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Amplification of the Structurally and Functionally Altered Epidermal Growth Factor Receptor Gene (c-*erbB*) in Human Brain Tumors

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By using Southern blot analysis, we found that in two cases of human glioblastoma multiforme, cells carried amplified c-*erbB* genes which bore short deletion mutations within the ligand-binding domain of the epidermal growth factor (EGF) receptor. The products of these mutated c-*erbB* genes were about 30 kilodalton (kDa) smaller than the normal 170-kDa EGF receptor, and the tumor cell membrane fractions containing the 140-kDa abnormal EGF receptor showed a significant elevation of tyrosine kinase activity without its ligand. In view of the similarity to the activated viral and cellular *erbB* genes in the avian system, these mutated and overexpressed EGF receptors might play a role in the onset or development of human glioblastoma cells.

The c-*erbB* gene is known to be a proto-oncogene which encodes the epidermal growth factor (EGF) receptor (4, 25, 30). The v-*erbB* gene product of avian erythroblastosis virus in chickens has a truncated form of EGF receptor and expresses a higher level of tyrosine kinase activity without its ligand (11). Thus, qualitative and quantitative alterations appear to be crucial for the activation of the c-*erbB* gene in chickens. In human tumors, amplification of the c-*erbB* gene has been reported in several squamous cell carcinomas and glioblastomas (3, 10, 13, 14, 17, 27, 29). However, it was not certain whether the c-*erbB* gene in those tumor cells was also structurally altered.

Since the activation of oncogenes in human brain tumors has not yet been extensively studied, we screened several transplantable brain tumors for the presence of abnormal proto-oncogenes, using Southern blot analysis (24).

Table 1 lists the tumors tested; six were glioblastomas, and two were ependymomas. The histological characteristics of these tumors have not drastically changed during serial passages in athymic nude mice.

Among 19 *onc* probes employed (*myc*, N-*myc*, *myb*, K-*ras*, N-*ras*, *sis*, *src*, *fps/fes*, *yes*, *mos*, *fos*, *ros*, *fms*, *fgr*, *abl*, *rel*, *raf/mil*, *erbB*, *erbB-2/neu*), no amplification or rearrangement was detected in the transplantable brain tumors except for the v-*erbB* probe (26); two glioblastomas, GL-3 and GL-5, carried an amplified c-*erbB* gene (data not shown). Recently Libermann et al. have reported that about one-third of glioblastomas in primary human brain tumors contain an amplified c-*erbB* gene (13). They also indicated that this gene amplification is associated with a possible DNA rearrangement, but the details were not examined due to the limited availability of primary tumors. The frequency of amplification of the c-*erbB* gene in transplantable glioblastomas examined here is similar to that of their report.

To examine the fine structure of the amplified c-*erbB* gene in glioblastomas, we used a human EGF receptor cDNA as a probe (kindly provided by I. Pastan, National Cancer Institute, Bethesda, Md.) (16, 28). *Eco*RI-digested DNAs of GL-3 and GL-5 cells carry a high copy number of the c-*erbB* gene (Fig. 1a), and the degree of amplification was almost the same as in the A431 squamous carcinoma cell line, which is known to have 20 to 30 copies of the c-*erbB* gene (17, 25). Furthermore, possible rearranged DNA fragments were detected in both GL-3 and GL-5 among 5- to 7-kilobase (kb) DNA fragments. These abnormal bands were more clearly observed by using smaller amounts of DNA (Fig. 1b). To localize the rearranged DNA fragments in the c-*erbB* gene, various portions of EGF receptor cDNA were purified and used as probes. The middle and 3′ probes (the pE62 clone [16] and the 1.3-kb *Ava*I-*Cla*I fragment of clone pE7 [28]), which cover the carboxyl one-third of the extracellular domain, the transmembrane domain, and the entire cytoplasmic region including the tyrosine kinase domain, showed no abnormal bands (data not shown). However, the adjacent upstream probe, 0.7 kb long, which corresponds to the central portion of the extracellular domain, detected rearranged *Hin*dIII DNA fragments in GL-3 and GL-5 (Fig. 2c): A431 cells contain an amplified DNA at a normal position (about 15 kb), whereas the sizes of the abnormal DNA fragments in GL-3 and GL-5, about 25 and 11 kb, respectively, were different from that of placenta DNA. It is of interest that in these two tumors only the rearranged c-*erbB* genes were amplified. This suggests that DNA rearrangement had occurred before gene amplification. We could not detect any abnormal bands of c-*erbB* in other brain tumors tested.

We found that a portion of the extracellular domain was lost in these two tumors. Figure 2b shows the hybridization of cellular DNAs with a 0.2-kb fragment which lies upstream of the 0.7-kb DNA. Although this region was amplified in A431 cells, strong bands were not detected in either GL-3 or GL-5. When this result was obtained, the possibility of recombination between the c-*erbB* gene and another DNA of unknown origin was considered to be most likely. However, this was not the case; the left panel of Fig. 2a shows the results of Southern blots probed with the synthetic 40-mer oligonucleotide corresponding to the 5′ terminus of the coding region in the c-*erbB* gene (25). GL-3 and GL-5, as

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TABLE 1. Characteristics of human brain tumors[a]

Tissue designation	Age	Sex	Source
Controls			
H-P			Human placenta
A431			Epidermoid carcinoma cell line
BALB/c			Nude mouse spleen
Brain tumors in nude mouse			
Epe-1-JCK	10	M	Ependymoma
GL-1-JCK	3	M	Ependymoma
GL-2-JCK	32	M	Glioblastoma multiforme
GL-3-JCK	47	M	Glioblastoma multiforme
GL-5-JCK	50	M	Glioblastoma multiforme
GL-7-JCK	67	F	Glioblastoma multiforme
GL-8-JCK	10	M	Glioblastoma multiforme
GL-10-JCK	72	F	Glioblastoma multiforme

[a] Histologically, the original tumors for GL-3 and GL-5 were both typical glioblastomas (astrocytoma, grade III or IV). These morphological features were essentially conserved during establishment of transplantable tumors. Nude mice bearing GL-5 tumors show a hypervolemic syndrome with unknown etiology.

well as A431, were found to carry amplified 5'-terminal coding sequences. These results strongly suggest that GL-3 and GL-5 have similar, but not identical, short deletions within the extracellular domain of this gene.

We next examined mRNA of the mutated c-*erbB* gene in these tumors. Poly(A)$^+$ mRNA was probed with EGF receptor cDNA by Northern (RNA) blot analysis (Fig. 3) (23). A431 cells contained three mRNA species, 10 kb, 5.8 kb, and an A431-specific 2.9 kb (17, 25). On the other hand, GL-3 and GL-5 showed two mRNA species of 9.5 and 5.3 kb, about 0.5 kb shorter than those of the two upper bands in A431. The decreased size of c-*erbB* mRNA in these glioblastomas is consistent with a deletion mutation in the DNA. Human brain tumors which bear no amplification of the c-*erbB* gene showed almost undetectable levels (at most 10-fold less) of c-*erbB* mRNA compared with those of GL-3 and GL-5.

To examine the gene product of this rearranged c-*erbB* gene, [^{35}S]methionine labeling of GL-5 cells and immunoprecipitation with anti-human EGF receptor monoclonal antibody (528IgG) (9) were carried out. In these tumor cells the normal EGF receptor of 170 kilodaltons (kDa) was not detected, and instead, an abnormal molecule of about 140 kDa was observed (Fig. 4a). Since the EGF receptor is known to have a tyrosine kinase activity in the presence of EGF (7, 11), the enzymatic activity of the 140-kDa product was examined (Fig. 4b). Membrane fractions of GL-3, GL-5, or placenta tissue, which is well known to express a considerable amount of normal EGF receptor (6), were used for in vitro phosphorylation reactions with or without EGF.

Fresh frozen tissues of GL-3, GL-5, and placenta were homogenized with a blender in buffer A (25 mM Tris hydrochloride [pH 7.4], 0.25 M sucrose), supplemented with a proteinase inhibitor (1% Trasylol), and centrifuged at 600 $\times$ *g* for 3 min. The supernatants were recentrifuged at 100,000 $\times$ *g* for 30 min. The pellet was suspended in modified buffer B (40 mM PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)]–NaOH [pH 7.2], 0.15 M NaCl, 10 mM $MgCl_2$, 3 mM $MnCl_2$, 1 mM dithiothreitol, 1% glycerol, 1% Trasylol) to prepare the membrane fraction. The membrane fraction was preincubated with or without EGF (Bethesda Research Laboratories) at 21°C for 30 min. Phosphorylation was initiated by the addition of [γ-^{32}P]ATP, performed at 0°C for 1 min, and stopped by addition of modified RIPA buffer (50 mM Tris hydrochloride [pH 7.5], 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 20 mM EDTA). After the phosphorylation reaction, portions of samples were immunoprecipitated with anti-human EGF receptor monoclonal antibody 528IgG (9). Total membrane fractions and the immunoprecipitates were electrophoresed on a 7.5% polyacrylamide slab gel in the presence of 0.4% sodium dodecyl sulfate as described by Laemmli (12).

Figure 4b shows the results of polyacrylamide gel electrophoresis of the total membrane fraction (1) after phosphorylation and of the immunoprecipitates obtained from the same phosphorylated membrane fraction by using 528IgG monoclonal antibody. It is clear that the normal EGF receptor in placenta was strongly phosphorylated only in the presence of EGF. On the other hand, abnormal gene products of 140 kDa in both GL-3 and GL-5 were found to be heavily phosphorylated even without the addition of EGF. An autocrine-type model to explain the phosphorylation of the 140-kDa protein in the absence of EGF seems unlikely because we could not detect EGF or EGF-like activity in the cell lysate of the GL-5 tumor with a kinase assay system using normal EGF receptors in human placenta (data not shown). Furthermore, ligand-binding experiments indicated that the EGF receptor molecules with high affinity were lost in the GL-5 tumor (Fig. 5).

Since cell membrane fraction usually contains a considerable amount of serine-threonine kinase activity and EGF receptor is known to have a tyrosine kinase activity, phosphoamino acid analysis of the 140-kDa protein was carried out. The amino acid residue in the GL-5 140-kDa protein phosphorylated in vitro in the absence of EGF was exclusively tyrosine. We obtained essentially the same results by using GL-3 cell membrane (data not shown).

From these results, we cannot completely rule out a possibility that the 140-kDa abnormal EGF receptor might be due to an altered glycosylation in tumor cells, or be derived from a minor species of c-*erbB* gene and not from the amplified and deletion-carrying c-*erbB* gene. However, our

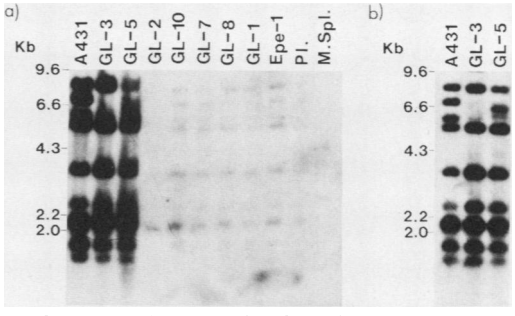

FIG. 1. Southern blot analysis of c-*erbB* gene in human brain tumors. (a) Total cellular DNA (10 μg) of human brain tumors, A431 human epidermoid carcinoma cell line, human placenta (Pl.), and nude mouse spleen (M. Spl.) were digested with restriction endonuclease *Eco*RI and examined by the Southern blotting method. The probe was the ^{32}P-labeled 2.3-kb DNA fragment of pE7 human EGF receptor cDNA. This 2.3-kb DNA fragment corresponds to the 3' two-thirds of the extracellular domain, the transmembrane domain, and the entire tyrosine kinase domain of the receptor. The size markers at the left are λ DNA fragments digested with *Hin*dIII. (b) A431 and glioblastoma GL-3 and GL-5 cellular DNAs (2 μg each) were analyzed essentially as described above.

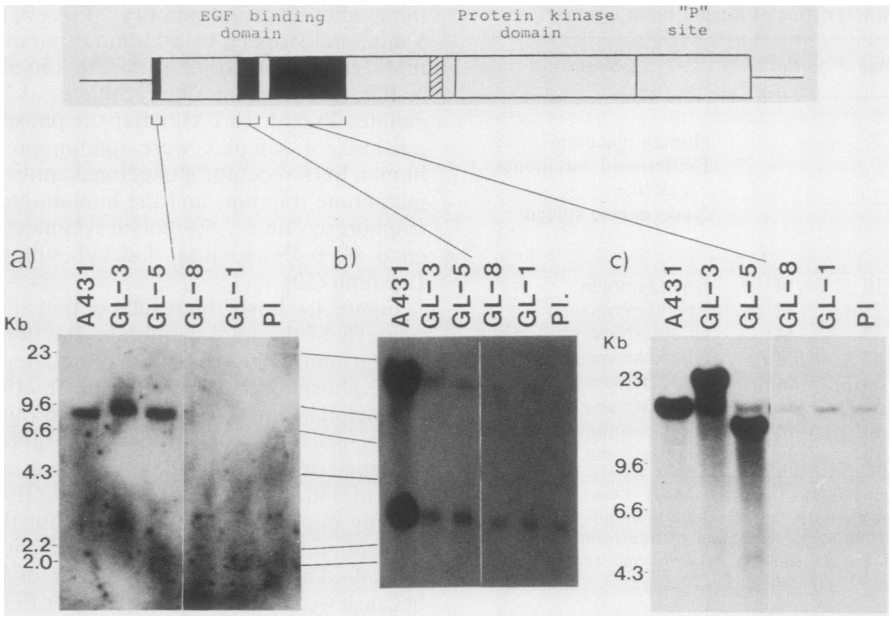

FIG. 2. Detection of a deletion mutation within the extracellular domain of EGF receptor gene in glioblastomas GL-3 and GL-5. Cellular DNA (10 μg) of A431 cells, human glioblastomas, and placenta (Pl) was digested with restriction endonuclease *Eco*RI (for panel a) or *Hin*dIII (for panels b and c) and examined by the Southern blotting method. The probes used were (a) a synthetic 40-mer oligonucleotide (5′-GATGCGACCCTCCGGGACGGCCGGGGCAGCGCTCCTGGCG-3′) corresponding to the amino-terminal region of EGF receptor; (b) the 0.2-kb *Cla*I-*Ava*I fragment of pE7 clone; and (c) the 0.7-kb *Ava*I-*Ava*I fragment of pE7 clone. The 40-mer oligonucleotide was labeled with ^{32}P by the use of T4 polynucleotide kinase, and other DNA fragments were labeled by nick translation. Hybridization was carried out under conditions of low stringency (30% formamide, 1 M NaCl, 37°C) for panel a and moderate stringency (50% formamide, 0.45 M NaCl, 37°C) for panels b and c. The positions of various probes on the EGF receptor are shown at the top. The hatched box and "P" site indicate the transmembrane domain and the region for autophosphorylation, respectively.

preliminary results indicated that the structure of a c-*erbB* cDNA obtained from GL-5 glioblastoma cells was consistent with a partial deletion within the extracellular domain. Thus, we suggest that the 140-kDa EGF receptor is encoded by the rearranged and amplified c-*erbB* gene. The cDNA analysis in detail will be published elsewhere.

In the chicken system, the v-*erbB* gene in the viral genome (26, 30) and the c-*erbB* gene activated by insertion of helper virus genome carry a large deletion in the extracellular domain of the EGF receptor (20). In addition, v-*erbB* is

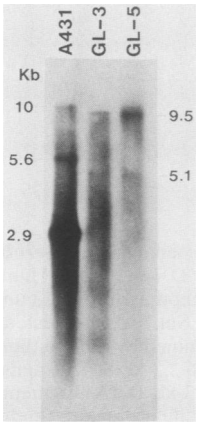

FIG. 3. Aberrant mRNA species of c-*erbB* gene in GL-3 and GL-5. Poly(A)$^+$ RNA was prepared from A431 cells and from GL-3 and GL-5. The samples (2 μg each) were electrophoresed and hybridized with pE7 probe as described in the legend to Fig. 1.

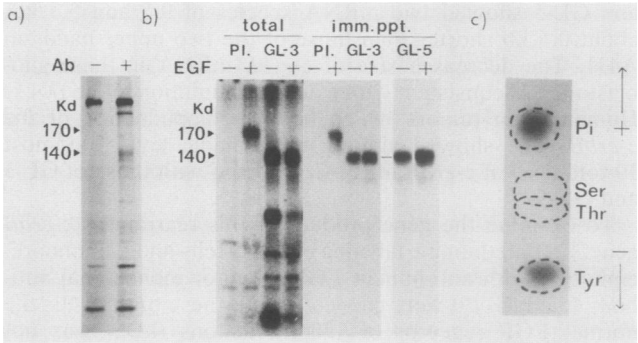

FIG. 4. [^{35}S]methionine labeling and ligand-independent phosphorylation of mutated EGF receptors. (a) A fresh GL-5 tumor was cut into tiny pieces and suspended in a culture medium. After pretreatment of tumor cells with serum-free minimal essential medium for 4 h, cells were labeled with [^{35}S]methionine for 10 h. Samples of cell lysate were mixed with protein A or with protein A plus monoclonal antibody 528IgG (Ab; 0.5 μg of protein), and the precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (b) Total membrane fractions of GL-3, GL-5, and placenta (Pl.) were preincubated with or without EGF and phosphorylated with [γ-^{32}P]ATP. The samples were immunoprecipitated (lanes imm.ppt.) with anti-human EGF receptor monoclonal antibody 528IgG (9) and electrophoresed on a polyacrylamide gel. (c) Identification of phosphoamino acid in the 140-kDa phosphoprotein of the GL-5 membrane fraction. The band of 140-kDa protein, phosphorylated without EGF as shown in panel b, was cut from the gel, electroeluted, and hydrolyzed. The hydrolysate was electrophoresed on a DEAE ion-exchange chromatography paper, DE81 (Whatman), with an electrophoresis buffer (pyridine-acetic acid-water, 1:10:89; pH 3.4). Authentic phosphoserine (Ser), phosphothreonine (Thr), and phosphotyrosine (Tyr) purchased from Sigma were added to the radioactive sample before electrophoresis.

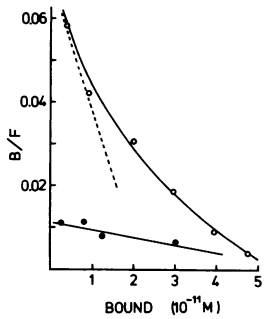

FIG. 5. Scatchard plot analysis of EGF binding to glioblastoma GL-5. [^{125}I]EGF binding activity was assayed by a method described by Hunts et al. (8). Briefly, frozen placenta and GL-5 tissue were homogenized in a buffer containing phenylmethylsulfonyl fluoride and filtered through a metal mesh. The concentration of protein was adjusted to 1 mg/ml. Portions of cell lysates were mixed with [^{125}I]EGF (6×10^{-11} to 1×10^{-8} M) and incubated on ice for 60 min. Bound [^{125}I]EGF was assayed by a filtration method with 0.45-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.), and the counts were subtracted from background (counts in the presence of 10^{-7} M unlabeled EGF). The K_d for the high-affinity receptor (broken line) of placenta (○) was 3×10^{-10} M, and the K_d for GL-5 (●) was 5×10^{-9} M.

known to have an altered structure at its carboxy-terminal region. In human tumors, many cases of squamous cell carcinoma and glioblastoma have been shown to carry amplified c-*erbB* genes; however, structural change of the gene has not yet been reported. As an exception, A431 cells are known to carry an amplified sequence of the extracellular domain in addition to the amplification of the whole c-*erbB* gene, and these cells secrete an abnormal product corresponding to the amino-terminal half of the EGF receptor (25). However, the physiological significance of this product in A431 cells is unknown at present. Thus, the glioblastoma GL-3 and GL-5 cells described here appear to be the first cases of human tumors in which the EGF receptor molecule is altered at both the gene and protein levels but still conserves the basic structure of the receptor.

The v-*erbB* gene product has been demonstrated to have an elevated tyrosine kinase activity in the absence of its ligand (11). Interestingly, GL-3 and GL-5 also showed a similar abnormality of kinase activity: a significant level of tyrosine kinase was detected without EGF. There appear to be at least two possibilities to explain the abnormal enzymatic activity of the 140-kDa EGF receptor: (i) that it is due to the deletion mutation within the extracellular domain shown in Fig. 2, and (ii) that it is due to critical point mutation(s) or very small deletion mutations in the genome that are undetectable by Southern blot analysis.

Other viral oncogenes derived from cell surface receptor or receptorlike genes, v-*fms* (21, 22), v-*ros* (15, 18, 19), and v-*kit* (2), were also shown to have truncated or mutated structures, and some of these gene products express uncontrolled high levels of tyrosine kinase activity (5, 21). It would be interesting to examine the biological activity of this mutated c-*erbB* gene on tumorigenicity in vitro and in vivo, especially in the central nervous system of animals, by using molecularly cloned cDNA molecules.

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LITERATURE CITED

1. **Akiyama, T., T. Kadooka, and H. Ogawara.** 1985. Purification of the epidermal growth factor receptor by tyrosine-sepharose affinity chromatography. Biochem. Biophys. Res. Commun. **131**:442–448.
2. **Besmer, P., J. E. Murphy, P. C. George, F. Qiu, P. J. Bergold, L. Lederman, H. W. Snyder, Jr., D. Brodeur, E. E. Zuckerman, and W. D. Hardy.** 1986. A new acute transforming feline retrovirus and relationship of its oncogene v-*kit* with the protein kinase gene family. Nature (London) **320**:415–421.
3. **Cowley, G., J. A. Smith, B. Gusterson, F. Hendler, and B. Ozanne.** 1984. The amount of EGF receptor is elevated on squamous cell carcinomas. Cancer Cells **1**:5–10.
4. **Downward, J., Y. Yarden, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ullrich, J. Schlessinger, and M. D. Waterfield.** 1984. Close similarity of epidermal growth factor receptor and v-*erbB* oncogene protein sequence. Nature (London) **307**:521–527.
5. **Feldman, R. A., L.-H. Wang, H. Hanafusa, and P. C. Bulduzzi.** 1982. Avian sarcoma virus UR2 encodes a transforming protein which is associated with a unique protein kinase activity. J. Virol. **42**:228–236.
6. **Hock, R. A., E. Nexo, and M. D. Hollenberg.** 1980. Solubulization and isolation of the human placenta receptor for epidermal growth factor-urogastrone. J. Biol. Chem. **255**:10737–10743.
7. **Hunter, T., and J. A. Cooper.** 1981. Epidermal growth factor induces rapid tyrosine phosphorylation of proteins in A431 human tumor cells. Cell **24**:741–752.
8. **Hunts, J., M. Ueda, S. Ozawa, O. Abe, I. Pastan, and N. Shimizu.** 1985. Hyperproduction and gene amplification of the epidermal growth factor receptor in squamous cell carcinoma. Jpn. J. Cancer Res. (Gann) **76**:663–666.
9. **Kawamoto, T., J. D. Sato, A. Le, J. Polikoff, G. H. Sato, and J. Mendelsohn.** 1983. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc. Natl. Acad. Sci. USA **80**:1337–1341.
10. **King, C. R., M. H. Kraus, L. T. Williams, G. T. Merlino, I. H. Pastan, and S. A. Aaronson.** 1985. Human tumor cell lines with EGF receptor gene amplification in the absence of aberrant sized mRNAs. Nucleic Acids Res. **13**:8477–8486.
11. **Kris, R. M., I. Lax, W. Gullick, M. D. Waterfield, A. Ullrich, M. Fridkin, and J. Schlessinger.** 1985. Antibodies against a synthetic peptide as a probe for the kinase activity of the avian EGF receptor and v-erbB protein. Cell **40**:619–625.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Libermann, T. A., H. R. Nusbaum, N. Razon, R. Kris, I. Lax, H. Soreq, N. Whittle, M. D. Waterfield, A. Ullrich, and J. Schlessinger.** 1985. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumors of glial origin. Nature (London) **313**:144–147.
14. **Libermann, T. A., N. Razon, A. D. Bartal, Y. Yarden, J. Schlessinger, and H. Soreq.** 1984. Expression of epidermal growth factor receptors in human brain tumors. Cancer Res. **44**:753–760.
15. **Matsushime, H., L.-H. Wang, and M. Shibuya.** 1986. Human c-*ros*-1 gene homologous to the v-*ros* sequence of UR2 sarcoma virus encodes for a transmembrane receptorlike molecule. Mol. Cell. Biol. **6**:3000–3004.
16. **Merlino, G. T., S. Ishii, J. Whang-Peng, T. Knutsen, Y.-X. Xu, A. J. L. Clark, R. H. Stratton, R. K. Wilson, D. P. Ma, B. A. Roe, J. H. Hunts, N. Shimizu, and I. Pastan.** 1985. Structure and localization of genes encoding aberrant and normal epidermal growth factor receptor RNAs from A431 human carcinoma cells. Mol. Cell. Biol. **5**:1722–1734.
17. **Merlino, G. T., X. Young-Hua, S. Ishii, A. J. L. Clark, K.**

Semba, K. Toyoshima, T. Yamamoto, and I. Pastan. 1984. Amplification and enhanced expression of the epidermal growth factor receptor gene in A431 human carcinoma cells. Science **224**:417–419.

18. **Neckameyer, W. S., M. Shibuya, M.-T. Hsu, and L.-H. Wang.** 1986. Proto-oncogene c-*ros* codes for a molecule with structural features common to those of growth factor receptors and displays tissue-specific and developmentally regulated expression. Mol. Cell. Biol. **6**:1478–1486.
19. **Neckameyer, W. S., and L.-H. Wang.** 1985. Nucleotide sequence of avian sarcoma virus UR2 and comparison of its transforming gene with other members of the tyrosine protein kinase oncogene family. J. Virol. **53**:879–884.
20. **Nilsen, T. W., P. A. Maroney, R. G. Goodwin, F. M. Rottman, L. B. Crittenden, M. A. Raines, and H.-J. Kung.** 1985. c-*erbB* activation in ALV-induced erythroblastosis: novel RNA processing and promoter insertion result in expression of an amino-truncated EGF receptor. Cell **41**:719–726.
21. **Rettenmier, C. W., J. H. Chen, M. F. Roussel, and C. J. Sherr.** 1985. The product of the c-*fms* protooncogene: a glycoprotein with associated tyrosine kinase activity. Science **228**:320–322.
22. **Sherr, C. J., C. W. Rettenmier, R. Sacca, M. F. Roussel, A. T. Look, and E. R. Stanley.** 1985. The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell **41**:665–676.
23. **Shibuya, M., J. Yokota, and Y. Ueyama.** 1985. Amplification and expression of a cellular oncogene (c-*myc*) in human gastric adenocarcinoma cells. Mol. Cell. Biol. **5**:414–418.
24. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Ullrich, A., L. Coussens, J. S. Hayflick, T. J. Dull, A. Gray, A. W. Tam, J. Lee, Y. Yarden, T. A. Libermann, J. Schlessinger, J. Downward, E. L. V. Mayes, N. Whittle, M. D. Waterfield, and P. H. Seeburg.** 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature (London) **309**:418–425.
26. **Vennström, B., L. Fanshier, C. Moscovici, and J. M. Bishop.** 1980. Molecular cloning of the avian erythroblastosis virus genome and recovery of oncogenic virus by transfection of chicken cells. J. Virol. **36**:575–585.
27. **Wong, A. J., S. H. Bigner, D. D. Bigner, K. W. Kinzler, S. R. Hamilton, and B. Vogelstein.** 1987. Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. Proc. Natl. Acad. Sci. USA **84**:6899–6903.
28. **Xu, Y.-H., S. Ishii, A. J. L. Clark, M. Sullivan, R. K. Wilson, D. P. Ma, B. A. Roe, G. T. Merlino, and I. Pastan.** 1984. Human epidermal growth factor receptor cDNA is homologous to a variety of RNAs overproduced in A431 carcinoma cells. Nature (London) **309**:806–810.
29. **Yamamoto, T., N. Kamata, H. Kawano, S. Shimizu, T. Kuroki, K. Toyoshima, K. Rikimaru, N. Nomura, R. Ishizaki, I. Pastan, S. Gamou, and N. Shimizu.** 1986. High incidence of amplification of the epidermal growth factor receptor gene in human squamous carcinoma cell line. Cancer Res. **46**:414–416.
30. **Yamamoto, T., T. Nishida, N. Miyajima, S. Kawai, T. Ooi, and K. Toyoshima.** 1983. The *erbB* gene of avian erythroblastosis virus is a member of the src gene family. Cell **35**:71–78.