Expression of Epidermal Growth Factor Receptors on Human Cervical, Ovarian, and Vulval Carcinomas

William J. Gullick,1 Judith J. Marsden, Nigel Whittle, Bruce Ward, Linda Bobrow, and Michael D. Waterfield

Protein Chemistry Laboratory [W. J. G., J. J. M., N. W., M. D. W.], interferon Laboratory [B. W.], and histology Unit [L. B.], Imperial Cancer Research Fund Laboratories, Lincoln’s Inn Fields, London WC2A 3PX, United Kingdom

ABSTRACT

We describe the properties of two monoclonal antibodies produced to a synthetic peptide consisting of residues 985 to 996 from the cytoplasmic domain of the epidermal growth factor (EGF) receptor. We have examined a group of ten human tumors including cervical, ovarian, and vulval carcinomas for expression of EGF receptors by immunohistological staining using one of these antibodies and another monoclonal antibody to the extracellular domain of the molecule. The tumors were examined using a sensitive amplified enzyme system and a less sensitive indirect staining method. There was generally a good correlation in staining intensity with the two monoclonal antibody reagents. Both antibodies showed strong staining of squamous cell carcinomas and usually weak or heterogeneous patterns with the adenocarcinomas. Samples of each tumor were solubilized in detergent and analyzed for the presence of functional EGF receptors by immunoprecipitation and autophosphorylation. Three of the squamous cell tumors gave labeled bands, M, 170,000, on sodium dodecyl sulfate-polyacrylamide gels. DNA was extracted from seven of the tumors and digested with two restriction endonucleases, and the fragments were analyzed on Southern blots using probes representing the extracellular and cytoplasmic domains of the molecule. The tumor DNA showed no apparent rearrangements or amplifications when compared to the EGF receptor gene in human placental DNA. These results suggest that there is a high level of EGF receptors on some squamous cell tumors.

INTRODUCTION

The EGF receptor is a M, 170,000 transmembrane glycoprotein found on many cell types (1–3). Its function is to bind the mitogen EGF and to transduce this signal across the cell membrane to the cytoplasm. EGF binding induces a variety of receptor mediated effects including ion transport, morphological changes, and ultimately mitogenesis in appropriate cell types (2, 3). The only intrinsic property of the EGF receptor described to date is tyrosine protein kinase activity (4, 5). Whether this activity alone is responsible for the pleiotropic effects that EGF binding can induce has not been determined.

Recently the human EGF receptor has been purified to homogeneity from normal human placenta and from the human vulval carcinoma derived cell line A431 (6). Direct sequence analysis of the EGF receptor has revealed extensive sequence homology with the transforming protein v-erb B present in several isolates of avian erythroblastosis virus (6). Comparison of the complete sequence of the human EGF receptor obtained by molecular cloning (7) with that of v-erb B showed that the latter was homologous only with the transmembrane and cytoplasmic domains of the EGF receptor and lacked the majority of the extracellular NH2-terminal sequences (6, 7). It has been hypothesized that expression of the v-erb B protein, which cannot bind EGF, in a constitutively active state contributes to uncontrolled growth and division of infected cells (6, 7). While this model remains to be proved it has stimulated examination of human malignant cell lines and primary tumors for their level of EGF receptor expression. A variety of different approaches have been taken including direct binding of iodinated EGF to breast cancer cells in culture (8), to human lung cancer cells (9), and to breast tumor biopsies (10) and by immunoperoxidase staining of cryosections of human malignant breast using a monoclonal antibody to EGF receptor (11). This antibody, called EGFRI, binds to the native folded external domain of the human and rat EGF receptors (12). It does not react with the v-erb B protein presumably since it recognizes sequences not present in the truncated molecule.

Using the same mAb Ozanne and co-workers surveyed the expression of EGF receptors on samples of several normal human tissues (13) and squamous lung cancers (14). The level of EGF receptor protein has also been determined by immunoprecipitation from detergent solubilized brain tumors (15, 16) and malignant cell lines (17) using monoclonal and polyclonal anti-EGF receptor antibodies. The organization of the gene(s) encoding EGF receptor has also been analyzed in detail using cDNA EGF receptor clones as probes on Southern blots (16, 17). The vulval carcinoma cell line A431, which has been studied extensively (18), possesses very high levels of EGF receptors per cell (2 × 106) and has manifold amplification of the gene(s) encoding EGF receptors (7, 19, 20). Libermann et al. (16) have shown that in some primary tumors of glial origin the EGF receptor gene is amplified.

The A431 cell line also synthesizes a truncated form of the EGF receptor comprising the external glycosylated EGF binding domain which is excreted into the growth medium (21, 22). It has, however, been difficult to examine cell lines or primary tumors for truncated EGF receptors representing the cytoplasmic domain since they would bind neither EGF nor the mAbs used to date. To address this problem we have made two mAbs to a synthetic peptide from the cytoplasmic domain of the EGF receptor. These mAbs are used together with EGFRI mAb to examine the immunohistological localization of EGF receptors on ten tumor samples of cervical, ovarian, and vulval origin. We also examined the tyrosine protein kinase activity of the EGF receptor by immunoprecipitation of solubilized tumor followed by

Received 4/29/85; revised 9/10/85; accepted 9/30/85.

1To whom requests for reprints should be addressed.
EGF RECEPTORS ON HUMAN TUMORS

Monoclonal Antibody Production. The peptide representing residues 985 to 996 of the human EGF receptor (7) with the sequence DVVDAYLIPQ was synthesized as described previously (23). It was coupled to keyhole limpet hemocyanin as follows. Peptide (5 mg) in 350 µl of phosphate buffered saline was mixed with keyhole limpet hemocyanin (5 mg) in 145 µl of PBS and 5 µl of 25% (v/v) glutaraldehyde (BDH, Poole, United Kingdom) was added, and the mixture was stirred at room temperature for 15 min. A further 2.5 µl of glutaraldehyde were then added and the reaction was continued for 15 min. A 1 mM solution of glycine-HCl (100 µl; pH 6.8) was then added and stirred for an additional 10 min to stop the reaction. The conjugate (500 µl) was mixed well with 3.9 ml PBS and 4.5 ml adjuvant (Cappel, Cochranville, PA). BALB/c mice, 6 weeks old, received, on day 0 200 µg of conjugate, mixed with complete Freund’s adjuvant, s.c. at several sites. The animals were boosted twice at 2-week intervals in the same way but using incomplete Freund’s adjuvant. Serum samples were taken from the tail on days 28 and 35 and were tested in ELISA assay against immunizing peptide and by immunoprecipitation of autophosphorylated EGF receptor as described below. The mouse used in the fusion described here received an i.p. injection of 200 µg of conjugate in PBS on day 39 and was sacrificed on day 42. The spleen cells were fused with NS-1 myeloma cells using polyethylene glycol 4000 (Merck, Darmstadt, West Germany) as described (24). The fused cell pellet was resuspended in 50 ml of medium and distributed into approximately 500 wells in 96-well tissue culture plates. After 14 days the supernatant from each well was tested for the production of immunoglobulin specific for the immunizing peptide by ELISA.

ELISA. A concentrated solution of peptide (10 mg/ml in PBS) was diluted to 10 µg/ml in 200 mw NaHCO3 buffer, pH 9.2, and 50 µl were added to wells of 96-well tissue culture plates. Plates from several manufacturers were tested; those from Nunc Inter Med (Kommun, Denmark) were found to give the best signal/noise ratio at a given serum dilution. Coating with the peptide alone had the advantage of excluding the contribution of anti-carrier and anti-glutaraldehyde antibodies to the color reaction. The peptide solution was left in the plates overnight at room temperature. The plates were then washed and 100 µl of substrate was added and the incubation was continued at 4°C for 10 min. The solution was cooled to 0°C, [γ-32P]ATP (Amersham, United Kingdom) was added to a final concentration of 10 µM and a specific activity of 5 Ci/µmol, and the reaction was allowed to continue for 10 min; 100 µl of this were then mixed with 50 µl of 10× concentrated hybridoma supernatant or 1 to 5 µg of purified mAb and incubated with agitation at 4°C for 1 h. A 1:1 suspension (20 µl) of Protein A-Sepharose (Pharmacia, Uppsala, Sweden) in PBS was added and the incubation was continued at 4°C for 30 min. The pellets were washed by centrifugation as described (26) and run on 7% polyacrylamide gels containing sodium dodecyl sulfate. The gels were stained, destained, dried, and then autoradiographed for approximately 18 h at −70°C using Kodak XR5 film.

Immunoprecipitation of v-erb B Protein. The chicken erythroblast cell line AV 6C2 infected with AEV ES4 virus was labeled with [35S]-methionine as follows. Logarithmically growing cells (5 x 10^6) were incubated for 1 h at 37°C in 1.0 ml of Dulbecco’s modified Eagle’s medium containing 10% of the normal methionine concentration, 10 mM 4-(2-hydroxyethyl) piperazineethanesulfonic acid buffer (pH 7.2), and 0.5 mM [35S]methionine (3000 Ci/mmol; Amershams). The cells were then washed with 10 ml Dulbecco’s modified Eagle’s medium at 0°C by gentle centrifugation. The cell pellet was lysed with 2 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 1% v/v Triton X-100, 150 mM NaCl, 25 mM benzamidine, 5 mM ethylene glycol bis[β-aminoethyl ether]-N,N,N',N''-tetraacetic acid, 0.1% bovine serum albumin, and phenylmethylsulfonyl fluoride, 50 ng/ml. Protein A-Sepharose (Pharmacia), 20 µl of a 1:1 suspension in PBS, was mixed with 2 µg of mAb F4 and incubated at room temperature for 10 min. The pellets were washed with 1.0 ml PBS by centrifugation. Erythroblast lysate (100 µl) was added, in either the presence or the absence of 5 mM peptide 2E, and the tubes were agitated at 4°C for 1 h. The pellets were washed as described (26) and sample buffer (50 µl containing β-mercaptoethanol was added. The samples were heated to 100°C for 2 min, then electrophoresed on 10% polyacrylamide gels containing sodium dodecyl sulfate, and treated with AMPLIFY (Amersham) before drying. The dried gels were exposed to Kodak XAR5 film for 16 to 18 h at −70°C using intensifying screens.

Tissue Preparation. Fresh samples of five ovarian, two cervical, and three vulval carcinomas were obtained from surgical excision biopsies. Several portions of each sample were immediately snap frozen and stored in liquid nitrogen, and one portion was routinely processed for conventional histological diagnosis. Similar preparations of normal skin and cervix were used as controls for the immunohistological studies.

Immunohistochemistry. Immunostaining was performed on frozen tissue sections using the indirect IAP and unlabeled antibody-enzyme (APAAP) techniques (27). Air dried cryostat sections (5 µm) were prefixed in cold (4°C) chloroform:acetone (1:1) for 5 min and incubated with ascites preparation of mAb EGF R1 and concentrated supernatant of mAb F4 at dilutions of 1:100 and 1:10, respectively. Subsequent layers consisted of sheep anti-mouse IgG (Fab2):alkaline phosphatase conjugate (1:50; Sigma) in the IAP test and rabbit anti-mouse immunoglobulin (Dakopatts, Denmark) at a final dilution of 1:25 in normal mouse serum followed by monoclonal (mouse) APAAP (1:10; Dakopatts) reagent in the unlabeled antibody-enzyme test. All antibody dilutions and intervening washes were made in 0.1 M PBS, pH 7.2. The red enzyme reaction was developed with Naphthol AS Bi phosphate and Fast Red TR in Veronal acetate buffer, pH 9.4, containing 1 mM levamisole to block endogenous alkaline phosphatase. Sections were counterstained in Mayer’s hemalum and examined by light microscopy.

Immunoprecipitation from Tumor and Phosphorylation of Human Tumor Extracts. This was done essentially as described (15) with the modifications that purified murine EGF, 4 µg/ml, was included in the phosphorylation mixture and the mAb used was EGF R1 at 5 µg/immunopre-
precipitate.

Isolation of DNA and Southern Blot Analysis. Samples of frozen tumors (approximately 100 mg) were homogenized in liquid nitrogen and the resulting powder was added directly to 10 ml of 100 mM Tris-HCl buffer, pH 7.6, containing 100 mM EDTA, 150 mM β-mercaptoethanol, and 1% (v/v) Sarkosyl. The solution was mixed gently for 2 h at 50°C and then overnight at 37°C with the addition of proteinase K (100 μg/ml; BRL, Cambridge, United Kingdom). The solution was then made 100 μg/ml with ethidium bromide and 90% (w/v) cesium chloride was added. Samples were centrifuged at 110,000 × g for 48 h in a Beckman 70 Ti rotor and the DNA band was isolated using a 15-gauge needle. The DNA sample was extracted with butanol and dialyzed extensively against 10 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA, and the volume was reduced by dehydration using Sephadex G-50 superfine (Pharmacia).

DNA (15 μg) was digested overnight in 400 μl buffer using 200 units of the indicated enzyme (Fig. 4) (BRL). After ethanol precipitation the samples were electrophoresed on 0.8% agarose gels at 2 V/cm and then transferred to Biodyne A transfer membranes (Pall Ultrafine Filtration Corporation, NY). The filter bound DNA was prehybridized and hybridized then transferred to Biodyne A transfer membranes (Pall Ultrafine Filtration Corporation, NY). The filter bound DNA was prehybridized and hybridized with 1 h in 30 mM sodium chloride, 3 mM sodium citrate, and 0.1% (v/v) sodium dodecyl sulfate at 50°C. Filters were subject to autoradiographic analysis using Kodak XAR5 with intensifying screens at −70°C for the indicated times.

RESULTS

Preparation of Anti-Synthetic Peptide mAbs. In order to develop reagents that could detect the cytoplasmic domain of the EGF receptor that were suitable for immunohistological examination of thin sections of tumors, we prepared mAbs to a synthetic peptide from the EGF receptor sequence. This peptide comprises residues 985 to 996 from the complete EGF receptor sequence (7) and is thus about 200 residues in length.

Polyclonal serum to this peptide raised in rabbits can recognize the native and denatured EGF receptor and v-erb B protein and reacts with the cytoplasmic domain of the molecule (23). We have produced two mAbs, designated F4 and D10, to this sequence which recognize only the appropriate peptide in ELISA (Chart 1). Both antibodies are of the IgG1 subclass (Table 1) and can immunoprecipitate native autophosphorylated human EGF receptor (Fig. 1A) and [35S]methionine labeled v-erb B (Fig. 1B) but have relatively low binding affinities (Table 1).

Immunohistology of Human Tumors. We obtained ten human tumors and examined them for their reaction with EGFR1 mAb to the extracellular domain and the F4 mAb to the cytoplasmic domain of the EGF receptor. The histological diagnosis and immunostaining results are shown in Table 2. Using a combination of the high sensitivity APAAP staining and the less sensitive IAP method, a clear distinction could be made between strong and weak reactivity patterns. The product of the alkaline phosphatase enzyme reaction is deposited as a red color. In general both antibodies produced stronger staining of the squamous cell carcinomas regardless of the anatomical state. Tumors 2, 3, 5, 9, and 10 (squamous cell carcinomas) were relatively strongly stained, although tumor 8 (adenocarcinoma), also gave strong staining with the F4 mAb. Tumors 1, 4, 6, and 7 (adenocarcinomas) gave weaker staining or were negative. Sections from

---

Table 1

<table>
<thead>
<tr>
<th>mAb</th>
<th>External/cytoplasmic domain of the EGF receptor</th>
<th>Subclass</th>
<th>Light chain</th>
<th>Affinity a</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR1 (12)</td>
<td>External</td>
<td>IgG2b</td>
<td>K</td>
<td>ND</td>
</tr>
<tr>
<td>F4</td>
<td>Cytoplasmic</td>
<td>IgG1</td>
<td>K</td>
<td>6 × 10^-4</td>
</tr>
<tr>
<td>D10</td>
<td>Cytoplasmic</td>
<td>IgG1</td>
<td>K</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Determined by radioimmunoassay using solubilized A431 membranes (26).

ND, not determined.

The binding affinity of D10 to native EGF receptor was too low to measure reliably.
tumors 3 and 5 (squamous cell carcinomas) and tumor 7 (adenocarcinoma), stained with both antibodies using the APAAP method, are shown in Fig. 2. Diffuse uniform staining was demonstrated on the tumor cells in both groups apart from sample 6 which contained foci of strong mAb EGFR1 positive cells.

**Immunoprecipitation and Phosphorylation from Tumor Extracts.** We next examined the ten tumors for the presence of functional EGF receptors using the system developed in Ref. 15. Pieces of each tumor were dissolved in detergent and the EGF receptor was precipitated by mAb EGFR1. The immunoprecipitates were incubated with EGF and carrier free [γ-32P]ATP so that any enzymically active EGF receptors would become auto-phosphorylated. Monoclonal antibody F4 was not used here because it is less sensitive than EGFR1 partly due to its lower affinity for the EGF receptor and partly because in binding close to the kinase domain it has an inhibitory effect on autophosphorylation on immunoprecipitates. This emphasizes that the radioactivity in the gel bands is a product of the amount of EGF receptor present and its autophosphorylating activity. Three tumors (tumors 3, 5, and 10; Fig. 3) gave labeled bands, M, 170,000, characteristic of the human EGF receptor. Tumor 10 (Fig. 3, track 10) also has a band at M, 150,000 which probably represents the breakdown product of the EGF receptor generated by a ubiquitous calcium activated neutral protease (29). In the tissue section studies these tumors were also strongly stained by the mAbs (Fig. 2; Table 2). The remaining tumors gave no apparent EGF receptor band by immunoprecipitation and showed variable immunohistological staining patterns (Table 2). This behavior may therefore reflect differences in the sensitivity of the assays or loss of enzyme activity during storage.

**Southern Blotting.** DNA was prepared from tumors 1 to 7 (Table 2) and digested with the restriction endonucleases EcoRI and HindIII. For comparison equal amounts of DNA from normal human placenta and from A431 cells [which are known to have amplified sequences coding for the EGF receptor (7, 19)] were included on the Southern blots. Two cDNA clones representing the 5' and 3' ends of the coding region of the human EGF receptor gene (Fig. 4A) were used to probe the blots (Fig. 4, B to E). The tumor DNA and normal placental DNA showed essentially the same pattern of fragments with each enzyme and each probe and gave similar intensities of hybridization. The DNA from A431 cells, however, gave a much greater extent of hybridization (Fig. 4) as reported previously (7, 17, 19, 20). There were, however, several small differences in fragment patterns that could be discerned on close examination of the autoradiographs. In Fig. 4E, the material digested with HindIII and probed with the cytoplasmic domain cDNA probe showed some differences in mobility between the 6-kilobase fragments. This behavior has, however, been observed when comparing DNA isolated from groups of normal individuals and thus probably represents polymorphism. An additional band of about 9 kilobases was present in Fig. 4D, track C, which was not present in normal DNA. This tumor (a squamous cell vulval carcinoma, tumor 3) displayed high levels of EGF receptor both by immunostaining (Fig. 2; Table 2) and by immunoprecipitation and autophosphorylation (Fig. 3, track 3). We are currently investigating whether this behavior represents significant rearrangement or polymorphism of EGF receptor coding sequences. These results, however, indicate that there has been no gross amplification of the EGF receptor genes in the tumors examined.

**DISCUSSION**

Little is known in detail about the pattern and mechanism of EGF receptor expression on human tumors and its relevance to the transformed state. In this work we have examined ten tumors

---

**Table 2.** Immunohistological analysis of tumor samples using monoclonal antibodies to the EGF receptor

<table>
<thead>
<tr>
<th>Tumor Location</th>
<th>Histological diagnosis</th>
<th>Immunoenzyme reaction with mAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFR1</td>
<td>F4</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Ovary</td>
<td>Serous papillary adenocarcinoma</td>
</tr>
<tr>
<td>2</td>
<td>Cervix</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>3</td>
<td>Vulva</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>4</td>
<td>Ovary</td>
<td>Infiltrating adenocarcinoma</td>
</tr>
<tr>
<td>5</td>
<td>Vulva</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>6</td>
<td>Ovary</td>
<td>Poorly differentiated adenocarcinoma</td>
</tr>
<tr>
<td>7</td>
<td>Ovary</td>
<td>Poorly differentiated adenocarcinoma</td>
</tr>
<tr>
<td>8</td>
<td>Ovary</td>
<td>Poorly differentiated adenocarcinoma</td>
</tr>
<tr>
<td>9</td>
<td>Vulva</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>10</td>
<td>Cervix</td>
<td>Undifferentiated squamous cell carcinoma</td>
</tr>
</tbody>
</table>

* NT, not tested.
for their localization and level of expression of EGF receptor protein and its intrinsic kinase activity and gene organization. As a first level of analysis we have localized EGF receptors on thin cryosections of selected normal and malignant tissue using mAbs to the extracellular and cytoplasmic domains of the molecule. Variable reactivity patterns were obtained using the IAP staining method and the more sensitive (APAAP) technique. Both mAbs exhibited strong staining of squamous cell carcinomas (13) and usually weak or heterogeneous patterns in the adenocarcinomas. A wider and more detailed study is at present under way, using the sensitive procedure described, to provide more information on EGF receptor expression on normal human tissues.

There was generally good correlation in staining intensity with the two mAbs which suggests that both reagents recognize the same molecule and no individual tumor within this group expresses exclusively truncated EGF receptors. It is not possible to detect by this method cells which express a low level of truncated EGF receptors (should such a situation exist in vivo as it clearly does in the A431 cell in culture). It would be informative to separate tumor derived EGF receptors by size and examine them by Western blotting with polyclonal serum to their localization and level of expression of EGF receptor. To date our attempts to achieve this have been unsuccessful, partly due to the small amount of protein that can be loaded on a single gel lane and partly due to the inefficiency of transfer of the high molecular weight form of the EGF receptor.

Immunoprecipitation and autophosphorylation of tumor extracts confirmed that several tumors possess immunohistologically contained functional EGF receptors. The detection of such a function is contingent on the receptor reacting with the mAb, possessing an active autophosphorylating activity and the sites at which phosphate may react covalently. The three major sites of EGF receptor autophosphorylation have been mapped (30) to the extreme carboxy terminus of the molecule. Nonetheless EGF receptor which lacks its COOH-terminal 20,000 daltons of sequence, cleaved off by the calcium activated protease, will be phosphorylated in an immunoprecipitate (Fig. 3, track 10, for instance) probably at a fourth site that has not yet been localized. Some tumors which were positive by immunostaining (Table 2) did not give visible immunoprecipitates. Whether this results from a difference in sensitivity of the assay, lack of kinase activity, or autophosphorylation sites is not clear.

DNA from the first seven tumors was analyzed by Southern blotting and showed no gross amplifications or rearrangements of the 5' or 3' EGF receptor coding sequences. Libermann et al. (15, 16) have clearly demonstrated that such changes have occurred in some glial cell derived tumors. However, not all tumors that had high levels of EGF receptor protein had amplified EGF receptor genes. In addition several cell lines with relatively high levels of EGF receptor protein showed little or no alteration in their EGF receptor gene structure (17). As stated by these authors gene amplification "is not the sole mechanism whereby cell types generate enhanced levels of EGF receptor protein." The presence of EGF receptors in these cell lines did, however, correlate with elevated levels of EGF receptor mRNA (17). Despite the good morphological preservation of the tumors studied in this work we could not obtain intact mRNA from them. In situ hybridization of EGF receptor specific nucleic acid probes to cryosections might be an alternative way to address this problem. It would also be desirable to develop probes that extend 5' to the sequence coding for the EGF receptor proteins signal sequence so that the structure of the normal and tumor transcriptional promoters could be analyzed.

Additional methods of increasing gene transcription include chromosomal translocations and polysomy, both found in A431 cells (31) and believed to be responsible for the activation of c-myc in Burkitt's lymphoma (32). Other mechanisms than those increasing transcription rates may be envisaged. Either increased mRNA stability or decreased EGF receptor degradation rates could lead to higher levels of EGF receptor protein. Indeed the EGF receptor in A431 cells appears to be degraded more slowly than in human fibroblasts (33) but since the EGF receptor gene is amplified in A431 cells it is difficult to say what effect this has on absolute receptor protein levels. Finally as suggested by the v-erb B model, alterations in receptor signaling activity may be important. Subtle structural changes such as point mutations are difficult to detect by the methods outlined above but could have significant effects on EGF receptor function. Such changes would not necessarily involve increased EGF receptor expression.

It should be emphasized that there is no direct evidence that overexpression or aberrant expression of EGF receptors is involved in the transformation of human cells. Transfection of cells with constructs of the EGF receptor gene by which the level of receptor protein can be varied, as has been done with c-src and v-src (34–36), would help to analyze this question. Examination of human tumors with high levels of EGF receptor expression for the levels of transcription of other oncogenes such as c-myc and c-fos would also be valuable. Meanwhile, however, examination of a wide spectrum of human tumors may aid in tumor classification (notably in the brain). A recent report (37) has indicated a correlation between invasiveness of human bladder tumors and a high level of EGF receptor expression suggesting that such analysis may be useful in predicting tumor behavior. Finally the relative levels of EGF receptors on some tumors versus normal tissues and the battery of anti-EGF receptor mAbs available (38) suggest that it might be worthwhile testing cytoxic antibody conjugates in model systems.

ACKNOWLEDGMENTS

We are grateful to Dr. R. Sainsbury for providing his unpublished results and to Audrey Becket for typing the manuscript.

REFERENCES

EGF RECEPTORS ON HUMAN TUMORS

Fig. 2. Immunohistological staining of frozen sections of human tumors. A, D, G & E stained sections; B, E, H, stained with EGFR1 mAb; C, F, I, stained with F4 mAb. The tumor numbers correspond to those in Table 2. Tumors 3 and 5, vulva, squamous cell carcinomas; tumor 7, ovary, poorly differentiated adenocarcinoma.
Fig. 4. Analysis of EGF receptor gene sequences by Southern blotting. The EcoRI subclones of the EGF receptor cDNA used as probes are shown diagramatically in A. Probe I corresponds to most of the external and transmembrane domains, while probe II represents the COOH terminus and part of the kinase domain (7). Genomic DNA (15 μg) was digested with EcoRI (B and C) or HindIII (D and E). The blots were hybridized with probe I (B and D) or probe II (C and E). Slight differences in intensity of hybridization signals can be accounted for by variations in the amount of DNA loaded. Tracks a to g correspond to tumors 1 to 7 (Table 2); track h, A431 cell DNA; track i, human placental DNA; track j, A431 cell DNA. Filters were autoradiographed at 70°C for 16 h except for tracks j, B and D, which were exposed for 2 h at -70°C.
Expression of Epidermal Growth Factor Receptors on Human Cervical, Ovarian, and Vulval Carcinomas

William J. Gullick, Judith J. Marsden, Nigel Whittle, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/1/285

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.