Characterization of insulin-like growth factor I and epidermal growth factor receptors in meningioma

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Receptors for insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) were localized and characterized in eight samples of human meningioma (four fibrous, two meningothelial, and two angioblastic types), using quantitative autoradiographic techniques. Effects of both growth factors on deoxyribonucleic acid (DNA) synthesis in the cultured meningioma cells were examined. High numbers of specific binding sites for both IGF-I and EGF were homogeneously present in tissue sections derived from fibrous and meningothelial types of meningiomas, whereas binding sites for these growth factors were not detectable in adjacent leptomeninges. While relatively large numbers of IGF-I binding sites were located in the wall of the intratumoral vasculature, the number of binding sites in the stromal component was lower in angioblastic-type meningiomas, including a low number of EGF binding sites detected only in the stromal portion. Scatchard analysis revealed the presence of a single class of high-affinity binding sites for both IGF-I and EGF in the meningiomas examined (dissociation constant \((K_d) = 0.6\) to 2.9 nM, and the maximum number of binding sites \((B_{max}) = 16\) to 80 fmol/mg for IGF-I; and \(K_d = 0.6\) to 4.0 nM, \(B_{max} = 3\) to 39 fmol/mg for EGF). Both growth factors increased the synthesis of DNA, in a dose-dependent manner, as measured by \(\text{H-thymidine incorporation.}

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These observations can be interpreted to mean that both IGF-I and EGF may be involved in the growth modulation of meningiomas, possibly through paracrine or autocrine mechanisms.

KEY WORDS meningioma • insulin-like growth factor • epidermal growth factor • brain neoplasm

MENINGIOMAS, typical mesenchymal tumors in the central nervous system, have been suggested to be sex-steroid hormone-sensitive. However, the functional significance of those receptors in meningiomas is unclear.

Growth factors may be involved in the transformation and/or proliferation of neoplastic cells. The epidermal growth factor (EGF) receptor has a significant sequence homology to the product of the erb-B oncogene; therefore, the contribution of the EGF to tumorigenesis must be considered. Amplification of the EGF receptor gene has been noted in cases of human glial origin tumors, and the increased expression of EGF receptors has been reported in cases of human squamous-cell lung cancers. Recently, Weisman, et al., characterized the receptors for EGF in human meningiomas and suggested the involvement of this growth factor in the proliferation and/or differentiation of meningioma cells.

Insulin-like growth factors (IGF-I and IGF-II) are well recognized as mitogenic polypeptides for various cell types in culture. The receptor for IGF-I as well as EGF has the intracellular tyrosine kinase domain, which is important in the regulation of cell proliferation. The production of IGF-like peptides or the enhancement of IGF messenger ribonucleic acid (mRNA) was noted in human neoplastic cells. Thus, not only EGF but also IGF-I may be involved in tumorigenesis and may be linked to paracrine or autocrine mechanisms related to tumor growth.

These growth factors as well as platelet-derived growth factor (PDGF) can act together to exert synergistic effects. It has been reported that EGF may synergize with IGF-I to promote the growth of BALB/
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c 3T3 cells. Therefore, the question was addressed whether IGF-I receptors were expressed in human meningiomas. In vitro quantitative autoradiographic techniques were used to localize and characterize the receptors for both IGF-I and EGF in human meningiomas. The influence of these growth factors on the synthesis of deoxyribonucleic acid (DNA) in meningioma cells was also examined.

Materials and Methods

Tumor Specimens and Materials

Fresh tumor samples were obtained from eight patients during surgery for removal of a meningioma. Immediately after excision, the tumor tissues were divided into two sections; one was prepared for pathological investigation, and an adjacent portion was immediately placed in liquid nitrogen and stored at -80°C for a maximum of 4 weeks. The frozen sections were cut 16 μm thick in a cryostat at -16°C, thaw-mounted onto gelatin-coated slides, and placed under vacuum overnight at 4°C.

Recombinant human IGF-I(Thr59) (> 95% pure), EGF (> 95% pure), and porcine insulin were used.* The IGF-I(Thr59) is identical to natural human IGF-I, except for substitution of threonine for methionine at position 59, and is equipotent to the native peptide in bioassays and binding to IGF-I receptors. Iodine-125-labeled IGF-I(Thr59) (125I-IGF-I; specific activity 267 μCi/μg) and EGF (125I-EGF; specific activity 153 μCi/μg) were also used.†

Binding Studies

Binding sites were labeled in vitro by incubation with 125I-IGF-I or 125I-EGF and quantified by autoradiography followed by computerized densitometry‡ and a comparison with 125I-standards.‡

IGF-I Binding Sites. Consecutive frozen sections from each specimen were preincubated for 15 minutes at 25°C in 25 mM Tris-HCl buffer, pH 7.5, containing 10 mM MgCl₂, 0.1% bovine serum albumin (BSA), and 1 mg/ml bacitracin, and then incubated for 120 minutes at 25°C in the same fresh buffer with 0.1 mM 125I-IGF-I in the presence of increasing concentrations of unlabeled IGF-I, porcine insulin, or EGF. After incubation, the sections were washed three times, for 1 minute each in 25 mM Tris-HCl buffer, pH 7.5, at 4°C, then rinsed in distilled water to remove the nonspecifically bound ligand. The sections were then dried under a stream of cold air, placed in x-ray cassettes together with ~2-~I labeled IGF-I, porcine insulin, or EGF. After incubation, the sections were washed and processed as described above.

Quantification of Binding Sites

After determination of the standard curve (in optical density × 100 vs. in disintegrations per min (dpm)/mg of standards), the optical densities corresponding to the tissue images were measured by computerized densitometry and interpolated in a straight line to obtain the corresponding dpm/mg bound to the tissue. Binding affinity (Kd) and capacity (Bmax) for IGF-I or EGF were obtained by Scatchard analysis of competition data, using the computer program LIGAND.19

Culture of Human Meningioma Cells

Immediately after removal of the tumor from Case 8, the specimen was minced into 1-sq mm pieces and incubated in 0.1% trypsin and 0.02% EDTA solution for 30 minutes at room temperature with constant shaking. Trypsin activity was inhibited by adding Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). After centrifugation at 1000 G, the cell pellet was resuspended in complete medium (DMEM supplemented with 10% FBS, penicillin, 5 U/ml, and streptomycin, 5 μg/ml) and the cells were mechanically dispersed. Cells were seeded in 75-sq cm tissue culture flasks and cultured at 37°C in 5% CO₂/95% air with 100% humidity until subconfluence occurred at 1 week.

3H-Thymidine Incorporation Studies

To test the mitogenic actions of IGF-I and EGF on the meningioma cells, 3H-thymidine incorporation studies were performed according to a modification of the method described by Russell, et al.20 The cultured meningioma cells were treated with 0.1% trypsin and 0.02% EDTA solution, suspended in DMEM containing 10% FBS, and plated onto 24-well tissue culture plates at a density of 3 × 10⁴ cells/well. The cells were cultured at 37°C for 48 hours, following which the medium was changed to serum-free DMEM and the preparation incubated for another 48 hours. The cultures were then incubated with varying concentrations of IGF-I, EGF, and 10% FBS after being washed with fresh DMEM; the control cultures were incubated with DMEM containing 0.1% BSA. After incubation for 22

* IGF-I(Thr59) obtained from AMGen Biologicals, Thousand Oaks, California; EGF obtained from Amersham, Arlington Heights, Illinois; and porcine insulin obtained from Sigma, St. Louis, Missouri.
† 125I-IGF-I and 125I-EGF obtained from Amersham, Arlington Heights, Illinois.
‡ Computerized densitometer, Model UHG-101, manufactured by Unique Medical Co., Tokyo, Japan.

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hours, \(^3\)H-methyl thymidine (0.5 \(\mu\)Ci/well) was added to the cultures and the incubation was continued for another 4 hours at 37\(^\circ\)C. The reaction was halted by aspiration of the incubating solution and washing the cells three times with ice-cold PBS. The cultures were then incubated with 5\% trichloracetic acid (TCA) at 4\(^\circ\)C for 20 minutes. The cultures were washed with fresh 5\% TCA, after which they were solubilized in 1\% sodium dodecyl sulfate. The resultant solution was added to 10 ml of complete counting cocktail and counted in a scintillation counter.

Results

Localization of IGF-I and EGF Binding Sites

The \(^125\)I-IGF-I binding sites were localized in all meningiomas tested. Nonspecific binding was less than 15\% of the total binding (Fig. 1). In both fibrous and meningothelial types of meningiomas, many specific IGF-I binding sites were present homogeneously (Bmax: 80, 64, 23, and 51 fmol/mg in four fibrous meningiomas, and 28 and 30 fmol/mg in two meningothelial meningiomas; Table 1), whereas there was no specific binding in adjacent leptomeningeal tissues. In the two angioblastic type meningiomas, a relatively greater number of specific IGF-I binding sites were mainly localized in the wall of the intratumoral vasculature (Bmax: 39 and 16 fmol/mg; Table 1 and Fig. 2). There were too few binding sites present in stromal areas to obtain reliable data.

There were also \(^125\)I-EGF binding sites present in all the meningioma specimens. The pattern of those distributions in tissue sections was similar to that of IGF-I binding, except for the angioblastic type where relatively low numbers of EGF binding sites were mainly localized in the stromal areas (Bmax: 10, 24, 27, and 39 fmol/mg in the fibrous meningiomas, 12 and 34 fmol/mg in the meningothelial meningiomas, and 3 and 4 fmol/mg in the angioblastic meningiomas) and the vasculature was devoid of binding sites (Table 1 and Fig. 2).

![Fig. 1. Autoradiographic localization of \(^125\)I-labeled insulin-like growth factor I (\(^125\)I-IGF-I) binding sites in the fibrous (left panels: Case 1) and angioblastic (right panels: Case 5) type of meningiomas. a: Typical sections with arrowhead showing the adjacent leptomeninges. H & E, \(\times\) 6. b: Autoradiographs corresponding to a, generated by incubating sections 16 \(\mu\)m thick with 0.1 nM \(^125\)I-IGF-I and leaving them apposed against \(^3\)H-Ultrofilm for 5 days. c: Sections adjacent to b, incubated with 0.1 nM \(^125\)I-IGF-I in the presence of 0.1 \(\mu\)M unlabeled IGF-I, showing nonspecific binding (less than 15\% of total binding). d: Photomicrographic appearance of each type of meningioma. H & E, \(\times\) 30.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Histological Subtype</th>
<th>IGF-I (Bmax fmol/mg, Kd nM)</th>
<th>EGF (Bmax fmol/mg, Kd nM)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>40, F</td>
<td>fibrous</td>
<td>80 (1.7) 10 (1.8)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>65, F</td>
<td>fibrous</td>
<td>64 (2.6) 24 (0.6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60, F</td>
<td>fibrous</td>
<td>23 (1.2) 27 (3.0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>62, F</td>
<td>fibrous</td>
<td>51 (2.9) 39 (1.1)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>69, M</td>
<td>angioblastic</td>
<td>39 (1.1) 3 (1.1)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>52, M</td>
<td>angioblastic</td>
<td>16 (0.6) 4 (0.9)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>51, F</td>
<td>meningothelial</td>
<td>28 (2.2) 12 (1.3)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>49, F</td>
<td>meningothelial</td>
<td>30 (1.6) 34 (4.0)</td>
<td></td>
</tr>
</tbody>
</table>

* EGF = epidermal growth factor; IGF-I = insulin-like growth factor I. Consecutive sections were incubated with 0.1 nM \(^125\)I-IGF-I (1,710,000 dpm added) or 0.1 nM \(^125\)I-EGF (920,000 dpm added) in the presence of increasing concentrations of unlabeled IGF-I or EGF. Bmax and Kd were determined by Scatchard analysis performed on displacement data using the computer program LIGAND. Values represent the mean of data obtained from two to three different sections derived from each specimen.
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Characterization of $^{125}$I-IGF-I and $^{125}$I-EGF Binding to Meningiomas

The binding of $^{125}$I-IGF-I to meningiomas was specific (Fig. 3). The unlabeled IGF-I inhibited the binding of $^{125}$I-IGF-I, in a dose-dependent manner, whereas EGF did not compete with $^{125}$I-IGF-I for the binding. Half-maximum displacement of the $^{125}$I-IGF-I binding occurred at an IGF-I concentration of approximately 3.0 nM. Insulin also competed for the $^{125}$I-IGF-I binding; however, approximately 1.0 μM insulin was required for half-maximum displacement. The Scatchard analysis, using the computer program LIGAND, revealed the presence of a single class of high-affinity binding sites for IGF-I in meningiomas (Kd: 1.7, 2.6, 1.2, and 2.9 nM in the fibrous type, 2.2 and 1.6 nM in the meningothelial type, and 1.1 and 0.6 nM in the angioblastic type; Table 1). The binding of $^{125}$I-EGF to meningiomas was also specific (Fig. 4). Unlabeled EGF had a high affinity for the $^{125}$I-EGF binding sites. The IGF-I had no potential to inhibit the binding of $^{125}$I-EGF. Half-maximum displacement of the $^{125}$I-EGF binding occurred at an EGF concentration of approximately 1.5 nM. Scatchard analysis revealed the presence of a single class of high-affinity binding sites for EGF in the meningiomas (Kd: 1.8, 0.6, 3.0, and 1.1 nM in the fibrous type, 1.3 and 4.0 nM in the meningothelial type, and 1.1 and 0.9 nM in the angioblastic type; Table 1).

$^3$H-Thymidine Incorporation Studies

The effect of IGF-I and EGF on $^3$H-thymidine incorporation into DNA of cultured meningioma cells derived from the patient in Case 8 was examined in a serum-free culture system. Both growth factors stimulated the synthesis of DNA in a dose-dependent manner in the meningioma culture (Fig. 5). The half-maximum responses occurred approximately at 0.1 nM for IGF-I.

![Fig. 3. Competition curves of insulin-like growth factor I (IGF-I) binding to meningioma in a typical experiment performed using consecutive tissue sections obtained from the Case 1 meningioma specimen. Points represent the average of data calculated from three different densitometry readings. The control value is defined as maximum binding in the absence of the unlabeled ligand. EGF = epidermal growth factor.](image)

![Fig. 4. Competition curves of epidermal growth factor (EGF) binding to meningioma in a typical experiment performed using consecutive tissue sections obtained from the same specimen (Case 1) as in Fig. 3. Points represent the average of data calculated from three different densitometry readings. The control value is defined as maximum binding in the absence of the unlabeled ligand. IGF-I = insulin-like growth factor I.](image)
and 0.01 nM for EGF. The median effective concentration for IGF-I-stimulated synthesis of DNA in meningioma cells was 17-fold lower than the affinity (Kd) for 125I-IGF-I binding to the tissue sections derived from the same meningioma specimen (Case 8). The EGF showed a significantly higher potency at a concentration lower than that of IGF-I; however, EGF appeared to be slightly inhibitory at the highest concentration tested (0.1 μM). Although the maximal stimulation of 3H-thymidine incorporation by individual growth factors was under 50% of the amount of 3H-thymidine incorporated in the presence of 10% FBS, the combination of IGF-I and EGF synergistically stimulated DNA synthesis and induced a nearly maximum level of 10% FBS at 10^-10 M for both growth factors (Fig. 6).

Discussion

These studies identified the presence of specific IGF-I binding sites in all the human meningioma tissues examined. Autoradiograms obtained from the tissue sections derived from fibrous and meningothelial types of meningioma indicated a possible co-localization of binding sites for IGF-I with those for EGF in the meningioma cells. Results from competitive binding studies suggested that 125I-IGF-I binding to meningiomas was consistent with the specific binding properties expected of IGF-I receptors. A single class of high-affinity binding sites for IGF-I has also been noted in other human-derived tissues such as fibroblasts, lymphocytes, cells of the mammary gland, and brain.

The binding affinities for IGF-I observed in meningiomas were of a similar concentration range (10^-10 to 10^-9 M) to those in other human tissues. These findings, together with the stimulatory effects of IGF-I and EGF on the synthesis of DNA in the cultured meningioma cells, provide additional evidence for the presence of functional receptors for these growth factors in human meningiomas, although the functional relevance of these receptors in meningiomas is unclear. A discrepancy between the IGF-I receptor affinity and the biological effects has also been noted in cells from the human mammary gland. The undetectable binding sites for both IGF-I and EGF in adjacent leptomeningeal tissue indicate the specific expression of the receptors for those growth factors in this lesion. The enhanced expression of the receptors for IGF-II but not for IGF-I has been detected in glioblastoma multiforme, determined based on membrane-binding methods. Expression of the receptors for IGF-I suggests that a contribution of IGF-I to tumorigenesis would have to be ruled out.

Weisman, et al., characterized the receptors for EGF in human meningiomas; they also noted that EGF stimulated the synthesis of DNA in cultured meningioma cells. The present findings are in general agreement with theirs, except that we detected a single class of EGF-binding sites, while they noted two classes of binding sites. This difference may be due to the different techniques used (autoradiography in thin whole-tissue sections in our study vs. binding to membrane preparations in their study). In addition, they demonstrated...
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the synergistic effect of EGF and PDGF on stimulation of the synthesis of DNA to nearly maximal levels, as induced by 10% FBS in cultured meningioma cells. We observed that IGF-I synergistically stimulated the synthesis of DNA in meningioma cells with EGF in a serum-free culture system, although only one meningioma was available for study. Synergism between IGF-I and EGF in DNA synthesis has been demonstrated in other cells. Leof, et al., reported that EGF was required during the traverse of only the first half of the G1/G0 cell cycle phase and the traverse of the final half of G0/G1, and commitment to DNA synthesis required the presence of IGF-I. Thus, EGF and IGF-I may have different mechanisms of action in meningioma cells, through different receptors. It has been reported that IGF-I regulates the progression of only the competent 3T3 cells, as induced by PDGF pretreatment; however, in our study the combination of IGF-I and EGF increased the synthesis of DNA in meningioma cells without pretreatment with PDGF, and similar effects were seen with 10% FBS on 3H-thymidine incorporation. This difference may relate to the fact that the meningioma cells used were nonquiescent ones. However, the possibility that the meningioma cells might produce PDGF-like peptides, as seen in glioma and sarcoma cells, would have to be ruled out.

An enhancement of IGF mRNA has been noted in cases of human colon carcinoma and liposarcoma. The secretion of an IGF-I-related protein by human breast cancer cells also was noted. The production of IGF-like peptide has been detected in tumor cyst fluid, cerebrospinal fluid, and tumor cytosol in patients with glial tumors. All this evidence indicates that autocrine or paracrine mechanisms constituted by these growth factors are involved in the proliferation and differentiation of meningioma cells.

Since the effect of these growth factors has been observed on the synthesis of DNA in meningioma cells, the investigations will be extended to examine the functional importance of receptors for IGF-I and EGF in meningiomas. The elucidation of the role of growth factors in the proliferation or differentiation of tumors should lead to improvement in adjuvant therapy. Antagonists to growth factor receptors or monoclonal antibody against those receptors could have clinical application in cases of inoperable or recurrent meningiomas.

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