MOLECULAR AND CELLULAR BIOLOGY, Mar. 1991, p. 1745–1750
0270-7306/91/031745-06$02.00/0

Vol. 11, No. 3

Adenovirus Type 5 E1A Gene Products Act as Transformation Suppressors of the *neu* Oncogene

DIHUA YU, KATHLEEN SCORSONE, AND MIEN-CHIE HUNG*

Department of Tumor Biology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

Received 12 October 1990/Accepted 22 December 1990

The adenovirus type 5 early region 1A (E1A) gene was introduced into *neu*-transformed B104-1-1 cells. Cells that expressed E1A possessed reduced transforming activity in vitro and reduced tumorigenicity in nude mice. These results demonstrate that the E1A gene products can act negatively to suppress the transformed phenotype in *neu*-transformed cells.

The *neu* oncogene is a transforming gene originally identified from rat neuro/glioblastomas (20). Subsequently, both the activated *neu* oncogene and its normal cellular counterpart, the normal *neu* gene, were cloned from rat and human libraries (2, 8, 11, 33). The *neu* gene encodes a 185-kDa transmembrane protein (p185) which is related to but distinct from the epidermal growth factor recepter. The *neu*-encoded p185 and the epidermal growth factor receptor have identical gross structural organization, including ligand-binding, transmembrane, and intracellular kinase domains, and also share extensive sequence homology; specifically, >80% of the amino acids in the tyrosine kinase domain are identical (1, 8, 19, 33). Recently, the ligand for the *neu*-encoded p185 protein has been functionally identified in rat cells and isolated from human breast cancer cells, which will facilitate a better understanding of the function of the *neu*-encoded p185 protein in normal and malignant cell growth and development (15, 35). The activated *neu* oncogene contains a single amino acid substitution in the transmembrane domain and has higher tyrosine kinase activity in comparison with its normal counterpart (3, 25). Our group has demonstrated that amplification of the *neu* proto-oncogene facilitates oncogenic activation by a single point mutation (12). The human homolog of the rat *neu* oncogene, also called *HER-2* or c-*erbB2*, has been shown to be amplified or overexpressed in 25 to 30% of human primary breast cancers and ovarian cancers (10, 21, 22, 37). Breast cancer patients with *neu* overexpression show a significantly lower overall survival rate and a shorter time to relapse than do patients without *neu* overexpression, suggesting that *neu* overexpression may be used as a prognostic factor (21, 22, 26, 27). Amplification or overexpression of the human *neu* gene has also been shown to correlate with the number of axillary lymph nodes positive for metastasis in breast cancer patients (21, 22). These studies strongly suggest that the *neu* oncogene may play an important role in malignant transformation and metastasis.

The primary function of the adenovirus E1A gene is to activate other adenovirus genes during a permissive viral infection by modifying the host cell transcriptional apparatus (4). The E1A protein also contributes to the transforming capabilities of adenovirus (32). Although both transcriptional activation and transcriptional repression of nonadenovirus genes by the E1A proteins have been reported (6, 9, 14, 18, 24, 28, 29), their functional significance and physiological impact are unclear in many cases. Recently, we have studied the effects of the E1A gene products on the promoter activity of the *neu* gene and found that E1A proteins can repress the expression of both the human and the rat *neu* oncogene at the transcriptional level (34, 36). Since both the *neu* gene and the E1A gene are well-known transforming oncogenes (13, 17, 20, 30), our previous findings raised an interesting question: Is it possible that the E1A proteins may act as transformation suppressors for the *neu*-transformed cells via transcriptional repression? We therefore undertook to develop a biological functional assay system to study this possibility. We introduced the E1A gene into *neu*-transformed B104-1-1 cells and generated B-E1A cell lines, derivatives of B104-1-1 that stably express the E1A gene products. We then compared the transformed phenotypes between the parental *neu*-transformed B104-1-1 cell line and the B-E1A cell lines. Our findings demonstrate that the E1A gene products can act as transformation suppressors of *neu* oncogene-transformed cells.

Generation of E1A-expressing stable transfectants. The B104-1-1 cell line, which is an NIH 3T3 cell derivative generated by transfection containing approximately 10 copies of mutation-activated genomic *neu* oncogene, has been shown to be highly transforming and tumorigenic (12, 20). Using the B104-1-1 cell line as a recipient line, we have cotransfected the adenovirus type 5 E1A-expressing plasmid pE1A (36) together with plasmid pSV2-neo, carrying the neomycin resistance marker gene, to generate E1A-expressing stable transfectants (7, 23). The G418-resistant clones were selected in medium containing 500 μg of G418 per ml for 2 to 3 weeks and expanded to cell lines, which were named B-E1A cell lines. The same strategy was used to select control cell lines, in which the pE1Apr plasmids, containing the E1A promoter without the E1A-coding sequence (36), were introduced into the *neu*-transformed B104-1-1 cells to generate the B-E1Apr cell lines, or the pE1A plasmids were introduced into NIH 3T3 cells to develop the N-E1A cell lines. To ensure that the exogenous E1A gene or E1A promoter DNA had integrated into the genome of the transfectants, DNA blot analysis with the E1A probe was performed. Figure 1a shows the results of Southern blot analyses for the parental cell lines and the representative stable transfectants used in this study. As expected, the parental B104-1-1 and NIH 3T3 cell lines did not contain any E1A DNA sequence, whereas the three B-E1A transfectants and the N-E1A transfectant had acquired the transfected

* Corresponding author.

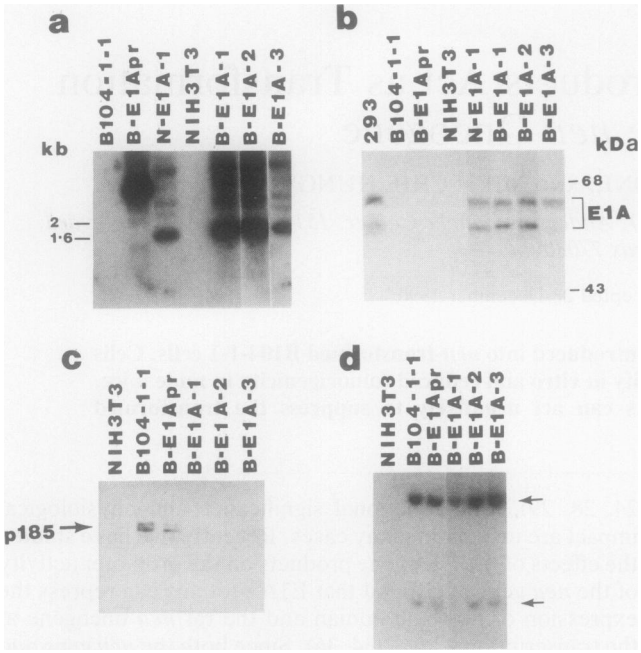

FIG. 1. (a) Southern blot analysis of NIH 3T3 cells, B104-1-1 cells, and their transfectants, using an *Eco*RI-*Sst*I E1A DNA probe. Samples (10 μg) of genomic DNA from the indicated cell lines were digested to completion with restriction endonucleases *Eco*RI and *Sst*I and subjected to electrophoresis on a 1% agarose gel. The DNAs were transferred to Nytran filter paper and hybridized with the E1A probe. The DNA markers are shown on the left. (b) Immunoblot analysis for E1A proteins in cell lysates of the indicated cell lines. A 50-μg amount of each sample was electrophoresed on a 10% sodium dodecyl sulfate-polyacrylamide gel prior to transfer to nitrocellulose. Filters were incubated with the primary antibody M73 against E1A. Positions of protein molecular weight markers and the E1A proteins are shown on the right. (c) Immunoblot analysis for the *neu*-encoded p185 proteins in cell lysates of the indicated cell lines. The primary antibody was monoclonal antibody 3 against p185. (d) Southern blot analysis of the indicated cell lines, using a rat *neu* DNA probe. The DNAs were digested with restriction endonuclease *Bam*HI.

E1A DNAs. The size of the lowest band (1.8 kb) was consistant with the length of the E1A-coding region cleaved from the pE1A plasmids by using restriction endonucleases *Eco*RI and *Sst*I. Noticeably, the three B-E1A transfectants acquired different copy numbers of the E1A gene. Integration of pE1Apr DNA in the B-E1Apr cell line could also be detected and be distinguished from that in B-E1A cell lines, since the E1A probe used in this study also has a portion of sequence from the E1A promoter that can hybridize with the pE1Apr DNA with a hybridization pattern different from that of the B-E1A cell lines (Fig. 1a). To confirm that the B-E1A and N-E1A transfectants actually produced E1A proteins, immunoblot analyses with anti-E1A antibodies were performed (Fig. 1b). The B-E1A and N-E1A transfectants expressed E1A proteins that comigrated with the E1A proteins from the 293 cell line, an established cell line that constitutively expresses the E1A proteins and hence was used as a positive control in this experiment. The negative controls, the parental B104-1-1 and NIH 3T3 cell lines as well as the B-E1Apr cell line, did not express E1A proteins. We had thus established three kinds of stable transfectants: (i) B-E1A transfectants (B104-1-1 transfectants harboring the E1A gene, which were used for comparisons with the B104-1-1 parental cell line to detect changes in transformed phenotypes); (ii) B-E1Apr transfectants (B104-1-1 transfectants containing E1A promoter sequence, which were used as a control cell line to make sure that the changes, if any, in transformed phenotypes in B-E1A transfectants were not due to transfection of the plasmid backbone or the pSV2-neo gene); and (iii) N-E1A transfectants (NIH 3T3 cells transfected with the E1A gene, which were used as another control cell line to determine the biological effects of E1A expression in nontransformed NIH 3T3 cells).

Expression of the *neu*-encoded p185 protein was reduced in B-E1A transfectants. We previously have shown that E1A proteins can repress expression of the *neu*-encoded p185 protein in transient transfection experiments (36). To examine whether expression of E1A in B-E1A stable transfectants can inhibit *neu* expression, immunoblot analyses for the *neu*-encoded p185 protein were performed (Fig. 1c; 16). The p185 proteins were virtually undetectable in all transfectants, as judged by using the horseradish peroxidase method. However, when the more sensitive [^{125}I]protein A detection method was used, slightly higher levels of p185 proteins could be detected in B-E1A-3 than in B-E1A-1 and B-E1A-2 cells (data not shown), which was likely due to a lower level of E1A protein in the B-E1A-3 line (Fig. 1b). The control B-E1Apr cell line expressed an amount of *neu*-encoded p185 protein comparable to that of the parental *neu*-transformed B104-1-1 cell line. Since p185 proteins were barely detectable in B-E1A transfectants, DNA blot analysis for the rat *neu* gene was done to ensure that the reduction in *neu*-encoded p185 protein level was not due to loss of the *neu* gene. Incorporation of the E1A gene into the genome did not alter the *neu* gene at the DNA level (Fig. 1d). Among the three B-E1A transfectants, B-E1A-2 and B-E1A-3 have levels of the *neu* gene comparable to that of the parental B104-1-1 cell line, whereas B-E1A-1 appears to have a lower level of the *neu* gene. This may be due to partial loss of the *neu* gene during the establishment of this transfected cell line. These results indicate that the reduction of *neu*-encoded p185 protein level in B-E1A transfectants is indeed due to the expression of E1A proteins.

To further confirm that the decreased expression of *neu*-encoded p185 protein in B-E1A stable transfectants is due to transcriptional repression of the *neu* gene by the E1A proteins, excess amounts (10 μg) of pNeuEcoRI-CAT plasmids, which contain the chloramphenicol acetyltransferase (CAT) reporter gene driven by the *neu* promoter and upstream sequences, were transfected into the B-E1A transfectants as well as the parental B104-1-1 cells, and CAT assays were performed after the transient transfection (36). The relative CAT activities in three B-E1A transfectants were reduced to 50 to 60% that of B104-1-1 cells (data not shown). Since the level of transiently transfected pNeuEcoRI-CAT reporter gene will be much higher than the level of stably integrated genomic *neu* oncogene (7), the decrease of CAT activity in the transient transfection CAT assay system is not as dramatic as the reduction of p185 protein in the B-E1A stable transfectants, which have only about 10 copies of genomic *neu* oncogene. Nevertheless, the results demonstrate that the E1A proteins expressed in B-E1A stable transfectants can repress *neu* gene promoter activity even in the presence of excess copies of reporter gene. Therefore, the reduced level of p185 protein in B-E1A transfectants is likely due to transcriptional repression of the *neu* gene by the E1A proteins in these cells. We thus chose the three B-E1A transfectants shown in Fig. 1 for further

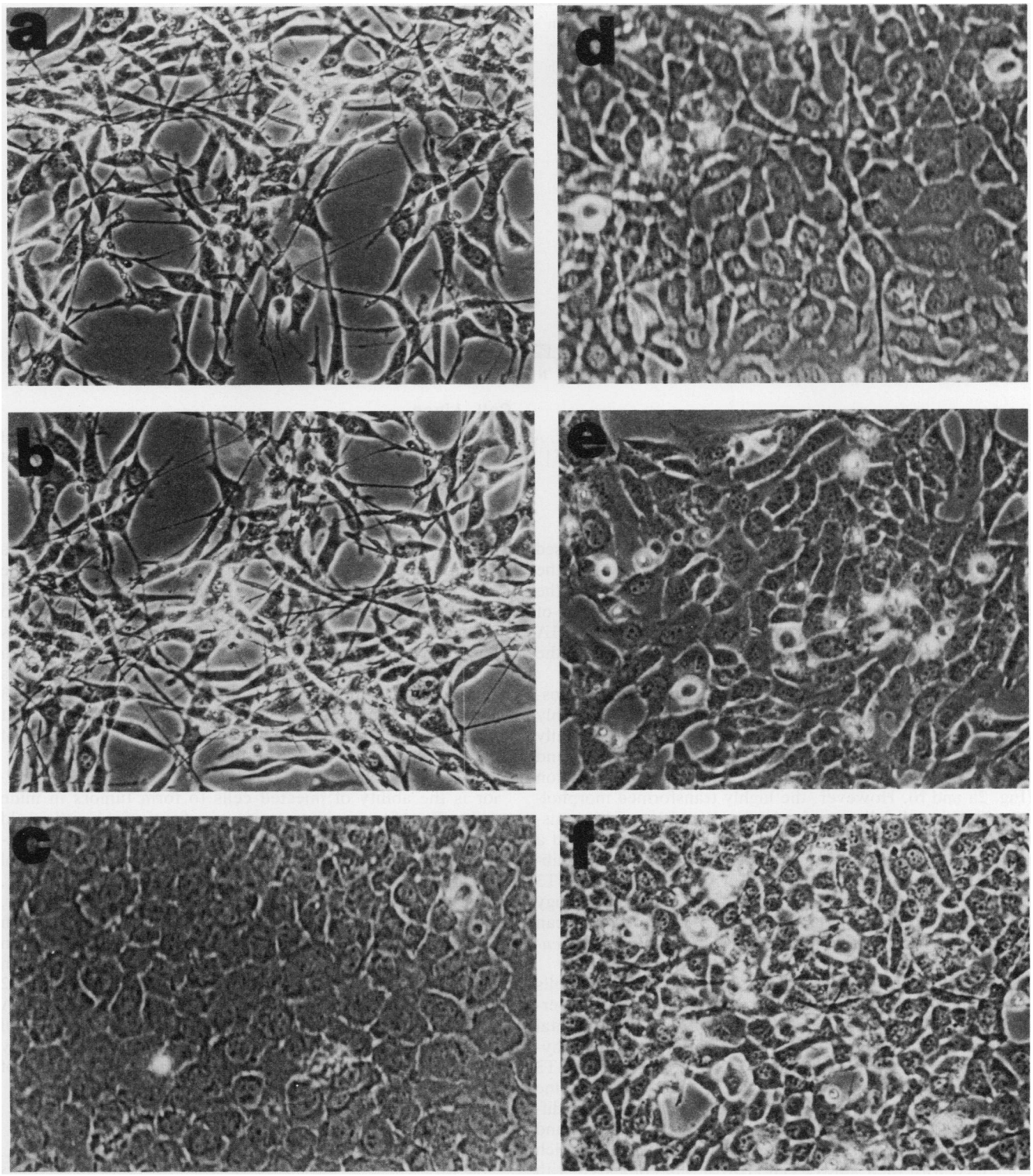

FIG. 2. Morphologic effects of E1A expression in *neu*-transformed B104-1-1 cells. (a) B104-1-1; (b) B-E1Apr; (c) N-E1A-1; (d) B-E1A-1; (e) B-E1A-2; (f) B-E1A-3. Magnification, ×130.

transformed phenotype assays because they represented three different subtypes of B-E1A transfectants: (i) B-E1A-1 had fewer copies of the *neu* gene than did B104-1-1 and high levels of the E1A gene; (ii) B-E1A-2 retained the same level of *neu* gene as B104-1-1 and high levels of the E1A gene; and (iii) B-E1A-3 contained the same amount of *neu* DNA as B104-1-1 but a low quantity of the E1A gene.

E1A expression in B-E1A transfectants can suppress the transformed phenotypes induced by *neu*. The transformed phenotypes of the *neu*-transformed cells include transformed

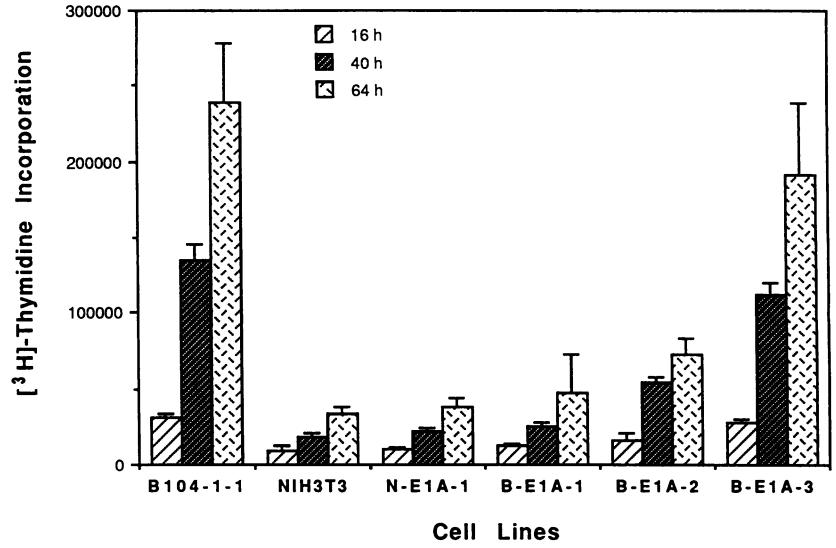

FIG. 3. [^{3}H]thymidine incorporation of the indicated cell lines. A total of 9×10^3 cells were plated in 96-well multiwell plates and cultured in cell culture medium. The cells received a 2-h pulse of 1 μCi of [^{3}H]thymidine per well at the indicated time points to label those cells that were synthesizing DNA prior to harvest.

morphology, non-contact-inhibited growth pattern, increased DNA synthesis rate, anchorage-independent growth, and the ability to induce tumors in *nu/nu* mice. To determine the effect of E1A expression on the transforming ability of *neu*-transformed B104-1-1 cells, we assayed the B-E1A transfectants as well as the control cell lines for all the above-mentioned transforming characteristics.

The biological effects of E1A expression on *neu*-transformed cells were first examined in culture. The highly transformed morphology of B104-1-1 cells was essentially unchanged after pE1Apr transfection. The B104-1-1 and B-E1Apr cells piled up and did not exhibit contact inhibition (Fig. 2a and b). However, the highly transformed morphology of B104-1-1 cells was markedly altered by pE1A transfection (Fig. 2d to f). The B-E1A transfectants exhibited a nontransformed, flattened morphology and a contact-inhibited growth pattern. Expression of E1A proteins in NIH 3T3 cells did not significantly alter the monolayered morphology (Fig. 2c). The results indicate that E1A gene products can specifically reverse the transformed morphology of *neu*-transformed cells.

A frequent parameter measured in growth-stimulated cells is [^{3}H]thymidine incorporation into DNA, and this is generally well correlated to overall DNA synthesis. The parental *neu*-transformed B104-1-1 cells had a high level of [^{3}H]thymidine incorporation, while the negative controls, NIH 3T3 and N-E1A cells, had very low levels of [^{3}H]thymidine incorporation (Fig. 3). [^{3}H]thymidine incorporation was different among the three B-E1A transfectants. B-E1A-1 and B-E1A-2 displayed a much lower DNA synthesis rate, which coincided with their slower cell growth rate compared with B104-1-1 cells (data not shown). This E1A-induced decrease in [^{3}H]thymidine incorporation was not as dramatic in the B-E1A-3 cell line, possibly because of the lower level of the E1A proteins. These data suggest that E1A proteins can block the effect of the *neu* oncogene on DNA synthesis and cell growth.

Anchorage-independent growth in soft agar is a good indicator of transforming ability for transformed cells. Transformed cells usually grow well in an anchorage-independent manner in soft agar, while nontransformed cells usually cannot grow in soft agar. To test the influence of the E1A proteins on anchorage-independent growth, we assayed B104-1-1 cells and the B-E1A transfectants for the ability to grow in soft agar (16). The *neu*-transformed B104-1-1 cells and B-E1Apr control cells formed soft agar colonies with much higher efficiency than did the B-E1A transfectants (Table 1). It is noteworthy that colony formation by NIH 3T3 and N-E1A-1 lines did not vary significantly, indicating that expression of E1A proteins did not enhance the efficiency of NIH 3T3 cells to grow in soft agar.

The most stringent experimental test of neoplastic behavior is the ability of injected cells to form tumors in nude mice. Therefore, in vivo repression of tumorigenicity would be a critical test for E1A-mediated transformation suppression in *neu*-transformed cells. When 10^5 cells of the parental B104-1-1 line were injected subcutaneously in nude mice, solid tumors developed by 8 days after injection; however, the same quantity of the B-E1A transfectants did not form tumors in nude mice until 12 to 26 days after injection, and in every case the tumors were much smaller than those from B104-1-1 cells (Table 2). Although the B-E1A-1 and B-E1A-2 transfectants contained comparable amounts of the E1A gene, the B-E1A-1 cells did not form detectable tumors until 20 days after injection, and their tumor volume was only 166 ± 78 mm^3 35 days after injection, probably because of

TABLE 1. Soft agar colony formation assay

Cells line	Formation of colonies (% efficiency)[a]
B104-1-1	14.4–18.3
B-E1Apr	12.7–15.5
NIH 3T3	<0.1
N-E1A-1	<0.1
B-E1A-1	0.2–0.4
B-E1A-2	2.3–3.1
B-E1A-3	2.6–4.5

[a] Number of soft agar colonies as percentage of 10^3 seeded cells.

TABLE 2. Tumorigenicity assay

Cell line	No. of tumors/6 animals injected						Tumor vol at day 16 (mm^3; mean ± SD)
	8[a]	10	12	14	20	26	
B104-1-1	6	6	6	6	S	S	8,240 ± 203
NIH 3T3	0	0	0	0	0	0	ND[c]
N-E1A-1	0	0	0	0	0	0	ND
B-E1A-1	0	0	0	0	5	6	ND
B-E1A-2	0	0	2	6	6	6	216 ± 53
B-E1A-3	0	0	4	6	6	6	481 ± 74

[a] Time (days) after injection.
[b] S, Sacrificed because of large tumor size.
[c] ND, No detectable tumor at the time of evaluation.

the lower level of *neu* gene in this line. On the other hand, both of the B-E1A-2 and B-E1A-3 transfectants contained the same level of the *neu* gene as did B104-1-1 cells; the tumorigenicity suppression effect on B-E1A-3 cells was not as strong as on B-E1A-2 cells, likely because of the lower level of the E1A gene in B-E1A-3 cells. The control animal injected with NIH 3T3 and N-E1A cells showed no evidence of tumor formation.

Previous studies of Wilms tumor cells and human prostate carcinoma DU145 cells demonstrated that reintroduction of chromosome 11 to Wilms tumor cells or restoration of the retinoblastoma gene to DU145 cells suppressed tumor formation but did not alter the cell morphology, growth rate, or colony-forming ability (5, 31). These data suggest that growth rate in culture and tumorigenicity in nude mice are separable phenomena. In our experiment, the B-E1A-1 and B-E1A-2 cells exhibited a slower growth rate and much weaker tumorigenic activity. Suppression of tumorigenicity cannot entirely be explained by their slower growth rate and decreased [^{3}H]thymidine incorporation. For example, the B-E1A-3 cells were similar in [^{3}H]thymidine incorporation and cell growth rate to B104-1-1 cells, while their tumorigenic activity was markedly suppressed. On the other hand, the growth rate of the B-E1A-1 cell line is about 50% that of the B104-1-1 cell line, whereas the tumor size from B-E1A-1 cells at 35 days after injection was about 5% that of the B104-1-1 cells at 16 days after injection. This observation strongly argues that E1A expression indeed suppresses tumor formation of *neu*-transformed cells, similar to the studies on the retinoblastoma gene mentioned above. Taken together, our results clearly demonstrate that introduction of the E1A gene into B104-1-1 cells suppresses all of the transformed properties of this cell line.

The adenovirus E1A gene is a well-known transforming oncogene that can substitute for the *myc* oncogene and simian virus 40 large tumor antigen gene in the *ras* cotransformation assay of primary embryo fibroblasts (13, 17, 30). Typically, cotransfection of the c-Ha-*ras* and adenovirus E1A genes into rat embryo cells resulted in a 10-fold-higher transformation frequency than transfection with the *ras* oncogene alone. However, our result in this report clearly shows that the adenovirus E1A gene can function as a transformation suppressor gene for *neu* oncogene-transformed NIH 3T3 cells. The apparent discrepancy in transforming ability between rat embryo cells transfected with *ras* plus E1A and NIH 3T3 cells transfected with *neu* plus E1A may be attributed to differences in the cellular functions of the proteins encoded by the two oncogenes (*ras* versus *neu*) as well as differences between the two systems. Nevertheless, our results clearly indicate that E1A can act as a transformation suppressor gene in the system described here.

The human *neu* proto-oncogene is known to play an important role in tumorigenicity in both animal models and human cancers (10, 21, 22, 37). Amplification or overexpression of the human *neu* proto-oncogene has been frequently found in human primary breast and ovarian cancers. We previously have shown that E1A proteins can also repress expression of the human *neu* gene product (34, 36). It will be important to examine whether this repression of *neu* expression may confer reduced transforming ability to the human breast and ovarian cancer cells that overexpress the *neu* oncogene, and these studies should establish E1A as a transforming suppressor gene for human cancer cells overexpressing the *neu* proto-oncogene.

We thank R. Arlinghaus, J. Klostergaard, G. Nicolson, and F. Strebel for helpful discussions and suggestions on the manuscript.

This work was supported by Public Health Service grant CA-45265 from the National Cancer Institute and grant 2527 from the Council for Tobacco Research U.S.A. Inc. Dihua Yu is the recipient of a Welch predoctoral fellowship (G1134).

REFERENCES

1. **Bargmann, C. I., M.-C. Hung, and R. A. Weinberg.** 1986. Multiple independent activations of the *neu* oncogene by a point mutation affecting the transmembrane domain of p185. Cell **45**:649–657.
2. **Bargmann, C. I., M.-C. Hung, and R. A. Weinberg.** 1986. The *neu* oncogene encodes an epidermal growth factor receptor-related protein. Nature (London) **319**:226–23.
3. **Bargmann, C. I., and R. A. Weinberg.** 1988. Increased tyrosine kinase activity associated with the protein encoded by the activated *neu* oncogene. Proc. Natl. Acad. Sci. USA **85**:5394–5398.
4. **Berk, A. J.** 1986. Adenovirus promoters and E1A transactivation. Annu. Rev. Genet. **20**:45–79.
5. **Bookstein, R., J.-Y. Shew, P.-L. Chen, P. Scully, and W.-H. Lee.** 1990. Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated RB gene. Science **247**:712–715.
6. **Borrelli, E., R. Hen, and P. Chambon.** 1984. Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. Nature (London) **312**:608–612.
7. **Chen, C. A., and H. Okayama.** 1988. Calcium phosphate-mediated gene transfer: a high efficient transfection system for stably transforming cells with plasmid DNA. Bio Techniques **6**:632–638.
8. **Coussens, L., T. L. Yang-Feng, Y. C. Liao, E. Chen, A. Gray, J. McGrath, P. H. Seeburg, T. A. Libermann, J. Schlessinger, U. Francke, A. Levinson, and A. Ullrich.** 1985. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with *neu* oncogene. Science **230**:1132–1139.
9. **Hen, R., E. Borrelli, and P. Chambon.** 1985. Repression of the immunoglobulin heavy chain enhancer by the adenovirus-2 E1A products. Science **230**:1391–1394.
10. **Hung, M.-C.** 1988. The *neu* proto-oncogene and breast cancer. Cancer Bull **40**:300–303.
11. **Hung, M.-C., A. L. Schechter, P.-Y. M. Churay, D. F. Stern, and R. A. Weinberg.** 1986. Molecular cloning of the *neu* gene: absence of gross structure alterations in oncogenic alleles. Proc. Natl. Acad. Sci. USA **83**:261–264.
12. **Hung, M.-C., D.-H. Yan, and X. Zhao.** 1989. Amplification of the proto-*neu* oncogene facilities oncogenic activation by a single point mutation. Proc. Natl. Acad. Sci. USA **86**:2545–2548.
13. **Land, H., L. F. Parada, and R. A. Weinberg.** 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (London) **304**:596–602.
14. **Lillie, J. W., and M. R. Green.** 1989. Transcription activation by

the adenovirus E1A protein. Nature (London) **338**:39–44.
15. **Lupu, R., R. Colomer, G. Zugmaier, J. Sarup, M. Shepard, D. Slamon, and M. E. Lippman.** 1990. Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and $p185^{erbB2}$. Science **249**:1552–1555.
16. **Matin, A., K.-L. Cheng, T.-C. Suen, and M.-C. Hung.** 1990. Effect of glucocorticoids on oncogene transformed NIH3T3 cells. Oncogene **5**:111–116.
17. **Ruley, H. E.** 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature (London) **304**:602–606.
18. **Sassone-Corsi, P., and E. Borrelli.** 1987. Promoter trans-activation of protooncogene c-fos and c-myc, but not c-Ha-ras, by products of adenovirus early region 1A. Proc. Natl. Acad. Sci. USA **84**:6430–6433.
19. **Schechter, A. L., M.-C. Hung, L. Vaidyanathan, S. Decker, J. Drebin, M. I. Greene, and R. A. Weinberg.** 1985. The *neu* gene: an erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. Science **229**:976–978.
20. **Shih, C., L. C. Padhy, M. Murray, and R. A. Weinberg.** 1981. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. Nature (London) **290**:261–264.
21. **Slamon, D. J., G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich, and W. L. McGuire.** 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. Science **240**:177–182.
22. **Slamon, D. J., G. Williams, L. A. Jones, J. A. Holt, S. G. Wong, D. E. Keith, W. J. Levin, S. G. Stuart, J. Udove, A. Ullrich, and M. F. Press.** 1989. Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science **244**:707–712.
23. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1**:327–341.
24. **Stein, R. W., and E. B. Ziff.** 1987. Repression of insulin gene expression by adenovirus type 5 E1a proteins. Mol. Cell. Biol. **7**:1164–1170.
25. **Stern, D. F., M. P. Kamps, and H. Cao.** 1988. Oncogenic activation of p185 neu stimulates tyrosine phosphorylation in vivo. Mol. Cell. Biol. **8**:3969–3973.
26. **van de Vijver, M., R. Bersselaar, P. Devilee, C. Cornelisse, J. Peterse, and R. Nusse.** 1987. Amplification of the *neu* (c-*erb*B-2) oncogene in human mammary tumors is relatively frequent and is often accompanied by amplification of the linked c-*erb*A oncogene. Mol. Cell. Biol. **7**:2019–2023.
27. **van de Vijver, M. J., J. L. Peterse, W. J. Mool, P. Wisman, J. Lomans, O. Dalesio, and R. Nusse.** 1988. *neu*-protein overexpression in breast cancer: association with comedo-type ductal carcinoma in situ and limited prognostic value in state II breast cancer. N. Engl. J. Med. **319**:1239–1245.
28. **Velcich, A., and E. Ziff.** 1985. Adenovirus E1A proteins repress transcription from the SV40 early promoter. Cell **40**:705–716.
29. **Webster, K. A., G. E. O. Muscat, and L. Kedes.** Adenovirus E1A products suppress myogenic differentiation and inhibit transcription from muscle-specific promoters. Nature (London) **332**:553–557.
30. **Weinberg, R. A.** 1985. The action of oncogenes in the cytoplasm and nucleus. Science **230**:770–776.
31. **Weissman, B. E., P. J. Saxon, S. R. Pasquale, G. R. Jones, A. G. Geiser, and E. J. Stanbridge.** 1987. Introduction of a normal human chromosome 11 into a Wilms' tumor cell line controls its tumorgenic expression. Science **236**:175–180.
32. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56**:67–75.
33. **Yamamoto, T. M., S. Ikawa, T. Akiyana, K. Semba, N. Normura, N. Miyajima, T. Saito, and K. Toyoshima.** 1986. Similarity of protein encoded by the human c-*erb*B 2 gene to epidermal growth factor receptor. Nature (London) **319**:230–234.
34. **Yan, D.-H., Chang, L.-S., and M.-C. Hung.** Oncogene, in press.
35. **Yarden, Y., and R. A. Weinberg.** 1989. Experimental approaches to hypothetical hormones: detection of a candidate ligand of the *neu* protooncogene. Proc. Natl. Acad. Sci. USA **86**:3179–3183.
36. **Yu, D., T.-C. Suen, D.-H. Yan, L.-S. Chang, and M.-C. Hung.** 1990. Transcriptional repression of the *neu* protooncogene by the adenovirus 5 E1A gene products. Proc. Natl. Acad. Sci. USA **87**:4499–4503.
37. **Zhang, X., E. Silva, D. Gershanson, and M.-C. Hung.** 1989. Amplication and rearrangement of c-erbB proto-oncogene in cancer of human female genital tract. Oncogene **4**:985–989.