Transferrin Receptor Expression in Intracranial Meningiomas

İntrakranial Menenjiomalarda Transferrin Reseptör Düzeyleri ve Prognastik Önemi

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Abstract: *Objective:* Recent studies of iron mechanism in the brain have indicated that there may be a relationship between transferrin receptor (TfR) expression and histological malignancy. TfR expression may also reflect cell proliferation. We investigated TfR staining intensity in intracranial meningiomas, and assessed whether this was related to recurrence and/or malignant proliferation. *Methods:* Immunohistochemical techniques were used to assess TfR expression in different types of intracranial meningiomas (*n=15*) that had been surgically removed. All were primary tumors. The specimens were semiquantitatively scored for staining intensity and proportions of cells stained, and the relationship between TfR immunoscore and recurrence/proliferation was subjectively evaluated. *Results:* High levels of TfR expression were observed in all the meningioma subtypes. Three of the 15 cases recurred, and two of these patients died due to intracranial pathology. *Conclusion:* Patients with meningiomas that have high TfR immunoscores must be carefully followed for recurrence and/or malignant transformation.

Key words: Meningioma, recurrence, transferrin receptor (TfR)

Özet: *Amaç:* Beyinde demir metabolizması ve fonksiyonları üzerinde son yıllarda yapılan çalışmalarda Transferrin reseptörleri (TfR) ile histolojik malignite arasında bir ilişki olduğu vurgulanmıştır. Bu çalışmada postoperatif olarak tanısı konmuş ve yüksek TfR boyanması gösteren meningiom olgularında boyanma yoğunluğu dikkate alınarak, bununla rekkürens ve malign transformasyon arasında bir ilişki olup olmadığı araştırılmıştır. *Yöntem ve Bulgular:* Çalışmada 15 primer intrakranial meningiom olgusunda TfR boyanmasını göstermek amacıyla immünohistokimya teknikleri kullanılmıştır. *Sonuç:* Tüm meningiom tiplerinde TfR belirgin olarak yüksek düzeyde boyanma göstermiştir. TfR yoğun boyanma gösteren olgulardan 3 tanesi nüks nedeniyle reopere edilmiş, rekkürens gösteren olguların 2 si tümöre bağlı nedenlerden dolayı kaybedilmiştir. TfR seviyeleri yüksek olan intrakranial meningiom olgularının rekkürens ve malign dejenerasyon açısından takip edilmeleri gereklidir.

Anahtar kelimeler: Meningioma, rekkürens, transferrin reseptörleri

INTRODUCTION

Iron is essential for oxygen transfer in the body. It is a cofactor for many enzyme systems that are specific to the brain, and is vitally important for brain cell function and viability (1,5,15,19,24,27). In recent years, more researchers have focused on exploring the mechanisms of iron function in the nervous

system. Transferrin (Tf) is an 80 KDa glycoprotein in plasma that is capable of binding iron, and functions as a carrier for iron to the cells via the bloodstream. Tf is recognized as an essential growth factor in cell proliferation, and both iron and Tf are required for DNA synthesis and cell division. A number of studies have demonstrated a close link between the quantity of transferrin receptors (TfRs) on the cell surface and the rate of cell proliferation (30). TfRs are not found in all brain regions, but large numbers are expressed in oligodendrocytes, cerebrospinal fluid, and capillary endothelial cells of the brain (4,13,14,15,23).

Various types of cell surface receptors, including epidermal growth factor receptors, interleukin receptors, and TfRs, have been identified on the surface of malignant brain tumor cells (10). The level of TfR expression in normal healthy brain tissue is insignificant compared to that in neoplastic tissue (3). Recent experimental findings have suggested that TfR expression is closely associated with degree of histological malignancy; thus, it may be an indicator of cell proliferation (8). *In vivo* studies have demonstrated the presence of TfR in the human melanoma cell line, and in a cell line derived from glioblastoma multiforme (GBM) (26).

Growing tumor cells and proliferating cells both exhibit higher levels of TfR expression than normal resting cells (3,8,26). Clinical studies have detected TfRs in most types of carcinomas, sarcomas, cerebral gliomas, non-glial tumors, meningiomas, and in Hodgkin's and non-Hodgkin's lymphoma (6,7,8). Meningioma is one of the most common brain tumors. Although most meningiomas are benign, recurrence after complete resection is relatively common. Various predictors of meningioma recurrence or malignant proliferation have been identified, including immunohistochemical markers such as Ki-67, neuroradiological findings, and degree of tumor removal. However, there are no detailed reports on how TfR expression relates to tumor proliferation and prognosis in meningioma patients. In this study, we immunohistochemically evaluated TfR expression in various types of intracranial meningiomas, and then subjectively assessed for links to outcome

MATERIALS AND METHODS

Fifteen surgically removed intracranial meningiomas were immunohistochemically studied. The subtypes varied, and all were primary tumors. In each case, the degree of tumor removal was scored according to the Simpson grading system (28). Histological classification and malignancy grading were performed according to the system recommended by the World Health Organization in 1993. Patients were followed for varying periods, and the case outcomes were categorized as no recurrence, recurrence, or exitus.

Histopathological and Immunohistochemical Methods

Tissue from each tumor was fixed in 10% formaldehyde solution, and the histological diagnosis was established from preparations stained with hematoxylin and eosin. For immunohistochemical determination of TfR levels, tissues were incubated in a 1:50 dilution of TfR mouse anti-cd73 antibody (Biogenex-mu-354-uc) in a DAKO Techmate 500-plus automated immunohistochemistry device with a microwave streptoimmunoperoxidase program. Staining intensities and proportions of positively stained cells were used as semiquantitative determinants of TfR immunoreactivity (18). Cases were graded "0" for no staining, "1" for mild staining, "2" for moderate staining, and "3" for intense staining. Regarding the proportions of peroxidase-stained cells, the cases were graded "0" if no cells were stained; "1" if the stained cells covered less than one-third of the processed area; "2" if the stained cells covered one- to two-thirds of the area; and "3" if the stained cells covered two-thirds or more of the area. For each case, these two scores were added together to give an overall "immunostaining score" between 0 and 6.

RESULTS

Table I lists the features and immunostaining scores for each of the 15 intracranial meningioma cases studied. The mean TfR immunoscore was 3.6±1.7 (mean±SD), and the follow-up times ranged from 2 weeks to 44 months (mean, 24.3 months).

The neoplasm recurred in three cases. In two of these patients (Cases No. 2 and No. 9), the primary and reccurent expressed very high levels of TfR. The times from initial surgery to first recurrence in these cases were 27 and 38 months, respectively.

As the table shows, both of these patients had very high TfR immunoscores when they died. One individual (Case No. 9) was a pediatric patient whose primary tumor was histopathologically diagnosed as meningotheliomatous meningioma. The first operation (Simpson grade 1) was in 1996, and the TfR immunoscore at that time was 3 (Figure 1). This patient required two other surgeries (Simpson grade 2) in 1997 and 1999 due to recurrence, and he died 2 months after his last operation. The final histopathologic diagnosis was malignant meningioma, and the TfR immunoscore for that mass was 5 (Figure 2).

The other patient (Case No. 2) was in herniation

Table I: Case features and transferrin receptor (TfR) immunoscores.

No	Age (year)	Sex	TfR score	Location	Histopathology	Resection Grade (Simpson)	Follow-up (month)	Outcome (*)
1	85	♂	3	Convexity	Transitional	1	29	0
2	64	♂	3	Ant. fossa	Meningotheliomatous	2	27	1
2 r	66	♂	5	Ant. fossa	Meningotheliomatous	3	0.5	2
3	56	♀	6	Post. fossa	Transitional	1	27	0
4	62	♀	3	Parasagittal	Angioblastic	1	33	0
5	48	♂	4	Middle fossa	Fibrous	1	31	0
6	69	♀	6	Convexity	Meningotheliomatous	2	25	0
7	55	♂	5	Ant. fossa	Angioblastic	1	25	0
8	62	♀	3	Falx	Transitional	2	17	0
9	7	♂	3	Convexity	Meningotheliomatous	1	38	1
9r	10	♂	5	Convexity	Malignant	2	2	2
10	43	♂	4	Tentorial	Transitional	1	10	0
11	38	♀	2	Convexity	Angioblastic	1	26	0
12	57	♀	4	Olfactory groove	Fibrous	1	23	1
12r	59	♀	2	Olfactory groove	Fibrous	1	26	0
13	46	♀	0	Parasagittal	Transitional	1	31	0
14	51	♀	2	Convexity	Transitional	1	29	0
16	48	♂	2	Ant. fossa	Fibrous	1	44	0

(*) 0: no recurrence, 1: recurrence, 2: death) ("r" indicates recurrence)

status on admission to hospital. His first operation (Simpson grade 2) took place in 1998, and the TfR immunoscore at that time was 3. Simpson grade 3 removal of a recurrent mass was performed 27 months later, but the patient died 2 weeks after this operation. The final histopathological diagnosis was meningotheliomatous meningioma and, as in Case No. 9, the final TfR immunoscore was 5.
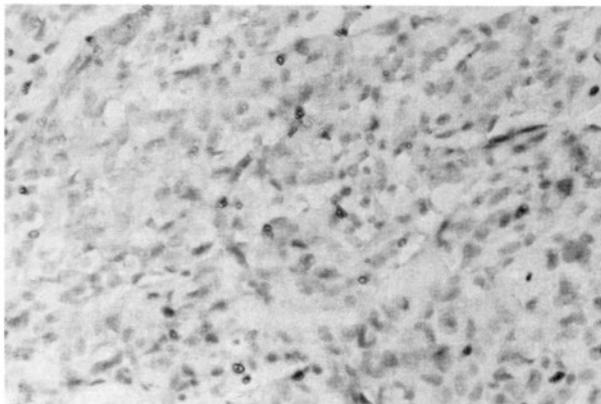
Figure 1: TfR expression in meningioma: This specimen from Case No. 9 shows mild TfR staining. (X40)

DISCUSSION

TfR is a specific cell surface antigen that is mainly expressed in tumoral tissue (11,25,26,31). As mentioned above, experimental studies have documented high levels of TfR expression on proliferating cells and cells that have undergone malignant transformation. Clinical studies
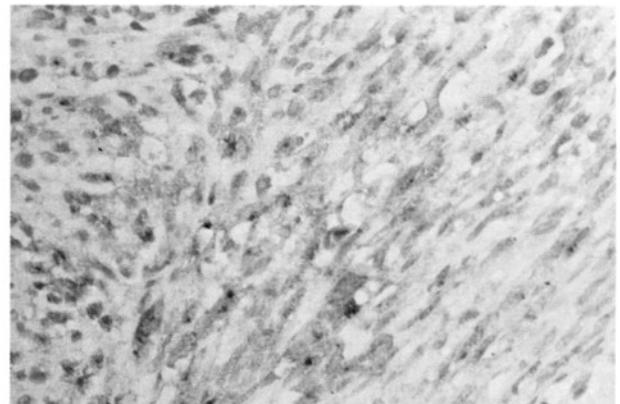
Figure 2: A specimen from the same patient obtained after recurrence 3 years later shows malignant transformation and intense staining. (X40)

have also yielded evidence that supports these links. Immunohistochemical investigations have detected TfRs on human surgical tissue from breast carcinomas, sarcomas, pituitary adenomas, GBM, and certain other tumor types (6,10,12,30). Recht and co-workers assessed 27 brain tumor biopsy specimens, and compared the numbers of TfRs in normal and neoplastic tissue (26). They found that GBM exhibited the most intense TfR staining, and reported that approximately 60% of the meningiomas showed less than 25% staining for TfR. A similar study revealed no detectable TfR expression in slow-growing brain tumors such as cerebellar astrocytomas and acoustic neuromas (10). Some investigators have found that the intensity of TfR staining correlates with tumor histopathology (12,25). In accord with this, several *in vitro and in vivo* studies have shown that GBM exhibits more intense TfR staining than other brain neoplasms (11).

Our immunohistochemical analysis demonstrated significant TfR expression in all types of meningiomas. Although we anticipated that we might see high levels of TfR expression in histologically atypical and anaplastic tissues, the intense staining in other meningioma subtypes was a surprise. As mentioned previously, most meningiomas are benign, but the rate of recurrence even after complete excision is considerable. The highest rates of meningioma recurrence are associated with incomplete tumor removal or higher degrees of histological aggressiveness (2,21,22). However, in all three of our cases that recurred, the patients had undergone Simpson grades 1 and 2 removal. Similar to what has been observed in many malignant neoplasms, our study results suggest that the levels of TfR expression in meningioma are directly related to recurrence and degree of malignancy.

Four of our patients had initial TfR immunoscores of 2 or less, and none of these individuals developed serious problems or tumor recurrence/malignant transformation. Three of the 15 cases recurred, and one of them showed malignant proliferation. Two of the 15 patients died at 2 weeks and 2 months, respectively, after removal of tumor material that was immunoscored as 5. Although positive TfR staining appears to reflect prognosis in meningioma cases, it is clearly not the only valuable indicator of recurrence and malignant proliferation. Simpson resection grade, peritumoral edema, tumor location, and tumor shape are some other important prognostic indicators. Nakasu et al. stressed the importance of radiological features for predicting malignancy in meningioma (21) The same study revealed a strong correlation between peritumoral edema and higher rates of proliferation and recurrence in meningioma cases. We recognize the relevance and significance of these conventional indicators, but believe that immunohistochemical markers should also be considered critical for assessing meningioma recurrence. Further, upregulation of TfR in brain tumors provides an alternative route for delivering anti-cancer drugs and/or toxins to the proliferating cells. Tumor-toxin targeted therapy in brain tumors, especially GBM and medulloblastoma, has been well documented (9,12,16,17,20,26,29,32). In our opinion, TfR upregulation is one of the best candidates for future meningioma immunotherapy.

Considering our patients' TfR findings in relation to follow-up findings, we observed that a considerable number individuals with higher TfR immunoscores had poor outcomes and required re-operation. However, due to the limited number of cases we studied and the relatively short duration of follow-up, we were unable to establish a statistical correlation between score and outcome. Nevertheless, based on these observations and results, we strongly recommend that meningioma patients with TfR expression scores of 3 or higher be carefully followed up for recurrence and/or malignant transformation. To further support and clarify our conclusion, future studies should include larger numbers of patients.

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REFERENCES

1. Aisen P: Entry of iron into cells: A new role for the transferrin receptor in modulating iron release from transferrin. Ann Neurol 32: 62-68, 1992
2. Bello L, Jianping Z, Demetrios CN, Jon FS, Roberto MV, David AC, Rona SC, Peter MB: avâ3 and avâ5 integrin expression in meningiomas. Neurosurgery 47(5): 1185-1195, 2000
3. Colombatti M, Massimo B, Lorena DA, Massimo AG, Guiseppe T: Sensitivity of human glioma cells to cytotoxic heteroconjugates. Int J Cancer 42: 441-448, 1988
4. Connor JR, Fine RE: Development of transferrin positive oligodendrocytes in the rat central nervous system. Journal of Neuroscience Research 17: 51-59, 1987
5. Connor JR, Brian SS, Paolo A, David AL, Peter LW: A quantitative analysis of isoferritins in selected regions of aged Parkinsonian and Alzheimer's diseased brains. Journal of Neurochemistry 65(2): 717-724, 1995
6. Faulk PW, Bai LH, Stevens PJ: Transferrin and transferrin receptors in carcinoma of the breast. The Lancet 23: 390-392, 1980
7. Feelders RA, Vreugdenhil G, Eggermont AM, Kuiper-

Kramer PA, Van Eijk HG, Swaak AJ: Regulation of iron metabolism in the acute phase response: interferon gamma and tumour necrosis factor alpha induce hypoferraemia, ferritin production and decrease in circulating transferrin receptors in cancer patients. Eur J Clin Invest 28(7):520-527, 1998

8. Gatter KC, Brown G, Strowbridge I, Woolston RE, Mason DY: Transferrin receptors in human tissues; their distribution and possible clinical relevance. J Clin Pathol 36: 539-545, 1983

9. Hall WA: Targeted toxin therapy for malignant astrocytoma. Neurosurgery 46(3): 544-552, 2000

10. Hall WA, Merrill MJ, Walbridge S, Youle RJ: Epidermal growth factor receptors on ependymomas and other brain tumors. J Neurosurg 72: 641-646, 1990

11. Hall WA, Godal A, Juell S, Fodstad O: In vitro efficacy of transferrin-toxin conjugates against glioblastoma multiforme. J Neurosurg 76: 838-844, 1992

12. Hall WA: Transferrin receptor on glioblastoma multiforme. (letter) J Neurosurg 74: 313-314, 1991

13. Hill JM, Ruff MR, Weber RJ, Pert CB: Transferrin receptors in rat brain: neuropeptide-like pattern and relationship to iron distribution. Neurobiology 82: 4553-4557, 1985

14. Hulet SW, Hess EJ, Debinski W, Arosio P, Bruce K, Powers S, Connor JR: Characterization and distribution of ferritin binding sites in the adult mouse brain. J Neurochem 72(2): 868-874, 1999

15. Huwyler J, Froidevaux S, Roux F, Eberle AN: Characterization of transferrin receptor in an immortalized cell line of rat brain endothelial cells, RBE4. J Recept Signal Transduct Res 19(1-4): 729-739, 1999

16. Johnson VG, Wrobel C, Wilson D, Zovickian J, Greenfield L, Oldfield EH, Youle R: Improved tumor-specific immunotoxins in the treatment of CNS and leptomeningeal neoplasia. J Neurosurg 70: 240-248, 1989

17. Laske DW, Youle RJ, Oldfield EH: Tumor regression with regional distribution of the targeted toxin TF-CRM 107 in patients with malignant brain tumors. Nat Med 3(12): 1362-1368, 1997

18. Lee CS, Redshaw A, Boag G: Nm 23-H1 protein immunoreactivity in laryngeal carcinoma. Cancer 77: 2246-2250, 1996

19. Loeffler DA, Connor JR, Juneau PL, Snyder S, Kanaley L, DeMaggio AJ, Nguyen H, Brickman CM, LeWitt PA: Transferrin and iron in normal, Alzheimer's disease and Parkinson's disease brain regions. Journal of Neurochemistry 65(2): 710-716, 1995

20. Martell LA, Agrawal A, Ross DA, Muraszko KM: Efficacy of transferrin receptor-targeted immunotoxins in brain tumor cell lines and pediatric brain tumors. Cancer Res 15;53(6): 1348-1353, 1993

21. Nakasu S, Nakajima M, Matsumura K, Nakasu Y, Handa J: Meningioma :proliferating potential and clinicoradiological features. Neurosurgery 37(6): 1049-1055, 1995

22. Nakasu S, Nakasu Y, Nakajima M, Matsuda M, Handa J: Preoperative identification of meningiomas that are highly likely to recur. J Neurosurg 90: 455-462, 1999.

23. Pardridge WM, Eisenberg J, Yang J: Human blood-brain barrier transferrin receptor. Metabolism 36(9): 892-895, 1987

24. Perl DP, Good PF: Comparative techniques for determining cellular iron distribution in brain tissues. Ann Neurol 32: 76-81, 1992

25. Prior R, Reifenberger G, Wechsler W: Transferrin receptor expression in tumours of the human nervous system: relation to tumour type grading and tumour growth fraction. Virchows Arch A Pathol Anat Histopathol 416(6): 491-496, 1990

26. Recht L, Torres CO, Smith TW, Raso V, Griffin TW: Transferrin receptor in normal and neoplastic brain tissue; implications for brain tumor immunotherapy. J Neurosurg 72: 941-945, 1990

27. Roelcke U, Leenders KL, von Ammon K, Radu EW, Vontobel P, Gunther I, Psylla M: Brain tumor iron uptake measured with positron emission tomography and 52Fe-citrate. J Neurooncol 29(2): 157-165, 1996

28. Simpson D. The recurrence of intracranial meningiomas after surgical treatment. J Neurol Neurosurg Psychiatry 20:22-39,1957

29. Singh M: Transferrin as a targeting ligand for liposomes and anticancer drugs. Curr Pharm Des 1;5(6): 443-451, 1999

30. Tampanaru SA, Stefaneanu L, Thapar K, Kontogeorgos G, Sumi T, Kovacs K: Transferrin and transferrin receptor in human hypophysis and pituitary adenomas. Am J Pathol 152(2): 413-422, 1998

31. Wen DY, Hall WA, Conrad J, Godal A, Florenes VA, Fodstad O: In vitro and in vivo variation in transferrin receptor expression on human medullablastoma cell line. Neurosurgery 36(6): 1158-1163, 1995

32. Zovickian J, Johnson VG, Youle RJ: Potent and specific killing of human malignant brain tumor cells by an anti-transferrin receptor antibody ricin immunotoxin. J Neurosurg 66: 850-861, 1987

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Prostaglandin D synthase (beta-trace) in meningeal hemangiopericytoma.

Kawashima M, Suzuki SO, Yamashima T, Fukui M, Iwaki T.

The level of prostaglandin D synthase (PGDS), a major protein constituent of cerebrospinal fluid (CSF), is altered in various brain diseases, including meningitis. However, its role in the brain remains unclear. PGDS is mainly synthesized in the arachnoid cells, the choroid plexus and oligodendrocytes in the central nervous system. Among brain tumors, meningiomas showed intense immunoreactivity to PGDS in the perinuclear region. Thus, PGDS has been considered a specific cell marker of meningioma.