Transferrin receptor in normal and neoplastic brain tissue: implications for brain-tumor immunotherapy

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The distribution of transferrin receptor (TfR) in normal human brain-tissue obtained at autopsy and in brain-tumor biopsy specimens from 27 patients was determined by immunohistochemistry using two specific murine monoclonal antibodies against human TfR. The tumors studied included 10 glioblastomas multiforme (GBM's), nine other glial tumors, and eight meningiomas. In normal brain, TfR was detected primarily in endothelial cells; rare glial cells also contained immunoreactive product. All tumors contained TfR-positive cells, although the intensity (number of cells stained) and pattern (focal vs. diffuse) of staining varied with the histopathological type of the tumor. Among gliomas, the most intense staining was seen in GBM's, especially in areas of pseudopalisading where virtually all cells were stained. A rough correlation between tumor grade, number of positively stained cells, and staining pattern was seen in the other astrocytic tumors. By contrast, all meningiomas demonstrated an identical and characteristic focal staining pattern. Considering the differential immunostaining for TfR between normal and neoplastic tissue, the authors conclude that TfR may be an appropriate target for monoclonal antibody-directed brain-tumor immunotherapy, especially in more malignant tumors such as GBM's.

KEY WORDS - transferrin receptor - immunohistochemistry - brain neoplasm - tumor cell proliferation

The possible development of a specific brain tumor immunotherapy has been bolstered in recent years by the advent of hybridoma technology. This methodology offers the opportunity to synthetically produce virtually unlimited amounts of specific monoclonal antibodies (MAb's), making it theoretically possible to create tumor-specific antibodies that could be selectively incorporated into tumor cells. Tumor cell death would then result from either the direct action of the tumor-specific MAb or its conjugation to a toxic substance. The desired effect would be a highly specific and sensitive tumor therapy. To realize this goal, however, there is a need to elucidate appropriate antigens that are either exclusively or (if this is not possible) differentially expressed in far greater quantities in brain tumors compared to normal central nervous system (CNS) tissue.

One antigen which has been suggested as a possible cellular target for such immunotherapy is the growth-associated membrane component transferrin receptor (TfR). Glioblastoma multiforme (GBM) cells in vitro express TfR and have been shown to be very sensitive to immunotoxins created from conjugating anti-TfR MAb's with intracellular toxins. However, TfR is not exclusively expressed on brain-tumor cells; previous studies have demonstrated its presence in normal neural tissues and cerebral endothelium. In order to be an effective and safe target for brain-tumor immunotherapy, TfR must be expressed in much greater quantities within the tumor compared to the surrounding normal brain. To assess this relationship, we studied the expression of TfR in various brain tumors and in normal postmortem brain tissue using an immunohistochemical method.

Materials and Methods

Brain-tumor specimens were obtained from 27 patients at the time of craniotomy. In addition, cerebral cortex and cerebellar tissue was taken within 6 hours after death from two patients who died of nonneurological causes. Cryostat sections 5 to 10 μm thick were immediately collected on gelatin-coated glass slides, air-dried for 2 hours at room temperature, then stored at −70°C until used. Prior to staining, the sections were fixed in acetone for 10 minutes at room temperature.
and then rehydrated for 10 minutes in phosphate-buffered saline, pH 7.4. Endogenous peroxidases were inhibited by pretreatment with 3% H$_2$O$_2$ for 10 minutes.

Two well-characterized murine immunoglobulin G1 (IgG1) anti-human TfR MAb's were used: 454A12* and 7D3.$^1$ In preliminary histological studies, identical staining was achieved irrespective of which MAb was utilized; therefore, they were used interchangeably.

For TfR staining, the sections were incubated overnight in 100 μl of a solution containing MAb diluted 1:1000 by 1% normal swine serum in Tris buffer with saline at 4°C. The sections were then washed twice with Tris-HCl buffer and incubated in a solution containing rabbit anti-mouse antibody† diluted 1:50 for 1 hour, washed in Tris-HCl again, and then reacted for 1 hour with 1:200 peroxidase antiperoxidase (mouse monoclonal origin).‡ After a final wash, the peroxidase reaction was developed with diaminobenzidine for 10 minutes, rinsed with water, and then counterstained with Harris hematoxylin, dehydrated, and mounted. Sections incubated with an irrelevant primary antibody instead of TfR served as negative controls. Normal trophoblastic placental tissue, which possesses very high concentrations of TfR, was used as a positive control.

The brain-tumor sections were examined by two observers (C.O.T. and T.W.S.) who were initially blinded as to the histological diagnosis of the neoplasm. The sections were evaluated for the presence, intensity, and pattern of TfR staining. Staining intensity was based on a qualitative assessment of the number of cells stained and was graded into three categories as follows: greater than 75% of cells stained; 25% to 75% of cells stained; and less than 25% of cells stained. Staining pattern was designated diffuse if positive and negative cells were distributed more or less randomly throughout the tissue, and focal if positive cells were arranged in clusters surrounded by large fields of negatively stained cells.

**Results**

In normal cerebellum and cortex, TfR immunoreactivity was observed primarily in endothelial cells (Fig. 1). Virtually all cerebral microvessels exhibited positive staining. Rare glial cells also showed positive immunostaining for TfR. No staining of neurons was observed.

The histological diagnoses of the 27 brain tumors included 10 GBM's, two anaplastic astrocytomas, three low-grade astrocytomas, four juvenile pilocytic astrocytomas, and eight meningiomas. The correlation of histopathology with the results of immunostaining is summarized in Table 1. All tumors contained cells that showed positive immunostaining for TfR in numbers far exceeding that seen in normal brain. The TfR reaction product was localized mainly to the perikaryon and processes of the tumor cells, with slightly more intense staining over the cytoplasmic membranes. Nuclear staining was generally not observed.

Among tumors of glial origin, the most intense staining was observed in GBM's. In nine (90%) of the 10 GBM's examined, TfR reaction product was present diffusely in almost all tumor cells; immunostaining was especially prominent in areas of pseudopalisading (Fig. 2). The only specimen of GBM in which this pattern of staining was not seen was obtained from a patient with a previously irradiated, recurrent dedifferentiated low-grade astrocytoma. In this case, immunoreaction product was seen in a focal pattern in less than 25% of cells.

In the other primary glial tumors, TfR staining was noted in fewer cells and the staining pattern was more often focal as compared to that of the GBM's. In two-thirds of these cases, intermediate numbers of cells

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* Monoclonal antibody 454A12 obtained from Cetus Corp., Emeryville, California.
† Anti-mouse antibody obtained from Dakopatts Co., Glos- trop, Denmark.
‡ Peroxidase antiperoxidase obtained from Sternberger-Meyer Immunohistochemicals, Jarrettsville, Maryland.

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<table>
<thead>
<tr>
<th>Histological Diagnosis*</th>
<th>No. of Cases</th>
<th>Staining Intensity†</th>
<th>Pattern</th>
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<tr>
<td>GBM</td>
<td>10</td>
<td>&lt;25%</td>
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<tr>
<td>AA Gr III</td>
<td>2</td>
<td>25%–75%</td>
<td>1</td>
</tr>
<tr>
<td>low grade</td>
<td>3</td>
<td>&gt;75%</td>
<td>2</td>
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<td>JPA</td>
<td>4</td>
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<tr>
<td>meningioma</td>
<td>8</td>
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* GBM = glioblastoma multiforme; AA Gr III = anaplastic astrocytoma without necrosis; low grade = fibrillary astrocytoma or oligodendroglioma; JPA = juvenile pilocytic astrocytoma.
† Staining intensity estimate based on the number of positively stained cells: > 75% of cells positively stained; 25% to 75% of cells positively stained; and < 25% of cells positively stained.

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**Fig. 1.** Photomicrograph of normal cerebral cortex stained for transferrin receptor (TfR). Only cerebral microvessels are immunostained for TfR. PAP-hematoxylin, × 440.
Transferrin receptor in brain-tumor tissue

Fig. 2. Photomicrograph of a glioblastoma specimen stained for transferrin receptor showing intense staining in the area of pseudopalisading. PAP-hematoxylin, × 146.

were stained; staining was particularly well visualized in the astrocytic processes (Fig. 3). Cells stained for TIR exceeded 75% in only two of the nine non-GBM glial neoplasms. In contrast to the glial tumors, where varied staining patterns were seen, all of the meningiomas exhibited a relatively stereotypical pattern of TIR staining. This was characterized by the presence of focal islands of intensely stained cells situated among fields of unstained tumor cells (Fig. 4). Immunoreactive product was observed in fewer cells in the meningiomas than in the glial neoplasms; in 62% of the meningiomas, less than 25% of tumor cells stained for TIR.

Discussion

Transferrin receptor is a cell-surface membrane disulfide-linked glycoprotein dimer of 180 kD which mediates cellular iron uptake by binding transferrin-iron complexes through endocytosis.\(^7,22,23\) In both the rat and human brain, TIR has been shown by immunohistochemistry to be expressed by capillary endothelial cells, where it may be involved in the transport of transferrin-bound iron into brain tissues.\(^13,20\) Furthermore, autoradiographical methods may visualize TIR in numerous brain sites distinct from iron-rich areas, implying the existence of a more complex and, as yet, poorly understood function for this receptor in the CNS.\(^12\)

Expression of TIR is also coordinately regulated with cell growth. Experimental studies have documented increased numbers of TIR on both proliferating cells and cells that have undergone malignant transformation.\(^8,18,26\) Clinical studies have correlated tumor TIR expression with outcome in a number of systemic neo-

Fig. 3. Photomicrographs of gliial tumors. PAP-hematoxylin, × 462. Left: Intermediate grade astrocytoma stained for transferrin receptor (TIR) showing positive staining of astrocytic fibrils. Right: Juvenile cerebellar pilocytic astrocytoma stained for TIR. The main component of immunostaining is also fibrillar.

Fig. 4. Photomicrograph of a meningioma specimen stained for transferrin receptor. Areas of intense immunostaining are seen amid negatively stained cells. This type of pattern was noted in all meningiomas examined. PAP-hematoxylin, × 146.
plasms, including breast cancer and lymphoma. 6,8,9,11,17, 18,24,28,30 In addition, immunotherapies directed against TIR have been effective in modulating tumor growth in a number of model systems. 1,10,13,25 The suggestion that TIR may also represent an effective target for brain tumor immunotherapy arises from the following observations: 1) malignant gliomas express TIR as determined by biochemical methods; 4,27 2) GBM cells in vitro are very sensitive to the effects of immunotoxins created from conjugation of the plant toxin ricin to transferrin or anti-TIR MAb's; 6,5,14,31 and 3) radioimmunoassay of malignant glioma and surrounding normal brain indicates that there is a much higher reactivity in tumor tissue for anti-TIR. 14

Our findings confirm previous observations that, in normal brain, the most prominent immunostaining is observed in endothelial cells. 13 Only rare glial cells, which appeared to be mainly oligodendrocytes, stained with anti-TIR MAb's. By contrast, TIR immunostaining is much more abundant in neoplastic tissue; all of the glial and meningeal tumors in our series had TIR-positive tumor cells. Both the number of positively immunostained cells and the patterns of cellular staining (focal vs. diffuse) roughly correlated with the histopathological tumor type and, in the case of gliomas, the grade of the tumor. Thus, the most intense staining was seen in the pseudopalisades that form around areas of necrosis in GBM's, whereas other glial tumors with a more benign histology had fewer positively stained cells and more often demonstrated a focal staining pattern. In contrast to the gliomas, the pattern of TIR immunoreactivity seen in all of the meningiomas was remarkably stereotyped. In each case, the proportion of positively stained cells was small, which is not surprising in view of the more benign clinical characteristics associated with nonmalignant meningiomas. 7 The importance of the striking focal staining pattern observed in this small series of meningiomas is less clear. It would be interesting to substantiate this finding in a larger series of specimens.

These preliminary findings also suggest that the assessment of TIR immunoreactivity may have value as an adjunct to other proliferative markers presently used, such as the nuclear antigen, Ki-67, and bromodeoxyuridine labeling. 7,19 Therefore, the characteristic TIR immunostaining patterns that occur as a function of particular tumor type, its tendency to immunostain large numbers of cells, and the relative simplicity of the staining technique are all potentially desirable qualities in a proliferation marker and warrant its further study.

Finally, all tumors showed much greater immunoreactivity for TIR than did normal brain tissue, suggesting that this membrane component may possess the necessary attributes of a target for brain-tumor immunotherapy. Therefore, while it is difficult to correlate immunostaining with the number of receptors per cell (which is a crucial determinant of immunotoxin efficacy 3,15), it would appear that enough differences exist between tumor and normal brain to warrant further study of TIR in brain-tumor immunotherapy. Because TIR is present in cerebral endothelial cells, however, the therapeutic index of anti-TIR MAb's as carriers of either toxins or radioligands may be greatest if delivered locally, either intrathecally or intratumorally.

References

Transferrin receptor in brain-tumor tissue

receptor and B-lymphoblast antigen — their relationship to DNA synthesis, histology and survival in B-cell lymphomas. Int J Cancer 33:173–177, 1984


Manuscript received October 3, 1989. This work was supported in part by a Joseph P. Healey grant and Grant CA39748 from the National Cancer Institute. Address reprint requests to: Lawrence Recht, M.D., Department of Neurology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Massachusetts 01605.