Oncogene (1997) 14, 1965–1971

Mapping of adenovirus 5 E1A domains responsible for suppression of *neu*-mediated transformation via transcriptional repression of *neu*

Hua Chen[1,2], Dihua Yu[1,2,3], G Chinnadurai[4], Devarajan Karunagaran[1,2] and Mien-Chie Hung[1,2]

[1]*Department of Tumor Biology,* [2]*Breast Cancer Basic Research Program, and* [3]*Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030;* [4]*Institute for Molecular Virology, Saint Louis University School of Medicine, 3681 Park Avenue, Saint Louis, MO 63110, USA*

Overexpression of *neu* (also known as c-*erb*B-2 or *HER*-2) commonly occurs in human cancer and is also known to enchance tumor metastasis and chemoresistance. Our earlier reports showed that the adenovirus 5 E1A can suppresss the *neu*-mediated transformation by repression of *neu*. Thus, E1A has the potential to be used as a therapeutic agent against the *neu*-overexpressing human cancers. However, a serious concern to this approach is that E1A is also capable of immortalizing primary culture cells and can co-operate with *ras* or E1B oncogenes to transform them. The E1A CR2 domain (amino acid residues 120 to 140) necessary for binding to RB is believed to be required for this oncogenic function. Here, we report that deletion of CR2 region did not affect E1A's capability to repress *neu*. Interestingly, deletion of the amino acid residues 4 to 25 or 40 to 80 completely disrupted E1A-mediated *neu* repression. By deleting the amino acid residues from 81 to 185, we have successfully generated a mini-E1A mutant that was sufficient to inhibit *neu* promoter activity and suppress *neu*-mediated transformation. The mini-E1A mutant does not contain the CR2 domain that is crucial for RB binding and immortalization, and hence, may serve as a more selective tumor suppressor, and a safer therapeutic agent. It may also be a useful tool to further investigate the molecular mechanism(s) of *neu* overexpression and E1A-mediated transcriptional repression in cancer cells.

Keywords: adenovirus 5 E1A domains; transcriptional repression; *neu* protooncogene; suppression; transformation

Introduction

The *neu* gene (also known as c-*erb*B-2, *HER*-2 or NGL) encodes a receptor tyrosine kinase belonging to epidermal growth factor receptor family (Bargmann *et al*., 1986a, b; Hung *et al*., 1986; Dougall *et al*., 1994). A single point mutation at the *neu* transmembrane domain was found in carcinogen-induced neuroblastoma (Padhy *et al*., 1982; Bargmann *et al*., 1986a, b), and the mutated *neu* was shown to be capable of generating tumors both in a nude mouse model (Hung *et al*., 1989; Dougall *et al*., 1994) and *neu*-transgenic mice (Muller *et al*., 1988). In human malignancy, *neu* overexpression (Van *et al*., 1987; Yokota *et al*., 1988; D'Emilia *et al*., 1989; Park *et al*., 1989; Slamon *et al*., 1989; Schneider *et al*., 1989; Zhang *et al*., 1989; Berchuck *et al*., 1990; Saya *et al*., 1990; Weiner *et al*., 1990; Hung *et al*., 1992; Shi *et al*., 1992) was found to be correlated significantly with poor prognosis (Van *et al*., 1987; Slamon *et al*., 1989; Berchuck *et al*., 1990; Weiner *et al*., 1990). Furthermore, *neu* overexpression was shown to be capable of enhancing metastatic potential and inducing chemoresistance in cancer cells (Yu *et al*., 1994; Tsai *et al*., 1995). Taken together, it may be stated that *neu* is a potent oncogene, and its overexpression plays a crucial role in the development of human malignancies (Yu *et al*., 1991, 1992).

We have previously reported that the adenovirus 5 E1A is capable of repressing *neu* expression and thereby suppressing the *neu*-mediated transformation (Yu *et al*., 1990, 1992, 1993). Hence, E1A may be considered as a targeting agent for the treatment of *neu*-overexpressing cancer. However, a serious concern to this approach is that E1A is also capable of immortalizing primary culture cells and it can co-operate with *ras* or E1B oncogene to transform them. The CR2 domain of E1A is known to be required for this oncogenic function as its deletion abolished the induction of cell immortalization by E1A (reviewed in Mymrick, 1996; Moran and Andrews, 1987; Chinnadurai, 1992). We have previously shown that the E1A mutant d1346 (deletion of nucleotides 859–907 from the wild type E1A), was unable to repress *neu* suggesting that this region, mistakenly interpreted at that time as the CR2 domain, may be required for *neu* repression (Yu *et al*., 1990) though it actually encodes the spacer region between CR1 and CR2. Abolishing or reducing the potential risk of immortalization and consequent transformation caused by the wild type E1A is expected to aid its use as a therapeutic agent. Hence, we attempted to delineate the structural domains of E1A said to be responsible for immortalization from those possibly involved in *neu* repression. To this end, in the present work, we successfully figured out the regions of E1A required and sufficient for *neu* repression and constructed a mini-E1A that does not contain the CR2 domain but is still capable of repressing *neu* expression and suppressing the *neu*-mediated transformation.

Results

The adenovirus 5 E1A N-terminal 80 amino acids are required for repression of neu *expression and suppression of foci formation induced by mutation-activated* neu

To map the region(s) of the E1A protein required for repression of *neu* expression, a series of E1A mutants

Correspondence: M-C Hung
Received 19 November 1996; accepted 13 January 1997

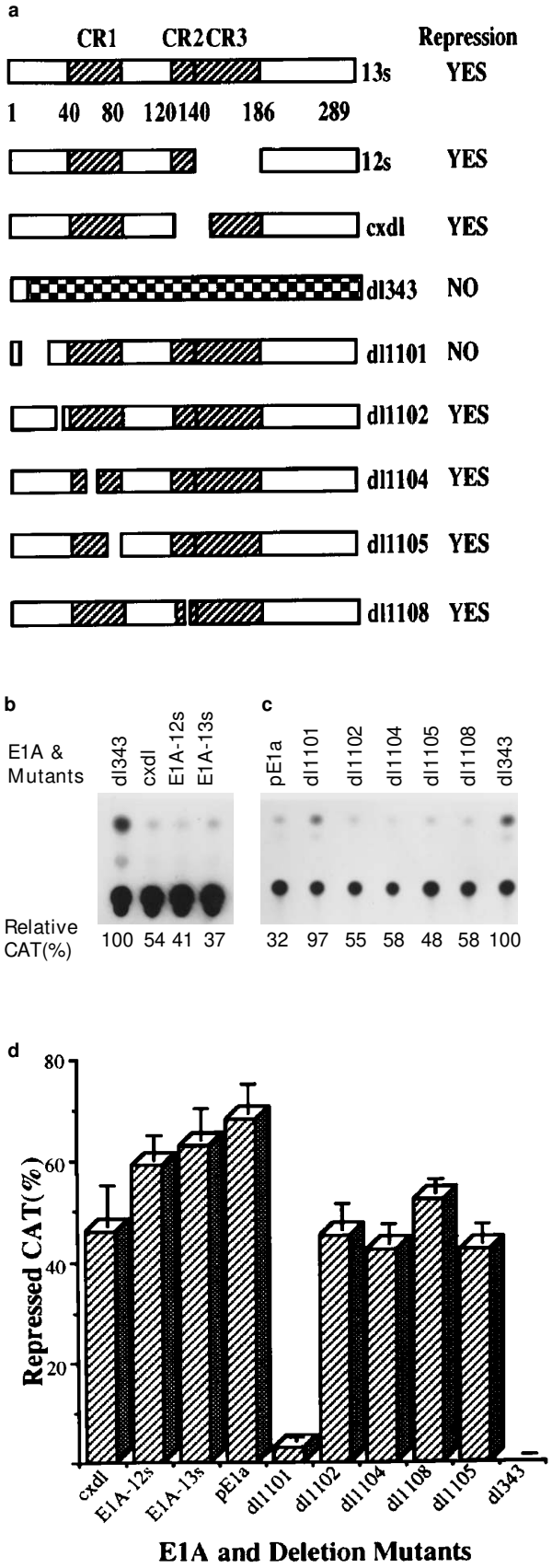

Figure 1 E1A domains required for transcriptional repression of *neu*. The structures of E1A and its mutants are shown schematically in (**a**). The hatched areas represent the conserved regions of E1A (CR1, CR2 and CR3). The discontinuous portions (gaps) depict the deleted regions. The experimental data are shown in (**b**) and (**c**). E1A plasmid, E1A frame shift plasmid (dl343), or E1A mutants (20 μg) were cotransfected into NIH3T3 cells along with pNeu-StuI-CAT (4 μg) and pRSVβ-gal (4 μg) The repressed CAT activities from multiple experiments are shown as relative CAT % and further diagrammed in (**d**). The standard deviations are shown by error bars

were examined for their ability to repress *neu* promoter activity. As shown in Figure 1, a mutant cxdl, in which the entire CR2 region was deleted, effectively repressed *neu* promoter activity as compared with wild type E1A (Yu *et al*., 1990), indicating that CR2 is not required for repression of *neu* expression. To further map whether the N-terminal and CR1 domains might be required for repression of *neu* promoter activity, we examined another set of mutants with the small deletion mutants in the N-terminal non-conserved domain and the CR1 domain for their ability to repress *neu*. The mutants used include dl1101 and dl1102 with deletion of amino acid residues 4 to 25 and 26 to 35 in N-terminal non conserved domain, respectively; and dl1104 and dl1105 with deletion of amino acid residues 48 to 60 and 70 to 80 in CR1 domain, respectively, Another mutant, dl1108, with deletion of amino acid residues 124 to 127 in the CR2 domain with a consequent loss of ability to bind the RB protein, was also tested to see whether RB-binding is critical for *neu* repression. All these E1A mutants were driven by the adenovirus 5 E1A promoter (Jelsma *et al*., 1988, 1989). When these mutants were transiently transfected in the NIH3T3 cells, all of them expressed the protein products at a level comparable to that of the wild type E1A (data not shown). As shown in Figure 1a, c, and d, the dl1101 mutant lost the ability to repress *neu*, whereas other mutant proteins still significantly repressed the promoter activity, suggesting that the adenovirus 5 E1A N-terminal (amino acid residues 4 to 25) is required for repression of *neu* expression, while some parts of the CR1 are dispensable, and confirmed that the RB-binding function is not required.

Encouraged by these results, we attempted to construct a mini-E1A mutant (by deleting the dispensable regions), which is still expected to repress the expression of *neu*. As shown in Figure 2a, the E1AN40 mutant, in which the regions from CR1 to CR3 were deleted, lost its ability to inhibit *neu* promoter activity. But the E1AN80 mutant, which contained the entire CR1 domain and was otherwise identical to E1AN40 mutant, still efficiently repressed the *neu* promoter activity, compared with the wild type E1A proteins. They expressed the proteins at a comparable level when they were transfected into NIH3T3 cells (Figure 2b. These results indicated that although some regions in CR1 (amino acid residues 48 to 60 or 70 to 80) are not required for repression of *neu* promoter activity, deletion of the entire CR1 region led to complete loss of the activity to repress *neu*.

To further examine whether these mini-E1A mutants are able to suppress the transformation phenotype induced by the mutation-activated *neu* oncogene, we performed the focus-forming assays by cotransfection of mini-E1A mutants and the genomic mutation-activated *neu* (c*Neu*-104) into NIH3T3 cells. As shown in Figure 3, the E1AN80 construct, like the wild type E1A, dramatically reduced the foci formation mediated by mutation-activated *neu*, while the E1AN40 construct did not suppress the foci formation.

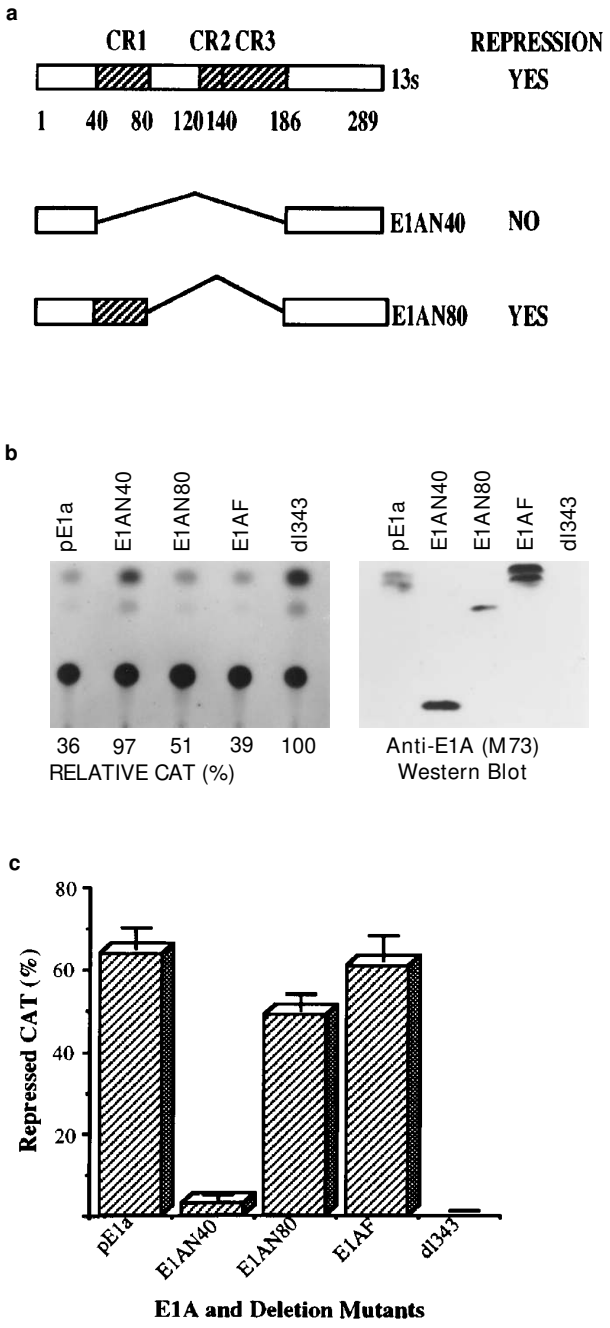

Figure 2 E1A domains sufficient for transcriptional repression of *neu*. The schematic structures of E1A and the mutants are shown in (**a**). The transfections were performed as described in Figure 1. The repression of *neu* promoter activity by mutant E1AN80 and the E1A proteins expressed by E1A and deletion mutants are shown in (**b**). The repressed CAT activities from multiple experiments are further diagrammed in (**c**), and the standard deviations are shown by error bars

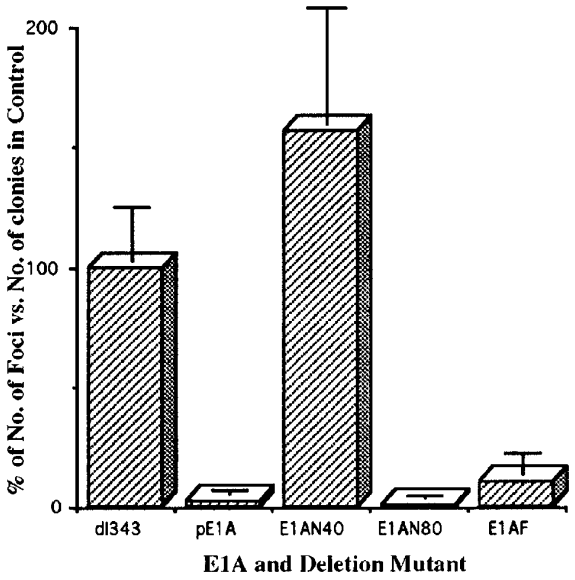

Figure 3 Suppression of activated rat *neu*-mediated foci formation by mini-E1A. One microgram of c*Neu*-104 was cotransfected into NIH3T3 cells along with 0.1 μg of the drug selection plasmid pSV2neo and 10 μg of the plasmids encoding E1A or deletion mutants. The number of foci formed for each transfection was normalized by dividing the foci number with G418-resistant colony numbers obtained from the same transfection. The results are shown as percentage of the normalized number of foci in each transfection versus that in control transfection (dl343). Data are the average from three independent experiments, and the standard deviations are shown by error bars

Reduction of neu*-encoded p185 level in the E1AN80 stable transfectants*

To test the mini-E1A mutant for its ability to down-regulate the *neu*-encoded p185 protein level and characterize its effects on transformation phenotypes, we also established stable transfectants of the mini-E1A mutant using B104-1-1 cells as recipient, which are NIH3T3 cells transformed by a genomic mutation-activated *neu* oncogene. To this end, we cotransfected the B104-1-1 cells with the E1AN80 construct and the pCMV-neo plasmid carrying the neomycin-resistance marker gene driven by the cytomegalovirus promoter. The G418-resistant clones were screened for E1A mutant protein expression and expanded into cell lines, which were designated as BEN80 cell lines. To rule out the possibility that the changes in any of the biological behavior of the transfectants were due to artificial cell manipulation, two E1AN80 expressing transfectants (BEN80.1 and BEN80.2) selected from individual clones, and one transfectant (BEN80.3) pooled from a single plate containing more than 20 individual clones, were used in the following experiments. A transfectant with the vector backbone containing the neomycin-resistant gene but without E1AN80 was also selected as a negative control, and designated Bneo. The expression of the E1AN80 mutants in these individual transfectants is shown in Figure 4b. BE1A1 is a previously established B104-1-1 transfectant expressing wild type E1A proteins with a consequent down-regulation of the p185 protein encoded by *neu* (Yu *et al*., 1990).

To determine whether expression of E1AN80 in BEN80 transfectants will affect the *neu*-encoded p185 expression, immunoblot analysis of *neu*-encoded p185 protein using monoclonal antibodies against *neu* (c-neu-Ab3) was performed. The p185 protein levels in all the mini-E1A transfectants were dramatically reduced compared to those of the control Bneo cell line and the parental B104-1-1 cell line (Figure 4a). The amount of protein loading is comparable as shown in Figure 4c by Western blot analysis using monoclonal antibodies against a β-actin protein. These results indicated that

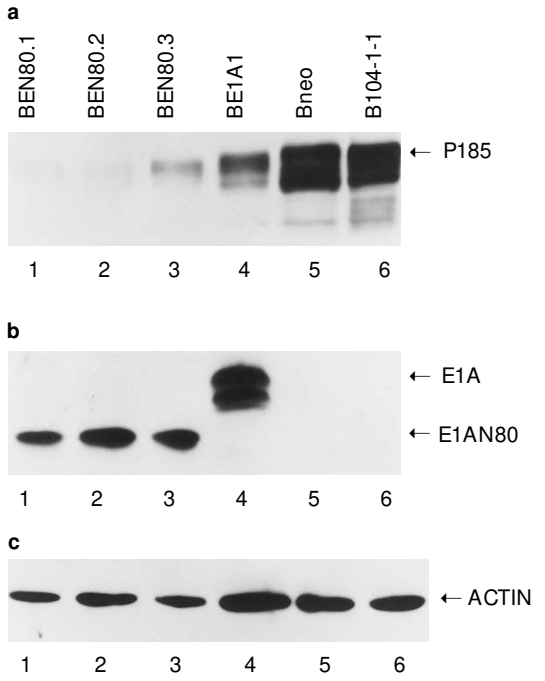

Figure 4 Reduction of the *neu*-encoded p185 level in mini-E1A stable transfectants. One hundred micrograms of protein was subjected to electrophoresis on 6% (**a**) or 8% (**b** and **c**) SDS–PAGE prior to transfer to nitrocellulose filters. Filters were incubated with the primary antibodies c-*neu*-Ab-3 against p185 (**a**), M73 against adenovirus E1A (**b**), and antibody against β-actin (**c**). BEN80 represents B104-1-1 cells transfected by E1AN80 mutants. BE1A1 is a previously established B104-1-1 transfected by the wild type E1A gene (23) (Yu *et al*., 1990)

the E1AN80 mutant is able to repress p185 expression, consistent with the fact that it inhibits the *neu* promoter activity.

Reversion of transformation phenotypes in E1AN80 tranfectants

To analyse if expression of E1AN80 would suppress *neu*-mediated transformation in the BEN80 cells, we characterized the transformation phenotype by *in vitro* assays. DNA sysnthesis rates and the cell growth rates were measured by [^{3}H]thymidine incorporation assay and MTT assay. The DNA synthesis rates and the growth rates of the BEN80 transfectants were lower than those of the control Bneo and parental cell B104-1-1 (Figure 5a and b). We then examined their anchorage-independent growth abilities by soft agar colonization assay. As shown in Figure 6, either the *neu*-transformed B104-1-1 cells or the control Bneo cells exhibited high efficiency to form soft agar colonies, whereas the colony-forming efficiencies of the three BEN80 transfectants were strikingly reduced. These data suggested that E1AN80 proteins can suppress the transformation mediated by mutation-activated *neu in vitro*, i.e. anchorage-independent growth. Taken together, these data indicated that the *in vitro* transforming phenotype of the B104-1-1 cells was largely reversed by transfection of the E1AN80 mutant.

E1AN80 mutant transfectants were then tested for their ability to suppress tumor formation *in vivo*. The tumorigenicity assays were conducted in nu/nu mice that were injected s.c. with 3×10^6 cells from BEN80

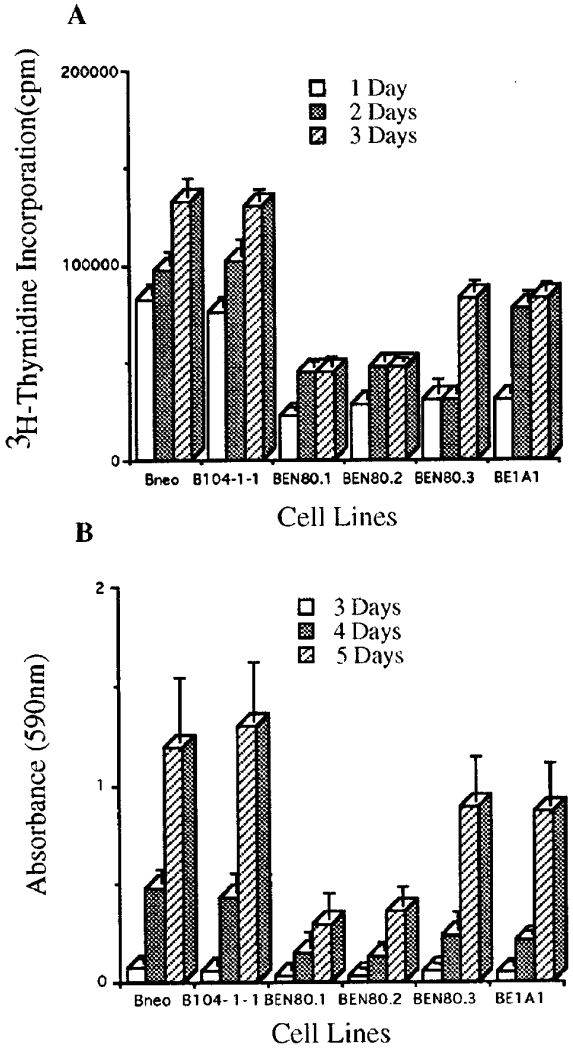

Figure 5 Reduced cell growth rate of mini-E1A stable transfectants. (**a**) [^{3}H]thymidine (1 μCi/well) was added to cells at the indicated time points to label those cells that were synthesizing DNA prior to harvest. Radioactivity of individual samples was counted by a scintillation counter; average c.p.m. were calculated from ten replicate samples. Experiments were repeated two times for each cell line. (**b**) MTT assays were performed as desribed (27) (Yu *et al*., 1993). At the indicated days after plating, average OD 590 nm values were calculated from ten replicate samples. Experiments were repeated two times for each cell line

transfectants, the control E1A wild type transfectant, Bneo cell line, and B104-1-1 cell, respectively (Figure 7). Mice injected with the parental B104-1-1 cells or with the control Bneo cells formed tumors, 2 days after injection, and had huge tumor burdens of 4000 mm^3 by 2 weeks post injection. However, mice injected with the same number of BEN80 transfectants did not form tumors in nude mice until 1 week after injection, and the tumor size was much smaller than the tumors formed from B104-1-1 and Bneo cells (Figure 7). These results clearly demonstrated that E1AN80 can suppress the tumorigenic potential of the B104-1-1 cells *in vivo*.

Discussion

Adenovirus E1A binds to various cellular regulators and mediates multiple cellular events. The present study extends and gives further credibility to our

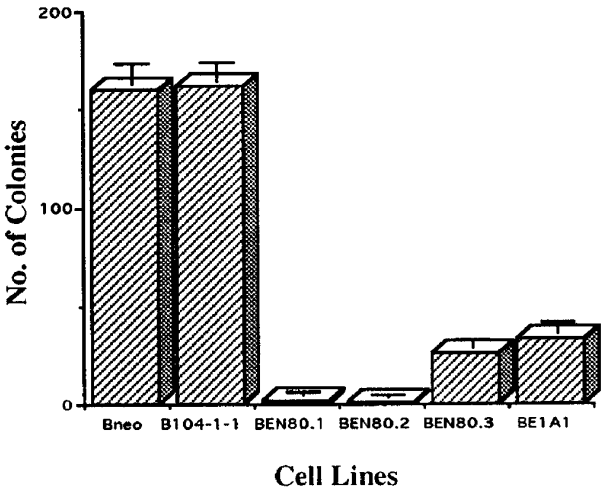

Figure 6 Reversion of activated *neu*-mediated transformation by the mini-E1A. Soft agar assays were performed as described in Materials and methods. The numbers of soft agar colonies from each cell line were calculated from four replicate samples and shown with standard deviation. Experiments were repeated for each cell line at least twice

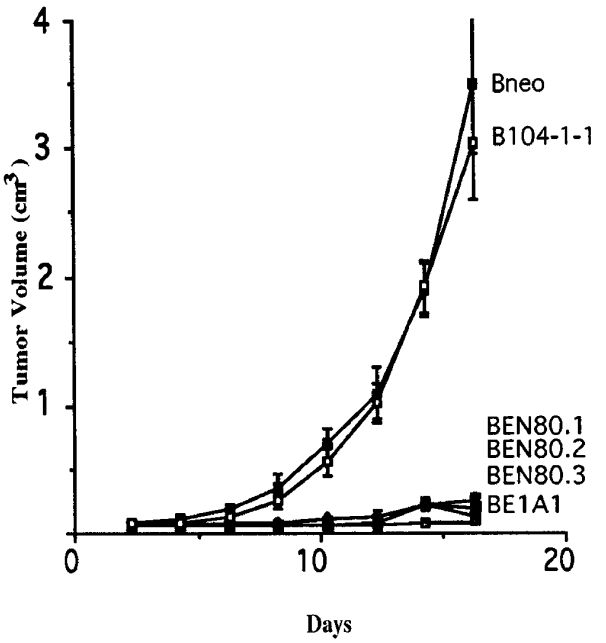

Figure 7 Suppression of tumor formation by mini-E1A. Viable cells (3×10^6) were injected into right and left flanks of female homozygous nu/nu mice. Five mice were injected for each cell line. Tumor volumes from mice injected with the indicated cell lines at the indicated days are shown with standard deviation

results published earlier which demonstrated that the wild type adenovirus 5 E1A is capable of repressing *neu* expression and thereby suppressing the *neu*-mediated transformation (Yu *et al*., 1990, 1992, 1993) supporting the use of E1A as a targeting agent (Zhang *et al*., 1995). Evidences supporting that E1A could suppress transformation, tumorigenicity and metastatic ability in several systems have accumulated over the years (Mymryk, 1996). The general mechanism by which E1A represses the expression of a number of cellular and viral genes appears to be through the interaction of its N-terminal non-conserved domain and CR1 domain with p300 (Mymryk, 1996). In line with this, the results from this study identified the adenovirus 5 E1A N-terminal non-conserved domain and CR1 domain as the E1A functional domains required for the repression of *neu* expression. The CR2 and CR3 domains are dispensable for *neu* repression as a mini-E1A mutant (E1AN80) with deleted CR2 and CR3 domains, was sufficient for transcriptional repression of the *neu* gene and suppression of the transformation phenotypes mediated by point mutation-activated *neu*. The E1A CR2 domain is known to bind with the RB family of proteins, leading to immortalization of the primary culture cells and in cooperation with *ras* or E1B oncogenes, E1A can lead to transformation (Whyte *et al*., 1989; Corbeil and Branton, 1994). Deletion of the CR2 domain or even a site mutation to knock out the RB-binding site on E1A is sufficient to abolish the immortalization function of E1A (Lillie *et al*., 1986; Moran *et al*., 1986; Zerler *et al*., 1986, 1987; Schneider *et al*., 1987; Kuppuswamy and Chinnadurai, 1987; Smith and Ziff, 1988; Jelsma *et al*., 1989; Whyte *et al*., 1989). Thus, the deletion of the CR2 and CR3 domains in the mini-E1A would abolish the potential risk of immortalization and consequent transformation caused by wild type E1A. The spacer region between CR1 and CR2 domains referred earlier (Yu *et al*., 1990) is not present in the E1AN80 mutant and so this region may not be directly involved in *neu* repression and perhaps, required for maintaining a correct conformation to allow *neu* repression by the wild type E1A. It is clear that the E1AN80 construct represents a mini-E1A mutant that still represses the *neu* expression and yet does not contain the domains needed for binding to the RB protein (CR2) or transactivating other promoters (CR3). Other known mechanisms of the anti-oncogenic properties of E1A include the induction of differentiation characteristics and accumulating the p53 tumor suppressor gene product (Braithwaite *et al*., 1990; Lowe and Ruley, 1993; Chiou *et al*., 1994; Mymrick *et al*., 1996). Although our mini-E1A construct essentially delinked the oncogenic regions of E1A from those of tumor suppressive regions, it is not clear whether specific sequences in the CR1 domain could be critical for *neu* repression or if deletion of the entire CR1 domain might cause conformational change and consequently prevent E1A from repression of *neu*. Nevertheless, our results do suggest that the mini-E1A (E1AN80) acts as a tumor suppressor for *neu*-mediated transformation, and it may be a safer therapeutic agent. Further investigations into the molecular mechanism(s) of E1A-mediated transcriptional repression of *neu* overexpression are in progress.

Materials and methods

Cell and plasmids

The B104-1-1 and NIH3T3 cell lines were grown in DMEM/F12 medium (GIBCO, Grand Island, NY) supplemented with 10% FBS. The stable transfectants were grown under the same conditions with the addition of G418 (800 μg/ml) to the culture medium. The deletion mutants cxdl, 12s, and 13s were generously provided by Dr Elizabeth Moran (The Fels Institute for Cancer Research and Molecular Biology, Temple University School of

Medicine, Philadelphia, PA). The deletion mutants dl1101, dl1102, dl1104, dl1105 and dl1108 were kind gifts from Dr Stanley T Bayley (Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1). The E1A gene fragments encoding the amino acid residues 1 to 40 (E1A N-terminal non conserved domain), 1 to 80 (E1A N-terminal non conserved domain and the CR1 domain), and 186 to 289 (E1A nuclear localization domain) were created by PCR using pE1A plasmid (Chang *et al*., 1989; Yu *et al*., 1990) as the template. The E1AN40 and E1AN80 mutants were generated by subcloning of the 1 to 40 or 1 to 80 PCR fragments together with the nuclear localization domain (186 to 289) into the vector pCDNAI (Invitrogen, CA), respectively. The E1AF was generated by subcloning of the genomic E1A gene into the pCDNAI vector. The pCMVneo plasmid was constructed by cloning the neo gene into pCDNAI vector. The following plasmids, which have been previously described, were used in this study: the *neu* promoter deletion-CAT constructs (Suen and Hung, 1990), c*Neu*-104 plasmid encoding genomic mutation-activated *neu* (Hung *et al*., 1989), pRSV-βgal (Edlund *et al*., 1985), and pSV2neo (Yu *et al*., 1993).

Transient transfections and CAT assay

The transfection assay was performed as previously described (Cheng and Okayama, 1988). NIH3T3 cells were cotransfected with 20 μg of plasmids encoding E1A or deletion mutants, 4 μg of plasmid p*Neu*-StuI-CAT and 4 μg of RSVβ-gal. Forty-eight hours after transfection, the cells were harvested and cell extracts were assayed for the β-gal activity (Norton and Coffin, 1985) and CAT activity (Gorman *et al*., 1982). Transfection experiments were repeated several times. The representative data are shown.

Focus-forming assay

The focus-forming assay was carried out as described previously (Yu *et al*., 1992). One microgram c*Neu*-104 was cotransfected into NIH3T3 cells with 10 μg of plasmids encoding E1A deletion mutants, E1A wild type protein, or E1A frame shift protein, respectively. The DNA mixture also contained 0.1 μg of the drug-selection plasmid pSV2neo, which served as an internal control for normalization of the transfection efficiency.

Stable transfection

B104-1-1 cells, which are NIH3T3 cells transformed by a mutation-activated genomic *neu*, were transfected with 10 μg of E1AN80 plasmid DNA along with 1 μg of pCMV-neo plasmid DNA. The cells were trypsinized and then split at a 1 : 10 ratio 48 h after transfection. After 4 to 6 weeks selection using medium containing 800 μg/ml of G418, individual G418-resistant colonies were cloned or pooled.

Immunoblotting

Immunoblot analyses were performed as previously described (Yu *et al*., 1990). The primary monoclonal antibodies used were M73 against the E1A proteins (a generous gift from Dr Ed Harlow, Massachusetts General Hospital, MA), c-*neu*-Ab-3 against the *neu*-encoded p185 protein (Oncogene Science, Inc, Manhasset, NY), and anti-β actin (Amersham, UK).

In vitro *cell growth and colony formation in soft agarose*

[^{3}H]thymidine incorporation assay and MTT assay were performed as previously described (Yu *et al*., 1993). The ability of different cell lines to grow in soft agarose was determined as previously described (Yu *et al*., 1992). Cells (10^3 cells/well, four wells for each cell line) were plated into 24-well plates in culture medium containing 0.35% agarose (BRL, Gaithersburg, MD) overlying a 0.7% agarose layer. The cells were then incubated at 37°C for 5 weeks, after which the plates were stained with *p*-iodonitrotetrazolium violet (1 mg/ml) for 48 h at 37°C. Colonies greater than 100 μ were counted for each dish and cell line.

In vivo *tumor growth*

Tumorigenicity assay was performed as previously described (Yu *et al*., 1993). 3×10^6 cells in 0.1 μl of PBS were injected s.c. into both the right and left flanks of female mice (Harlan-Sprague Dawley, Inc, Indianapolis, IN). The growth of tumors was monitored every other day for 2 to 3 weeks.

Acknowledgements
We are grateful to Dr Elizabeth Moran and Dr Stanley T Bayley for providing the E1A mutant constructs and Dr Ed Harlow for the E1A monoclonal antibody. We thank Duen-Hwa Yan and Yujiao Zhang for helpful discussions and suggestions on the manuscript. This research was supported by grants R01-CA58880, R01-CA60858 (to M-CH), core grant 16672 from the National Cancer Institute, and Nellie Connally Breast Cancer Research Fund from M D Anderson Cancer Center (to M-C H).

References

Bargmann CI, Hung MC and Weinberg RA. (1986a). *Cell*, **45,** 649 – 657.
Bargmann CI, Hung MC and Weinberg RA. (1986b). *Nature*, **319,** 226 – 230.
Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, Clarke PD and Marks P. (1990). *Cancer Res*., **50,** 4087 – 4091.
Braithwaite A, Nelson C, Skulimowski A, McGovern J, Pigott D and Jenkins J. (1990). *Virology*, **177,** 595 – 605.
Chang LS, Shi Y and Shenk TJ. (1989). *Virol*., **63,** 3479 – 3488.
Chen CA and Okayama H. (1988). *BioTechniques*, **6,** 632 – 638.
Chinnadurai G. (1992). *Oncogene*, **7,** 1255 – 1258.
Chiou SK, Tseng CC, Rao L and White E. (1994). *J. Virol*., **68,** 6553 – 6566.
Corbeil HB and Branton PE. (1994). *J. Virol*., **68,** 6697 – 6709.
D'Emilia J, Bulovas K, D'Ercole K, Wolf B, Steele GJ and Summerhayes IC. (1989). *Oncogene*, **4,** 1233 – 1239.
Dougall WC, Qian X, Peterson NC, Miller MJ, Samanta A and Greene MI. (1994). *Oncogene*, **9,** 2109 – 2123.
Edlund T, Walker MD, Barr BJ and Ratter WJ. (1985). *Science*, **230,** 912 – 916.
Gorman C, Moffat LF and Howard BH. (1982). *Mol. Cell. Biol*., **2,** 1044 – 1051.
Hung MC, Schechter AL, Chevray PY, Stern DF and Weinberg RA. (1986). *Proc. Natl. Acad. Sci. USA*, **83,** 261 – 264.
Hung MC, Yan DH and Zhao XY. (1989). *Proc. Natl. Acad. Sci. USA*, **86,** 2545 – 2548.

Hung MC, Zhang X, Yan DH, Zhang HZ, He GP, Zhang TQ and Shi DR. (1992). *Cancer Lett*., **61,** 95–103.
Jelsma TN, Howe JA, Evelegh CM, Cunniff NF, Skiadopoulos MH, Floroff MR, Denman JE and Bayley ST. (1988). *Virology*, **163,** 494–502.
Jelsma TN, Howe JA, Mymryk JS, Evelegh CM, Cunniff NF and Bayley ST. (1989). *Virology*, **171**, 120–130.
Kuppuswamy M and Chinnadurai G. (1987). *Virology*, **159,** 31–38.
Lillie JW, Green M and Green MR. (1986). *Cell*, **46,** 1043–1051.
Lowe SW and Ruley HE. (1993). *Genes Dev*., **7,** 535–545.
Moran E and Mathews MB. (1987). *Cell*, **48,** 177–178.
Morn E, Grodzicker T, Roberts RJ, Mathews MB, and Zerler B. (1986). *J. Virol*., **57,** 765–775.
Muller WJ, Sinn E, Pattengale PK, Wallace R and Leder P. (1988). *Cell*, **54,** 105–115.
Mymryk JS. (1996). *Oncogene* **13,** 1581–1589.
Norton PA and Coffin JM. (1985). *Mol. Cell. Biol*., **5,** 281–290.
Padhy LC, Shih C, Cowing D, Finkelstein R and Weinberg RA. (1982). *Cell*, **28,** 865–871.
Park JB, Rhim JS, Park SC, Kimm SW and Kraus MH. (1989). *Cancer Res*., **49,** 6605–6609.
Saya H, Ara S, Lee PS, Ro J and Hung MC. (1990). *Mol. Carcinog*., **3,** 198–201.
Schneider JF, Fisher F, Goding CR and Jones NC. (1987). *EMBO J*., **6,** 2053–2060.
Schneider PM, Hung MC, Chiocca SM, Manning J, Zhao XY, Fang K and Roth JA. (1989). *Cancer Res*., **49,** 4968–4971.
Shi D, He G, Cao S, Pan W, Zhang HZ, Yu D and Hung MC. (1992). *Mol. Carcinog*., **5,** 213–218.
Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J and Ullrich A. (1989). *Science*, **244,** 707–712.
Smith DH and Ziff E. (1988). *Mol. Cell. Biol*., **8,** 3882–3890.
Suen TC and Hung MC. (1990). *Mol. Cell. Biol*., **10,** 6306–6315.
Tsai CM, Yu D, Chang KT, Wu LH, Perng RP, Ibrahim NK and Hung MC. (1995). *J. Natl. Cancer Inst*., **87,** 682–684.
Van dVM, Van dBR, Devilee P, Cornelisse C, Peterse J and Nusse R. (1987). *Mol. Cell. Biol*., **7,** 2019–2023.
Weiner DB, Nordberg J, Robinson R, Nowell PC, Gazdar A, Greene MI, Williams WV, Cohen JA and Kern JA. (1990). *Cancer Res*., **50,** 421–425.
Whyte P, Williamson NM and Harlow E. (1989). *Cell*, **56,** 67–75.
Yokota J, Yamamoto T, Miyajima N, Toyoshima K, Nomura N, Sakamoto H, Yoshida T, Terada M and Sugimura T. (1988). *Oncogene*, **2,** 283–287.
Yu D and Hung MC. (1991). *Oncogene*, **6,** 1991–1996.
Yu D, Hamada J, Zhang H, Nicolson GL and Hung MC. (1992). *Oncogene* **7,** 2263–2270.
Yu D, Matin A and Hung MC. (1992). *J. Biol. Chem*., **267,** 10203–10206.
Yu D, Suen TC, Yan DH, Chang LS and Hung MC. (1990). *Proc. Natl. Acad. Sci. USA*, **87,** 4499–4503.
Yu D, Wang SS, Dulski KM, Tsai CM, Nicolson GL and Hung MC. (1994). *Cancer Res*., **54,** 3260–3266.
Yu D, Wolf JK, Scanlon M, Price JE and Hung MC. (1993). *Cancer Res*., **53,** 891–898.
Zerler B, Moran B, Maruyama K, Moomaw J, Grodzicker T and Ruley HE. (1986). *Mol. Cell. Biol*., **6,** 887–899.
Zerler B, Roberts RJ, Mathews MB and Moran E. (1987). *Mol. Cell. Biol*., **7,** 821–829.
Zhang X, Silva E, Gershenson D and Hung MC. (1989). *Oncogene*, **4,** 985–989.
Zhang Y, Yu D, Xia W and Hung MC. (1995). *Oncogene*, **10,** 1947–1954.