Alpha-1-antitrypsin in human brain tumors

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This study was undertaken to confirm the presence of alpha-1-antitrypsin (α₁-Aₜ) in human brain tumors and to attempt to elucidate its significance. Seventy-seven consecutive unselected patients with various brain tumors were entered in this study. The α₁-Aₜ and α₂-macroglobulin contents of the tumor extracts were qualitatively assessed by Ouchterlony immunodiffusion techniques. Plasminogen activator (PA) activity was assayed electrophoretically on sodium dodecyl sulfate gels. The patients were divided into two groups according to the positivity of their tumors to α₁-Aₜ. Sixty-eight percent of the tumors were positive for α₁-Aₜ, and all specimens were negative for α₂-macroglobulin. Clinical and biological parameters obtained in all study patients failed to show statistically significant differences between the two groups with the exception of PA activity (p = 0.001), the peritumoral edema as seen on computerized tomography, and the preoperative serum fibrinogen level. These three parameters were higher in the group with specimens positive to α₁-Aₜ.

This study supports the hypothesis that α₁-Aₜ is produced primarily by tumor cells in proportion to the regional proteolytic and inflammatory activity, and may protect the tumor cells.

KEY WORDS • brain neoplasm • alpha-1-antitrypsin • alpha-2-macroglobulin • plasminogen activator activity • protease

In recent years, there has been rapidly increasing evidence that tumor cell proteinases play an important role in tumor invasion. In support of this theory are the many observations demonstrating the ability of tumor cells in culture to degrade extracellular proteins and the findings of increased levels of proteinases capable of digesting connective tissue matrix component within the tumor environment. High concentrations of proteinases such as plasminogen activator (PA) substance(s) were shown to be produced by cultured cells derived from brain tumors and are abundantly found in human brain tumors (R Sawaya and R Highsmith, in preparation).

As a consequence of these studies, interest has arisen in regard to the distribution of proteinase inhibitors in tumors. In a previous paper, we were able to demonstrate a proteolytic inhibitory activity associated with brain tumors. Proteolytic inhibitory activity may be related to the presence of specific proteins, most of which inhibit fibrinolysis. These include alpha-1-antitrypsin (α₁-Aₜ), α₂-antiplasmin, α₁-antichymotrypsin, α₂-macroglobulin, and antithrombin III. We have previously postulated that α₁-Aₜ was the most likely inhibitor to be associated with brain tumors. This study was therefore undertaken to confirm the presence of α₁-Aₜ in human brain tumors and to attempt to elucidate the significance of its presence.

Clinical Material and Methods

Seventy-seven consecutive unselected patients (mean age ± standard error of the mean: 52.79 ± 1.8 years) with various brain tumors were entered in this study. Clinical and biological parameters were obtained on all study subjects including age, sex, duration of the symptoms, Karnofsky index, plasma fibrinogen level, prothrombin time, partial thromboplastin time, platelet count, sedimentation rate, and histological diagnosis. The tumors included benign non-neoplastic central nervous system disorders such as granulomas as well as other brain tumors such as neuroblastoma, pituitary adenoma, and lymphoma.

Computerized tomography (CT) scans were used to identify tumor volume according to the following formula: volume = π (a.b.c)/6 where a, b, and c represent the three axes of the tumor mass. Peritumoral brain edema was defined as a low-attenuation zone surrounding the mass lesion and was graded from 0 (no edema) to 5+ (marked edema) where the low-density area ex-
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TABLE 1
Summary of findings in the two study groups*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Group I</th>
<th>Group II</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>age (yrs)</td>
<td>52.01 ± 2.4</td>
<td>54.59 ± 2.68</td>
<td>NS</td>
</tr>
<tr>
<td>sex (% males)</td>
<td>47</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>duration of symptoms (days)</td>
<td>327.89 ± 69</td>
<td>222.13 ± 64.99</td>
<td>NS</td>
</tr>
<tr>
<td>Karnofsky index</td>
<td>81.22 ± 2.36</td>
<td>83.63 ± 3.05</td>
<td>NS</td>
</tr>
<tr>
<td>size of tumor (cm)</td>
<td>26.09 ± 4.2</td>
<td>39.2 ± 12.0</td>
<td>NS</td>
</tr>
<tr>
<td>PTT (sec)</td>
<td>11.85 ± 0.20</td>
<td>11.39 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>25.83 ± 0.56</td>
<td>26.23 ± 1.07</td>
<td>NS</td>
</tr>
<tr>
<td>platelets (/cu mm)</td>
<td>324.857 ± 15.85</td>
<td>290.556 ± 23.04</td>
<td>NS</td>
</tr>
<tr>
<td>fibrinogen (mg/100 ml)</td>
<td>374.43 ± 30.38</td>
<td>263.46 ± 19</td>
<td>p = 0.025</td>
</tr>
<tr>
<td>peritumoral edema†</td>
<td>2.05 ± 0.25</td>
<td>1.076 ± 0.32</td>
<td>p = 0.049</td>
</tr>
</tbody>
</table>

* Group I tumors (53 cases) were positive for anti-a1-antitrypsin (a1-AT) and Group II tumors (24 cases) were negative for anti-a1-AT. Values are means ± standard error of the means. PT = prothrombin time; PTT = partial thromboplastin time; ESR = electrolyte sedimentation rate; PAA = plasminogen activator activity; NS = not significant.

† Edema score: 0 = no edema; 5+ = marked edema.

Results

The patients were separated into two groups according to the reactivity of their tumors to sheep anti-human a1-AT. Group I was composed of 53 patients with positive anti-a1-AT (68.6%), and Group II of 24 patients with negative anti-a1-AT reactivity (31.4%). All specimens were negative for a2-macroglobulin. No statistically significant differences were observed between the two groups with regards to age, sex, Karnofsky index, duration of symptoms, size of tumor, prothrombin time, partial thromboplastin time, platelet count, and electrolyte sedimentation rate (Table 1).

Marked differences in a1-AT were noted among the various histological groups of brain tumors (Table 2). The difference in a1-AT reactivity reached statistical significance for the following groups: acoustic neuroma versus meningioma, metastasis versus meningioma, and acoustic neuroma versus miscellaneous brain tumors. No statistical difference in a1-AT positivity was found between malignant glioma and low-grade glioma.

Discussion

This study demonstrates that immunologically detectable a1-AT is present in both malignant and nonmalignant brain tumors. Alpha-1-antitrypsin has a broad range of enzyme inhibitory activity exceeded only by a2-macroglobulin. It is the major serine protease inhibitor of human plasma. Serine proteases represent the largest group of mammalian enzymes, and are characterized by a serine residue at their active site. As several lines of evidence suggest that proteases may play an important role in the tumor-host relationship, maintenance of a proper balance between protease and protease inhibitors might be essential in the regulation of tumor growth. In fact, in the group of tumors with detectable a1-AT in our study, the mean value for the PA activity was significantly higher than that for the group with nondetectable a1-AT. It is well known that PA activity is found in a high concentration in many tumor cells, and that PA activity is either at

TABLE 2
Alpha-1-antitrypsin (a1-AT) positivity in various tumor types

<table>
<thead>
<tr>
<th>Histological Diagnosis</th>
<th>No. of Cases</th>
<th>a1-AT Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acoustic neuroma</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>metastasis*</td>
<td>12</td>
<td>91.6</td>
</tr>
<tr>
<td>glioblastoma</td>
<td>20</td>
<td>78.6</td>
</tr>
<tr>
<td>low-grade glioma</td>
<td>7</td>
<td>71.4</td>
</tr>
<tr>
<td>meningioma</td>
<td>22</td>
<td>50.0</td>
</tr>
<tr>
<td>miscellaneous†</td>
<td>6</td>
<td>33.3</td>
</tr>
</tbody>
</table>

* Metastasis: nine lung carcinomas, two unknown, and one kidney carcinoma.
† Miscellaneous = one each of: pituitary adenoma, ependymoma, sarcoid granuloma, demyelinating process, lymphoma, and adult neuroblastoma.
the beginning of a protease cascade or is itself the active agent. The generation of plasmin is an important event in the breakdown of complex extracellular matrices. Plasminogen activator is capable of activating a latent collagenase that has long been suspected of being involved in invasion and metastasis, and is capable of promoting angiogenesis crucial to the spread and survival of the tumors. Several experiments have suggested that antifibrinolytic, anticoagulant, or antiplatelet agents decrease both tumor cell survival and the incidence of successful tumor cell implantation.

On the other hand, the role and significance of protease inhibitors such as α1-AT in tumors are not known with certainty. Local concentrations of active protease inhibitors, including α1-AT, may be important in modulating tumor proteinases, and in this manner affecting the neoplastic spread. Moreover, they can interfere with tumor promotion, perhaps by modulating the oxygen radical response. Indeed, many synthetic and natural antiproteinases have the ability to retard the oxygen radical response. 13,17 Indeed, many synthetic and natural antiproteinases have the ability to retard tumor growth. Conversely, evidence suggests that α1-AT is also an important factor in the inflammatory and immune responses. It is known that α1-AT plays an important role in inhibiting the cytotoxic reactions of lymphocytes including antibody-dependent cell-mediated cytotoxicity, T cell-mediated cytotoxicity, and natural killer activity. It also modulates the activity of a number of inflammatory pathways controlling an excessive inflammatory response.

Because α1-AT inhibits serine esterases released from host leukocytes (some of which participate in immune cytolysis of tumor cells), the production of α1-AT may compromise a vital host defense mechanism. These findings, together with the known importance of protease activities in cell functions and the mitogenic activity of some proteases on lymphocytes, indicate the need for further investigation into the relationship of these activities to each other.

The source of α1-AT in brain tumors is unknown. A number of normal tissues and cells contain α1-AT, including liver, 22 histiocytes and mast cells, 22 gastrointestinal tract, 11 lung, 52 and pancreatic islet cells. 35 Synthesis of α1-AT was reported by yolk sac, hepatocellular carcinoma, 56 certain ovarian malignancies, undifferentiated liver sarcomas, gastric carcinoid, 36 benign and malignant "fibrohistiocytic" tumors, and osteoclastomas. Akatsuka, et al., using immunoelectron microscopy, have localized α1-AT to the endoplasmic reticulum and Golgi apparatus of human tumors transplanted in nude mice. It is, therefore, logically possible to hypothesize that α1-AT is actively produced in the region of the tumor either by the neoplastic cells or by the supportive and/or reactive cells. In support of this hypothesis is the fact that several other neoplasms have been shown to produce α1-AT, especially since all somatic cells of an individual are of the same basic genetic composition. The lack of detectable α2-macroglobulin suggests that the presence of α1-AT is not the result of a passive transfer from the serum. Similarly, the lack of differences in several blood parameters between the two groups is evidence against the possibility of a nonspecific acute-phase reaction. However, the higher fibrinogen level in Group I (patients positive for anti-α1-AT) remains unexplained. In addition, the significant association of α1-AT positivity with a higher brain edema score is of interest, since edema itself may be influenced by the local hyperfibrinolytic activity of the tumor (R. Sawaya and R. Highsmith, in preparation). Finally, it remains to be determined whether α1-AT is the product of the tumor itself or of the reactive cells as a response to the abnormally high proteolytic activity generated by the tumor.

On the basis of the data currently available, we hypothesize that α1-AT is produced primarily by tumor cells in proportion to the regional proteolytic and inflammatory activity. The role of α1-AT appears to be protective to the tumor cells and immunosuppressant to the host. Additional studies will be required to further our understanding of the complex relationship that exists between proteases and protease inhibitors, and the role they play in the growth and invasiveness of the neoplastic tissue.

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