Expression and activation of epidermal growth factor receptors in meningiomas

RONA S. CARROLL, PH.D., PETER M. BLACK, M.D., PH.D., JIANPING ZHANG, M.S., MATTHIAS KIRSCH, M.D., IVONA PERCEC, A.B., NELSON LAU, AND ABHIJIT GUHA, M.S.C., M.D., F.R.C.S.(C)

Neurosurgical Laboratories and Brain Tumor Center, Brigham and Women’s Hospital, Boston, Massachusetts; The Children’s Hospital, and Dana Farber Cancer Institute, Boston, Massachusetts; Department of Surgery, Harvard Medical School, Boston, Massachusetts; Programme in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Ontario, Canada; and Division of Neurosurgery and Surgical Oncology, Toronto Hospital, University of Toronto, Toronto, Ontario, Canada

Previous studies have demonstrated expression of epidermal growth factor receptors (EGFRs) in human cerebral meningiomas. However, the activation status of the EGFRs and whether they activate cytoplasmic mitogenic signaling pathways are not known. In this study, using Northern blot analysis and the polymerase chain reaction, the authors report expression of epidermal growth factor, transforming growth factor–α, and EGFR messenger RNA in 27 meningioma specimens. Using Western blot and immunohistochemical analyses of the meningioma samples, the authors demonstrate that the EGFRs expressed by these meningiomas are activated. These activated EGFRs interact with and phosphorylate Shc, an SH2 domain–containing adapter protein that is important in transducing mitogenic signals from EGFRs to the nucleus via activation of the Ras signaling pathway. These results support the concept that activation of EGFRs in human meningiomas by autocrine/paracrine stimulation may contribute to their proliferation.

KEY WORDS • meningioma • epidermal growth factor receptor • Northern blot analysis • immunohistochemistry • Western blot analysis

Meningiomas constitute the second most common primary central nervous system (CNS) neoplasm, with biological characteristics that distinguish them from most other intracranial tumors. They are primarily benign mesenchymal tumors that, in contrast to the more common gliomas, can be surgically cured if total tumor removal can be achieved with acceptable risks. Meningiomas usually arise from the meninges, grow slowly, and push the adjacent brain while maintaining a discrete tumor–brain interface. This interface may be breached, leading to brain infiltration in rare cases in which malignant transformation to a meningial sarcoma has occurred. The cell of origin is controversial, but meningiomas are thought to arise from the meningothelial cells that comprise the arachnoid villi of the meninges. Although associated with some familial cancer syndromes such as neurofibromatosis-2, the vast majority of meningiomas occur sporadically in patients 50 to 60 years of age. The incidence of intracranial meningiomas is approximately twofold higher in females, a ratio that is even higher in the less frequently occurring spinal meningiomas. In addition, the growth of meningiomas in women appears to be accelerated during the luteal phase of the menstrual cycle and during pregnancy. Numerous growth factors have been implicated in meningioma proliferation.

Aberrant stimulation by growth factors, their cognate receptors, and the intracellular signal transduction pathways they use are implicated in the transformation and/or proliferation of a variety of neoplastic cells. Epidermal growth factor (EGF) is a polypeptide hormone that acts through activation of its cognate receptor (EGFR) and stimulates proliferation of a wide variety of cells in vitro and in vivo. Its biological actions include enhanced ion transport, stimulation of endogenous protein phosphorylation, changes in cellular morphology, and stimulation of DNA synthesis. The EGFRs are expressed by a wide variety of normal and neoplastic cells, including those of the CNS. The EGFR is the protooncogene (c-erb-B1) counterpart of the constitutively activated v-erb-B1 oncogene, implicating the potential for EGFR to play an important role in tumorigenesis. The EGFR is a single-chain transmembrane glycoprotein that possesses an integral tyrosine kinase domain with several tyrosine autophosphorylation sites. These sites are activated by phosphorylation on receptor dimerization due to binding of EGF or other members of the EGF family, including transforming growth factor–α (TGFα) and amphireg-
Several additional members of the EGFR family exist, including c-erb-B2, c-erb-B3, and c-erb-B4, with which heterodimerization is possible; their biological sequelae are presently unclear.

Possible mechanisms of aberrant EGFR activation leading to cellular transformation can occur by several means, including truncation of extracellular domains, which causes ligand-independent activation (the mechanism of v-erb-B), and overexpression of the normal EGFR, which leads to ligand-dependent autocrine/paracrine stimulation. Elevated expression or activity of EGFR has been reported in a number of human cancers, including squamous cell carcinoma and neoplasms of the bladder, breast, and brain. Increased levels of EGFRs have resulted from gene amplification, enhanced transcription, translation, or decreased receptor turnover. Amplification and/or overexpression of EGFRs has been reported in glioblastomas (the most malignant astrocytoma and the most common type of primary CNS neoplasm in adults) and may contribute to the malignant phenotype by receptor activation through constitutively activated truncation mutants or by an autocrine mechanism. In support of this hypothesis, unpublished results from the laboratory of one of the authors (A.G.) documented that levels of activated Ras–guanosine triphosphate (GTP) are elevated and are important for the proliferation of human malignant astrocytoma cell lines, although they do not harbor activating Ras mutations. The authors further demonstrated that in malignant astrocytoma cells, elevation of Ras–GTP, which is an important intracellular signal transduction pathway for a variety of receptor tyrosine kinases, is due to proliferative signals from the EGFR and other receptor tyrosine kinases that these cells overexpress.

In 1987, Weisman, et al., characterized the EGFR in meningiomas and suggested the involvement of this growth factor in the proliferation and/or differentiation of meningioma cells. They also demonstrated the enhancing effect of EGF on DNA synthesis and cell growth in primary cultures of meningioma cells. In the present study we investigated the expression and activation status of the EGFR by means of Northern blot analysis, Western blot analysis, polymerase chain reaction (PCR), and immunohistochemical analysis in a large number of primary human meningioma specimens.

Materials and Methods

Tissue Samples

For Northern and Western blot analyses 27 meningioma specimens were obtained in patients at the time of craniotomy and were immediately snap frozen in liquid nitrogen at the University of Toronto Nervous System Tumor Bank. A portion of each specimen was removed for neuropathological diagnosis and the tumor was classified using standard criteria as syncytial, transitional, or fibroblastic. Nonneoplastic tissue in patients undergoing temporal lobectomy for uncontrollable seizures was obtained for use as a comparison.

Ribonucleic Acid Isolation and Northern Blot Analysis

Total RNA was isolated according to the method of Chirgwin, et al. The frozen tumor samples were homogenized using a polytron in 4 M guanidinium isothiocyanate buffer. The lysates were centrifuged for 10 minutes at 3000 rpm and the supernatant was layered over a 5.7-M cesium chloride gradient and ultracentrifuged at 38,000 rpm for 16 hours at 22°C. The RNA pellet was dissolved in 0.3 M sterile sodium acetate and the RNA ethanol was precipitated. Twenty micrograms (A260) of total RNA for each sample was subjected to electrophoresis in a 1.8% formaldehyde gel and diffusion blotted onto a nylon membrane. The RNA was crosslinked to the nylon membrane with ultraviolet light. At 42°C, the blots were prehybridized for 2 hours in 50% formamide, 5 × SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7), 10 × Denhardt’s solution, 50 mM NaPO4, 1% sodium dodecyl sulfate (SDS), 10 μg/ml Sigma free acid, and degraded herring sperm DNA and then hybridized overnight in 50% formamide, 5 × SSC, 1 × Denhardt’s solution, 20 mM NaPO4, 0.5% SDS, 5% dextran sulfate, 20 μg/ml Sigma free acid, and degraded herring sperm with 106 cpm/ml of a 32P-labeled complementary (c)DNA probe. Northern blots were sequentially hybridized to detect EGFR, EGF, and TGFα mRNA, as well as β-actin. The blots were washed at room temperature in 1 × SSC/1% SDS for 15 minutes, 0.5% SSC/0.5% SDS for 15 minutes, and twice in 0.1 × SSC/0.1% SDS, and again at 50°C in 0.1% SSC/0.1% SDS for 30 minutes and then subjected to autoradiography.

The following cDNA probes were used for hybridization: 1) a 2.4-kb Clal human EGFR insert; 2) a 0.5-kb Hind III–EcoRI human TGFα; 3) a 3.8-kb human EGF cDNA; and 4) a 1.8-kb human β-actin fragment. The cDNA fragments were labeled with phosphorus-32 using random primer translation to achieve a specific activity of 0.5 to 1 × 108 cpm/μg DNA.

Western Blot Analysis

Each frozen tissue specimen was crushed in a porcelain mortar and transferred to a Dounce tissue grinder containing 3 ml of cold lysis buffer (20 mM hydroxethyl piperazine ethanesulfonic acid, pH 7.4; 1% Nonidet P-40, 1% glycerol, 2.5 mM ethylene glycol tetraacetic acid, 2.5 mM ethylendiamine tetraacetic acid, 0.9% NaCl, 0.9% NaCl, 0.9% NaCl, 0.9% NaCl, 0.9% NaCl, 0.9% NaCl). The protein lysates were centrifuged to remove any cellular debris, quantified (using protein analysis), and stored at −70°C. For all Western blot analyses, the protein lysates were first enriched for EGF or Shc by immunoprecipitation with a human EGF–specific antibody (Ab:EGFR directed against the COOH terminus [residues 996–1022], 1:100) or a polyclonal antibody generated against the NH-terminal portion of Shc (Ab:Shc). The antibody was added directly to the protein lysate and incubated for 2 hours at 4°C with constant rocking. Protein A–Sepharose CL-4B resin was then added for 1 hour at 4°C. Each sample was subsequently rinsed four times with lysis buffer. The samples were reconstituted in Laemmli sample buffer, boiled for 5 minutes, and analyzed using SDS-polyacrylamide gel (7.5% acrylamide) electrophoresis (PAGE).

After PAGE, the gels were transferred to Immobillon-P and blocked at room temperature by using 5% nonfat milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 (10 mM Tris, pH 8, and 0.9% NaCl [TBST]) for 3 hours. The first antibody was diluted in 5% bovine serum albumin/TBS/0.1% Tween-20 and the antibodies were incubated overnight at 4°C. The first antibodies included 1) the anti-EGFR antibody (Ab:EGFR), which was generated against the residues 996 to 1022 of the human EGFR and which recognizes the activated and nonactivated forms of the EGFR (1:2500 in TBST); 2) an anti-activated EGFR antibody (Ab:EGFR*) against human tyrosine–phosphorylated EGFR, which recognizes only the activated form of the receptor (1:1000 in TBST); and 3) a rabbit antibody against human EGFR, clone 4G10 (Ab:4G10: 1:2500 in TBST). After rinsing in TBST, the blots were incubated with the secondary anti–mouse horseradish peroxidase–conjugated antibody (1:1000 in 5% nonfat milk/TBST) for 1 hour at room temperature. The blots were then rinsed again in TBST and detection was performed using a Western detection system according to the manufacturer’s instructions. The blots were exposed to Kodak XAR film for 1 hour at −70°C. The presence of Shc in the lysates, the Shc immunoprecipitates were run on a 10% SDS gel and incubated with Ab:Shc, and the signal was detected using electrogenerated chemiluminescence. The same blots were probed for Shc phosphorylation using Ab:4G10.
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Immunohistochemical Analysis

Immunohistochemical staining was performed using the same EGFR antibodies that were used to perform the Western blot analyses (Ab:EGFR and Ab:EGFR*). Cultured A431 human epidermoid carcinoma cells were stimulated with 100 ng EGF/ml for 5 minutes and subsequently used as a positive control for the expression of both EGFR and EGFR* as detected by immunocytochemical analysis. After stimulation, the cells were trypsinized and counted. Approximately 10,000 cells were spun onto lysine-coated glass slides in a centrifuge, fixed in acetone for 10 minutes at 20°C, washed twice in PBS with 0.1% Triton-X, deparaffinized, and then quenched in methanol and 4.5% H2O2 for 5 minutes. The slides were microwaved for 5 minutes in 10 mM sodium citrate to demask the antigens. Then the slides were permeabilized with 0.3% Triton-X 100 in PBS. The following steps used the Vectastain Elite ABC Kit and the Vector VIP Substrate Kit for peroxidase. Briefly, after incubation with blocking horse serum for 30 minutes, the primary antibody (10 ng/ml) was added and the slides were incubated for 1 hour at room temperature, followed by binding to a secondary antibody–immunoglobulin G horse antibody for 30 minutes, and then binding to a preformed avidin–biotinylated horseradish peroxidase macromolecular complex. Slides were washed twice between each step for 5 minutes in PBS. The chromogen Vector VIP resulted in a dark purple membranous and cytosolic staining of positive cells with a wash time of 15 minutes. Tissue sections and slides were counterstained with 1% methyl green in 0.1 M acetate buffer, dehydrated in ethanol, and mounted with coverslips. Formalin-fixed, paraffin-embedded surgical specimens were deparaffinized, quenched in methanol with 4.5% H2O2 for 5 minutes, and treated as described earlier for the A431 cells.

Polymerase Chain Reaction

Two micrograms of tumor RNA was reversed transcribed at 37°C for 1 hour by using an oligo(dT)12–18 primer in a total volume of 20 μl. Each reaction mixture included 1 μl reverse transcription (RT) buffer (75 mM KCl, 50 mM Tris–HCl, pH 8.3, 1.3 mM MgCl2, 4 μl 2.5 mM deoxynucleotides, 40 pmol oligo(dT)12–18 primer, 1 mM dithiothreitol, 20 U RNasin, and 40 U SuperScript RNAse H-minus RT). After the incubation was completed, the reaction mixture was boiled for 10 minutes. Amplification was performed in a final volume of 30 μl containing 8 μl RT sample (corresponding to 200 ng total RNA), 1.5 mM/L of each of upstream and downstream primer; 2.5 mM/L MgCl2, 0.4 mM/L each of deoxyadenosine triphosphate, deoxyctydine triphosphate, deoxyguanosine triphosphate, and deoxuthymidine triphosphate, 2.5 U Taq DNA polymerase, 3 μl 10× Taq buffer, and autoclaved distilled water. Amplification was performed for 35 cycles, denaturing at 94°C for 1 minute once, followed by 94°C for 2 minutes, annealing at 55°C for 2 minutes, and elongation at 72°C for 5 minutes. Control samples for each RNA from which RT had been omitted were run in parallel. Thirty microliters of each PCR sample was subjected to electrophoresis on a 1.2% agarose gel for analysis of amplification results, followed by denaturation, neutralization, and transfer by diffusion blotting to the nylon membrane. The blot was then hybridized for 24 hours at 42°C with a specific cDNA probe–labeled random primer,7 corresponding to the PCR-amplified product. The blot was washed and subjected to autoradiography. The primers for EGF (5′-CCT TAT GAG GAG TCG AGC AGA -3′ and 5′-CCT GCC TCC ATG AAG TTG GTT -3′) along with the amplification reaction were expected to yield a 192-bp DNA molecule. The primers spanned two introns between exon 22 and exon 24 of human EGFR genomic DNA.

Sources of Supplies and Equipment

The Centrifuge SW 50.1 rotor was supplied by Beckman Instruments, Palo Alto, CA. Duralon nylon membrane and Stratalinker ultraviolet light were supplied by Stratagene, La Jolla, CA. Human TGFα was kindly provided by R. Derynck; human EGF cDNA by Graeme I. Bell; and human β-actin fragment by Larry Kedes. The Ab:EGFR was supplied by Transduction Laboratories, Lexington, KY, and the Shc was generously donated by Dr. Tony Pawson. Protein A–Sepharose CL-4B resin came from Pharmacia, Piscataway, NJ. The Immobilon-P came from Millipore, Bedford, MA, and Clone 4G10 from Upstate Biotechnology Inc., Lake Placid, NY. The Phototope-horseradish peroxidase Western detection system was supplied by New England Biolabs, Beverly, MA. The A431 human carcinoma cells and the 2.4-kb Clal human EGFR insert came from American Type Culture Collection, Rockville, MD, the EGF from Collaborative Biomedical Products, Bedford, MA, and the lysine-coated glass slides from Sigma Chemical Co., St. Louis, MO. The cytospin centrifuge was provided by Shandon, Pittsburgh, PA, and the Vectastain Elite ABC Kit and Vector VIP Substrate Kit for peroxidase by Vector Laboratories, Burlingame, CA. SuperScript RNAse H-minus RT came from Life Technology, Inc., Gaithersburg, MD, and Taq DNA polymerase and RNAse from Promega, Madison, WI.

Results

Northern Blot and PCR Analysis

Twenty-seven meningiomas were examined for the presence of EGFR, EGF, and TGFα by means of Northern blot analysis. For EGFR we detected one RNA species of approximately 10 kb under highly stringent washing conditions in all meningioma specimens (Fig. 1). This is a similar pattern to that previously reported for A431 cells, a human epidermoid carcinoma cell line. The level of EGFR mRNA varied among the specimens and there was no relationship between the level of receptor expression and age, sex, or histological type (Table 1). A normal 4-kb transcript was observed for TGFα in all specimens (Fig. 2). There was no relationship between the level of TGFα expression and patient age, sex, or the tumor histological subtype. No amplification of the genes was detected in any of the tumors examined. For EGF we did not observe a signal in any of the meningioma specimens in which Northern blot analysis was performed (data not shown). However, we were able to detect a signal in all but two of the meningioma specimens examined using PCR with specific EGF primers (Fig. 3 and Table 1).

Western Immunoblot Analysis

All meningioma samples were enriched by immunopre-
cipitation with a human EGFR antibody and subjected to SDS-PAGE. To verify the specificity of nonactivated and activated EGFR antibodies, a Western immunoblot analysis was performed using HER 14 cells in the presence or absence of EGF (Fig. 4). The meningioma samples were all run in triplicate and each separate gel was then immunoblotted with Ab:EGFR, Ab:EGFR*, and Ab:4G10 (Fig. 5). For the Ab:EGFR, a strong band of 180 kD, representing normal EGFRs, was expressed by all the meningioma specimens. There were no significant differences in the intensity of the band among the tumor samples. A 180-kD band was also observed with Ab:4G10 in all the specimens, implying that at least a portion of the EGFRs expressed by the meningiomas was activated. This was further verified using Ab:EGFR*, which only recognizes the activated EGFR. The 180-kD bands denoting activated EGFR, by the Ab:4G10 or activation-specific Ab:EGFR* immunoblots, were much weaker than the band detected by Ab:EGFR, which recognizes both forms of the receptor (activated and inactivated). Western immunoblotting using the Ab:Shc demonstrated expression of all three Shc isoforms by all of the meningioma samples (Fig. 6). The 52- and 46-kD isoforms were the major Shc isoforms, with the larger 66-kD isoform being variably expressed and phosphorylated. These characteristics of the nontransforming 66-kD Shc isoform, in contrast to

![FIG. 2. Representative Northern blot demonstrating expression of the 4-kb TGFα mRNA transcript in eight meningioma specimens (upper) and reprobed with β-actin (lower), demonstrating equivalent total RNA loading in each lane.](image)

**TABLE 1**

Results of Northern blot and PCR analysis in 27 meningiomas

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<th>Tumor Type</th>
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Abbreviations: den = densitometry; ND = not done; neg = negative; NS = not suitable; pos = positive.

![Fig. 3. Detection of EGF mRNA by RT-PCR in meningiomas. Upper: Ethidium bromide–stained 1% agarose gel of 10 meningioma specimens, demonstrating the products of RT-PCR reaction containing EGF primers. Size markers are a 100-bp ladder. Lower: Autoradiograph of amplified EGF cDNA obtained by RT-PCR, transferred from the agarose gel shown in upper, and probed with a radiolabeled EGF cDNA probe.](image)
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The smaller two species, which do transform transfected fibroblasts, have been previously recognized. 37 Tyrosine phosphorylation of Shc, as detected in the meningioma specimens (Fig. 6), results in phosphorylation of Tyr#317, which is the binding site of the Grb2 SH2 domain, implicating activation of the Ras signaling pathway. For five nonneoplastic brain specimens a weak band was seen with Ab:EGFR, and no band was observed with Ab:EGFR* (data not shown).

Immunohistochemical Analysis

Twenty-one meningiomas were examined for their expression of the EGFR protein by immunohistochemistry using the same two antibodies that were used for the Western blot analysis: Ab:EGFR and the activation-specific Ab:EGFR* (Fig. 7). Two specimens were not suitable for immunohistochemistry because the paraffin sections were too small for analysis. Staining with Ab:EGFR was very heterogeneous in 10 meningiomas. There were areas that exhibited very strong staining alternating with areas that showed faint staining. In these 10 cases, staining was especially strong around whorls in some meningiomas, whereas others exhibited predominant vascular endothelial cell staining or a combination of both patterns. Nine of the 19 meningiomas showed a more homogeneous staining pattern with strong expression of the EGFR protein. For all specimens, staining with activation-specific Ab:EGFR* was much weaker than that with Ab:EGFR. Nine meningiomas revealed only a very faint staining pattern with the Ab:EGFR*, whereas 10 specimens demonstrated moderately strong staining. There were four cases in which focal staining was observed around cell whorls. The pattern of endothelial cell staining was similar to that observed using Ab:EGFR.

Discussion

Expression of a growth factor and its cognate ligand by a tumor cell does not automatically imply a causal role for tumor cell proliferation by autocrine/paracrine stimulation. In this study we demonstrated that meningiomas express abundant EGFRs both at the mRNA and protein levels. The EGFRs can be activated by their natural ligands, EGF and especially TGFα, expressed by the meningiomas. Similarly, high levels of expression of other growth factors and/or growth factor receptors such as platelet-derived growth factor, have been demonstrated in meningiomas,6,33 in keeping with the fact that several genetic aberrations may be involved in the pathogenesis of these tumors. However, in this study we go further and demonstrate that the EGFRs expressed by meningiomas are activated. This is demonstrated by analyzing flash-frozen meningioma lysates by means of Western immunoblotting with Ab:4G10 and an activation-specific antibody directed toward the phosphorylated intracellular domain of EGFR, Ab:EGFR*. In addition, using immunohistochemical analysis with Ab:EGFR*, we demonstrated that the activated EGFRs were localized to the meningothelial tumor cells.

All meningiomas expressed the normal 10-kb mRNA EGFR transcript shown on Northern blot analysis and expressed the 180-kD EGFR protein detected by Western immunoblot analysis, regardless of patient characteristics, tumor location, or histological subtype. Previous studies using ligand-binding techniques51,52,67,69 have reported a wide range of positivity for the presence of EGFR in meningiomas, varying from approximately 30 to 100%. The discrepancies in the literature may be accounted for by the different techniques used in each of the studies. Some
studies used equilibrium binding assays in tissue specimens, whereas others used Scatchard analysis on cellularly derived microsomes. In the equilibrium binding experiments, receptor quantities were expressed in different units, and the authors did not define the quantity of the receptor they considered “significant.” In our study, the presence of EGFRs was determined by using Northern and Western blot analyses, which are very different from, and much more sensitive than, most techniques used in previous studies that determined EGFR levels using ligand binding. These discrepancies could account for the variations in the literature regarding the expression levels of EGFRs in meningiomas.

Using the same tumor specimens, protein lysates that were immunoprecipitated with the nonactivation-specific Ab:EGFR and Western blotted with the activation specific Ab:EGFR* demonstrated a 180-kD signal. This band, which recognizes only activated or phosphorylated EGFR, was observed in all meningioma specimens examined, indicating that binding of EGF to its receptor sends mitogenic signals to the nucleus. Immunohistochemical analysis using the activation and nonactivation EGFR antibodies demonstrated strong immunopositivity in the presence of Ab:EGFR compared to a much weaker signal in the presence of the activation-specific Ab:EGFR*. The EGFR expression was especially strong in the whorls of meningothelial cells, which are pathogenic for meningiomas, and was also prominent in tumor cells around vascular channels and in the actual vascular endothelial cells themselves. The expression was patchy in some tumor specimens, as has previously been reported.27-35 In contrast, other studies of meningiomas have suggested that distribution of EGFRs was more uniform, especially when compared with the heterogeneous expression of EGFRs in glioblastomas.36 These differences in the staining patterns among the various studies are probably the result of the different epitopes recognized by the primary EGFR antibodies. In our study the Ab:EGFR was directed against the COOH-terminal region outside the kinase domain, whereas in previous reports the antibody was directed against the carbohydrate region or extracellular domain.

The two endogenous EGFR ligands, EGF and TGFα, were both detected in all meningioma specimens. Expression of EGF mRNA was minimal and undetectable using Northern blot analysis (data not shown) but was present in the majority of specimens, as shown by PCR analysis. In contrast, TGFα mRNA was much more abundant and was detected in all meningioma specimens using Northern blot analysis. These results suggest that meningiomas have the ability to express both ligands for the EGFR but that TGFα is predominant. High levels of TGFα expression by meningiomas have been previously noted, indicating that TGFα may be a predictor of increased recurrence and of less than an optimum response to other therapeutic interventions postsurgery.35

There have been a few studies that have tried to answer the question of functionality of the EGFRs on meningioma proliferation by examining the effects of EGF stimulation on meningioma cell lines in culture. Most studies demonstrate an increase in DNA synthesis in a dose-dependent manner, as measured by [3H]thymidine incorporation following addition of exogenous EGF.29,67 It has also been shown that addition of progesterone to meningioma cells in culture increases their sensitivity to EGF stimulation, suggesting that there may be some interactions between sex steroid hormones and EGF-mediated proliferation. In this study we demonstrated that a portion of the EGFRs expressed by meningiomas are activated or phosphorylated. In addition, we demonstrated that in many meningioma specimens, Shc, a nonenzymatic adapter protein that activates the Ras signaling pathway, is tyrosine phosphorylated or activated. The Ras-Raf-microtubule-associated protein kinase signaling pathway is pivotal in transducing mitogenic signals from many receptor tyrosine kinases, including activated EGFR expressed in meningiomas.38,39,53,55,59 Ras is located in the inner cell membrane in its guanosine diphosphate (GDP)–bound inactive form and is activated by recruitment of guanine nucleotide exchange factors such as mammalian Son of Sevenless (mSos) to the cell membrane.1,7,24,47,58 This mSos is associated through its proline rich COOH-terminal tail with the SH3 (src-homology-3) domain of another adapter-signaling protein called Grb2.
This Grb2 can directly bind to the tyrosine-phosphorylated sites of activated receptors located at the cell membrane through its SH2 (src-homology-2) domain, thereby bringing mSos in proximity to Ras-GDP. In addition, this can also be accomplished by the binding of the SH2 domain of Grb2 to the major tyrosine phosphorylation site (Tyr#317) of another adapter protein called Shc, which interacts with the receptors. The Tyr#317 of Shc is

Fig. 7. Photomicrographs. A: Immunohistochemical detection of the EGFR using the activation-independent antibody, Ab:EGFR, shows strong staining of meningioma and vascular endothelial cells. B: Staining for the activated EGFR using the activation-sensitive antibody, Ab:EGFR*, reveals diffuse light staining of meningioma and endothelial cells. C: A negative control omitting the primary antibody. D: Staining of the meningioma specimen using H & E. Original magnification × 100.
phosphorylated on binding of Shc through its COOH-terminal SH2 domain or through its more recently described NH-terminal phosphotyrosine-binding domain, to the activated receptor. Therefore, the presence of tyrosine-phosphorylated Shc in the majority of meningiomas specimens suggests that the Ras-Raf-microtubule-associated protein kinase signaling pathway is being activated in these tumors. Many receptor tyrosine kinases use this signaling pathway; hence, ongoing experiments are required to prove that the activated EGFRs expressed by meningiomas are activating the major mitogenic signaling pathway mediated by activation of the Ras pathway.

Conclusions

These studies demonstrate that not only are EGFRs expressed by human meningioma specimens, but a portion of these EGFRs are activated. Activation of the EGFRs is not a result of any mutations of the EGFR, but we speculate it is secondary to autocrine/paracrine stimulation by their endogenous ligands, EGF and especially TGF. Growth of meningioma specimens suggests that the Ras-Raf-TGF pathway is an important mitogenic pathway. In addition, we provide preliminary evidence that the activated EGFRs in meningiomas are capable of initiating activation of the Ras signaling pathway, a major mitogenic cascade used by many receptor and nonreceptor tyrosine kinases. Understanding the molecular pathogenesis of meningiomas may lead to biological therapies to supplement surgery in the management of these common CNS tumors.

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Address reprint requests to: Rona S. Carroll, Ph.D., Neurosurgical Laboratories, Brigham and Women’s Hospital, 221 Longwood Avenue, Room 121, Boston, Massachusetts 02115.

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