Estramustine-Binding Protein and Specific Binding of the Anti-Mitotic Compound Estramustine in Astrocytoma

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ABSTRACT

Estramustine-binding protein (EMBP) is a M, 46,000 heterodimeric protein originally isolated from prostastic tissue. It has a demonstrated high affinity for, and selective binding of, estramustine, which is a derivative of 17β-estradiol and nonnitrogen mustard with antimitotic activity. In this study, we have analysed the expression of an EMBP-like protein in astrocytoma specimens. Immunohistochemistry revealed a pronounced reactivity for EMBP in astrocytoma grades III-IV as well as in metastatic prostatic adenocarcinoma used as positive control. In astrocytoma grades I-II, the expression was weak. The EMBP-like protein was quantified by radioimmunoassay in astrocytoma tumor tissue with higher concentrations in malignant astrocytoma, grades III-IV, compared to grades I-II tumors. Western immunoblotting of immunopurified estramustine-like protein under nonreducing conditions revealed an immunoreactivity corresponding to M, 138,000 and 200,000, indicating a different structure of EMBP in astrocytoma compared to prostatic tissue. Specific binding and the presence of saturable binding sites for 3H-labeled estramustine were demonstrated in astrocytoma tissues expressing EMBP-like protein. Scatchard plot analysis showed a Kd at ~30 nM, which suggests a binding affinity for estramustine in the same range as previously reported for EMBP in the prostate. Moreover, the number of estramustine binding sites/g tumor as calculated from the Scatchard plots was well correlated with the EMBP levels determined in the radioimmunoassay. In conclusion, an EMBP-like protein is expressed in astrocytoma. This protein may be responsible for the specific binding of estramustine in the tumor tissue. Whether this specific binding of estramustine is of importance for the cytotoxic effect in glioma cells remains to be evaluated.

INTRODUCTION

The treatment of primary malignant supratentorial brain tumors is disappointing. In malignant astrocytoma, the extent of surgery and postoperative irradiation have been shown to increase survival (1, 2), and adjuvant chemotherapy may improve survival in selected patients, but only marginally (3, 4).

EMP3 (Estracyt; Emcyt), a combination of 17β-estradiol phosphate and nonnitrogen mustard used in the treatment of prostatic carcinoma, has recently gained interest in the management of malignant glioma. The metabolites of EMP have been shown to exert specific antiproliferative effects in glioma cells in vitro (5–7). The main mechanism of action seems to involve the microtubule system (8–12) with arrest cytotoxic effect in glioma cells remains to be evaluated.

As a derivative of 17β-estradiol and nonnitrogen mustard with antimitotic activity, EMP seems to be facilitated by a specific protein, EMBP, which is proposed to be responsible for the binding and accumulation of the active metabolites of EMP, EaM and EoM (17–20). A specific binding of EaM to EMBP in human prostatic tissue has been demonstrated with an affinity of Kd 35 nM (21). Previously, an EMBP-like protein has been demonstrated in cultured human glioma cells (5) and astrocytoma tissue from patients (22, 23). In the clinical situation, EaM and EoM have been detected in malignant brain tumors after oral administration of EMP, with an accumulation of EaM in the tumor tissue (23).

In this study, the presence of an EMBP-like protein was investigated in a series of astrocytomas using different immunological techniques. Immunohistoblotting was used for partial characterization of the EMBP-like protein in malignant astrocytoma tissue. The presence of high affinity binding sites for EaM was investigated in tissues expressing the EMBP-like protein using 3H-labeled EaM in an in vitro binding assay.

MATERIALS AND METHODS

Tumor Tissue. Paraffin-embedded sections from 51 patients with astrocytoma (18 astrocytomas, grades I-II; 33 astrocytomas, grades III-IV) were obtained at surgery for spinal metastasis, as well as normal rat prostatic tissue. The tumor histopathology was reviewed, and the diagnosis was made according to Kernohan (24). Prostatic carcinoma tissue from six patients, obtained at surgery for spinal metastasis, as well as normal rat prostatic tissue were used as positive controls. For quantitative analysis of EMBP, partial characterization, and analysis of specific binding of EaM, astrocytoma tissues were collected during surgery. The tissue samples were immediately frozen in liquid nitrogen and stored at −70°C until analysed. Precautions were taken not to denature the tissues during sampling.

Immunohistochemical Detection of EMBP. The presence of an EMBP-like protein in sections from tumor specimens was demonstrated by the indirect antibody peroxidase technique on formalin-fixed, paraffin-embedded sections using the avidin-biotin-peroxidase technique (25, 26). The sections were cut 5 μm thick, deparaffinized in xylene, and rehydrated in graded ethanol. Endogenous peroxidase activity was blocked by the addition of H2O2 in methanol (0.3% v/v) for 15 min. The primary antibody, a mouse monoclonal antibody raised against purified rat EMP and with demonstrated cross-reactivity with human EMP (5, 27, 28), was added at a concentration of 0.1 mg/ml in PBS containing 1% w/v BSA to the sections for 1 h. Rabbit anti-mouse avidin-biotin-peroxidase-antiperoxidase complexes (Vectorstain; Vector Laboratories, Inc., Burlingame, CA) were added after sequential washings in PBS. The staining reaction was developed in dimethyl sulfoxide/ethanol/benzidine, followed by counterstaining with hematoxylin and mounting in glycerol-gelatin. In order to serve as negative controls, all tumors were processed concomitantly as above but with the primary monoclonal antibody omitted. Negative serum controls were done using nonimmune mouse IgG added to the sections instead of the anti-EMBP mAb. Rat prostatic tissues were used as positive controls.

The immunohistochemical staining was evaluated by two of the authors (T. B. and J. B.) by counting the proportion of cells positively stained for EMBP and estimating the intensity of staining semiquantitatively, defined as missing (0), low (1), moderate (2), or high (3). In the case of divergent opinions, the mean was used.

RIA. Ten astrocytoma specimens (grade II, n = 2; grade III, n = 6; and grade IV, n = 2) obtained at surgery were used for quantitative analysis of EMP in a RIA. Homogenization of tumor tissue was performed in 50 mM

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3 The abbreviations used are: EMP, estramustine phosphate; EMBP, estramustine-binding protein; EaM, estramustine; EoM, estromustine; PBS, phosphate-buffered saline; BSA, bovine serum albumin; mAb, monoclonal antibody; RIA, radioimmunoassay; SDS, sodium dodecyl sulfate.
sodium phosphate, 10 mM EDTA, and 10 mM NaCl containing 0.2% w/v BSA (pH 7.4) using an Ultra-Turrax homogenizer (4 x 5 s with 20-s cooling intervals). The homogenate was centrifuged for 20 min at 2000 x g (4°C), and the supernatant was collected for determination of EMBP. The RIA was carried out as described previously (29) using a polyclonal rabbit antiserum raised against purified rat EMBP at a dilution of 1:5000 and with purified rat EMBP as tracer and standard. The chloramine-T technique (30) was used for radiiodination with specific activity ranging from 50 to 100 μCi/μg protein. A standard curve ranging from 0.1 to 20 pmol/ml of purified rat EMBP was obtained. One hundred μl of the sample, the tracer, and the antiserum were incubated at room temperature for 2 h. For separation of free and bound antigen, sheep anti-rabbit-IgG-coated particles were added, and the test tubes were slowly rotated overnight. Thereafter, the samples were centrifuged at 3000 x g for 5 min, washed three times in saline, and counted in a gamma counter. The concentration of EMBP-like protein in astrocytoma was obtained from the rat-EMBP standard curve and was expressed as pmol/g tumor tissue or pmol/mg protein, assuming equal amounts of bound antibody per antigen molecule in rat EMBP and in astrocytoma EMBP-like protein. Protein was determined by the method of Lowry et al. (31).

Partial Purification of EMBP. An immunosorbent was prepared to enrich cross-reactive components in a sample of astrocytoma grade III (no. 1) for analysis in a Western immunoblot. Briefly, 2.5 mg of the anti-EMBP mAb was coupled to an N-hydroxysuccinimide-activated HiTrap resin (1 ml; Pharmacia Biotech, Sollentuna, Sweden) in 0.2 M sodium bicarbonate-0.5 M sodium chloride at pH 8.3, according to the instructions given by the manufacturer. Blocking of unreacted sites was performed by sequential washings in 0.5 M ethanolamine (pH 8.3) and 0.1 M sodium acetate (pH 4.0), where both buffers contained 0.5 M sodium chloride. The coupling yield was about 97% and with minimal antibody leakage from the column. The antigen-binding capacity was estimated to about 0.1 mg, as determined by loading of rat EMBP to the column.

The astrocytoma sample was poured through PD-10 columns (Pharmacia Biotech) and equilibrated in PBS; the excluded volume was filtered on 0.2 μm filters. The sample was loaded onto the immunosorbent by recirculation overnight and washed until baseline absorbance at 280 nm was reached; finally, retained protein was eluted with 0.1 M glycine-HCl buffer (pH 2.5) and with 0.1 M sodium carbonate buffer (pH 11.5), interrupted by washing in PBS in between. Eluted fractions were immediately neutralized to near physiological pH, the buffer was changed to PBS, and finally the samples were concentrated and frozen until further use.

Western Blot Analysis. Gel electrophoresis in the presence of SDS was run with denaturated samples on 8–25% gradient gels (PhastGel Gradient 8–25) using the PhastSystem (Pharmacia Biotech) according to the instructions given by the manufacturer. Transfer to 0.45 μm nitrocellulose was carried out using the PhastTransfer kit (Pharmacia Biotech). The membranes were blocked for 30 min in 3% w/v BSA in Tris-buffered saline (TBS; pH 7.5), washed three times for 5 min in TBS containing 0.05% v/v Tween-20 (TTBS) and incubated overnight with rabbit polyclonal anti-EMBP serum, diluted 1:2000 in TTBS containing 1% v/v nonimmune goat serum. After washing three times for 5 min with TTBS, incubation for 30 min was performed with goat anti-rabbit immunoglobulin conjugated with horseradish peroxidase (Bio-Rad, Richmond, CA) diluted 1:4000 in TTBS. Finally, immunodetection was made using the enhanced chemiluminescence system from Amersham. In the negative controls, the primary antisemur was replaced by nonimmune rabbit serum accordingly diluted (i.e., 1:2000).

Determination of Estramustine Binding Characteristics. Three astrocytoma grade III specimens (nos. 1, 2, and 3) previously investigated for the expression of EMBP-like protein using RIA were selected for binding analysis. Extracts from the astrocytoma specimens were prepared from minced frozen tissue by homogenization for five times 10 s in 5–7 volumes of 0.25 M sucrose, 50 mM Tris, 10 mM KCl, and 1.5 mM MgCl2 (pH 7.4) containing 8 mM 3-[3-chomiodipropyl]diethylylamino]-1-propanesulfonate and protease inhibitors (EDTA, phenylmethylsulfonyl fluoride, pepstatin, and leupeptin). The extracts were obtained by collecting the supernatant after high-speed centrifugation of the homogenates for 1 h at 105,000 x g. In order to avoid underestimation of the EMBP binding sites, endogenous ligand(s) that might occupy the binding sites were removed by aceton precipitation, mainly according to the method described by Ichii (32). Briefly, the tumor extracts (1 ml) were added drop-wise to 9 volumes of ice-cold aceton in the "cold room." The precipitated protein was recovered by centrifugation for 30 min at 10,000 x g. The supernatant was discarded, and the precipitate was washed with 10 volumes of ice-cold diethyl ether and centrifuged as above. The supernatant was discarded, and the remaining ether was evacuated under a stream of nitrogen gas. Finally, the precipitate was dissolved in 2 ml TEN buffer (50 mM Tris, 1 mM EDTA, and 10 mM NaCl, pH 7.5) and clarified by centrifugation as above. The delipidated samples were immediately frozen at -30°C pending further use.

Aliquots of the delipidated extracts were incubated in duplicates with serially diluted [3H]Estramustine at a final concentration ranging from 1 to 544 nM. Briefly, 10 μl of the radioligand dissolved in ethanol were added to 150-μl sample and incubated for 30 min at 30°C. The incubation was stopped by placing the samples in an ice-bath for 5 min. The protein-bound radioactivity was assessed by filtration of the incubate (100 μl) through Sephadex G-25 medium (Pharmacia Biotech) packed in 2-ml plastic syringes. Twenty-five μl were transferred directly into counting vials for determination of total radioactivity. The apparent dissociation constant, Kd, and the number of available binding sites, Bmax, were calculated according to Scatchard (33).

RESULTS

Immunohistochemistry. The results from immunohistochemical staining for EMBP-like protein is presented in Table 1. In all positive astrocytoma cells, the immunostaining was encompassed within the cytoplasm. Occasionally, the cells stained were randomly scattered in the tumor tissue, and the staining intensity varied considerably between different cells in the same tumor. In low grade astrocytomas (grades I-II), there was generally a weak staining of the tumor cells (Fig. 1A). High grade astrocytomas (grades III-IV) showed a higher staining intensity also within the cytoplasm of the cells and usually with a higher proportion of cells stained compared to low grade astrocytomas (grades I-II; one-way analysis of variance; P < 0.001 and P < 0.05, respectively; Table 1; Fig. 1, A and B). In many tumors, there was a moderate positive reaction in the majority of cells with a strongly stained subpopulation scattered in the tumor (Fig. 1C). In astrocytomas with areas of necrosis, there was usually a high staining intensity in the necrotic area.

All positive controls were positively stained and served as reference for evaluation of staining intensity. The negative controls were negative, as well as the negative serum controls, excluding nonspecific immunoreactivity (data not shown). In metastatic prostatic carcinoma, a variation in the proportion of cells positively stained was also observed, as well as a variation in the staining intensity. As in astrocytoma, the staining was located in the cytoplasm of the tumor cells, leaving the stroma unstained (Fig. 1D). RIA. A comparison between RIA analysis and immunohistochemical detection of the EMBP-like protein in the same astrocytoma tumors is presented in Table 2. The RIA values suggested an increase in EMBP levels with higher malignancy grade of the tumor. The
Table 2 Immunohistochemical staining for EMBP and concentrations of EMBP in astrocytoma tumors expressed in binding sites/g tumor tissue and binding sites/mg protein as detected by radioimmunoassay.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cells (%)</th>
<th>Intensity</th>
<th>pmol/g tumor tissue</th>
<th>pmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytoma II</td>
<td>2</td>
<td>55 (50-60)</td>
<td>2.0 (2-3)</td>
<td>0.028 (0.026-0.030)</td>
</tr>
<tr>
<td>Astrocytoma III</td>
<td>8</td>
<td>68 (50-85)</td>
<td>1.8 (1-3)</td>
<td>0.047 (0.010-0.116)</td>
</tr>
<tr>
<td>Astrocytoma IV</td>
<td>2</td>
<td>65 (65-65)</td>
<td>2.0 (2-3)</td>
<td>0.117 (0.110-0.124)</td>
</tr>
<tr>
<td>All astrocytoma</td>
<td>12</td>
<td>64 (50-85)</td>
<td>2.1 (2-3)</td>
<td>0.056 (0.010-0.124)</td>
</tr>
</tbody>
</table>

* Means and ranges are given.

concentration of EMBP-like protein was, as an average, 2.3 (0.4–7.0) pmol/g tumor tissue or 0.06 (0.01–0.12) pmol/mg protein.

Western Blot Analysis. The EMBP-like protein in astrocytoma no. 1 was enriched by immunoaffinity chromatography on an anti-EMBP immunoabsorbent. The adsorbed protein was eluted both at acidic and alkaline pH (Fig. 2). The crude astrocytoma extract as well as the fractions eluted at pH 2.5 and 11.5, respectively, were analysed by immunoblotting after SDS gel electrophoreses under denaturing conditions (Fig. 3). Purified rat EMBP showed the typical two bands corresponding to subunits S1 (M, 19,300) and S2 (M, 22,100). In addition, there was a weak specific band at M, 35,700, which is often

Fig. 1. Microphotographs demonstrating immunoreactivity for the EMBP-like protein in homogeneous staining of an astrocytoma grade III specimen from patient no. 1 (A); heterogeneous staining in a specimen of astrocytoma grade III (C); and staining of metastatic prostatic carcinoma (positive control) with invasive growth adjacent to the vertebral bone (D); bar, 50 μm.
EMBP/estramustine in astrocytoma

igated in order to assess the binding affinity and the concentration of saturable EaM sites. The Scatchard plot analysis for binding of EaM to the astrocytoma specimen no. 1 is shown in Fig. 4. In all three samples, a high degree of nonspecific or low-affinity binding was obtained, which had to be subtracted in order to calculate the binding characteristics for the high-affinity sites. The results from the \textit{in vitro} binding experiments are summarized in Table 3. An apparent dissociation constant, $K_d$, of approximately 30 nM was obtained for the high-affinity sites. The number of EaM binding sites were also calculated and expressed as pmol bound EaM per g tumor tissue. These values were comparable to the RIA levels of the EMBP-like protein, which suggest that EaM is bound to this protein with a stoichiometric relation of 1:1. This finding demonstrates that the two methods used for determination of EMBP-like protein (RIA and \textit{in vitro} binding assay) are well correlated.

The astrocytoma specimen no. 1 was also subject to immunohistochemical analysis (Fig. 1B), demonstrating a staining intensity for EMBP of 2+ and with 80% of the cells displaying immunoreactivity.

DISCUSSION

Using immunohistochemistry and different immunochemical and ligand-based techniques, the present study demonstrates the existence of an EMBP-like protein in astrocytoma tumors with similar epitopic

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Fig. 2. Enrichment of EMBP-like protein from a human astrocytoma extract (patient no. 1) by chromatography on immobilized mouse anti-rat EMBP mAb. Arrows, the start of elution at low pH (pH 2.5) and high pH (pH 11.5), respectively.

Fig. 3. Partial characterization of the immunoabsorbent fractions (Fig. 2, patient no. 1) by Western blot analysis after SDS electrophoresis in 8–25% gradient gels under nonreducing conditions. Samples were purified rat prostate EMBP \textit{(rat-EMBP)}, crude astrocytoma extract \textit{(starting material)}, and immunoabsorbent fractions after elution at pH 2.5 and pH 11.5. Negative controls \textit{(neg.)} were incubated in the absence of rabbit anti-rat EMBP. Dashes, the positions of subunits S1 and S2 of the rat prostate EMBP as well as the two main specific bands visualized in the astrocytoma samples. The molecular masses were calculated from Rainbow markers 12,300–200,000 (Amersham, Solna, Sweden) run in the same gels (data not shown).

seen when rat EMBP is overloaded in gels and when the more sensitive enhanced chemiluminescence technique is used for development.\textsuperscript{4} In the astrocytoma extract before (starting material) and after immunoaffinity chromatography (pH 2.5 and 11.5, respectively), specific staining was observed as two bands with a molecular mass of 138 and 200 kilodaltons, respectively. Other bands most probably represent nonspecific staining or cross-reactivity with preimmune serum since they were also visualized in the negative controls.

In \textit{Vitro} Binding of EaM. The binding of EaM to extracts from three astrocytoma specimens with high EMBP expression was inves-

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\textsuperscript{4} Unpublished observation.
EMBP-like protein in human astrocytoma tissue compared to what
compared to the concentrations in serum and tumor tissue (23). This
glandular lumina (35). The high degree of EMBP reactivity found in
of EMBP-like protein seem to increase with the higher grades of malig
necrotic astrocytoma tissue can possibly be explained by staining of

characteristics as have been described in rat and human prostate (29,
34, 35). Most interestingly, the expression of the EMBP-like protein
seems to increase with the higher grades of malignancy of the astro
cytomas. This observation is in accordance with what has been found
in prostatic carcinoma in which well-differentiated tumors had lower
expression of EMBP than moderately and poorly differentiated tu
mors (20, 36, 37).

It is known that intracellular proteins and protein structure may un
dergo developmental changes during cell maturation (38—40) as well as
in anaplastic deformation (41, 42). The expression of glial fibrillary acidic
protein and glutamine synthetase is known to decrease with the grade of
anaplasia in astrocytoma (41, 43). It is plausible to assume that the
expression of some proteins may even be of prognostic importance, as
seems to be the case for the protein S-100 in astrocytoma (44). In
prostatic carcinoma, it is clearly demonstrated that EMP treatment de
creases the expression of EMBP in the tumor (20, 36). It has been
suggested that the expression of EMP could be a predictor of the clinical
effect expected from EMP therapy for this tumor (20, 36). Our findings
that immunohistochemical expression as well as the quantitative analysis
of EMBP-like protein seem to increase with the higher grades of malign
nancy could indicate a similar role of the EMBP-like protein in astrocy
toma. In addition, EMP could be of importance not only as a predictor
of treatment but also as a prognostic marker. The validity of the immu
nohistochemical analysis was enhanced by the use of negative controls
and irrelevant mouse immunoglobulins to minimize the problem of
nonspecific immunoreactivity. The concentrations of EMBP-like protein
in malignant astrocytoma (grades III-IV) were in about the same range as
has been found in prostatic carcinoma (0.05—0.43 pmol/mg protein; Ref.
34), indicating a similar concentration of binding sites for estramustine in
these two malignancies. This condition was supported by our data from
immunohistochemistry comparing astrocytoma with metastatic prostatic
carcinoma.

EMBP-like protein in astrocytoma seems to be located in the
cytosol, which is consistent with what has been earlier observed in
primary human prostatic carcinoma (35, 36) and also in metastatic
prostate carcinoma cells, as shown in this study. In normal rat pro
state, however, EMBP displays a staining pattern typical for a secretory
protein with a high expression of the protein found in the prostatic
glandular lumina (35). The high degree of EMBP reactivity found in
necrotic astrocytoma tissue can possibly be explained by staining of
intracellular protein from tumor cell debris. In this context, it is of
interest to recall the observation that the concentrations of Eam and
EoM detected in the fluid of glioma cysts were generally lower
compared to the concentrations in serum and tumor tissue (23). This
may reflect the absence of EMBP-like protein in astrocytoma cystic
fluid. Thus, there is, so far, no evidence that EBM is extruded out of
the glioma cells.

Western immunoblotting indicated a different structure of the
EMBP-like protein in human astrocytoma tissue compared to what
has been found in normal prostatic tissue and prostatic carcinoma
from humans and rats (29, 45, 46). In the rat, EMBP consists of two
closely related subunits, S1 and S2, with a mass of 18 and 20
kilodaltons, respectively (29). A similar composition of subunits was
indicated for EMBP in human prostatic carcinoma (46). In astrocy
toma, the immunoreactivity was observed at 138 and 200 kilodaltons.
The basis for this structural difference between rat EMBP and astro
cytoma EMBP-like protein is presently not known and will be the
subject of further analysis.

Although a high degree of nonspecific or low-affinity binding was
observed, this study unequivocally demonstrates the presence of high
affinity binding sites for EaM in all astrocytoma specimens analysed.
These specimens exhibited high expression of EMBP in the RIA and
a strong strengthening intensity in the immunohistochemical analysis. The
Kg value calculated for these sites was approximately 30 nm, which is
in the same range as previously shown for the binding of EaM to
EMBP in the rat prostate (Kg, 10—30 nm; Ref. 47) and in human
benign hyperplastic prostate specimen (Kg, 35 nm; Ref. 21). The fact
that the concentration of EaM binding sites and the levels of EMBP-
like protein determined in the RIA were well correlated indicates that
EaM is bound to the EMBP-like protein in astrocytoma. Moreover,
our finding suggests a stoichiometric ratio of 1:1 for EaM and EMBP-
like protein, which is consistent with the data of Heys and Bossyns
(48) that prostate EMBP carries only one binding site for EaM. It is
of great interest that an uptake of EaM has been demonstrated in the
clinical situation (23). In one of the patients (no. 1 in this paper), the
uptake and accumulation of EaM after a single oral dose of 280 mg
EMP was 13 ng/g tissue 12 h after drug administration, while at the
same time no EaM was detectable in serum. Thus, a very high
concentration gradient in the tumor versus serum was obtained in this
patient (23). It is also of interest to recall the observation that the
expression of EMBP-like protein is considerably lower in normal
brain tissue compared to astrocytoma tissue (22). The recent report on
the absence of cytotoxic effect of estramustine on normal human glial
cells compared to glioma cells further supports the concept that
EMBP-like protein may play an important role for the cytotoxicity of
EaM in glioma (49).

In conclusion, the present study demonstrates by various immuno
logical techniques the presence of an EMBP-like protein in human
astrocytoma. The expression displayed a heterogeneity with different
degrees of staining scattered in the tissue. Quantitative analysis using
RIA showed concentrations in astrocytoma at levels comparable to
those in prostatic carcinoma. The expression of this EMBP-like protein
seems to be positively correlated with increasing malignancy.
Western blot analysis showed two specifically stained bands, although
at different positions than observed for rat EMP, indicating a dif
ferent structure of this protein in astrocytoma. A binding affinity for
EaM similar to that in human prostate was demonstrated in astro
cytoma. These results, together with earlier findings of the cytotoxic effect in glioma cell lines and a high uptake of the metabolites of EMP in astrocytoma, emphasize the possibility of a clinical effect of EMP in the treatment of malignant glioma.

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