell Culture. All cells were obtained from ATCC and grown in Dulbecco's modified Eagle's medium (DMEM)/10% heat-inactivated fetal bovine serum.

cDNA Cloning. Total cellular RNA was extracted from A-431 (subclone A3), and MDA-MB-361 cells, and poly(A)$^+$ RNA was isolated. First-strand cDNA synthesis (11) was performed on A-431-A3 RNA with reverse transcriptase by using a 72-fold degenerate primer mixture based on the amino acid sequence (in one-letter code) YMIMVKCWMI (ARRD1). A cDNA library was constructed in λgt10, and 3.0×10^5 recombinants were screened in duplicate on nitrocellulose filters. Filters were first probed at low stringency with ^{32}P-labeled ARRD2, a 96-fold degenerate oligonucleotide. The filters were then stripped and reprobed at high stringency with ^{32}P-labeled EGFR300, a cDNA fragment corresponding to amino acids 863–944 of EGF-R (12). Of 69 clones that differentially hybridized to ARRD2 and EGFR300, three were found to encode an EGF-R-related protein (HER3), six clones encoded HER2 protein, and 10 clones spanned various regions of EGF-R. One HER3 clone was used as a probe to screen a λgt10 cDNA library derived from oligo(dT)-primed MDA-MB-361 RNA, and three additional cDNA clones were isolated—one with a 4.5 kilobase (kb) insert (pHER3-3b). To obtain the 5′-cDNA clone, we used the polymerase chain reaction (PCR) as follows. Single-stranded cDNA, synthesized from MDA-MB-361 RNA with S729ERR as primer, was dA-tailed and used as template for PCR amplification by using S720ERR, XSCT17, and XSC as primers (13). All cDNA clones and several PCR-generated clones were sequenced on both strands by using T7 polymerase with oligonucleotide primers (14).

The oligonucleotide mixtures (including their degeneracy or length and corresponding amino acid residues) used for priming and screening the λgt10 library were as follows (sequences in parentheses are in one-letter amino acid code):

ARRD1 5′-ATCATCCARCAYTTDACCATDATCATRTA-3′,
(72-fold, YMIMVKCWMI)
ARRD2 5′-GCCATCCAYTTDATNGGNAC-3′,
(96-fold, VPIKWMA)
S729ERR 5′-TCGTCGACTCCTTCACCACTATCTCA-3′,
(26-mer, EIVVKD)
S720ERR 5′-TGGCGTCGACCTATCTCAGCATCTCGGT-3′,
(28-mer, DRDAEIV)
XSCT17 5′-GACTCGAGTCGACATCGATTTTTTTTTTTTTTT-3′,
(35-mer)
XSC 5′-GACTCGAGTCGACATCG-3′,
(17-mer).

Abbreviations: EGF, epidermal growth factor; EGF-R, EGF receptor; TGF-α, transforming growth factor α; HER, human EGF receptor; PCR, polymerase chain reaction; nt, nucleotide(s).

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34309).

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

Expression of HER3 in COS Cells. The complete 4.1-kb *HER3* coding sequence was reconstructed and inserted into a pCDM8 (Invitrogen, San Diego) -based expression vector. The resulting plasmid (cHER3x) was grown in competent *Escherichia coli* MC1061/P3 bacteria (15) and was introduced into COS-1 cells by using the DEAE-dextran method (15).

Immunoblotting. Cell pellets were harvested 72–96 hr after transfection; one hundred μg of protein was separated by using 7% SDS/PAGE and analyzed by immunoblotting with polyclonal antisera generated against synthetic peptides (16) specific to the kinase domain of HER3 (PPDDKQLLYSEAKT, amino acids 841–854; GAEPYAGLRLAEVPD, amino acids 889–903; sequences shown using single-letter code).

RESULTS

Amphiregulin Purified from MCF-7 Cells Is Not a Ligand for HER2/neu. EGF-R shares structural homology with HER2/neu, the protein product of *ERBB2* protooncogene: 83% amino acid identity exists in the cytoplasmic kinase domain, and 42% homology is present in the extracellular ligand-binding domain, where 49 of 50 cysteines are conserved (12, 17). Therefore, we sought to establish whether amphiregulin interacted with this putative receptor and homolog of EGF-R, because the ligand for HER2/neu has not been identified. Experiments were performed on cells expressing high levels of human (SKBR-3 and BT474) HER2 or rat (B104-1-1 and DHFR-G8) neu protein. Direct binding, *in vitro* kinase assays, proliferation assays, *in vivo* phosphorylation, and receptor down-regulation assays (18) all showed no association between the 78-amino acid form of amphiregulin and HER2/neu (G.D.P., M.S., Y. Yarden, G.J.T., and R. Weinberg, data not shown). The ligand for HER2/neu remains elusive.

Isolation of cDNA Clones Encoding an EGF-R-Related Gene. Northern (RNA) blot analysis of A-431 and human placental RNA provides evidence for the existence of other EGF-R-related transcripts (12, 17). A subclone of the A-431 cell line (A-431-A3) was isolated after growth in soft agar and was characterized to have 30-fold-increased sensitivity to amphiregulin inhibition as compared with the parental line, yet had no alteration in the number or affinity of EGF-Rs as measured by binding competition assays (7). Poly(A)$^+$ RNA was prepared from these cells, and a cDNA library was constructed by specific priming with a 72-fold degenerate oligonucleotide complementary to a stretch of 10 amino acids unique to the kinase domains of EGF-R and HER2/neu. A 96-fold degenerate oligonucleotide probe, based on an upstream region of 7 amino acids conserved in EGF-R and HER2/neu, was used to screen the cDNA library at low-stringency conditions. EGF-R clones were eliminated by rehybridization at high stringency with a short EGF-R cDNA probe spanning the same region of the kinase domain. Sixty-nine clones were partially sequenced, revealing three clones that encoded a closely related, yet distinct, member of the ERBB/EGF-R subfamily of receptor tyrosine kinases. A survey of several human tumor cell lines identified the breast carcinoma cell line, MDA-MB-361, to be a more abundant source of the 6-kb transcript than A-431-A3 cells, so it was used to isolate overlapping cDNA clones spanning the entire coding sequence of *HER3*. The HER3 transcript was also detected in several primary breast carcinomas and in normal colon, kidney, breast, and brain RNA (data not shown).

The nucleotide sequence for *HER3* contains an open reading frame coding for 1342 amino acids beginning with a consensus-initiating methionine at nucleotide (nt) 199 (Fig. 1). The amino acids downstream of this methionine have the characteristics of a signal sequence; the mature protein is predicted to begin at Ser-20, followed by 1323 amino acids with a calculated M_r of 146,000. The 4026-nt coding region is flanked by 198 nt of 5′ and 755 nt of 3′ untranslated sequences. No poly(A) tail is present, suggesting this is a partial cDNA sequence.

Structural Domains of HER3. HER3 has all the structural features of receptor tyrosine kinase (19), with a single hydrophobic stretch of 32 amino acids characteristic of a transmembrane region that separates the sequence into a 612-residue extracellular ligand-binding domain and a 677-amino acid COOH-terminal cytoplasmic domain strongly homologous with other members of the tyrosine kinase family (Figs. 2 and 3). The ligand-binding domain can be divided into four subdomains (I–IV), including two cysteine-rich regions (II, IV) that conserve all 48 cysteines present in the corresponding regions of the EGF-R (12) and two flanking domains (I and III) that may define specificity for ligand binding (21). HER3 shares 40–50% identity with EGF-R and 40–45% identity with HER2/neu in each of these subdomains (12, 17). There are 10 potential *N*-linked glycosylation sites in the extracellular domain of HER3, conserving 5 of 12 potential sites in EGF-R and 3 of 8 sites in HER2/neu. The cytoplasmic domain includes the following: a stretch of basic residues flanking the membrane-spanning region; a consensus ATP-binding site (Gly-Xaa-Gly-Xaa-Xaa-Gly-Xaa$_n$-Lys), and sequences homologous to other members of the tyrosine kinase family (22); an acidic helical motif similar to the domain of EGF-R that confers receptor internalization and ligand-dependent calcium influx (23); and a hydrophilic COOH-terminal tail containing 13 tyrosines (Fig. 3). The kinase domain of HER3 is most similar to EGF-R and HER2/neu (60 and 62%) and shared lower homology (26–34%) with representatives of other classes of tyrosine kinases (Fig. 2). EGF-R and HER2/neu have 83% amino acid sequence identity between their kinase domains, suggesting that, in this domain, they are more closely related to each other than they are to HER3.

The cytoplasmic domain of HER3 also has several unique features (Fig. 3). Protein kinase C-induced phosphorylation of EGF-R occurs primarily on Thr-654, whereas Thr-669 has been implicated as a major site for phosphorylation of EGF-R in response to EGF (24, 25). Both of these residues are conserved in HER2/neu, but both are altered in HER3 (Ala-657 and Asp-672). Recent studies using EGF-R mutants have shown that preventing phosphorylation at either residue has no effect on EGF binding; however, in certain cell lines, phosphorylation of EGF-R Thr-654 by phorbol esters appears to block EGF-induced mitogenesis (24, 25). HER3 already contains these "mutations" and may lack this negative control mechanism induced by protein kinase C.

Two additional atypical amino acid changes are present in the HER3 kinase domain (Fig. 3). A sequence comparison of protein kinases reveals certain residues that are either highly or completely conserved (22). HER3 contains most of these amino acids including Gly-697, Gly-699, Val-704, Lys-723, Asn-820, Asp833–Phe834–Gly835, Glu-862, Asp-874, Gly-879, and Arg-936; however, HER3 has nonconservative substitutions at Cys-721, His-740, and Asn-815. The latter two amino acids are present as glutamate and aspartate, respectively, in all known protein kinases—serine/threonine as well as tyrosine kinases (Figs. 2 and 3). Strict conservation of these residues suggests that they are important for catalytic activity, and the amino acid changes at these positions in HER3 indicate it might have altered kinase activity.

The COOH-terminal 353 amino acids of HER3 contain 13 tyrosines, several of which are flanked by numerous charged residues. These features are characteristic of the autophosphorylation domains of EGF-R (26) and HER2/neu (27), yet this region of HER3 shares no significant primary sequence homology to either of these two family members and is ≈30–50% longer. The sequence Tyr-Glu-Tyr-Met is repeated

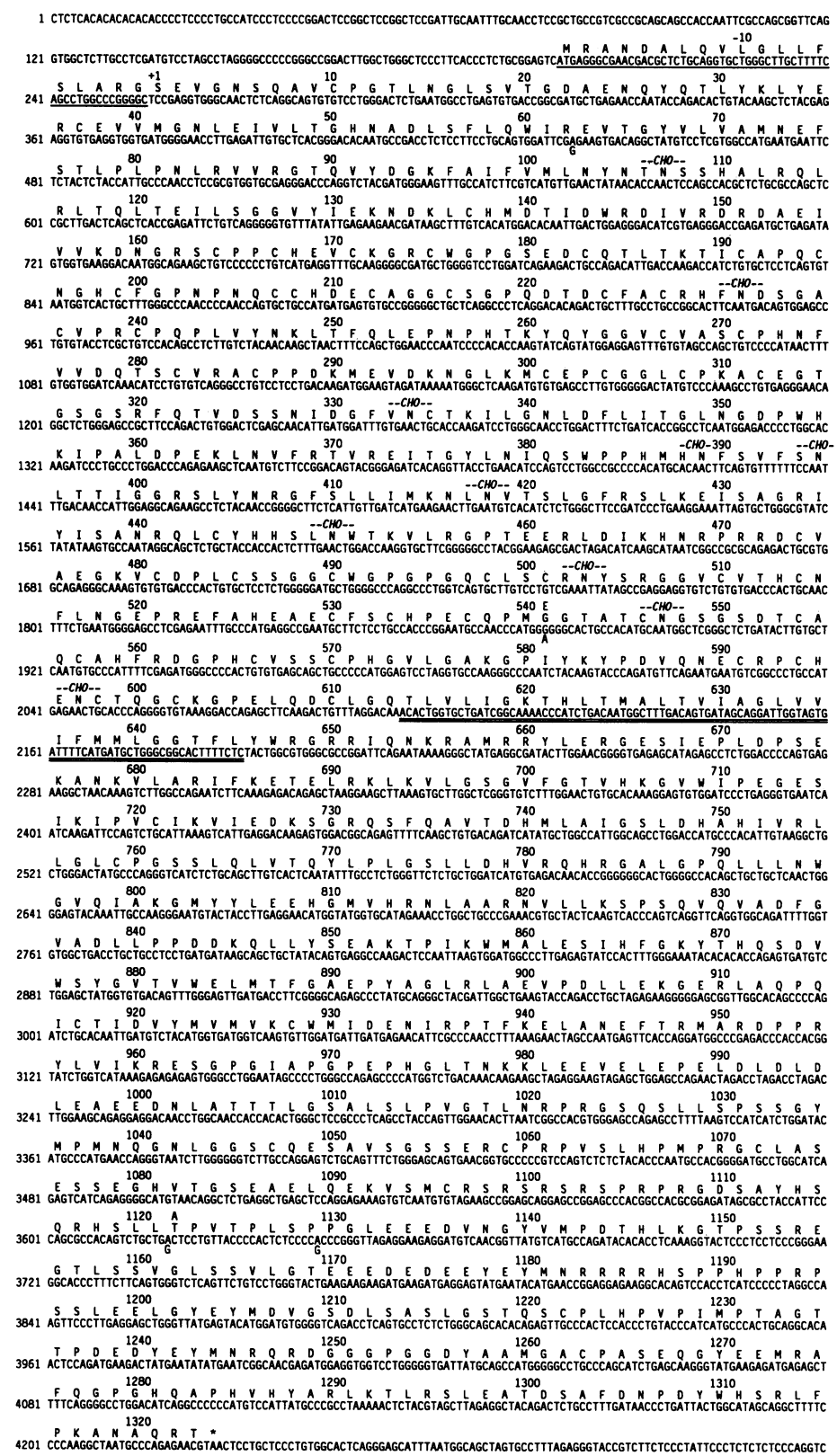
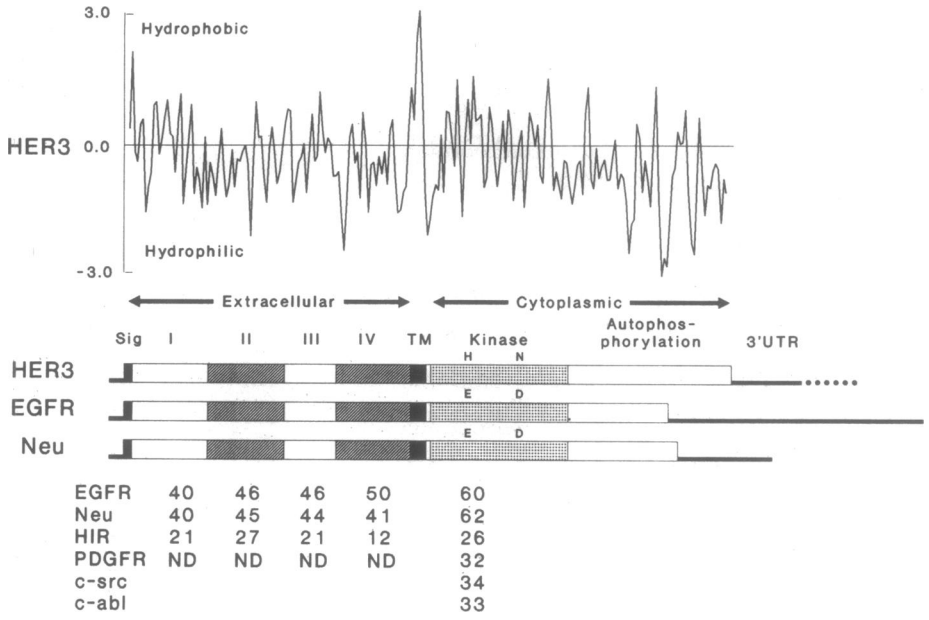


FIG. 1. Nucleotide sequence and deduced amino acid sequence of human HER3 (1342 residues). Nucleotides are numbered at left, and amino acids (in one-letter code) are numbered above the sequence. The 19-residue signal sequence is singly underlined, and the predicted NH_2 terminus of the mature protein is indicated by +1. The transmembrane domain is doubly underlined at amino acids 614–645. Potential N-linked glycosylation sites (Asn-Xaa-Ser or Asn-Xaa-Thr) are denoted with -CHO-. No poly(A) tail was present in the clones. This sequence represents a consensus based on cDNA and PCR clones isolated from MDA-MB-361 RNA and was used in subsequent expression constructs. Clonal sequence differences are listed below the consensus residue, including Ade-441 to guanine in one of four PCR clones, Gua-1877 to adenine from one A-431 cDNA clone, Ade-3619 to guanine in one cDNA clone, and Ade-3642 to guanine in one cDNA clone.

three times in this domain of HER3, possibly the result of gene duplication.

Expression of HER3 in COS Cells. To determine the biochemical properties of HER3, we inserted its complete coding sequence into an expression vector under the control of the cytomegalovirus immediate-early promoter (15). This vector (cHER3x) was transfected into COS cells, and the transiently expressed protein was detected by immunoblot analysis using antisera specific to the cytoplasmic domain of HER3. The recombinant protein migrated with an apparent



EGFR	40	46	46	50	60
Neu	40	45	44	41	62
HIR	21	27	21	12	26
PDGFR	ND	ND	ND	ND	32
c-src					34
c-abl					33

FIG. 2. Hydropathy profile (20) of HER3 and comparison of protein domains for HER3 (1342 amino acids), EGF-R (1210 amino acids), and HER2 (Neu) (1255 amino acids). Signal peptide and transmembrane domains are represented by filled boxes, the cysteine-rich extracellular subdomains are hatched, and the cytoplasmic tyrosine kinase domain is stippled. Amino acids unique to the HER3 kinase domain (histidine, asparagine) compared with other protein kinases (glutamic and aspartic residues) are indicated using the single-letter code. The percent amino acid sequence identity between HER3 and representatives of other classes of tyrosine kinases are indicated. Sig, signal peptide; I, II, III, and IV, extracellular domains; TM, transmembrane domain; 3′ UTR, 3′ untranslated region; HIR, insulin receptor; PDGFR, platelet-derived growth factor receptor; ND, not determined.

M_r of 160,000, slightly less than the EGF-R (Fig. 4). The abundance of EGF-R in COS cells complicated efforts to assess whether EGF-like ligands bind HER3. Stable expression of HER3 in cells lacking EGF-R will allow us to determine whether EGF, TGF-α, or amphiregulin interact with this putative receptor.

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HER3   -19  MRANDAL--QVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLP
EGFR   -24  ••PSGTAGAAL•A••AA•CPA•RALEEKK••Q••S•K•TQL•TF•DHFLS•QRMFNN•••L•••••TYVQR•Y•••••KT•Q••A••••I•L•TVERI•
NEU      1  MELAALCRW•••LA•LPPGAASTQ----T••DMK•RLPASP•THLDM•RH••QG•Q••Q••••LTYLPT••S•••••D•Q••Q••••I•H•QVRQV•

HER3    80  LPNLRVVRGTQVYDGKFAIFVMLNYNT-NSS--------HALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVRDRDAEIVVKD--N-GRSCPPC
EGFR    77  •E••QII••NMY•ENSY•LA•LS••DA-•KT---------G•KE•PMRN•Q•••H•A•RFSN•PA••NVES•Q•••••SSDFLSNMSM•FQ•HLG••QK•
NEU     96  •QR••I•••••LFEDNY•LA•LD•GDPL•NTTPVTGASPGG••E•Q•RS•••••K•••L•QR•PQ••YQ•••L•K••FHKNNQLALTLIDT•RS•A•H••

HER3   168  HEVCK-GRCWGPGSEDCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEPNPHTKYQYGGV
EGFR   167  DPS•PN•S•••A•E•N••K•••I•••Q••S•R•R•KS•SD•••NQ••A••T••RES••LV••K•R•EAT•KDT••PLML••PT•Y•MDV••EG••SF•AT
NEU    196  SPM••GS••••ES•••••S••R•V••GG•A-R•K••L•TD•••EQ••A••T••KHS••L••L•••H••I•ELH••ALVT••TD••ESM•••EGR•TF•AS

HER3   267  CVASCPHNFVV-DQTSCVRACPPDKMEVD-KNGLKMCEPCGGLCPKACEGTG--SGSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDP
EGFR   267  ••KK••R•Y••T•HG••••••GA•SY••MEED•VRK•KK•E•P•R•V•N•I•IGEFKDSLSINAT••KH•K•••S•S•D•HI•PVAFR••SFTHT•P•••
NEU    295  ••TA••Y•YLST•VG••TLV••LHNQ••TAED•TQR••K•SKP•ARV•Y•L•MEHLREVRA•T•A••QE•AG•K••F•S•A••PESFD•••ASNTAP•Q•

HER3   363  EKLNVFRTVREITGYLNIQSWPPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGRIYISANRQLCYHHSLNWTKVLRGPTEER
EGFR   366  QE•DILK••K••••F•L••A••ENRTDLHA•E••EI•R••TKQHGQ•••AVV-S••I••••L•••••••D•DVI••G•KN•••ANTI••K•LF-•TSGQK
NEU    395  •Q•Q••E•LE••••••Y•SA••DSLPDL•••Q••QV•R••I•H•GAY••TLQ-G•GISW••L•••R•LGS•LAL•HH•TH••FV•TVP•DQLF-•NPHQA

HER3   463  LDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSCRNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQPMGGTATCNGSGSDTCAQCAHFRD
EGFR   464  TK•IS••GENS•K•T•Q••HA•••PE•••••E•RD•V••••V•••RE••DK•KL•E••••••VENS••IQ•••••L•QAMNI••T•R•P•N•I••••YI•
NEU    493  •LHTA•••EDE••G••LA•HQ••ARRALL•S••T••VN•SQFL••QE••EE•RV•Q•L•••YVNARH•LP•••••••QN•SV••F•PEA•Q•VA•••YK•

HER3   563  GPHCVSSCPHGVLGAKG--PIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKTHLTMALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRR
EGFR   564  •••••KT••A••M•ENN-TLVW••A•AGHV•HL••P•••Y••T••G•EG•PTN-GPK•PSIATG•VGALLLL•••AL-----IG•FM•R•H•VR••TL••
NEU    593  P•F••AR••S••KPDLSYM••W•F••EEGA•Q••PI•••HS•VDLDDKG•PAEQRASPLTSIVSAVVG--IL•••VLGVVF•I-•IK•RQQKIR•YT•••

HER3   661  YLERGESIEPLDPS-EKANKVLARIFKETELRKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLC
EGFR   658  L•QER•LV•••T••G•AP•QA•L••L••••FK•I••••••A••••Y••L••••••KV••••A••ELREATSPKANKEIL•EAYVMA•V•NP•VC••••I•
NEU    690  L•QET•LV•••T••GAMP•QAQM••L•••••••V••••••A••••Y••I•••D••NV••••A•••LRENTSPKANKEIL•EAYVMAGVGSPYVS••••I•

HER3   760  PGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWM
EGFR   758  LT•TV••I••LM•F•C•••Y••E•KDNI•S•Y••••C•••••••N•••DRRL•••D•••••••V•T•QH•KIT•••L•K••GAEE•EYHAEGG•V•••••
NEU    790  LT•TV•••••LM•Y•C••••••EN••R••S•D••••CM••••••S•••DVRL•••D•••••••V•••NH•KIT•••L•R••DI•ETEYHADGG•V•••••

HER3   860  ALESIHFGKYTHQSDVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDPPRYLVI
EGFR   858  •••••LHRI•••••••••••••••••••SK••D•IPAS•ISSI•••••••P••P••••••••I••••••••ADS••K•R••II••SK•••••Q•••••
NEU    890  •••••LRRRF•••••••••••••••••••K••D•IPAR•I••••••••••P••P••••••••I••••••••SEC••R•R••VS••S••••••Q•FV••

HER3   960  KRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSSGYMPMNQGNLGGSCQESAVSGSSERC
HER3  1060  PRPVSLHPMPRGCLASESSEGHVTGSEAELQEKVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPDTHLKGTPSSREGTLS
HER3  1160  SVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAA
HER3  1260  MGACPASEQGYEEMRAFQGPGHQAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT
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FIG. 3. Protein sequence comparison between members of the human EGF-R family. Sequences are displayed using the single-letter code, and identical residues are denoted with dots. Gaps were introduced for optimal alignment and are shown by a dash. Signal sequences are bounded by single lines, transmembrane domains are bounded by double lines, and sequences used to derive the probes and primers (ARRD1, ARRD2) used for cloning are underlined. Cysteine residues are marked with stars, the potential ATP-binding site is shown with circled crosses (Gly-Xaa-Gly-Xaa-Xaa-Gly beginning at residue 697 and Lys-723), COOH-terminal tyrosines are denoted with open triangles, and additional residues of HER3 referred to in the text are marked with arrows (Ala-657, Cys-721, His-740, and Asn-815). Neu, HER2/neu.

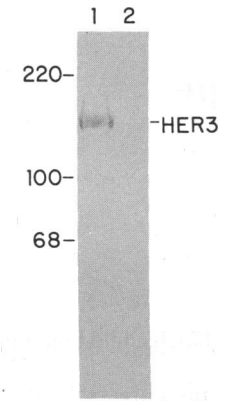

FIG. 4. Immunoblot analysis of the recombinant HER3 transiently expressed in COS cells. Lanes: 1, HER3-transfected COS cells; 2, cDM8 mock-transfected COS cells. Note presence of a 160-kDa band.

DISCUSSION

Previous biochemical and biological evidence suggested that members of the EGF/TGF-α/amphiregulin family are not functionally equivalent (3, 28). For example, TGF-α has an activity comparable to that of EGF in many *in vitro* assays: TGF-α and EGF bind with similar affinity to EGF-R, resulting in autophosphorylation of EGF-R; both ligands induce similar changes in intracellular pH and calcium levels; and both transduce comparable proliferative signals in many cell types; however, TGF-α appears more potent than EGF in several *in vivo* assays, including the promotion of bone resorption, wound healing, and angiogenesis (28). Likewise, amphiregulin can be distinguished from EGF/TGF-α based on EGF-R binding studies and by a number of biological assays (3).

The identification of another member of the EGF-R subclass of receptor tyrosine kinases reveals a greater repertoire by which the EGF/TGF-α/amphiregulin family of ligands might exert their diverse growth regulatory signals. Conceivably, some differences in biological activity between amphiregulin and EGF/TGF-α may result from the ability of amphiregulin to interact differentially with members of the EGF-R family. Because EGF-R and HER2/neu have been shown to act synergistically (29), HER3 may interact with either of these two receptors. Moreover, the disparity in response to these ligands may be reflected in the less conserved cytoplasmic region of HER3 compared with EGF-R and HER2/neu. The COOH-terminal region of EGF-R appears to act as a competitive inhibitor for exogenous substrate phosphorylation and thereby serves to fine-tune its kinase activity (26, 27). The more conserved homology between the cytoplasmic regions of EGF-R and HER2/neu, as opposed to HER3, suggests these two receptors share a more similar function, whereas the distinctiveness of HER3 indicates it may be subject to different regulation and might signal an alternate biochemical response.

During review of this manuscript, the sequence of a cDNA encoding ERBB3 was reported (30). The amino acid sequence of ERBB3 is identical to that of HER3 except for two residues; both Gly-541 and Gly-1045 of HER3 were deduced to be glutamic acid (Glu-541, Glu-1045) in ERBB3. We have noted four polymorphic purine residues among our HER3 cDNA clones (see Fig. 1). Two of these differences alter the deduced amino acid sequence (Glu-541 and Ala-1122), with one change corresponding to a difference between the HER3 and ERBB3 sequences.

The isolation of HER3 cDNA will facilitate the investigation on its function in normal cells and in various proliferative disorders including neoplasia. Site-directed mutagenesis and expression of modified HER3 in mammalian cells will make possible the unraveling of the biochemical mechanism of its putative ligand(s), including amphiregulin. The generation of chimeric receptors consisting of different domains of the three EGF-R family members as well as other receptor tyrosine kinases will further help to define the signal-transduction pathways used by these receptors and their cognate ligands. Conceivably, the multiplicity of receptor and ligand types provides an organism with a better means to regulate homeostasis under various physiological and pathological conditions.

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