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Overexpression of ERBB2 in human mammary epithelial cells signals inhibition of transcription of the E-cadherin gene

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ABSTRACT **Overexpression of the ERBB2 receptor in transfectants of a human mammary epithelial cell line (MTSV1-7) is associated with a reduced ability to undergo morphogenesis *in vitro* and with a decreased level of expression of the E-cadherin and α_2 integrin genes. The inhibition of expression of the adhesion molecules has been shown to be at the level of transcription by using nuclear run-on assays and by following transcription of a reporter gene fused to 5′ sequences of the E-cadherin gene. To relate the effects on gene transcription to a functional ERBB2 protein, signaling from the receptor was inhibited by the antibody 4D5, which blocks phosphorylation of ERBB2 on tyrosine residues and association of the protein with the GRB2/Sem5 protein. After treatment with the antibody 4D5, the ERBB2 transfectants regain the ability to form three-dimensional structures in collagen gels and the rates of transcription of the genes encoding the E-cadherin and the α_2 integrin subunit are restored to the levels seen in MTSV1-7neo cells. These results demonstrate that the inhibition of morphogenesis and transcription of specific adhesion molecules in human mammary epithelial cells can be affected by signals generated by the ERBB2 receptor and suggest a role for ERBB2 overexpression in tumor progression and metastasis.**

Several protooncogenes have been characterized as active participants in signal transduction pathways that lead to downstream effects on gene transcription. The *ERBB2* protooncogene was initially identified through its homology to the gene coding for the epidermal growth factor receptor (*EGFR*) (1) as a transmembrane tyrosine kinase that undergoes autophosphorylation in overexpressing cells (2). However, the signals generated by the p185-ERBB2 receptor have not been well characterized, possibly due to uncertainties regarding its ligand (3–7).

The *ERBB2* gene is overexpressed in a proportion of breast (8) and ovarian carcinomas (9), and overexpression appears to be indicative of a poor prognosis, suggesting that this protooncogene may be involved in malignant progression. To understand how the aberrant expression of an oncogene or a protooncogene may affect malignant progression, it is crucial to consider effects on parameters that relate to metastasis. One group of molecules thought to play a major role in all stages of metastasis are the cell adhesion molecules, which mediate cell–cell and cell–matrix interactions. In the case of carcinomas, the first stage in invasion necessitates a separation of the epithelial cells from each other and from the basement membrane and is likely to be accompanied by a decrease in the level of expression or function of molecules involved in these interactions.

The E-cadherin molecule, which is expressed at the surface of epithelial cells, has been found to play a central role in maintaining epithelial cell morphology through homotypic Ca^{2+}-dependent interactions (10). Significantly, a reduction in E-cadherin expression is found in a proportion of breast cancers, and this can be correlated with increased invasiveness (11). To directly examine the effects of overexpression of ERBB2 on cell morphology and on the expression of adhesion molecules in human mammary epithelial cells, we have developed a nontumorigenic cell line MTSV1-7 from luminal epithelial cells cultured from milk (12). MTSV1-7 cells make three-dimensional structures in collagen gels and express high levels of the adhesion molecules characteristic of normal luminal epithelial cells, including E-cadherin and the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins. Transfectants of MTSV1-7 have been developed that overexpress ERBB2, and these show a reduced ability to undergo morphogenesis *in vitro* (13). Here we demonstrate that the effect on morphogenesis is correlated with downregulation of expression of the E-cadherin gene at the level of transcription. A reduced level of transcription of the gene coding for the α_2 integrin subunit is also observed. The effects on transcription are specifically dependent on a functional ERBB2 receptor and can be reversed if ERBB2 signaling is abolished by treatment with the antibody 4D5 (14). The regulation of gene expression reported here defines *ERBB2*-generated signals affecting transcription. Moreover, this effect of *ERBB2* suggests a mechanism whereby ERBB2 could affect the metastatic process and malignant progression.

MATERIALS AND METHODS

Culture and Immunofluorescent Staining of Cell Lines. The development of the MTSV1-7 transfectants and the media used for their culture and for the SKBR3 cells has been reported (13). For immunostaining, cell monolayers were fixed with 3.7% formaldehyde for 10 min and then stained with monoclonal antibodies to either the α_2 integrin subunit (HAS4) or the human E-cadherin (HECD-1) as described (13).

Antibodies and Antisera. The antibodies used were generously provided as follows: 4D5 (ERBB2) was from Genentech; HAS4 (α_2 integrin subunit) was from F. Watt (Imperial Cancer Research Fund); HECD-1 (E-cadherin) was from M. Takeichi (Kyoto University); 9G6 (ERBB2) was from F. van Leeuwen (Stanford University); 21N antiserum (ERBB2) was from W. Gullick (Imperial Cancer Research Fund), and the anti-GRB2 antiserum was from J. Downward (Imperial Cancer Research Fund). The anti-phosphotyrosine antibody PY20 was from Amersham.

Collagen-Induced Morphogenesis. Morphogenesis was assessed as described (15). Where indicated, cells were preincubated with antibody 4D5 (25 μg/ml) for 15 min at room temperature before embedding and the antibody was added at the same concentration to the medium during the growth period.

Northern Blot Hybridization. Total cellular RNA (30 μg) was size fractionated, transferred to Hybond-N membrane, and hybridized with 50 ng of [α-^{32}P]dCTP-labeled probes (13, 16). For detecting the transcript coding for the α_2 integrin

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Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CAT, chloramphenicol acetyltransferase.

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subunit a 3.6-kb fragment of 5′ sequence of a human cDNA clone was used; for that coding for the E-cadherin, two fragments covering the entire mouse E-cadherin cDNA were used. To control for RNA loading, the blots were probed with a 125-bp fragment of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

Nuclear Run-On Assays. The rates of transcription were determined by nuclear run-on assays essentially as described by Hotchin and Watt (17) with the following modifications. Transcriptional elongation (using 1×10^8 nuclei per reaction) was allowed to proceed for 30 min at 37°C in the presence of 200 μCi of [^{32}P]UTP (specific activity, 800 Ci/mmol; 1 Ci = 37 GBq; Amersham) per reaction. Isolated labeled transcripts were then hybridized to 5 μg of linearized plasmid DNA from vectors containing cDNA sequences encoding GAPDH, α_2, α_3, and β_1 integrin subunits or E-cadherin immobilized on nitrocellulose filters.

Transfection of Reporter Gene Constructs and Enzyme Assays. The chloramphenicol acetyltransferase (CAT) constructs containing the E-cadherin regulatory sequences (p0.2CAT and p6.5CAT) were kindly provided by J. Behrens. The control plasmid, pGCAT-A, was obtained from T. Frebourg and O. Brison (18) and the pSVCAT construct was from Promega. pJ3ΩLuc containing the luciferase gene driven from the simian virus 40 promoter was obtained from S. Goodbourn (Imperial Cancer Research Fund). Transient transfections with reporter gene constructs were performed on cells (plated 18–24 hr previously) using the calcium phosphate coprecipitation method (19). Ten micrograms of CAT plasmid together with 2 μg of pJ3ΩLuc was used for each 60-mm dish (duplicate dishes for each sample). Transfection was carried out for 12 hr and CAT activity was assayed 48 hr after precipitate removal (20). Where indicated, antibody 4D5 was added to the medium at 25 μg/ml after precipitate removal and incubated with the cells for 48 hr.

Immunoprecipitation and Western Blot Analysis. For detection of the ERBB2 or GRB2 protein, cells were washed three times with 1 mM sodium orthovanadate in phosphate-buffered saline (PBS) and lysed in HNET buffer (50 mM Hepes, pH 7.5/100 mM sodium chloride/1 mM EGTA/1% Triton X-100) containing 1 mM dithiothreitol, 1 mM orthovanadate, 10 mM sodium pyrophosphate, and 1 mM phenylmethylsulfonyl fluoride. After clarification by centrifugation, the protein concentration of the lysates was estimated with the Bio-Rad protein assay kit (Bio-Rad). For precipitation of p185-ERBB2 (and associated proteins) 50 μg of protein was incubated with antibody 9G6 for 4 hr at 4°C and the immune complexes were collected using 7 μg of rabbit anti-mouse antiserum conjugated to protein A-Sepharose beads. The beads were washed three times with lysis buffer containing 0.1% Triton X-100 and boiled in sample buffer before loading on SDS/7.5% polyacrylamide gels and blotting onto Hybond-C membranes (Amersham). For direct detection of proteins by Western blotting, 50 or 100 μg of protein was electrophoretically separated on SDS/polyacrylamide gels and transferred onto Hybond-C [in the case of E-cadherin, cells were washed in complete PBS and lysed in Laemmli's sample buffer (21) containing 1 mM calcium chloride]. Immunoblots were first blocked with either 5% bovine serum albumin and 0.1% Tween 20 in PBS (when probing with antiserum 21N to ERBB2 or the antibody PY20) or 5% semi-skimmed milk and 0.1% Tween 20 (when probing with antibody HECD-1 or the anti-GRB2 antiserum) and then probed with the respective antibodies; the immune complexes were detected using iodinated secondary antibodies (Amersham).

RESULTS

Decreased Expression of E-Cadherin in ERBB2 Transfectants of MTSV1-7 Cells. Three ERBB2 transfectants expressing different levels of the p185-ERBB2 receptor were developed by transfecting the human *ERBB2* cDNA into an immortalized human mammary epithelial cell line, MTSV1-7 (12). Of the three transfectants (ce1, ce2, and ce3), ce1 expressed the highest level of the protooncogene and ce3 expressed the lowest level (13). When cultured in collagen type 1 gels, MTSV1-7 cells form three-dimensional ball-like structures, which can with time develop branches reminiscent of mammary epithelial morphogenesis *in vivo*. In contrast, the ERBB2 transfectants exhibit a reduced ability to form structures in collagen gels, which correlates with the level of expression of ERBB2 and inversely correlates with the level of expression of the $\alpha_2\beta_1$ integrin (13). Fig. 1 illustrates this effect on *in vitro* morphogenesis for the highest ERBB2 expressor, ce1 (Fig. 1*c*).

Analysis of E-cadherin expression in the ERBB2 transfectants by Western blotting showed a clear decrease in protein levels, as compared to the parental MTSV1-7 line or the neo transfectant. Fig. 2*A* shows this result and also shows E-cadherin expression in the breast cancer cell line SKBR3, which overexpresses ERBB2. Fig. 2*B* demonstrates that the decrease in expression of E-cadherin in the transfectants and in SKBR3 cells is also seen at the level of mRNA. The correlation between the level of expression of the protooncogene and the reduction in expression of E-cadherin mRNA is very clear, with the highest ERBB2 expressor ce1 expressing only 12% of the message expressed in the parental cell line or control neo transfectant. This dramatic reduction in expression of E-cadherin probably explains the change in morphology seen in the ce1 cells, which, when subconfluent, exhibit a fibroblast-like morphology (13). The level of E-cadherin mRNA expressed by the SKBR3 cell line (60% of the MTSV1-7 cell line) corresponds to that seen in the ce2 transfectant (52% of the parental MTSV1-7 cell line), which shows similar levels of ERBB2 expression (13). The reduction in levels of mRNA in ce3, the lowest ERBB2 expressor, is small but significant.

Transcription of the E-Cadherin Gene Is Inhibited in ERBB2 Transfectants of MTSV1-7. To analyze whether the decrease in the level of expression of E-cadherin mRNA is at the level of transcription, the rate of transcription was analyzed in ce1 cells and in the control transfectant MTSV1-7neo. Fig. 3 (lanes A and B) shows the results of a nuclear run-on experiment measuring the rate of transcription of the E-cadherin gene in the two cell lines as compared to that of the GAPDH house-

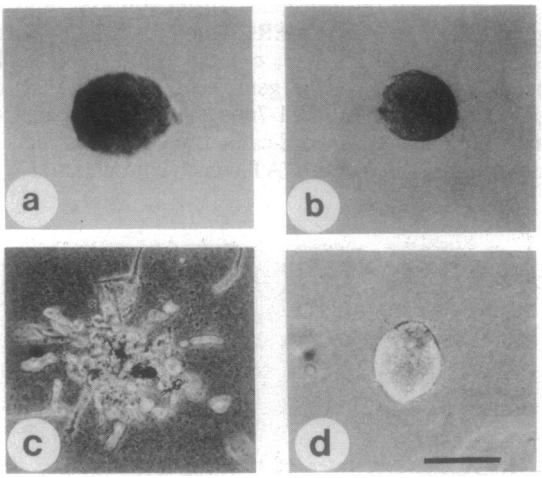

FIG. 1. Restoration of the ability of ce1 cells to undergo morphogenesis on treatment with the monoclonal antibody 4D5. Cells were grown in collagen type 1 gels fed with either normal medium (*a–c*) or medium containing monoclonal antibody 4D5 (25 μg/ml) (*d*) and structures were photographed after 12 days in culture. (*a*) MTSV1-7. (*b*) MTSV1-7neo. (*c* and *d*) ce1. (Bar = 100 μm.)

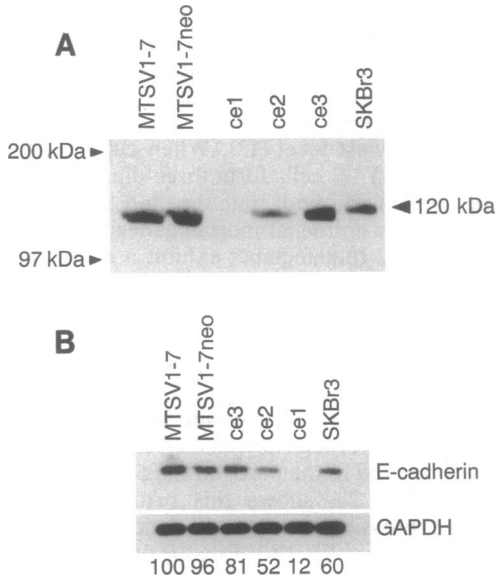

FIG. 2. E-cadherin expression in MTSV1-7, the transfected cell lines, and SKBR3 cells. (*A*) Immunoblot analysis. One hundred micrograms of total cellular protein was fractionated on a SDS/7.5% polyacrylamide gel and blotted onto Hybond-C membrane; E-cadherin was detected with antibody HECD-1. (*B*) Northern blot analysis. Thirty micrograms of RNA from the cell lines was separated in a 1.3% agarose-formaldehyde gel, blotted onto Hybond-N membrane, and probed with an E-cadherin or a GAPDH probe. The level of RNA (%) for the transfectants and for the SKBR3 cells as compared with the parental MTSV1-7 cell line was calculated from numbers obtained by densitometric scanning and is indicated under each lane.

keeping gene. Transcription rates for the subunits of the major collagen-binding integrins expressed by MTSV1-7 cells ($\alpha_2\beta_1$ and $\alpha_3\beta_1$) were also measured. Fig. 3 clearly shows a specific and dramatic decrease in the rate of transcription of the E-cadherin as well as the α_2 integrin gene in ce1 cells, while no reduction was seen in the rates of transcription of the α_3, β_1 and GAPDH genes. The levels of transcription of the α_2 integrin subunit and the E-cadherin genes were also found to be low in the SKBR3 cells (data not shown).

The regulatory sequences governing tissue-specific expression of the E-cadherin gene have been coupled to a reporter gene and analyzed by transient-transfection assays (22). To analyze further the effect of ERBB2 expression on transcription of the E-cadherin gene, constructs of the E-cadherin promoter fused to the CAT gene were transfected into the ERBB2 and control MTSV1-7neo transfectants, and CAT activity was measured. Two E-cadherin–CAT constructs were used in the assays—the p0.2CAT, which contained −178/+17

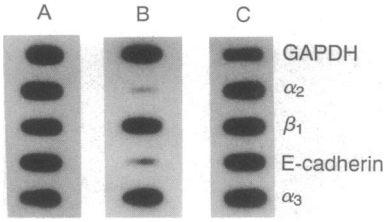

FIG. 3. Analysis of rates of transcription of the α_2, β_1, α_3, E-cadherin, and GAPDH (control) genes in the MTSV1-7neo transfectant (lane A) and the ce1 transfectant treated with (lane C) or without (lane B) the 4D5 antibody for 48 hr. [^{32}P]UTP-labeled transcripts from the MTSV1-7neo transfectant and ce1 transfectant treated with or without the 4D5 antibody were hybridized to 5 μg of the respective linearized plasmid DNA probes bound to nitrocellulose membrane.

of the E-cadherin genomic sequence, and a longer construct, p6.5CAT, containing the genomic fragment −6400/+92 (22). Fig. 4*A* shows a striking inverse correlation between the level of ERBB2 expression and CAT activity in the cells transiently transfected with the E-cadherin–CAT constructs with transcription being reduced by >90% in the highest ERBB2 expressor (ce1) and by 25% in the lowest expressor (ce3). As observed by Behrens *et al.* (22), the short fragment of the E-cadherin promoter was much more effective in driving transcription of the CAT gene than the long fragment. However, a reduction in CAT transcription was also seen, particularly in ce1 cells, even with the longer fragment.

Inhibition of p185-ERBB2 Receptor Signaling by the Antibody 4D5. A direct link between the effects on gene expression and p185-ERBB2 receptor signaling was established by using the monoclonal antibody 4D5, which has been shown to inhibit autophosphorylation of the p185-ERBB2 receptor in breast cancer cell lines (14). Antibody 4D5 also inhibits tyrosine phosphorylation of p185-ERBB2 in ce1 cells as shown in Fig. 5, which shows the level of expression of the receptor (Fig. 5*A*) and the level of its tyrosine phosphorylation (Fig. 5*B*) after 4D5 treatment. The inhibition of tyrosine phosphorylation is obvious within a few hours, and, by 36 hr, phosphorylation is only 19% of that seen in untreated cells. In contrast, no effect on the level of receptor expression is seen until after 36 hr of antibody treatment and even then the reduction is small (13%). As was seen with the SKBR3 cells (23), inhibition of autophosphorylation of the ERBB2 receptor in the ce1 cells by the 4D5 antibody was accompanied by inhibition of anchorage-independent growth (data not shown).

Intracellular proteins containing specific SH2 domains, have been shown to bind to tyrosine-phosphorylated regions

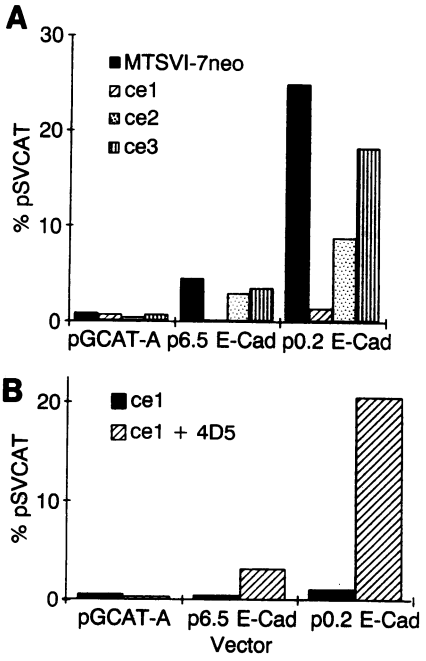

FIG. 4. (*A*) Expression of the CAT gene from E-cadherin promoter sequences in the ERBB2 transfectants and MTSV1-7neo. (*B*) Increase in activity of E-cadherin promoter sequences in ce1 cells when cultured in the presence of antibody 4D5 (25 μg/ml) for 48 hr. Cells were transfected with pJ3ΩLuc and one of the CAT reporter constructs: pGCAT-A (promoterless CAT construct), pSVCAT (simian virus 40 enhancer–promoter CAT construct), p6.5 CAT (containing 6.5 kb of mouse E-cadherin 5′ upstream sequence), or p0.2 E-cadherin (containing the −178/+17 E-cadherin promoter fragment). CAT activities are expressed as the ratio CAT activity (cpm/2 hr)/luciferase activity (arbitrary units of chemiluminescence). Ratios obtained are expressed as a percentage of that obtained with pSVCAT.

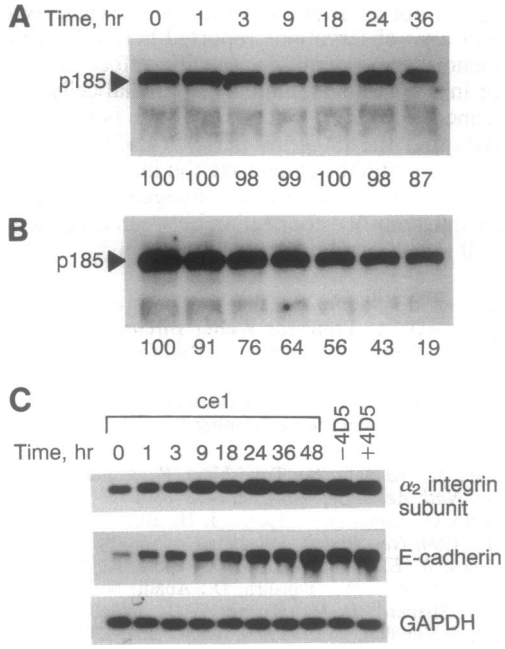

FIG. 5. Effect of treatment with antibody 4D5 (25 μg/ml) on relative level of expression of ERBB2 (*A*), tyrosine phosphorylation of ERBB2 (*B*), and levels of E-cadherin and α_2 integrin mRNA (*C*). ce1 cells were treated with the 4D5 antibody (25 μg/ml) for the times indicated. (*A*) Total cell lysate (100 μg of protein) was separated by electrophoresis and p185-ERBB2 levels were detected by immunoblotting with antiserum 21N. (*B*) p185-ERBB2 protein was immunoprecipitated from 100 μg of protein with the 9G6 antibody; immune complexes were separated by gel electrophoresis and immunoblotted with antibody PY20. In *A* and *B*, bound antibody was detected with ^{125}I-labeled secondary antibodies and bound radioactivity was quantitated by densitometric scanning of autoradiograms and expressed as a percentage of that seen in untreated cells. (*C*) RNA (30 μg) was fractionated in a 1.3% agarose-formaldehyde gel, blotted onto Hybond-N membrane, and probed with an α_2 integrin, E-cadherin, or GAPDH probe. Lanes −4D5 and +4D5, MTSV1-7 cells in the absence and presence of 4D5 antibody.

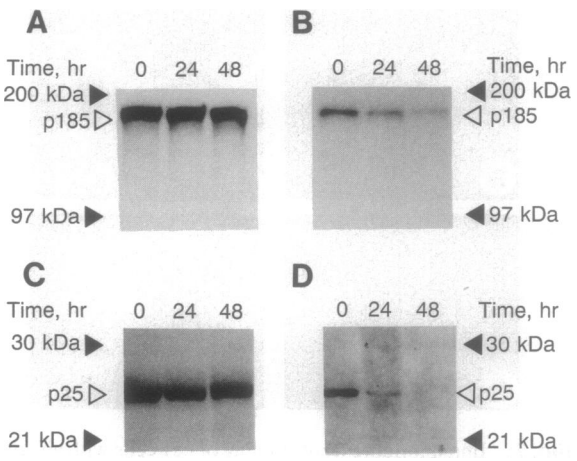

FIG. 6. Decrease in the level of GRB2 protein complexed to p185 ERBB2 in ce1 cells after treatment with antibody 4D5. ce1 cells were treated with the 4D5 antibody (25 μg/ml) for the times indicated before analyzing for total expression of ERBB2 protein (*A*), level of tyrosine phosphorylation of p185-ERBB2 (*B*), total level of GRB2 protein (*C*), and level of GRB2 complexed to p185 ERBB2 (*D*). (*A*, *B*, and *D*) p185-ERBB2 was immunoprecipitated from 50 μg of total lysate using antibody 9G6. Washed complexes were separated on SDS/polyacrylamide gels, transferred to nitrocellulose membranes, and probed with either the 21N antiserum (*A*), antibody PY20 (*B*), or the GRB2 antiserum (*D*). (*C*) Fifty micrograms of total cell lysate was separated by SDS/PAGE, blotted onto Hybond-C membrane, and probed with the GRB2 antiserum.

of growth factor receptors (24), thus providing a mechanism by which receptor activation can be coupled to intracellular signaling pathways. One such protein, GRB2/Sem5, has been implicated in signaling from the activated epidermal growth factor receptor (25, 26). We now find that the GRB2/Sem5 protein coprecipitates with the p185-ERBB2 receptor in ce1 cells and that the interaction of the proteins is inhibited by 4D5 treatment. Fig. 6 shows that as the level of phosphorylation of the p185-ERBB2 receptor decreases, the amount of GRB2 that coprecipitates with the receptor decreases, even though the total levels of ERBB2 and GRB2 present in the cell are essentially unaltered. These results confirm that signals generated by the ERBB2 receptor are inhibited by the 4D5 antibody.

Restoration of Morphogenesis and Levels of Transcription of Adhesion Molecules in the ce1 Cells Treated with Antibody 4D5. Since treatment with antibody 4D5 inhibits signaling from the p185-ERBB2 receptor, the effect of antibody treatment on *in vitro* morphogenesis and expression of adhesion molecules in ce1 cells was examined. Treatment with the antibody was indeed very effective in restoring the ability of ce1 cells to form ball-like structures in collagen gels (Fig. 1*d*) and to express high levels of E-cadherin and the α_2 integrin subunit. Fig. 5*C* shows the time course of the increase in mRNA expression for both genes. An increase in mRNA levels begins within 1 hr of addition of the antibody and the levels continue to increase as phosphorylation of the receptor decreases, becoming similar to the levels in the control MTSV1-7neo transfectant by 24–36 hr. Nuclear run-on analysis illustrated in Fig. 3 (lane C) shows that the increase in mRNA levels is due to an increase in the rates of transcription of the genes, which reach those seen in the neo transfectant after 48 hr of treatment with the 4D5 antibody (compare lanes A and C). Furthermore, in ce1 cells transfected with the E-cadherin promoter–CAT constructs, treatment with the 4D5 antibody for 48 hr resulted in a dramatic increase in the level of CAT expression (Fig. 4*B*), the transcription of the shorter construct being increased >20-fold. The restoration of expression of the E-cadherin and the α_2 integrin subunit could be demonstrated at the protein level by immunofluorescence as illustrated in Fig. 7. These results demonstrate unequivocally that inhibition of transcription of the E-cadherin and α_2 integrin genes is the end result of signals generated by a functional ERBB2 receptor.

DISCUSSION

The *ERBB2* gene is expressed at low levels by most tissues but shows a dramatic increase in expression in 20–30% of breast cancers (8). Since overexpression of ERBB2 is associated with a poor prognosis and is expressed by those *in situ* breast tumors that are more likely to become invasive (27), the protooncogene may be involved in the early stages of malignant progression. The decreased level of expression of the E-cadherin gene, which is seen in some breast cancers, is also likely to be associated with development of the invasive phenotype at an early stage, when cell adhesion needs to be reduced as part of the metastatic process (11). The results presented in this study demonstrate that in ERBB2 transfectants of a human mammary epithelial cell line (MTSV1-7), transcription of the E-cadherin gene is reduced as is the morphogenetic potential of the cells. Moreover, the effects on gene transcription and on morphogenesis could be shown to be dependent on a functional ERBB2 receptor.

The major collagen-binding integrins expressed by human mammary epithelial cells and MTSV1-7 cells are the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins, and these have also been reported to be

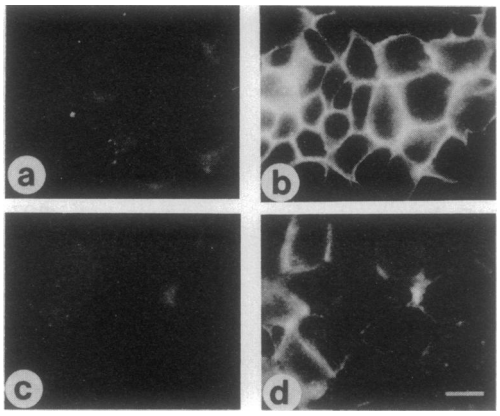

FIG. 7. Immunofluorescent staining of cel cells with an antibody to the α_2 integrin subunit (*a* and *b*) or an antibody to the human E-cadherin (*c* and *d*). Cells were cultured in the presence of normal medium (*a* and *c*) or were cultured for 36 hr in the presence of monoclonal antibody 4D5 at a concentration of 25 μg/ml (*b* and *d*) before staining with the monoclonal antibodies. (Bar = 7 μm.)

downregulated in some breast cancers (28, 29). Moreover, the $\alpha_2\beta_1$ integrin has been shown to play a crucial role in the collagen-induced morphogenesis of MTSV1-7 cells (15). The reduced transcription of the α_2 integrin subunit seen in the ERBB2 transfectants could result in reduced adhesion of the cells to the extracellular matrix and thus amplify the dissociative effect of the loss of E-cadherin expression and enhance the invasive process.

To demonstrate that the effects on morphogenesis and transcription of the adhesion molecules were dependent on signals generated by the p185-ERBB2 receptor in the MTSV1-7 transfectants, the 4D5 antibody was used. This antibody acts as an antagonist and inhibits phosphorylation of the receptor in overexpressing breast cancer cell lines and, as we now demonstrate, in ERBB2 transfectants. Phosphorylated tyrosines play an important role in signal transduction from membrane receptor tyrosine kinases through their interaction with molecules containing SH2 domains (22). While some SH2-containing proteins have a catalytic function, others appear to act as adaptor proteins, which link the phosphorylated membrane receptor with a protein(s) that has a catalytic function. In this context, the GRB2/Sem5 protein has been shown to transmit signals from the phosphorylated epidermal growth factor receptor to the ras pathway through its interaction with the guanine exchange protein SOS (26). We have now shown that the GRB2/Sem5 protein also interacts with the phosphorylated ERBB2 receptor in the MTSV1-7 transfectants and that the interaction does not occur after treatment with the 4D5 antibody.

The reversion of the phenotype in the ERBB2 transfectants treated with antibody 4D5 is dramatic. The cells no longer show anchorage-independent growth, their ability to form structures in collagen gels is restored, and rates of transcription of the E-cadherin and α_2 integrin genes are increased. The changes in these parameters seen in the transfectants can therefore be directly related to the overexpressed and functional p185-ERBB2 receptor.

The effects on the expression of adhesion molecules could relate ERBB2 receptor function to the early stages of malignant progression of breast cancers *in vivo*. Preliminary studies in our laboratory indicate that similar effects on the expression of E-cadherin and the α_2 integrin subunit (reversible by antibody 4D5) are seen in breast cancer cell lines overexpressing ERBB2, suggesting that ERBB2 regulation of these adhesion molecules is not an artefact of transfected cells. Whether or not the *in vitro* observations reported here reflect an *in vivo* phenomenon, they identify a specific effect of the ERBB2 receptor in the luminal mammary epithelial cells from which breast cancers develop. Since E-cadherin is not expressed in fibroblasts and the integrins perform different functions in these cells, our studies emphasize the importance of defining cell phenotype in the analysis of oncogene or protooncogene function and identify a connection between a signal generated at the cell membrane and gene expression.

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