Characterization of Insulin-like Growth Factor 1 in Human Primary Brain Tumors

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Abstract

Insulin-like growth factor 1 (IGF-1) is involved in the regulation of brain development and has been suggested as an autocrine stimulator of brain tumor cell proliferation. This study demonstrates the expression of IGF-1 in tumor tissue from human gliomas and one esthesioneuroblastoma. Using immunohistochemistry, expression of an IGF-1-like peptide was localized in tumor cells of 6 of the 9 gliomas examined as well as the esthesioneuroblastoma. From one anaplastic oligodendroglioma (which showed strong IGF-1 immunostaining) the IGF-I transcripts were characterized after isolation of mRNA following amplification using the reverse transcriptase-polymerase chain reaction. Two IGF-1 complementary DNA results from alternative splicing of the IGF-1 primary transcript were identified. These transcripts encode two different precursor proteins which correspond to Ea IGF-1 and Eb IGF-1. The significance of IGF-1 alternative mRNA splicing pathways remain to be determined.

Introduction

Gliomas are the most common primary tumors of the central nervous system in adults. At present the prognosis for patients with the malignant forms of these tumors is poor; however, an understanding of the cellular mechanisms underlying the loss of normal growth control mechanisms in glioma cells may lead to improved therapy. Growth factors play an essential role in the regulation of normal cellular growth and differentiation and are believed to be involved in tumorigenesis. Autocrine, juxtacrine, or paracrine mechanisms may be involved in tumor cell growth. IGF-1 has been implicated as an autocrine growth stimulator for several tumor types, e.g., human breast carcinoma cells (1) and small cell lung tumor cells (2). IGF-1 is integrally involved in the normal growth and differentiation of the nervous system and has been suggested as a putative autocrine stimulator of tumor growth in the central nervous system (3-5). Expression of IGF-1 receptors has been shown in human glioma (3, 6-8). However, it is unclear whether IGF-1 is produced in these tumors or what its function would be. Multiple transcripts from the IGF-1 gene are seen in malignant gliomas (5). The IGF-1 gene consists of 6 exons which may be alternatively spliced to result in different transcripts. These include Ea IGF-1 (exons 1-4 and 6) and Eb IGF-1 (exons 1-5) (9). The IGF-1 transcripts and peptides in gliomas have not been characterized. To maintain an autocrine loop hypothesis it is essential to establish the synthesis of both the growth factor and its receptor by the tumor cells. The transcripts must be characterized in detail to exclude mutations and overexpression. In the present study we demonstrate high levels of IGF-1 expression in human malignant glioma cells, and we have characterized the transcripts for the IGF-1 variants found. The complete amino acid sequence of two IGF-1 precursor proteins expressed have been deduced from the cDNA sequence isolated from the glioma mRNA.

Materials and Methods

MATERIALS. Tumor material removed at operation was collected and frozen at -85°C for periods of up to 1 year. The tumors were histopathologically classified according to the WHO classification and malignancy grading system (10) with the addition of the criteria of Burger et al. (11) to differentiate between anaplastic astrocytomas and glioblastomas.

Immunohistochemistry. The tissue was embedded in paraffin (Histowax). Sections of 4 μm were placed on chrome-alum-treated glass slides, dried at 37°C overnight, dewaxed, and rehydrated. Sections were blocked with phosphate-buffered saline (pH 7.4) containing 2% bovine serum albumin and 2% normal goat serum. Primary and secondary antisera were centrifuged at 12,000 rpm for 15 min before use. A polyclonal antiserum K37 (12) raised in rabbit Emmenbrucke, Switzerland was applied to the sections. After a second set of buffer washes, visualization was obtained with streptavidin-fluorescein-isothiocyanate (1:30; Bioscience Products). Controls for the specificity of the reactions were performed both by replacement of the primary antiserum with nonimmune rabbit serum and by preabsorption of the primary antiserum with hIGF-1 (0.4, 4, 40, and 400 μg peptide/ml diluted antiserum). As “positive” controls, sections of rat endocrine pancreas known to contain IGF-1 immunoreactive cells (12) were processed in parallel with each incubation series. Photographs were taken with a Zeiss Axioshot.

cDNA Amplification and Sequencing. Tumor tissue from the patient with anaplastic oligodendroglioma was removed from the frontal lobe of the brain and immediately placed in liquid nitrogen. Total RNA was isolated by acid guanidinium thiocyanate-pheno1-chloroform extraction (13). Polyadenylated RNA was separated by three cycles of oligodeoxynucleotide cellulose chromatography (type 3; Collaborative Research). RNA concentration and purity were estimated on the basis of absorbance measurements at 260 and 280 nm. Five μg polyadenylated RNA were heated at 65°C for 5 min and then cooled on ice, followed by single-stranded cDNA synthesis with an oligodeoxynucleotide primer (cDNA kit; Amersham). One-fifth of the cDNA reaction was amplified by PCR (14) with AmpliTag DNA polymerase (Perkin Elmer Cetus) in two steps: first, with primers designed for the S'-region of EcoRI sites common to both the IGF-1 mRNAs (Met-1 5'-ATAGAATTCT-ATGG-GAAAATACGACGTCTTCC and Gln-6 5'-ATAGAATTCT-CGGTCTCT-TGGCATCTCCGTTG)T), and second, with specific primers with EcoRI sites for the Ea IGF-1 cDNA (Asp-6 5'-ATAGAATTCT-GACATGCACGACCCAGAA and exon 6 5'-ATAGAATTCT-GACCATCTACCACTC-CAGG) and the Eb IGF-1 cDNA (Asp-6 5'-ATAGAATTCT-GACATGCACGACCCAGAA and exon 5 5'-ATAGAATTCT-TTCTCTGGCTTCTATGTTG) sequences (9). The PCR program was 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min followed by a final extension at 72°C for 5 min. The products were run on a 1% agarose gel to confirm the sizes (Fig. 1). Then the products were reamplified to provide material for subcloning into a plasmid vector to the EcoRI site. The inserts were sequenced by the dideoxy chain termination method using Sequenase version 2.0 (United States Biochemical Co.) according to the manufacturer's instructions.

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The abbreviations used are: IGF-1, insulin-like growth factor 1; PCR, polymerase chain reaction; cDNA, complementary DNA.

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Results and Discussion

As summarized in Table 1, IGF-1-like immunoreactivity was found in the tumor cells of 6 of the 9 gliomas which encompassed tumors of malignancy grades II–IV. The immunoreactions obtained were extinguished by preabsorption with recombinant human IGF-1, whereas they were not reduced by preabsorption with IGF-2 or insulin. The IGF-1 staining was localized intracellularly in the cytoplasm of tumor cells and not in the nucleus, the extracellular matrix, or the fibrous stroma (Fig. 2). No IGF-1 immunoreactivity could be detected in the glioma of malignancy grade II, i.e., the astrocytoma. In the gliomas of malignancy grade III, i.e., the anaplastic astrocytomas and the anaplastic oligodendroglioma, intense IGF-1 immunoreactivity was observed in 2 cases, the presence of IGF-1 immunoreactivity was observed in one case, and no IGF-1 immunoreactivity was observed in one case. In the glioblastomas of malignancy grade IV intense IGF-1 immunoreactivity was found in one case, the presence of IGF-1 immunoreactivity in 2 cases, and no IGF-1 immunoreactivity in one case.

A larger number of gliomas should be investigated to determine if there is a correlation between elevated expression of IGF-1 and increasing malignancy grade. Intense IGF-1 immunostaining was also observed in the single esthesioneuroblastoma examined. In this case the immunoreactivity was similarly distributed in the cells.

The IGF-1 immunoreactive peptide in the anaplastic oligodendroglioma was characterized. As shown in Fig. 2, the tumor cells in this glioma strongly expressed IGF-1. mRNA was isolated from the tumor tissue and by using PCR two IGF-1 cDNAs were identified and their nucleotide sequences determined (Fig. 3). The nucleotide sequences correspond to Ea IGF-1 and Eb IGF-1 cDNA, which arise from the alternative splicing of exon 5 or exon 6 into the IGF-1 transcript (9). Ea IGF-1 encodes a precursor protein of 153 amino acids including an E peptide of 35 amino acids, and Eb IGF-1 encodes a precursor protein of 195 amino acids containing an E peptide of 77 amino acids. As shown in Fig. 3, the sequence and length of the E peptides in the two precursor proteins differ. The amino acid sequence of the mature IGF-1 protein is identical to that reported in other tissues. This is the first report of a complete characterization of IGF-1 in human brain tumors. The nucleotide sequence is identical to that found in normal human fetal brain (15), thus demonstrating that the IGF-1 gene transcript is not modified in one malignant glioma.

Table 1 Summary of IGF-1-like immunoreactivity observed in the tumor cells of human malignant gliomas as well as in one esthesioneuroblastoma (astrocytoma, anaplastic astrocytoma, anaplastic oligodendroglioma, glioblastoma, and esthesioneuroblastoma)

<table>
<thead>
<tr>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>A(1)</td>
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<tr>
<td>AA(3)</td>
<td>1</td>
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<tr>
<td>AO(1)</td>
<td>1</td>
<td>2</td>
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<td>GB(4)</td>
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<tr>
<td>ENB(1)</td>
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* Number in parentheses, number of tumors in the diagnostic category.
+ Immunostaining was classified as intense (++), present (+), or undetectable (−).
A, astrocytoma; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GB, glioblastoma; ENB, esthesioneuroblastoma.
Glioma expresses both Ea IGF-1 and Eb IGF-1 mRNA, as do hepatoma cells (16) and smooth muscle tumor cells (17). The mechanisms governing the splicing of the IGF-1 mRNA precursor and the functional significance of the Ea IGF-1 and Eb IGF-1 precursor proteins are unclear at present. The E peptide of IGF-1b contains several amino acid sequence in this E peptide which is biologically active by itself, with growth-promoting effects on both normal and malignant human bronchial epithelial cells. In the rat, there is different regulation of the expression of the two IGF-1 mRNAs. Eb IGF-1 mRNA is present in low abundance relative to Ea IGF-1 mRNA in all tissues (19). The proportion of the two transcripts is relatively similar in the different tissues with the exception of the liver, where the proportion of Eb IGF-1 mRNA relative to Ea IGF-1 mRNA is 2–4 times greater than in other tissues. Quantitative studies of the relative expression levels of these IGF-1 RNA species in tumor cells remain to be carried out. The present identification of both IGF-1 mRNAs in brain tumors raises the question of whether differential expression may be important in autocrine stimulation of tumor cell growth. The characterization of glioma IGF-1 may lead to new therapeutic strategies similar to those proposed by Trojan et al. (20), who used antisense cDNA to inhibit IGF-1 transformation of rat glioblastoma cells in a combined in vivo/in vitro system. Recently, Pietrzkowski et al. (21) have demonstrated that analogues of IGF-1 which are inhibiting the autophosphorylation of the IGF-1 receptor are capable of inhibiting the proliferation of several types of cells including prostate carcinoma cells and simian virus 40-transformed cells. However, the T98G cells studied, which are derived from a human glioma, did not respond to the inhibitory effect of IGF-1 peptide analogues.

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References


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