Expression of erbB-4/HER4 Growth Factor Receptor Isoforms in Ovarian Cancer

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ABSTRACT

Immunohistochemical expression of erbB4 protein was identified in 93% (49 of 53) of ovarian cancers using the HFR-1 antibody (targeted to the cytoplasmic domain of the erbB4 receptor) and in 89% (47 of 53) of ovarian cancers using the H4.77.16 antibody (targeted to the extracellular domain). Tumors of serous histology were more likely to express a higher level of erbB4 than endometrioid tumors, and for stage III serous tumors, long-term survival was associated with moderate to high coexpression of erbB4 and erbB2. Within ovarian cancer cell lines, high erbB4 expression was associated with cisplatin resistance. Using reverse transcription-PCR, the presence of multiple isoforms of erbB4 mRNA was identified in both ovarian primary tumors and cell lines. Splice variants in the juxtamembrane (JM-a and JM-d) and cytoplasmic (CT-a and CT-b) regions were identified in mRNA of both cell lines and primary tumors. The use of an anti-erbB4 blocking antibody suggested that erbB4 was not the mediator of the growth stimulatory effects of neuregulin in ovarian cancer cells and indeed could potentially antagonize this effect.

INTRODUCTION

The Mr 180,000 erbB4 (HER4) protein (1) is the most recently identified member of the erbB receptor family that includes the EGF receptor (erbB1), erbB2, and erbB3. Cloned in 1993, it shares extensive homology with erbB3 in its extracellular domain, and within its cytoplasmic domain, it has 77–79% identity with the EGF receptor and erbB2. Both erbB4 and erbB3 are activated by the neuregulin (or heregulin) family of ligands (2, 3), whereas erbB4 can also be activated by betacellulin (4), heparin-binding EGF (5), and epiregulin (6). The activated receptors interact with a variety of signaling pathways that ultimately may lead to cell division, growth inhibition, differentiation, and chemotaxis.

Recent studies have demonstrated that overexpression of both the EGF receptor and erbB2 are associated with poor prognosis in ovarian cancer (7–10). The erbB3 receptor is also present in the majority of ovarian cancers (11, 12), but similar to erbB4, little is known of its expression and function in this disease. erbB4 has been shown to be expressed in many adult and fetal tissues, including the lining epithelia of the gastrointestinal, urinary, reproductive, and respiratory tracts as well as the skin, skeletal muscle, circulatory, endocrine, and nervous systems (13). The adult ovarian surface epithelium is weakly positive, whereas the stroma is nonreactive (13).

Recently, splice variants of erbB4 have been identified that encode sequence differences in the juxtamembrane and cytoplasmic regions of the protein, and these are likely to possess differing functions (14–19). Sequencing of full-length erbB4 cDNAs revealed the existence of two isoforms that differed by insertion of either 23 or 12 alternative amino acids in the extracellular JM region (14). Although both isoforms (JM-a and JM-b) are activated by erbB4 ligands, they are variably expressed in human tissues and are differentially processed in response to phorbol ester, consistent with representing protein kinase C-activated cleavable and noncleavable forms (14, 15). In addition, two novel JM splice variants (JM-c and JM-d) have been identified in medulloblastoma cells, and these represent isoforms lacking and including, respectively, both the a and b sequences (16). A second modification has been identified in the cytoplasmic portion of the receptor representing a deletion of 48 bp (17–19). Within this region, a PI3-K binding site could mediate cell survival and chemotaxis (19).

Although ovarian carcinoma cells have occasionally been used in studies examining the function of the erbB4 protein (20–22), no systematic study has yet described the expression of this receptor in this disease or obtained information on its function. To address this, we have explored the expression of erbB4 protein in primary cancers and cell lines and have then examined which splice variants are present. The availability of blocking antibodies now allows some testing of its function.

MATERIALS AND METHODS

Cell Lines. The PE01, PE01CDH, PE04, PE06, PE014, and PE016 cell lines were developed at the Edinburgh Medical Oncology Unit (23). The OVCAR-3, OVCAR-4, and OVCAR-5 cell lines were kindly donated by Dr. T. Hamilton (Fox Chase Institute, Philadelphia, PA). The 41 M, OAW-42, 59 M, and A2780 cell lines were obtained from the European Tissue Collection (Porton Down, United Kingdom). The SKOV-3, CAOV-3, and SW626 cell lines were obtained from the American Type Culture Collection (Manassas, VA). All cell lines were routinely cultured at 37°C, 90% humidity, and 5% CO2 in RPMI 1640 (Life Technologies, Inc., Paisley, United Kingdom) containing 10% heat-inactivated FCS, 100 μg/ml streptomycin, and 100 IU/ml penicillin.

Tumor Samples. Fresh primary ovarian tumor tissue was obtained from 53 patients with epithelial ovarian cancer at initial debulking surgery, transferred to liquid nitrogen, and then formalin fixed and embedded in paraffin. Tumor histology was assessed on paraffin-embedded sections and classified according to WHO criteria. Tumor histologies were classified as follows: serous adenocarcinoma (29 tumors), endometrioid adenocarcinoma (18 tumors), and clear cell carcinomas (6 tumors). Information on stage was available for 48 patients and on grade of differentiation for 49 patients.

Immunohistochemistry. Sections (3 μm) were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by incubating sections in 3% H2O2 for 30 min. Sections were immersed in citric acid buffer (0.005 M, pH 6.0) and microwaved for 3 × 5 min. Slides were washed in 0.05 M Tris/NaCl buffer (pH 7.6) and then incubated in 20% FCS for 10 min. Primary antibodies were added for 1.5–2 h. For the detection of erbB4, two antibodies were used, HFR-1 and H4.77.16. The immunogen for the HFR-1 antibody was the synthetic peptide corresponding to amino acids 1249–1264 (RSTLQIIH-DYLQEST; Ref. 13), whereas the immunogen for H4.77.16 was the extra-
cellular fragment corresponding to amino acids 1–625 (24). H4.77.16 (Ab-1) was obtained from Neomarkers, Inc. (Fremont, CA). To detect erbB2, CB-11 (1:40 dilution; Neomarkers) was used. After primary antibody incubation, sections were washed in Tris/NaCl buffer. A streptavidin-biotin multilink method (StrAviGen Multilink kit; Biogenex, San Ramon, CA) was used for detection of reactivity. Sections were stained with secondary multilink antibody (1:20 dilution for 30 min), followed by horseradish peroxidase-labeled streptavidin complex (1:20 dilution for 30 min). Diaminobenzidine tetrachloride was used as chromagen and applied for 5 min. Sections were lightly counterstained in hematoxylin, dehydrated, and mounted. A section of human skin was used as a positive control because this tissue has reported previously to show a characteristic pattern of erbB-4 reactivity (13). Negative controls for each tumor section were included in all runs by replacing the primary antibody with Tris buffer. Reactivity was measured using a semiquantitative scale of 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), relative to the positive control section. Slides were assessed by two observers independently, and assessment was concordant in 90% of readings. Where there were differences in the magnitude, these were reviewed by the two observers to obtain an agreed score.

RT-PCR. Total cellular RNA was extracted from cells in log phase growth using TRI reagent (Sigma, Poole, United Kingdom). Samples were treated with 20 units/50 µl of DNase I (Boehringer Mannheim, East Sussex, United Kingdom) to remove genomic DNA contamination. RNA was then re-extracted using a phenol/chloroform protocol. Reverse transcription was performed with a first-strand cDNA synthesis kit (Boehringer Mannheim) using the oligo dT primer provided. One µg of RNA yielded 20 µl of cDNA, of which 2.5 µl was used for each subsequent PCR reaction with each primer pair. PCR reactions were performed in a final volume of 25 µl containing the following: 1 X PCR buffer, 1.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate mixture, 1.25 units of Taq polymerase (Imperial Cancer Research Fund Clare Hall, South Mimms, United Kingdom), 400 nm of each primer (Imperial Cancer Research Fund). The PCR conditions for actin were as follows: step 1, 94°C for 2 min; step 2 consisted of 35 cycles of 94°C for 30 s, 57°C for 45 s, and 72°C for 45 s; and step 3, 72°C for 5 min. The annealing temperature was modified for each primer pair to optimize final yield: for erbB4-JM and erbB4-CT primers, 52°C; for total erbB4, 61°C.

The following primers were used: total erbB4, AGTTTTCAAGGATGCTCTTACCTGTTGGCG and ACTGGCCCTCGAGACCCTTCTG; CT, CTGATCGACCACTAGTCCCGGTGC; JM, CTGACGTTAGGAGATTGTGGGCTGT; erbB4-CT primers, 52°C; for total erbB4, 61°C.

Fig. 1. Diagrammatic representation of the erbB4 receptor and the regions of DNA where alternate splicing gives rise to the variant isoforms. Alternate splicing in the JM region gives rise to four possible sequences: JM-a alone, JM-b alone; JM-c (wherein both JM-a and JM-b are absent); and JM-d (where both JM-a and JM-b are present). Alternate splicing in the cytoplasmic domain can produce either a sequence (CT-a) that encodes a PI3-K binding site or a region (CT-b) that lacks this sequence. Sites to which antibodies (H4.77.16, H4.72.8, HFR-1, and Ab-2) and PCR primers (JM, CT, and Total erbB4) are targeted are indicated, as are regions in the molecule encoding tyrosine kinase activity (TK) and cysteine-rich sites.

RESULTS

erbB4 Expression in Primary Ovarian Tumors. erbB4 immunoreactivity was investigated in 53 ovarian carcinomas. Two antibodies targeting erbB4 were used: HFR-1, which detects a region in the cytoplasmic domain of the receptor; and H4.77.16, which detects an...
The extracellular domain (Fig. 1). Positive cytoplasmic staining was observed in 93% of the tumors using the HFR-1 antibody, 19% of which stained strongly, 42% moderately, and 32% weakly (Table 1). Using the H4.77.16 antibody, 89% of tumors were positive with 13% demonstrating strong staining, 27% moderate staining, and 49% weak staining (Table 1). In all cases, staining was relatively homogeneous throughout the tumor, with virtually all (>90%) tumor cells showing a similar intensity of staining. Both antibodies gave very similar staining results for individual tumors (P < 0.003 by paired t test), and examples are shown in Fig. 2.

Table 1  erbB4 expression in primary ovarian cancer

<table>
<thead>
<tr>
<th>Staining intensitya</th>
<th>HFR-1</th>
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a Homogeneous staining was observed in epithelial cells, and the staining intensity is shown.
b x2 test.
c x2 test for trend (serous versus endometrioid).

Differential expression was observed between the two predominant histologies of ovarian adenocarcinoma with serous adenocarcinomas more likely to express higher levels of erbB4 than endometrioid tumors (for HFR-1 staining, P = 0.026; for H4.77.16 staining, P = 0.002, x2 for trend; Table 1). Neither stage nor grade of differentiation could be associated with the erbB4 expression level (Table 1). Using semiquantitative RT-PCR to measure total erbB4 and γ-actin, a ratio of erbB4:γ-actin mRNA was obtained, and the difference in erbB4 expression between serous and endometrioid tumors was again apparent (P = 0.017, Mann-Whitney; Fig. 3).

For the serous adenocarcinoma group, 23 tumors were stage III and moderately poorly differentiated. Because histology, stage, and grade are major determinants of survival in this disease, this was the only group wherein the effect of erbB4 on survival could be assessed while these other prognostic variables remained constant. Although the expression of erbB4 alone did not relate to survival in this group, the 5 long-term survivors (>2.5 years) in this group of 23 had moderate-to-high expression of both erbB4 and erbB2 in contrast to only 7 of 18 who survived for <2.5 years (P = 0.037, Fisher’s test), suggesting that the coexpression of both receptors might relate to good prognosis in this subtype.

erbB4 Expression in Ovarian Carcinoma Cell Lines. Western blot analysis using the Ab-2 antibody indicated variation of erbB4 expression within a series of 16 ovarian carcinoma cell lines, with the PE04, PE06, OVCAR-3, and OVCAR-4 cell lines expressing high levels of erbB4; OAW42 and A2780 expressing moderate levels of erbB4; and SKOV-3 and PE014 cells expressing low levels of erbB4 (Fig. 4). The other 8 cells lines (PE01, PE01CDDP, PE016, OVCAR-5, SW626, 41 M, 59 M, and CAOV3) expressed levels that were undetectable by this method.

Three of these cell lines (PE01, PE04, and PE06) were developed directly from material obtained at three different time points from a

A. HFR-1

B. H4.77.16

Fig. 2. Immunohistochemical expression of erbB4 in an ovarian cancer section. A, HFR-1 antibody. B, H4.77.16 antibody. Note that the epithelial cells within these sections are diaminobenzidine positive (brown). The counter stain is hematoxylin (blue). ×400.
single patient. We observed that the cell lines (PE04 and PE06), which were obtained after clinical resistance to cisplatin treatment had developed, possessed markedly higher levels of erbB4 than in the initial cell line (PE01), which was sensitive to cisplatin, although this increase was not apparent in an in vitro-derived cell line (PE01 CDDP).

Of the other 12 lines investigated (from 12 independent patients), treatment data have been reported for 11 of these (no data on SW 626). Of these 11, only 3 cell lines (OVCAR-3, OVCAR-4, and OAW42) had been obtained from patients after exposure to cisplatin, and all 3 had been obtained at a stage at which the patients had developed clinical resistance to this drug. These 3 lines again were the highest expressors of erbB4, suggesting a possible link between cisplatin treatment/resistance and increased erbB4 expression.

**RT-PCR Analysis of erbB4 Isoforms in Ovarian Cancers and Cell Lines.** Several splice variants of erbB4 have been reported recently, which are predicted to initiate different signaling events and may have differing functionality. It was therefore of interest to define which forms were present in ovarian cancers and cell lines. mRNA from these tissues was isolated, subjected to reverse transcription, and analyzed by primers flanking the variable regions.

For the JM region, the expected product size for the JM-a-derived cDNA was 375 bp; for the JM-b cDNA, 345 bp; and for the JM-d cDNA, 414 bp. After separation on a 2.5% agarose gel, bands corresponding to JM-a and JM-d were observed in the cell line panel (Fig. 5A). As a control, the same reverse transcription products were probed with primers targeted to γ-actin, and a 250-bp product was identified in all samples (Fig. 5C). The identities of these amplified RT-PCR...
products (JM-a and JM-d) were confirmed by cloning the cDNA products into a P-Gem-T Easy vector and sequencing the inserts (Table 2).

RT-PCR products were identified in 5 of 10 cell lines (Fig. 5A). The cell lines PE06, OVCAR-3, and OAW42 expressed both JM-a and JM-d isoforms, whereas SKOV-3 and 41 M cells expressed the JM-a isoforms. The JM-d isoform was clearly a minority product (approximately 10–20% intensity) compared with the JM-a form. Neither expression was observed under these PCR conditions in the PE01, PE01 CDDP, PE016, OVCAR-5, or SW626 cell lines. No expression of the JM-b or JM-c isoforms was detected in any of the 10 lines.

A panel of 24 primary ovarian tumors was then examined, and JM-a was detected in 18 of 24 tumors (Fig. 6A). A band consistent with JM-d was also observed as a minor component in several of these tumors (Fig. 6A).

A similar analysis was undertaken with primers flanking the cytoplasmic site that had demonstrated variability previously. In the cell line panel, RT-PCR products consistent with the CT-a and CT-b isoforms were identified (Fig. 5B). The identities of these were confirmed by direct sequencing. The same 5 cell lines that had shown expression for the JM-a primers were positive for both the CT-a and CT-b isoforms, and for 4 of these cell lines (PE06, OVCAR-3, 41 M, and OAW42), the CT-a (full-length) isoform was predominant; however, for SKOV-3, the CT-b form was predominant. For the series of 24 carcinomas, the same 18 tumors demonstrating JM-a expression showed both CT-a and CT-b expression, and weak expression of both forms was observed in 4 additional tumors (Fig. 6B). Expression was predominant for CT-a in 7 tumors, for CT-b in 7 tumors, and was equivalent for both isoforms in 8 tumors.

**Effect of erbB4 Blocking Antibody on Growth and Signaling.** To test whether the erbB4 receptor might mediate a growth function, the effects on the growth of cell lines of an antibody (H4.72.8) that blocks the binding of NRG1β to the erbB4 receptor (24) were investigated. Four cell lines were studied: PE01, PE06, PE01 CDDP, and SKOV-3. Both PE01 (which expresses a low-to-undetectable level of erbB4) and PE06 (which expresses a high level of erbB4) are growth stimulated by NRG1β, whereas PE01 CDDP (which expresses a low level of erbB4) is growth inhibited by NRG1β. Growth of SKOV-3 cell lines is unaffected by addition of NRG1β.

NRG1β alone stimulated growth of the PE01 cell line. In the presence of the antibody, this growth effect was not blocked but enhanced (Fig. 7). In the absence of NRG1β, the addition of the blocking antibody also markedly enhanced growth of the PE01 cell line, perhaps consistent with growth inhibition exerted through the erbB4 receptor by NRG1β present at low levels in the charcoal-stripped serum or generated via an autocrine route in the cells. For PE06 cells, the antibody produced a small increase in growth in the presence of NRG1β but had no effect in the absence of added factor (Fig. 7).

Growth of SKOV-3 cells was unaffected by the addition of NRG1β (Fig. 7). Addition of the antibody either in the presence or absence of added growth factor did not influence growth. The PE01 CDDP cell line is growth inhibited by NRG1β. This was enhanced slightly by addi-

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**Table 2. Partial nucleotide sequences of erbB-4-related PCR products cloned from PE06 cells**

| Product | Partial sequence
|---------|-------------------
| JM-a   | ACC AGTTTGTATA CCGAGATGGA
| JM-d   | GATGGATAGG TGTAACGGT CCCACTAATC ATGACTGCAT TTACTACCCA TGGACGGGCC
| CT-a   | GGCCATTCCA CTTACCACA AGAATTGACT CGAATAGGAA CCAGTTTGTA TACCGAGATG GA
| CT-b   | 3141 TTCCAGAGCA AGAATTGACT CGAATAGGAA CCAGTTTGTA TACCGAGATG GA

* Partial sequences that demonstrate variability and positions of nucleotides are shown based on the GenBank entry for erbB4 mRNA (L07868). Boldface, variable region.

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**Fig. 6. RT-PCR analysis of erbB4 isoforms in primary ovarian cancers.** A, expression of the erbB JM-a and JM-d isoforms. Expression of JM-a was detected in 18 of 24 cancers. Samples 91, 96, 5, 9, 77, and 99 were negative; all others showed a clear PCR product. B, expression of the erbB4 CT isoforms. Expression of both CT-a and CT-b was detected in 20 of 24 cancers. Lanes 96 and 5 were negative, and Lanes 91 and 77 were borderline. C, expression of γ-actin. All 24 samples showed a clear product. H2O, water control, and sample numbers are shown. Amplified PCR products were detected using a 2.5% gel stained with ethidium bromide for UV visualization.
were obtained in a repeat experiment.

The phosphorylation of both p44 (ERK1) and p42 (ERK2; Fig. 8A) was increased tyrosine phosphorylation of both erbB2 (Fig. 8A) and phosphorylation of both p44 (ERK1) and p42 (ERK2; Fig. 8B). This was partially reversed (~50%) by coinoculation with H4.72.8, demonstrating the involvement of erbB4 in NRG1β signaling.

**DISCUSSION**

There has been considerable interest in the roles of the erbB receptor family of growth factors and their interactions in cancer growth and progression. Overexpression of both erbB1 (EGF receptor) and erbB2 have been associated with aggressive behavior in a range of solid cancers including ovarian cancer (12–15). The present study demonstrates that erbB4 receptors are present in the majority of ovarian carcinomas. Within our series of ovarian cancers, 90% of tumors were positive, indicating the prevalence of expression of this receptor in this disease. The nature of the staining was predominantly cytoplasmic, consistent with a previous report of HFR-1, although nuclear reactivity has also been observed (26), and expression levels varied from strong to absent. Srinivasan et al. (13) have analyzed previously erbB4 immunostaining with HFR-1 in a small series of 10 ovarian cancers and found that, compared with normal ovarian tissues, expression was reduced in 5 cancers, expressed at a normal level in 4 cancers, and increased in 1 cancer. We investigated expression in 5 normal ovaries and found only weak staining in surface epithelial cells (data not shown). Expression changes of erbB4 have been observed in other cancer types with reduced expression compared with normal having been reported in prostate (27) and pancreatic (28) cancers and increased expression in childhood medulloblastoma (29), thyroid (30), and gastric (31) cancers. The one ovarian stromal tumor examined to date is the granulosa cell tumor, and 10 of 12 tumors expressed erbB4 at moderate-to-high levels in >50% of cancer cells (32). Several reports have described expression of erbB4 in breast cancer, and higher levels of expression have been associated with a more differentiated phenotype (26, 33). We did not find any clear association with grade of differentiation; however, within our series of ovarian cancers, expression levels did differ among the histological subtypes. Serous tumors tended to have a higher level of expression than endometrioid cancers, and this was evident at both the protein and mRNA level. Expression of erbB4 when analyzed alone was not obviously associated with survival; however, coexpression with erbB2, the preferred heterodimer for all of the erbB receptors, did suggest an association with improved prognosis. The same coexpression of receptors has been associated with reduced survival in childhood medulloblastoma (29).

In a series of ovarian cancer cell lines, erbB4 expression was again variable, and increased expression appeared to be associated with prior platinum treatment and/or resistance. Increased expression of EGF receptor, erbB2, and erbB3 has been observed previously in drug-resistant breast cancer cell lines (34), but we are not aware of any study finding an association between increased erbB4 and drug resistance. Further studies are required to assess whether and how this increased erbB4 expression influences platinum sensitivity.

RT-PCR analysis indicated the presence of multiple isoforms of erbB4 within these ovarian cancer systems. The JM isoforms are differentially expressed in human tissues with, for example, the cerebellum expressing both forms, whereas the heart expresses only JM-b (14). Only the JM-a form undergoes cleavage by phorbol ester, and this might result in another level of regulation of the activities of the different ligands for erbB4 (15). All of the cell lines and tumors expressing erbB4 possessed the JM-a rather than the JM-b isoform. In addition, several cell lines and primary tumors expressed the JM-d form, which contains both exons. There has been no report on the

**A. Anti-phosphotyrosine**

**B. Anti-phospho ERK**

*Fig. 8. Effect of an anti-erbB4 antibody (H4.72.8) on erbB2 (p185) tyrosine phosphorylation (detected using an anti-phosphotyrosine antibody; A) and p42 and p44 ERK phosphorylation (using an anti-phosphoERK antibody; B) in PE01 cells after NRG1β stimulation. Cells were treated as described in “Materials and Methods” with either NRG1β (10−9 M) alone or a combination of NRG1β and H4.72.8 for 5 days. Cell lysates were then prepared for Western blot analysis. In the presence of H4.72.8, densitometric analysis indicated an ~50% reduction in intensity. Similar data were obtained in a repeat experiment.*
expression or function of the JM-d molecule, other than it can also be found in medulloblastoma cells (16). Although no other study has formally reported its presence in ovarian cancer cells, a recent GenBank entry (AT798478) describing the variable sequence found within the JM-d form was obtained from mRNA derived from a pool of five human ovarian cancers, confirming its presence in this disease.

We observed that all cell lines that expressed the “full-length” CT-a isoform contained the CT-b isoform also, and this is consistent with the initial report of this isoform (17). The CT-b differs from CT-a in lacking 48 bp, which encode a PI3-K binding site. Both isoforms have been demonstrated recently to be functional NRG receptors, and consistent with expectation, CT-b does not bind or activate PI3-K in a ligand-dependent manner (18). When the isoforms were transfected separately into NIH 3T3 cells, NRG1β-stimulated cells contained either CT-a or CT-b, suggesting that the PI3-K site was not critical for a growth response, but the ability to induce survival and chemotaxis was markedly reduced in the CT-b transfact compared with the CT-a transfact (19). Consistent with the involvement of the PI3-K site in the latter two processes, Akt was phosphorylated in the CT-a but not the CT-b transfact (19).

The relative levels of the two isoforms varied among the cell lines and primary tumors that we studied, potentially allowing a variety of signaling options. In the initial report (17) of these isoforms were two ovarian cell lines, OVCAR-3 and SKOV-3. The former cell line was reported positive, and the latter was reported negative, by initial RT-PCR but positive by Southern blotting of the amplified cDNA. In the present study, we found both cell lines to be positive, but SKOV-3 cells differed thus far because they expressed more CT-b than CT-a. Because we identified erbB4 expression in SKOV-3 cells by two other sets of primers and also by Western analysis and because it has also been detected by others (21), it is likely that our technique is more sensitive. Our study indicates that multiple transcripts of erbB4 exist in ovarian cancer cells, and just as alternate transcripts of erbB3 have been identified in ovarian cancer (35), this adds further complexity to the signaling possibilities that might arise from erbB receptor interactions. The retention of these multiple forms in ovarian cancers and in other tissues (17, 18) does suggest that they have functionally important roles.

The function of erbB4 appears to vary, depending on its context. Although erbB3 has been shown to have an important role in cardiac and neural development (36), its role in different cancers is at present unclear. For example, in breast cancer, erbB4 has been proposed to mediate a proliferative function because down-regulation of erbB4 using a ribozyme hammerhead strategy inhibited colony formation of PE01 cells differing thus far because they expressed more CT-b than CT-a. Because we identified erbB4 expression in SKOV-3 cells by two other sets of primers and also by Western analysis and because it has also been detected by others (21), it is likely that our technique is more sensitive. Our study indicates that multiple transcripts of erbB4 exist in ovarian cancer cells, and just as alternate transcripts of erbB3 have been identified in ovarian cancer (35), this adds further complexity to the signaling possibilities that might arise from erbB receptor interactions. The retention of these multiple forms in ovarian cancers and in other tissues (17, 18) does suggest that they have functionally important roles.

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In conclusion, this is the first systematic analysis of erbB4 protein expression in ovarian cancer, and we have demonstrated the presence of multiple mRNA isoforms of this receptor within this disease. Highest expression is associated with the serous subtype and with platinum resistance, and the receptor appears to program for minor growth effects. Further studies are required to explore its interaction with other members of the erbB receptor family.

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