Growth hormone receptor expression and function in meningiomas: effect of a specific receptor antagonist

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Object. This study was undertaken to explore the effects of growth hormone (GH) and the GH-stimulated peptide insulin-like growth factor–1 (IGF-1) on the growth rate of meningiomas.

Methods. Polymerase chain reaction and ribonuclease protection assays were used to demonstrate that GH receptor messenger RNA was present in all 14 meningioma specimens studied, regardless of tumor grade. Both wild type (GHRwt) and a previously described exon 3 deletion isoform (GHRd3) of the GH receptor were identified in individual tumor specimens. The importance of the GH receptor was assessed using a GH receptor antagonist (B2036). Blockade of the GH receptor with B2036 reduced serum-induced DNA synthesis, as measured by thymidine incorporation, by 8 to 33% (mean 20%) in primary meningioma cultures. Tumors that expressed the GHRwt and GHRd3 isoforms, or a combination of the two, were all responsive to antagonist treatment. The importance of IGF-1 in stimulating meningioma cell growth was also assessed. It was found that IGF-1 increased thymidine incorporation in primary meningioma cultures in a dose-dependent manner: 1 ng/ml, 5 ng/ml, and 10 ng/ml resulted in increases in thymidine incorporation of 21%, 43%, and 176%, respectively, over baseline values.

Conclusions. In these studies the authors demonstrate that activation of the GH/IGF-1 axis significantly increases the growth rate of meningiomas. Blockade of the GH receptor on tumor cells inhibited tumor growth. If these findings are confirmed in animal studies, agents that downregulate the GH/IGF-1 axis might represent a potential adjuvant therapy in the management of patients with meningioma.

Key Words • meningioma • growth hormone receptor • receptor antagonist • insulin-like growth factor 1

The synthesis of insulin-like growth factor–1 (IGF-1) in the liver and many other tissues is stimulated by growth hormone (GH). A combination of the direct effects of GH and the indirect effects of IGF-1 produce the many physiological changes associated with growth. For example, GH deficiency in children is associated with substantially decreased growth velocity, delayed puberty, and decreased final height, whereas an excess of GH (acromegaly) produces accelerated linear growth and gigantism. In adults with excess GH, bone growth in areas such as the calvarium, mandible, and phalanges predominates because of epiphyseal fusion. Soft tissue and visceral growth (heart, kidneys, lungs, liver, thyroid) is also common.

Several decades of basic and clinical studies have contributed to the identification of GH and IGF-1 as potent inducers of cell growth in many neoplastic tissues. In patients with acromegaly, an increased incidence of several types of neoplasms has been observed. For instance, Ron, et al.,27 found that 1041 men with acromegaly who were followed for an average of 8.3 years had a 3.1-fold increase in the risk of colon cancer as compared with a group of normal controls; others have noted a similar relationship between acromegaly and the development of both benign and malignant colonic polyps.11,12 Nabarro22 has reported an increased incidence of breast neoplasms in women with acromegaly and IGF-1 has been identified as a potent growth factor for breast carcinoma cell lines.16

Both of the foregoing are relatively rare tumors; however, the development of meningiomas in patients with acromegaly has been reported in the medical literature on a number of occasions.1,5 In one large series19 it was found that 1.5% of patients with acromegaly who had not undergone cranial irradiation also had a meningioma, leading the authors to hypothesize an association between GH/IGF-1 and meningioma development. Additional cases have been reported in individuals exposed to ionizing radiation.11,20 Although the GH receptor status of these tumors has not been studied, IGF-1 receptors have been identified in approximately 75% of meningioma specimens by using a variety of techniques including binding assays and receptor messenger (m)RNA measure-
ments. Insulin and IGF-1 (with insulin presumably functioning through the IGF-1 receptor) have been demonstrated to increase DNA synthesis significantly in primary meningioma cultures.

In this study, we investigated the role of the GH/IGF-1 axis in stimulating meningioma growth. Initial experiments were performed to determine if meningiomas expressed GH receptor mRNA. The functional importance of the GH receptor in primary meningioma cultures was evaluated using a recently developed specific receptor antagonist to block GH action. The GH receptor antagonist used in this study (B2036) is similar to those previously described, in that it is a natural analog of GH that has been used in this study (B2036) is similar to those previously described, in that it is a natural analog of GH that has been used in this study (B2036) is similar to those previously described, in that it is a natural analog of GH that has been used in this study (B2036) is similar to those previously described, in that it is a natural analog of GH that has been used in this study (B2036) is similar to those previously described, in that it is a natural analog of GH that has been used in this study.

Materials and Methods

Specimen Collection/Primary Culture Establishment

Fourteen tumor specimens that had been collected from 14 different patients were included in this study. The age, gender, histological type, and tumor location in each of the patients from whom a surgical specimen was obtained are listed in Table 1. In summary, nine tumors were benign, three were atypical, and two were malignant; specimens obtained from women and men (seven each) were equally represented. The ages of the patients ranged from 39 to 80 years, with a mean of 57.7 years.

After surgical removal, a portion of each tumor was submitted for characterization at the time of surgery. Other portions were dispersed by treatment with Dispase I for 15 to 30 minutes at 37°C. One million cells were then plated in a 100-mm tissue culture dish in low-glucose Dulbecco’s minimum essential medium (DMEM) with 10% fetal bovine serum (FBS), penicillin, and streptomycin. The cells were grown to confluence and then harvested, aliquoted, and stored in liquid nitrogen for future use. In six instances, enough tissue was obtained at resection so that a portion of the tumor remained in excess of what was required to establish a primary culture. In these cases, the remaining portion of the tumor specimen was frozen immediately at −80°C.

TABLE 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Histological Finding</th>
<th>Location</th>
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<tr>
<td>5</td>
<td>52</td>
<td>M</td>
<td>malignant</td>
<td>cavernous sinus</td>
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<td>malignant</td>
<td>spinous process metastasis (T10-L3)</td>
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<td>8</td>
<td>49</td>
<td>F</td>
<td>benign</td>
<td>parasagittal</td>
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<td>9</td>
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<td>F</td>
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<td>frontal</td>
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<td>43</td>
<td>F</td>
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<td>convexity</td>
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<td>75</td>
<td>M</td>
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<td>olfactory groove</td>
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<td>61</td>
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<td>frontal</td>
</tr>
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<td>15</td>
<td>63</td>
<td>F</td>
<td>benign</td>
<td>convexity</td>
</tr>
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<td>sphenoid wing</td>
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<td>F</td>
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<td>F</td>
<td>benign</td>
<td>convexity</td>
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</tbody>
</table>

K. E. Friend, R. Radinsky, and I. E. McCutcheon

The IOMM-Lee cell line has been previously described and was kindly provided by Dr. W. H. Lee. These control cells, which were derived from a male patient with a metastatic malignant meningioma, were grown in the same media as the primary meningioma cultures: low-glucose DMEM, 10% FBS, penicillin, and streptomycin.

Thymidine Incorporation

Thymidine incorporation experiments were performed using a modified version of a previously described methodology. Briefly, dispersed meningioma cells (3.5 × 10⁶ cells/well) were seeded into tissue culture plates (0.32 cm²/well) and grown overnight in low-glucose DMEM, 10% FBS, penicillin, and streptomycin. The culture medium was then replaced with serum-free medium for a 3-day period. Growth factors or serum were subsequently added for 20 hours. Where indicated, a GH receptor antagonist, B2036, was added to the media along with serum. A total of 3.7 × 10⁶ Bq methyl-[3H]thymidine (185 GBq/mmol) was added to each well for 4 hours. The cells were harvested with trypsin, rinsed on glass filter strips. The amount of radioactive activity present in each specimen was quantified using a scintillation counter.
Growth hormone receptor in meningioma

**Fig. 1.** Assays showing expression of GH receptor in meningioma tumors and primary cultures in exons 7 through 10. Upper: Gel showing RNA-PCR detection (35 cycles) of GH receptor mRNA in six surgically removed meningioma specimens. The 100-bp size marker (M) is shown in the first lane, with the first double-intensity band indicating 600 bp. The observed PCR product is consistent with the predicted size of the amplification product (513 bp). Lower: Gel showing RNA-PCR detection of GH receptor mRNA in RNA isolated from 11 primary meningioma cultures and the IOMM-Lee cell line.

**Data Analysis**

All thymidine incorporation experiments were performed in triplicate. Results are expressed as the mean ± standard error. The data for each treatment group were compared with the others by using a paired t-test on commercially available software. Probability values are reported throughout the text. Values of less than 0.05 were considered to represent significant differences between treatment groups.

**Sources of Supplies and Equipment**

The Dispase I was purchased from Boehringer Mannheim, Indianapolis, IN. The DMEM and UDG cloning kit were supplied by Gibco BRL, Gaithersburg, MD. The B2036 was acquired from Sentinel Drug Development Corp., Austin, TX, and the [methyl-3 H]thymidine from Amersham, Buckinghamshire, England. The glass filter strips were obtained from Cambridge Technology, Inc., Watertown, MA. The statistical software (StatView) was purchased from Abacus Concepts, Inc., Berkeley, CA.

**Results**

**Expression of GH Receptor mRNA**

All the meningioma specimens were analyzed for GH receptor mRNA by using PCR. Two areas of the receptor were analyzed. A downstream region including part or all of exons 8 through 10 was studied initially. As can be seen in Fig. 1, GH receptor PCR products were easily visible on ethidium bromide staining in both the meningioma tumor specimens and the primary cultures after 35 cycles of PCR. The only specimen that did not express GH receptors was the immortalized IOMM-Lee meningioma cell line. All other specimens, which included benign, atypical, and malignant tumors, expressed easily detectable amounts of receptor mRNA. The same region of the GH receptor mRNA was analyzed using an RNase protection assay to provide a more quantitative measurement. Five tumor specimens were analyzed (Fig. 2 upper left); a β-actin probe was used to normalize the amount of template RNA in each specimen (Fig. 2 upper right). The ratio of GH receptor mRNA to β-actin mRNA varied approximately 90-fold between meningioma specimens, but they all contained readily detectable amounts of GH receptor mRNA. The RNA isolated from cell lines generated from several other types of tumors (osteosarcoma, colon cancer, prostate cancer) did not express GH receptor (Fig. 2 lower left), even though these tumors expressed abundant amounts of β-actin (Fig. 2 lower right).

The existence of an alternatively processed variant of the GH receptor mRNA in which exon 3 is deleted (GHRd3) has been described. This isoform has been observed in both normal and malignant tissues and is thought to be specific to the individual, arising from a genetic polymorphism that is inherited in simple mendelian fashion. To determine the percentage of meningiomas expressing the GHRd3 isoform compared with full-length GH receptor mRNA (wild type [GHRwt]), a second PCR experiment was performed in which primers including part or all of exons 2 through 5 were used. Some tumors and cell lines expressed GH receptor transcripts that included exon 3, whereas others expressed GHRd3 (Fig. 3). Most individual specimens contained one particular receptor isoform rather than a combination of both.

**Thymidine Incorporation**

Increasing the amounts of serum in the media increased the growth rates of the primary meningioma cultures in a dose-responsive manner, as measured by thymidine incorporation. The mean thymidine incorporation for the primary meningioma cultures in serum-free conditions was 2740 counts/minute, which increased to 5624 counts/minute with 1% serum and 11,722 counts/minute with 10% serum. The difference between each of these groups was significant (0% compared with 1%, p = 0.0188; 0% compared with 10%, p = 0.0177; 1% compared with 10%, p = 0.0192). The addition of B2036 reduced the mean thymidine incorporation in all groups (Fig. 4). Both concentrations of B2036 tested (1 μg/ml and 10 μg/ml) provided approximately the same efficacy; the decrease ranged from 8 to 33%, with a mean reduction of 20%. The decrease was statistically significant in the group that received 10% serum (0 μg/ml compared with 1 μg/ml B2036, p = 0.019; 0 μg/ml B2036 compared with 10 μg/ml B2036, p = 0.014).

Decreases were observed in tumors regardless of the type of GH receptor isoform expressed. The mean decreases in thymidine incorporation observed in the GHRwt- and GHRd3-expressing tumors were 16% and 21%, respectively. The only tumor expressing both isoforms exhibited a 32% decrease. The amount of GH receptor mRNA present as measured by an RNase protection assay did not seem to have a direct correlation with response to...
B2036 (Table 2). Tumors containing both high and low levels of mRNA exhibited decreased thymidine incorporation after GH receptor antagonist administration. Although the direct actions of GH are important in many tissues, in most instances the growth-stimulating actions of IGF-1 are significantly more important. To assess the role of IGF-1 in these primary meningioma cultures, this factor was added to serum-free culture media in several different concentrations. Again, thymidine incorporation was used as a marker for cell proliferation. A dose-dependent response was observed, with progressively increasing amounts of IGF-1 being associated with higher levels of thymidine incorporation (Fig. 5). The mean percentage increase from baseline (no IGF-1) was 21% with 1 ng/ml, 43% with 5 ng/ml, and 176% with 10 ng/ml. Statistical analysis indicated that the difference between the 0 ng/ml and 10 ng/ml groups was significant (p = 0.025), as were the differences between the 10 ng/ml group and the 5 ng/ml (p = 0.026) and 1 ng/ml groups (p = 0.038). These results demonstrate that both GH and IGF-1 are capable of stimulating the growth of primary meningioma cultures.

Discussion

Although GH and IGF-1 are potent inducers of cell growth in many normal and neoplastic tissues, information about the mechanisms by which these compounds...
influence meningioma growth was limited prior to these studies. Our experiments demonstrate that mRNA transcripts encoding the GH receptor are quite prevalent in meningiomas. All the clinical specimens examined had readily detectable amounts of mRNA; primary cultures from benign, atypical, and malignant tumors were included in the analysis. Both the full-length GH receptor (GHRwt) and the splice variant in which exon 3 is deleted (GHRd3) were present, although most individual tumor specimens expressed one particular receptor isoform rather than a combination of both. In the immortal cell line, there was no detectable GH receptor mRNA, indicating that loss of differentiation as evidenced by immortalization may be associated with loss of GH receptor expression.

Exon 3 of the GH receptor encodes a 22–amino acid portion of the extracellular domain of the receptor. This region is not highly conserved across species,31 and the ligand-binding capacities of the GHRwt and GHRd3 have not been shown to be substantially different.2,30,33 Accordingly, our studies indicate that tumors expressing the GHRwt, GHRd3, or a combination of both respond to GH receptor antagonist administration. A previous study of a Hutterite pedigree demonstrated that 10% of the individuals expressed GHRd3 in a homozygous manner.31 In our data there was no noticeable correlation between the histological grade of the meningioma and the type of GH receptor isoform present.

The functional importance of the GH receptor was studied using the antagonist. The direct effects of GH receptor blockade were assessed by adding B2036 to serum present in the culture media. A modest but significant decrease (mean 20%) was observed. This indicates that GH receptor blockade can reduce meningioma growth rates despite exposure of the primary cultures to the complicated milieu of growth factors present in serum. This is an experimental model that is likely to be most reflective of the tumor environment. The B2036 was equally effective in tumors expressing the GHRwt or GHRd3 isoforms.

As noted previously, within the GH/IGF-1 axis, the actions of IGF-1 tend to be the most significant in regard to stimulating growth. In our studies, IGF-1 was observed to increase thymidine incorporation significantly. At the highest dose studied, 10 ng/ml, a 176% increase was observed. These experiments confirm the results of a previous study in which IGF-1 was also noted to have a significant ability to stimulate the growth of meningiomas.14 In several types of neoplasms, IGF-1 has also been demonstrated to inhibit apoptosis as well as stimulate growth.5

When used in in vitro studies such as these, GH receptor antagonist compounds like B2036 offer investigators the ability to examine the effects of GH receptor blockade alone. When administered in vivo, they are capable of producing a number of other potentially significant physiological events, and the full clinical utility of these compounds would likely depend on actions at multiple sites. These include direct actions on the tumor itself and indirect actions on the host tissues enveloping the neoplasm. Circulating IGF-1 concentrations would also be decreased because of GH receptor blockade in liver tissue.

The primary treatment for meningiomas clearly remains tumor resection. However, in some instances additional therapies are required. A number of pharmacolog-
logical agents have been proposed as possible adjunctive therapies. Interferon has been demonstrated to be an effective cytostatic agent in some tumor models. Agents that interfere with angiogenesis have also been demonstrated to block tumor growth in animals. Some studies have demonstrated that chemotherapeutic agents such as hydroxyurea can be effective in primary culture and in meningioma transplant models. Induction of apoptosis appears to be a primary mechanism of action for hydroxyurea. Our studies indicate that the GH/IGF-1 axis may also represent a target for meningioma therapies. However, that target is relatively broad, because GH may affect tumor cells either directly through receptors on the cell membrane or indirectly through IGF-1 generated in the liver and released into the general circulation. The effects of a GH receptor antagonist on tumor growth in vivo are thus not entirely clear, and would reflect its hepatic action as well as its action on the tumor cells themselves. Clearly, before conclusions are made on the potential utility of such agents as adjuvant therapy in patients with meningiomas, studies of their effect in animal models of that tumor must be undertaken.

Conclusions

These studies have demonstrated that GH receptor mRNA is ubiquitously expressed in meningiomas. Blockade of the GH receptor by using B2036 significantly decreases the growth rates of many primary meningioma cultures, whereas IGF-1 administration significantly increases it. The effect of administering GH receptor antagonists would likely be substantially greater in experiments in which both the direct (tumor) and indirect effects (decreased host IGF-1 production) of the compound could be monitored. On our current observations about the importance of the GH/IGF-1 axis in the growth of meningiomas, further in vivo analysis of compounds that can downregulate this axis seems warranted.

References


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