Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8582–8586, October 1991
Biochemistry

Characterization of a neu/c-erbB-2 protein-specific activating factor

(*neu* oncogene/tyrosine kinase/growth factor)

KUNIO DOBASHI*†, JAMES G. DAVIS*‡, YASUNORI MIKAMI*, JONATHAN K. FREEMAN*§, JUNJI HAMURO*¶, AND MARK I. GREENE*||

*Division of Immunology and the Center for Receptor Biology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, ‡David Mahoney Institute of Neurological Sciences, University of Pennsylvania, and §Harrison Department of Surgical Research, Hospital of the University of Pennsylvania, Philadelphia, PA 19104-6082

Communicated by Peter C. Nowell, June 17, 1991

ABSTRACT **The *neu* oncogene encodes a tyrosine kinase with growth factor receptor-like properties. A neu protein-specific activating factor (NAF) was partially purified from medium conditioned by the transformed human T-cell line ATL-2. NAF was able to stimulate the tyrosine-specific kinase activity of the neu protein ($p185^{neu}$), induce dimerization and internalization, and increase the growth of cells bearing the neu protein. The effects of NAF were mediated by an interaction with the $p185^{neu}$ extracellular domain. NAF had no effect on the epidermal growth factor receptor kinase activity and no effect on cells that express that receptor. Further analysis of NAF and of other recently described neu protein-activating activities should help clarify the role of the neu protein in cell growth and transformation.**

The rat *neu* gene encodes a 185-kDa transmembrane tyrosine kinase that shows significant structural similarity to the epidermal growth factor receptor (EGFR) (1–3). The rat *neu* gene product has been identified in two forms: the protooncogenic form is termed $p185^{c\text{-}neu}$ and the oncogenic form is termed $p185^{neu}$. $p185^{neu}$ becomes activated in chemically induced neuroglioblastomas by a point mutation in the transmembrane region that results in constitutively increased neu tyrosine kinase activity (4–7). The c-*erbB-2* gene (8, 9) is the human homologue of the rat *neu* gene, and aberrant c-erbB-2 protein expression has been implicated in a number of human adenocarcinomas, including those of the breast (10–12), ovaries (12), salivary gland and digestive tract (13), skin (14), kidney (13), pancreas (15), and lung (16). Its growth factor receptor-like attributes, tissue-specific and developmentally regulated expression pattern (17, 18), and involvement in neoplasia suggest that the p185/c-erbB-2 protein plays a role in normal and abnormal growth and differentiation of the cells in which it is expressed and that a cognate ligand exists for this protein.

Although the *neu* gene has been studied for nearly 10 years now, identification of candidate ligands for p185 have only been reported in the last 2 years. Though several endogenous p185 modulatory factors may exist, the effects of the ligand for p185 might be most comparable to those of EGF and platelet-derived growth factor (PDGF) on their receptors, since these receptors are the transmembrane tyrosine kinases most closely related to p185 (19, 20). According to these criteria, the primary ligand for p185 should increase the autophosphorylation/kinase activity of p185, induce p185 dimerization and internalization, and affect the growth of cells that express p185. Several reports have described preparations that contain p185-activating activity (21–23). Each of these activities/factors conforms to these criteria to a different extent. We report here the characterization of a purified neu protein-specific activating factor (NAF) secreted by the transformed human T-cell line ATL-2 (24) that meets all of these expected criteria.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cells. PN-NR6 and PT-NR6 cells express high levels of $p185^{c\text{-}neu}$ (protooncogenic neu protein) and $p185^{neu}$ (transforming, oncogenic neu protein), respectively. NE-19 cells are NR6 cells transfected with a human EGFR gene and express EGFR at high levels. These cell lines were constructed and maintained as described (25).

***In Vitro* Kinase Assay.** All chemicals were from Sigma unless otherwise indicated. The indicated amounts of the appropriate factor were added to aliquots of the appropriate cell lysate. These mixtures were subjected to immunoprecipitation with either anti-p185 or anti-EGFR antibodies prior to kinase reaction and SDS/PAGE analysis (25).

Preparation of ATL-2 Conditioned Medium. Mycoplasma-free cultures of human ATL-2 cells were maintained in RPMI 1640 with 10% fetal bovine serum. ATL-2 cells were harvested and washed twice with phosphate-buffered saline before resuspension in the serum-free medium at a density of 3×10^5 cells per milliliter. After 3 days of growth in serum-free RPMI 1640 medium supplemented with 2 mM L-glutamine, the ATL-2 cells were pelleted, and the resulting supernatant was filtered (0.45 μm) and designated ATL-2 conditioned medium.

NAF Sample Preparation. Details of this partial purification of NAF are described elsewhere (26). In brief, ATL-2 conditioned medium was concentrated 100-fold (from 1 liter to 10 ml) using YM2 Diaflo membranes (Amicon). The concentrated conditioned medium was filtered (0.45 μm) and then applied to a DEAE-SW column (Waters) that had been preequilibrated with 10 mM Tris Cl (pH 8.1). Concentrated conditioned-medium proteins representing 1 liter of original ATL-2 conditioned medium per HPLC run were adsorbed to the column and then eluted with a linear gradient of 0–400 mM NaCl at a flow rate of 4 ml/min. Fractions were assayed using the *in vitro* kinase assay (described above) and 10% of the appropriate DEAE fraction. The fractions that increased the tyrosine kinase activity of $p185^{c\text{-}neu}$ in a dose-dependent manner were concentrated and then subjected to C_{18} reverse-phase chromatography (Waters) with elution by a linear

Abbreviations: EGF, epidermal growth factor; EGFR, EGF receptor; PGDF, platelet-derived growth factor; NAF, neu protein-specific activating factor.

†Present address: First Department of Internal Medicine, Gunma University, Maebashi-shi, Japan.
¶Present address: Basic Research Institute, Ajinomoto Co., Inc., Kawasaki, Japan.
||To whom reprint requests should be addressed.

gradient of 2-propanol against 0.1% trifluoroacetic acid. All the fractions were dialyzed against RPMI 1640 to remove the 2-propanol and assayed using the *in vitro* kinase assay and a 1% concentration of the appropriate fraction. We mixed the three fractions representing proteins eluted in this range and used this semipurified sample, referred to herein as the NAF sample, in the experiments described.

Western Blotting. PN-NR6 ($p185^{c\text{-}neu}$+/EGFR−) and NE-19 (p185−/EGFR+) cells were incubated for 10 min at 37°C with or without factors, lysed, and then subjected to immunoprecipitation with anti-p185 antibody 7.16.4 and anti-EGFR carboxyl-terminus antibodies (provided by Stuart Decker, Rockefeller University), respectively. Western blot analysis was performed as described (27).

Crosslinking/Dimerization Assay. PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−) (3×10^6 per 10-cm culture well) were incubated with the indicated amounts of NAF (percent by volume) at 37°C for 10 min, then exposed to crosslinking reagents, lysed, and subjected to immunoprecipitation with monoclonal anti-p185 antibodies (27). Monomeric and dimeric forms of $p185^{c\text{-}neu}$ were detected with polyclonal anti-p185 intracellular domain antibodies referred to as DBW-2.

Internalization/Down-Modulation Assay. Cells were cultured overnight in Dulbecco's modified Eagle's medium (DMEM) containing insulin, transferrin, and selenous acid (ITS, Collaborative Research), incubated with NAF or EGF for the indicated time, and subjected to immunoprecipitation with 7.16.4 or anti-EGFR antibody (monoclonal antibody 425, which recognizes the extracellular domain of human EGFR, was provided by M. Herlyn of the Wistar Institute) at 2 μg/ml. Immunoprecipitates were then assayed as described (21).

Proliferation Assay. Subconfluent cells were trypsinized and then were suspended in DMEM containing 10% fetal bovine serum and placed in a 96-well plate (10^4 cells per well). Following cell attachment overnight, the medium was replaced with DMEM supplemented with ITS. Incubation was continued in this serum-free medium for an additional 48 hr. Thereafter, cells were exposed to appropriate factor for 16 hr. Cells received [^{3}H]thymidine (0.5 μCi/ml; 1 Ci = 37 GBq) for 6 hr prior to harvest.

Anchorage-Independent Growth Assay. Anchorage-independent growth capability was determined by assessing the colony-forming efficiency of cells suspended in soft agar. When NAF sample was added to soft-agar cultures, it was incorporated into the top layer only. Colonies of >0.5-mm diameter were counted using a dissecting microscope at 7 days.

RESULTS

NAF Sample Preparation. ATL-2 conditioned medium generated from mycoplasma-free ATL-2 cultures was concentrated 100-fold with an Amicon YM2 ultrafiltration membrane (1000-Da cutoff) and then subjected to fractionation by anion-exchange (DEAE) HPLC (26). Fractions were assayed for neu-activating activity by the immune-complex kinase assay described in *Materials and Methods*. DEAE active fractions were pooled, concentrated, and then subjected to C_{18} reverse-phase HPLC (26). Active fractions obtained from the second chromatographic step were pooled and designated as NAF. This enriched form of NAF was used in the characterization studies described below.

Effect of NAF on neu Protein Kinase Activity. Human c-erbB-2 and rat $p185^{c\text{-}neu}$ autophosphorylation activity was stimulated in a dose-dependent manner by the addition of NAF (Fig. 1*a*). NAF (1% by volume) increased the p185 band intensity 2- to 4-fold compared with the control lane when quantified by scanning densitometry. The exogenous substrate histone 2B (Boehringer Mannheim), as well as $p185^{c\text{-}neu}$, was phosphorylated in a dose-dependent manner by the addition of NAF (Fig. 1*b*).

Other known growth-modulatory factors were tested for ability to activate p185 in our assay. Since EGFR activation results in transphosphorylation and activation of p185 (28–30), it was necessary to rule out the possibility that EGF played a role in the neu activation in certain of these experiments. With human A431 epidermoid carcinoma cells (which express high levels of EGFR but no p185), EGFR autophosphorylation was increased by the addition of EGF but was unaffected by the same dilutions of NAF (Fig. 1*c*) that increased the neu protein kinase activity. These data indicated that there was no EGFR-activating activity in the

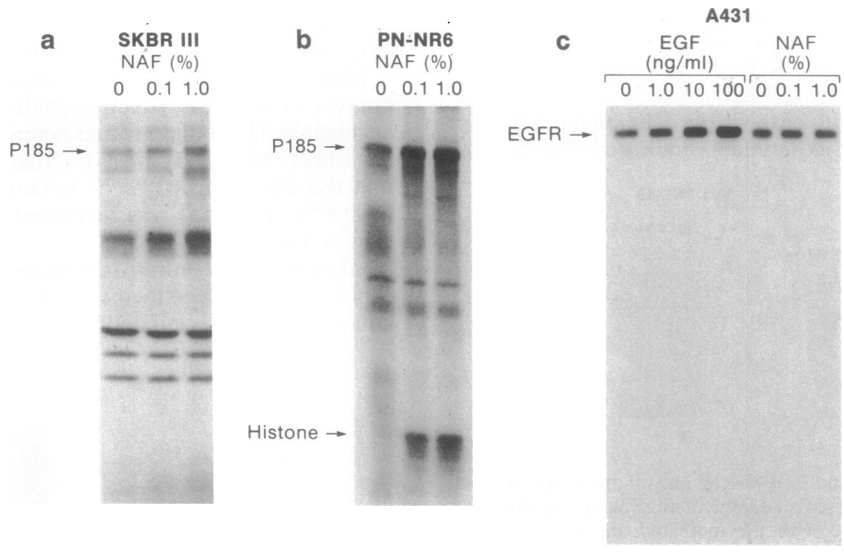

FIG. 1. Effect of NAF and EGF on the kinase activity of immunoprecipitated human c-erbB-2, rat p185, and human EGFR proteins in the *in vitro* kinase assay. Various amounts of NAF (percent by volume) or EGF (ng/ml) were added to cell lysates prior to immunoprecipitation and kinase reaction. The cell lysates were derived from human SKBRIII cells (p185+/EGFR+), rat PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−), and human A431 cells (p185−/EGFR+). (*a*) Effect of NAF on human c-erbB-2 protein kinase activity. (*b*) Effect of NAF on rat $p185^{c\text{-}neu}$ kinase activity and on exogenous substrate histone 2B (Boehringer Mannheim) phosphorylation. (*c*) Effect of EGF and NAF on human EGFR kinase activity.

NAF preparation and that the EGFR-mediated activation of p185 had not contributed to the observed effects of NAF on c-erbB-2 protein activity in the SKBRIII cell lysate. The interpretation of the PN-NR6 data is not complicated by this issue, since these cells do not express any EGFR. We also tested factors known to be secreted by the ATL-2 cell line (31–34), to eliminate the possibility that any of these factors individually were responsible for the NAF-mediated increase in p185 activity. Interleukin 1, interleukin 6, and adult T-cell leukemia-derived factor (all provided by Ajinomoto, Japan), transforming growth factors α and β (both from ICN), and PDGF purified from human platelets (Sigma) had no effect on the kinase activity of $p185^{c\text{-}neu}$ in the *in vitro* kinase assay (data not shown).

Effect of NAF on p185 Phosphotyrosine Content. Western blot analysis revealed that the amount of phosphotyrosine in $p185^{c\text{-}neu}$ from PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−) was increased by addition of NAF in a dose-dependent manner, whereas EGF had no effect under the same conditions (Fig. 2*a*). The amount of phosphotyrosine detectable in EGFR from NE-19 cells (p185−/EGFR+) was increased by addition of EGF but was not increased by NAF addition (Fig. 2*b*). NAF affected the tyrosine kinase activity of $p185^{c\text{-}neu}$ expressed in the NR6 cell background (PN-NR6 cells) yet, at the indicated dilutions, had no effect on the EGFR expressed in the same cellular background.

Effect of NAF on p185 Is Mediated by an Interaction with the p185 Extracellular Domain. Several experimental strategies were employed to demonstrate that NAF interacts with the putative extracellular ligand-binding domain of p185. Two monoclonal antibodies specific for distinct epitopes on the extracellular domain of p185 were examined for their ability to block the effects of NAF on p185. Preincubation of PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−) with purified p185 extracellular domain-specific monoclonal antibody 7.16.4 (35) blocked the NAF-induced activation of p185, while an irrelevant, isotype-matched monoclonal antibody, 9BG5 (36), did not (Fig. 3*a*). Monoclonal antibody 7.9.5 (35) recognizes a distinct extracellular domain of p185 and partially blocked the NAF-induced activation of p185. Similarly, preincubation of cells with suramin, which has been shown to block binding of EGF and PDGF to their receptors, blocked the NAF-induced activation of p185 (data not shown). In addition, the effect of NAF on a p185 extracellular-domain deletion mutant, p185-D4 (which lacks a 523-base-pair sequence corresponding to amino acids 475–648; J.K.F., J. N. Myers, and M.I.G., unpublished work), was assessed. NAF was not able to increase the kinase activity of this p185 deletion mutant (Fig. 3*b*). Collectively, these experiments indicated that NAF's

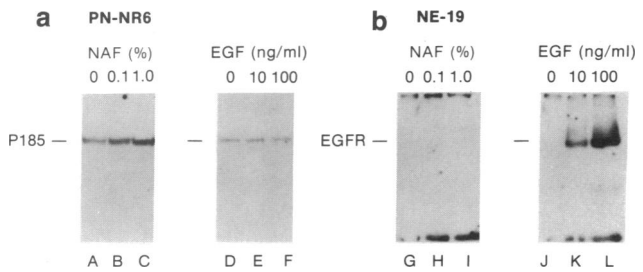

FIG. 2. Effect of NAF and EGF on the phosphotyrosine content of p185 and EGFR expressed in identical fibroblast backrounds. Intact cells were incubated with the indicated amounts of NAF (percent by volume) or EGF (ng/ml) at 37°C for 8 min and then subjected to Western blot analysis with monoclonal anti-phosphotyrosine antibody PY-20 (ICN). (*a*) Effect of NAF (lanes A–C) and EGF (lanes D–F) on the phosphotyrosine content of $p185^{c\text{-}neu}$ derived from PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−). (*b*) Effect of NAF (lanes G–I) and EGF (lanes J–L) on the phosphotyrosine content of EGFR from NE-19 cells (p185−/EGFR+).

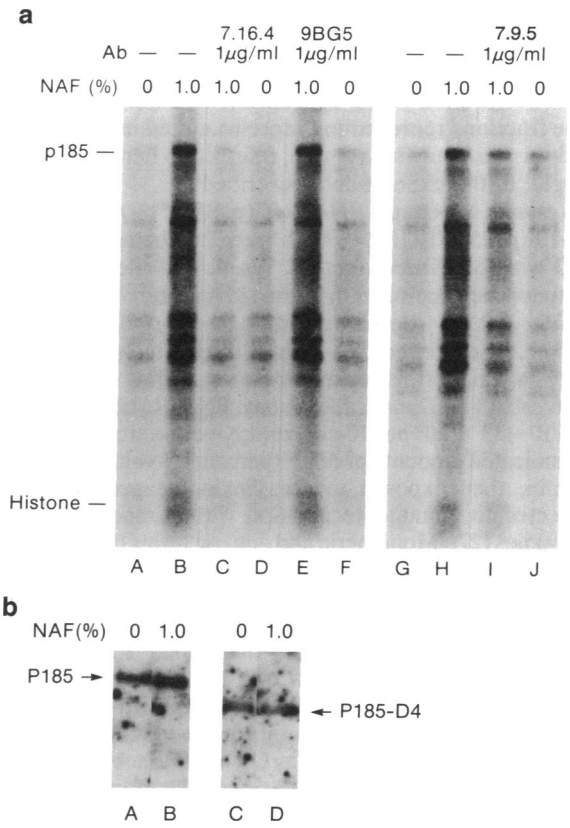

FIG. 3. Requirement for p185 extracellular domain. (*a*) PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−) were preincubated without antibody (Ab) (lanes A, B, G, and H), with high-affinity anti-p185 extracellular domain antibody 7.16.4 (lanes C and D), or with 7.9.5, which recognizes a distinct ectodomain on p185 (lanes I and J), or with an irrelevant isotype-matched antibody, 9BG5 (lanes E and F). Cell lysates were treated with or without 1% NAF before immunoprecipitation and *in vitro* kinase assay. (*b*) Western blot analysis using antibodies specific for phosphotyrosine was done on both the p185-D4 deletion mutant (lanes C and D) and unmutated $p185^{c\text{-}neu}$ (lanes A and B) expressed in NR6 cells that were untreated (lanes A and C) or treated with 1% NAF (lanes B and D).

effects on p185 occurred through its interaction with discrete parts of the neu protein extracellular domain.

Effect of NAF on p185 Dimerization. Receptor tyrosine kinases are induced by their cognate ligands to form receptor aggregates. Homodimeric and heterodimeric species of p185 have been described (27, 37). Crosslinking studies revealed that the amount of $p185^{c\text{-}neu}$ homodimers in PN-NR6 cells ($p18^{c\text{-}neu}$+/EGFR−) was increased with exposure to NAF (Fig. 4, lane B).

Internalization/Down-Modulation of p185 by NAF. In response to exposure to their cognate ligands, receptor tyrosine kinases are down-regulated from the cell surface. The ability

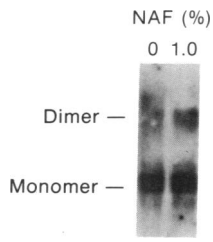

FIG. 4. Effect of NAF on p185 dimerization. PN-NR6 cells were incubated with or without 1% NAF, exposed to cross-linking reagents, and lysed. Immunoprecipitated p185 monomer and dimer were analyzed by Western blotting.

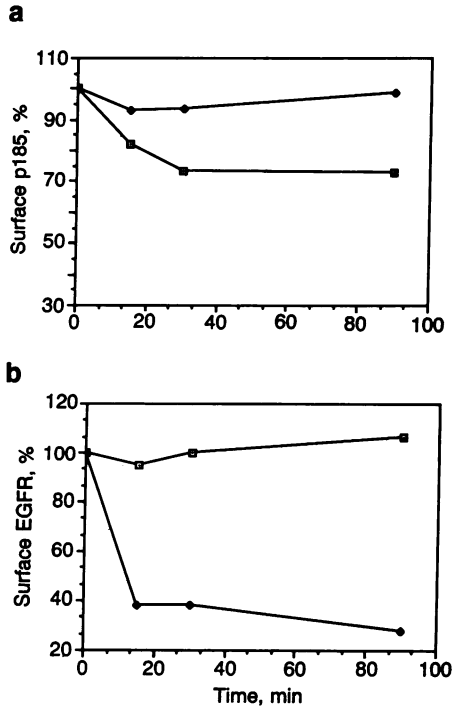

FIG. 5. Effect of NAF (□) and EGF (◆) on p185 and EGFR internalization/down-modulation. (*a*) Rat p185 internalization in PN-NR6 cells. (*b*) EGFR internalization in NE-19 cells.

of NAF to down-modulate $p185^{c\text{-}neu}$ was assessed. Surface expression of $p185^{c\text{-}neu}$ on PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−) was decreased by 30% at 30 min and by 40% at 90 min after the addition of NAF, whereas EGF had no effect on the internalization of $p185^{c\text{-}neu}$ in these cells (Fig. 5*a*). Surface EGFR on NE-19 cells (p185−/EFGR+) was down-modulated by the addition of EGF but was not down-modulated by NAF (Fig. 5*b*).

Effect of NAF on Cell Growth. The effect of NAF on cell growth was assessed by [^{3}H]thymidine incorporation and soft-agar colony growth assays. NAF (1% by volume) increased the relative levels of DNA synthesis in cultures of PN-NR6 cells ($p185^{c\text{-}neu}$+/EGFR−) and of PT-NR6 cells ($p185^{neu}$+/EGFR−) but did not affect NE-19 cells (p185−/EGFR+) (Fig. 6). PN-NR6 cells do not ordinarily form colonies in soft agar. NIH 3T3 transfectants that overexpress EGFR form colonies in soft agar only upon addition of EGF (38). NAF increased the soft-agar growth capability of PN-NR6 cells but had no effect on NE-19 cells (Table 1). Conversely, EGF increased the soft-agar growth capability of

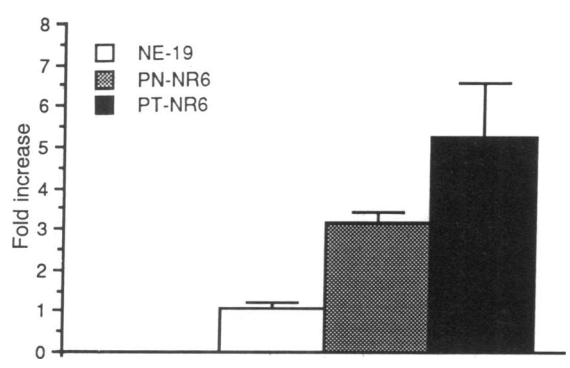

FIG. 6. Effect of 1% NAF on [^{3}H]thymidine incorporation by NE-19 (p185−/EGFR+), PN-NR6 ($p185^{c\text{-}neu}$+/EGFR−), and PT-NR6 ($p185^{neu}$+/EGFR−) cells. Results (mean ± SEM, n = 3) are expressed relative to the cell line-matched untreated control.

Table 1. Effect of NAF and EGF on the growth of PN-NR6, NE-19, and NR6 cell lines in soft agar

Factor	Conc.	No. of colonies		
		PN-NR6	NE-19	NR-6
—	—	0	0	0
NAF	0.1%	6	0	0
	1.0%	25	0	0
EGF	10 ng/ml	0	22	0
	100 ng/ml	0	30	0

Colony growth was determined as described in *Materials and Methods*. NAF concentration is given as percent by volume.

NE-19 cells but had no effect on the neu-bearing PN-NR6 cells. The parent NR-6 cells (p185−/EGFR−) were not affected by EGF or by NAF. Thus, the growth-promoting effects of NAF were seen only in cells that expressed $p185^{c\text{-}neu}$.

DISCUSSION

After the neu protein-activating activity was detected in ATL-2 conditioned medium, a characterization scheme to examine this activity's effect on the neu protein was developed based on a presumed similarity between NAF and the well-characterized ligands for the prototypic members (the EGF and PDGF receptors) of this family of tyrosine kinases. Based on these criteria, a ligand for the neu protein might activate its intrinsic tyrosine kinase activity, lead to neu protein dimerization and internalization, and have an impact on the growth of cells that express the neu protein. NAF interacts with the neu extracellular domain, resulting in neu-specific kinase activation, neu dimerization and internalization, and subsequent potentiation of the growth of neu protein-bearing cells only, and thus meets all of these proposed biological criteria.

Several lines of evidence support the conclusion that NAF is neu protein-specific. The kinase, internalization, and cell growth studies using NAF and the NR6, PN-NR6, and NE-19 cell lines permitted the comparison of NAF's effects on p185 and the EGFR expressed in identical cellular backrounds. In each of these studies, the effects of NAF were limited to those cells that expressed $p185^{c\text{-}neu}$ (PN-NR6 cells), whereas the effects of EGF were limited to those cells that expressed the EGFR (NE-19 cells). This indicated that the NAF sample did not contain any EGFR-activating activity when assayed at the same dilutions used to significantly increase neu kinase activity.

In addition, some of the physical characteristics of the NAF in ATL-2 conditioned medium were examined. This activity was heat-stable (unaffected by 30 min at 100°C) and sensitive to the protease chymotrypsin (26). Fractions from gel filtration chromatography of concentrated ATL-2 conditioned medium on a Protein-Pak 125 column (Waters) were analyzed. Most of the dose-dependent neu-activating activity eluted in the 8- to 24-kDa range (data not shown). Similarly, most of the neu-activating activity passed through a 30-kDa-cutoff membrane (data not shown). Although there was evidence of larger forms, most of the neu-activating activity contained in the ATL-2-conditioned medium appeared to exist as one or multiple species of <30 kDa.

Recently, several groups have described factors that interact with the neu protein (21–23). The neu-activating activity associated with *ras*-transformed fibroblasts (21) was able to induce p185 kinase activity, internalization, and the proliferation of p185-expressing cells. Another neu-activating activity, identified in bovine kidney extracts, was mitogenic for p185 expressing cells and was able to decrease the half-life of surface $p185^{c\text{-}neu}$ (23). A glycoprotein derived

from the human MDA-MB-453 breast cancer cell line (22) activated both p185 and EGFR, displayed a molecular mass of 30 kDa, and inhibited the growth of human p185 (c-erbB-2)-overexpressing cells. The more prominent specificity of NAF for the neu protein compared with the EGFR, the distinct low molecular weight estimates, and its ability to induce p185 dimerization suggest that NAF may be distinct from the other neu-activating factors recently described.

Differences in the source of all of these neu protein-activating activities, in the nature of the purification strategies for each, and in the types of assays used to examine these activities make it difficult to compare and distinguish these factors completely at this time. Ultimately, the similarities and differences between these molecules will be revealed with the cloning and sequencing of these factors. These factors should help clarify the role of the neu protein in cell growth and transformation and may also provide the basis for the generation of molecular interventions aimed at the human malignancies in which the c-erbB-2 protein has been implicated.

We wish to thank Gail Massey, Maria Hellman, Caroline Shim, Allen Chen, and Wayne Rackoff for technical assistance; John Reed, J. Carl Oberholtzer, and Glen Gaulton for critical discussion of the data and manuscript. This work was supported by grants from the National Institutes of Health, the National Cancer Institute, the Council for Tobacco Research, and the Lucille Markey Charitable Trust.

1. Padhy, L. C., Shih, C., Cowing, D., Finkelstein, R. & Weinberg, R. A. (1982) *Cell* **28**, 865–871.
2. Schechter, A. L., Hung, M.-C., Vaidyanathan, L., Weinberg, R. A., Yang-Feng, T. L., Francke, U., Ullrich, A. & Coussens, L. (1985) *Science* **229**, 976–978.
3. Bargmann, C. I., Hung, M.-C. & Weinberg, R. A. (1986) *Nature (London)* **319**, 226–230.
4. Bargmann, C. I., Hung, M. C. & Weinberg, R. A. (1986) *Cell* **45**, 649–657.
5. Stern, D. F., Kamps, M. P. & Cao, H. (1988) *Mol. Cell. Biol.* **8**, 3969–3973.
6. Bargmann, C. I. & Weinberg, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5394–5398.
7. Weiner, D. B., Kokai, Y., Wada, T., Cohen, J. A., Williams, W. V. & Greene, M. I. (1989) *Oncogene* **4**, 1175–1178.
8. Coussens, L., Yang-Feng, T. L., Chen, Y. L. E., Gray, A., McGrath, J., Seeburg, P. H., Libermann, T. A., Schlessinger, J. & Franke, V. (1985) *Science* **230**, 1132–1139.
9. Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, N., Saito, T. & Toyoshima, K. (1986) *Nature (London)* **319**, 230–234.
10. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. & McGuire, W. L. (1987) *Science* **235**, 177–182.
11. King, C. R., Kraus, M. H. & Aaronson, S. A. (1985) *Science* **229**, 974–976.
12. Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A. & Press, M. F. (1989) *Science* **244**, 707–712.
13. Yokota, J., Yanamoto, T., Toyoshima, K., Terada, M., Sugimura, T., Battifora, H. & Cline, M. J. (1986) *Lancet* **i**, 765–767.
14. Maguire, H. C., Jaworsky, C., Cohen, J. A., Hellman, M., Weiner, D. B. & Greene, M. I. (1989) *J. Invest. Dermatol.* **92**, 786–790.
15. Williams, T. M., Weiner, D. B., Greene, M. I. & Maguire, H. C., Jr. (1990) *Pathobiology* **59**, 46–52.
16. Kern, J. A., Sachwartz, D. A., Nordberg, J. E., Weiner, D. B., Greene, M. I., Torney, L. & Robinson, R. A. (1990) *Cancer Res.* **50**, 5184–5191.
17. Kokai, Y., Cohen, J. A., Drebin, J. A. & Greene, M. I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8498–8501.
18. Press, M. F., Cordon-Cardo, C. & Slamon, D. J. (1990) *Oncogene* **5**, 953–962.
19. Hunter, T. & Cooper, J. (1985) *Annu. Rev. Biochem.* **54**, 897–930.
20. Yarden, Y. & Ullrich, A. (1988) *Annu. Rev. Biochem.* **57**, 443–478.
21. Yarden, Y. & Weinberg, R. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3179–3183.
22. Lupu, R., Colomer, R., Zugmaier, G., Sarup, J., Shepard, M., Slamon, D. & Lippman, M. E. (1990) *Science* **249**, 1552–1555.
23. Huang, S. S., Koh, H. A., Konish, Y., Bullock, L. D. & Huang, S. J. (1990) *J. Biol. Chem.* **265**, 3340–3346.
24. Maeda, M., Shimuzu, A., Ikuta, K., Okamata, H., Kashiwara, M., Uchiyama, T., Honjo, T. & Yodoi, J. (1985) *J. Exp. Med.* **162**, 2169–2174.
25. Dobashi, K., Weiner, D. B. & Greene, M. I. (1989) *DNA* **8**, 723–732.
26. Davis, J. G., Hamuro, J., Shim, C., Samanta, A., Greene, M. I. & Dobashi, K. (1991) *Biochem. Biophys. Res. Commun.*, in press.
27. Wada, T., Qian, X. & Greene, M. I. (1990) *Cell* **61**, 1339–1347.
28. Stern, D. & Kamps, M. P. (1988) *EMBO J.* **7**, 995–1001.
29. King, C. R., Borello, I., Bellot, F., Comoglio, P. & Schlessinger, J. (1988) *EMBO J.* **7**, 1647–1651.
30. Kokai, Y., Dobashi, K., Weiner, D. B., Myers, J. N., Nowell, P. C. & Greene, M. I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5389–5393.
31. Okada, M., Maeda, M., Tagaya, Y., Taniguchi, Y., Teshigawara, K., Yoshiki, T., Diamantstein, T., Smith, K. A., Uchiyama, T., Honjo, T. & Yodoi, J. (1985) *J. Immun.* **135**, 3995–4003.
32. Tagaya, Y., Okada, M., Sugie, K., Kasahara, T., Kondo, N., Hamuro, J., Matsushima, K., Dinarello, C. A. & Yodoi, J. (1988) *J. Immun.* **140**, 2614–2620.
33. Teshigawara, K., Maeda, M., Nishino, K., Nikaido, T., Uchiyama, T., Tsudo, M., Wano, Y. & Yodoi, J. (1985) *J. Mol. Cell. Biol.* **2**, 17–26.
34. Yodoi, J., Teshigawara, K., Nikaido, T., Fukui, K., Noma, T., Honjo, T., Takigawa, M., Sasaki, M., Minato, N., Tsudo, M., Uchiyama, T. & Maeda, M. (1985) *J. Immunol.* **134**, 1623–1630.
35. Drebin, J. A., Link, V. C. & Greene, M. I. (1988) *Oncogene* **2**, 273–277.
36. Lee, P. W. K., Hayes, E. C. & Joklik, W. C. (1981) *Virology* **108**, 134–146.
37. Weiner, D. B., Liu, J., Cohen, J. A., Williams, W. V. & Greene, M. I. (1989) *Nature (London)* **339**, 230–231.
38. DiFiore, P. P., Pierce, J. H., Fleming, T. P., Hazan, R., Ullrich, A., King, C. R., Schlessinger, J. & Aaronson, S. A. (1987) *Cell* **51**, 1063–1070.