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CD44 Expression in Human Meningiomas: an Immunohistochemical Analysis

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CD44 molecules play an important role in cell adhesion, interaction between cells and extracellular matrix and cellular invasive growth potential. The aim of the study was to evaluate CD44 expression in atypical and benign meningiomas. The tumor specimens were obtained from 61 patients (27 males and 34 females; aged 19 - 78). The analysed material included 10 atypical meningiomas (G2), and following subtypes of benign meningiomas (G1): 21 transitional, 17 meningothelial and 13 fibroplastic. Paraffin tissue sections were immunostained using CD44 antibody (DAKO). The expression of CD44 was multifocal and diffuse. The strong expression was observed in atypical meningiomas, no expression in fibroplastic meningiomas and moderate expression in the other subtypes of benign meningiomas. Statistically significant differences were revealed between the expression of CD44 in G2 and G1 tumors. The results of the study support the thesis on the role of mutated CD44 molecule in the invasive growth potential of neoplastic cells.

Introduction

The CD44 molecule is a polymorphic integral membrane glycoprotein, which plays an important role in cell-to-substrate and cell-to-cell interactions including lymphocyte homing to specific lymph node tissue [13].

In the recent years, numerous investigations have concentrated on CD44 isoform expression in malignant tumors, because CD44 has been linked to tumor progression [10, 12, 14, 16, 18, 22].

Several CD44 isoforms have been identified. A complex gene occupying a stretch of 60 - 80 kb in the human chromosome 11p13 encodes the CD44 molecules. The CD44 gene contains at least 21 exons, ten of which can be alternatively spliced. The variant molecules differ in the middle region, located on the external side of the cell membrane [7, 14, 15]. Altered expression of CD44 molecules has been observed in many types of neoplasm [1, 4, 5, 10, 12, 16, 18].

There is a little data presenting CD44 expression using immunohistochemical method in meningiomas and correlated to histological subtypes [1, 3]. Rooprai et al. have demonstrated results of CD44 expression in meningiomas using immunohistochemical, immunocytochemical and flow cytometry techniques [19].

The aim of this study was to document the pattern of the expression of CD44 standard molecule in the common histological types of benign (G1) and atypical (G2) meningiomas.

Material and Methods

The study included 61 patients with meningiomas. Twenty-seven patients were males and 34 females with the age ranging from 19 to 78 years (the mean age was 54 years). There were 10 atypical meningiomas (G2) and 51 benign subtypes (G1): transitional - 21, fibroplastic - 13 and meningothelial - 17 cases. Histological diagnosis was performed according to the criteria of the WHO 2000 (Table 1).

The tissue specimens were fixed in 10% buffered formaldehyde and routinely processed for paraffin embedding.

For special staining 61 tissue blocks were selected. Three - four-μm thick sections (one or two specimens of central zone in tumor) were cut and mounted on poly-L-lysine-coated glass slides (Polysine^{TM} Microslides; MENZEL-GLASER). Monoclonal Mouse Anti-Human Phagocytic Glycoprotein-1 CD44 (1:40, Clone: DF1485, Isotype: IgG1, kappa) (DAKO) and Universal Streptavidin-Biotin System with DAB were used. The staining was performed strictly according to the producer's instructions. A microwave oven was used in order to reveal the antigens.

Immunostaining was evaluated semiquantitatively as the percentage of positive tumor cells (10 per cent - negative; <50 per cent, >50 per cent; >80 per cent - uniform positive staining) and as membranous or cytoplasmic [18].

Morphometric investigation was performed by means of a computer image analysis system. Positive cells number to all counted cells number ratio was calculated for each sample. A minimum of 1000 cells per specimen were counted at 400x magnification. The immunointensity was also subclassified into four groups in comparison with inter-

TABLE1

Sex and age and histological characteristics of the study groups

Histological type WHO	No	Grade	Age range	Age mean	Sex M / F
Atypical meningioma	10	G2	35 - 72	56.5	5 / 6
Meningothelial meningioma	17	G1	33 - 76	54.7	7 / 10
Transitional meningioma	21	G1	19 - 78	50.2	10 / 10
Fibroplastic meningioma	13	G1	30 - 76	54.4	4 / 9
Total	61		19 - 78	54.0	26 / 32

nal controls (the latter were infiltrating lymphocytes): negative - 0, weak - 1, moderate - 2, and strong - 3 staining [14, 20].

A previously studied skin cancer biopsy specimen known to be positive for CD44 proteins was used as a positive control sample. The same biopsy specimen processed without the primary antibody was used as negative control sample.

Statistical methods

Values of examined indices in the groups of neoplasms varying in malignancy (grade) were compared as well as correlation between patients' age and sex was estimated. For whole sample and for each group there were following parameters assigned: numbers of cases (n), mean value (ME), its standard error (SE), standard deviation (SD) and maximum (MAX) and minimum value (MIN). To test significance of differences between mean values in various groups Mann-Whitney test (for 2 groups) and Kruskal-Wallis test (for more than 2 groups) were used.

Results

The results of immunohistochemical staining for glycoprotein CD44 in meningiomas are presented in Table 2. Expression of CD44 was found in 7 out of 10 atypical meningiomas, in 8 out of 21 transitional meningiomas and in 11 out of 17 meningothelial meningiomas (Figs. 1A - 1C).

All the 13 fibroplastic meningiomas were CD44 negative.

The localization of reaction product was seen on the cell membranes. The extracellular matrix was immunonegative.

In the majority of meningiomas, diffusely distributed moderate and strong membranous staining for CD44 was observed with exception for negative expression in the fibroplastic meningiomas in this series (Table 2).

TABLE 2

CD44 expression in meningiomas

Histological type WHO	Number of cases	CD44 expression	Number of cases % of the CD44s-positive cells
Atypical meningioma G2	10	Strong +3	3 <10% 1 <50% 4 >50% 2 >80%
Meningothelial meningioma G1	17	Moderate +2	6 <10% 9 <50% 2 >50%
Transitional meningioma G1	21	Moderate +2	8 <10% 6 <50% 5 >50% 2 >80%
Fibroplastic meningioma G1	13	Negative 0	13 - 0%

Weak immunoreactivity for CD44 was not observed in meningioma cells. The reaction intensity was stronger in the atypical meningiomas (G2) than in the benign ones (G1).

The difference between the expression of CD44 in atypical meningiomas (G2) and in benign subtypes (G1) was statistically significant ($p<0.001$).

Expression of CD44 in meningiomas is shown on the Figure 2. The overlapping of extreme values of atypical and benign meningiomas is visible except there is no overlapping of atypical and fibroplastic meningiomas.

Diffusely distributed strong membranous staining was seen in all atypical meningiomas. Relatively strong immunoreactivity with CD44 was observed in the central sites but there was no reaction in the peripheral regions. We did not investigate the surrounding cerebral tissue because of the lack of such material.

The expression of CD44 was especially prominent in some individual cells embedded in abundant connective tissue stroma or on cells abutting on the preserved islands of neural tissue. In the normal arachnoids villi and brain tissue as well as in the regions distant from the meningiomas there was no expression of CD44.

Moreover, the reaction for CD44 was observed in some macrophages, lymphocytes and fibroblasts of tumor stroma. The diffuse intracytoplasmic deposits of chromogen were also found in macrophages.

Discussion

The present study has demonstrated the expression of CD44 proteins in meningiomas.

CD44 is a transmembrane glycoprotein expressed on virtually all cell types where it acts as a receptor for hyalu-

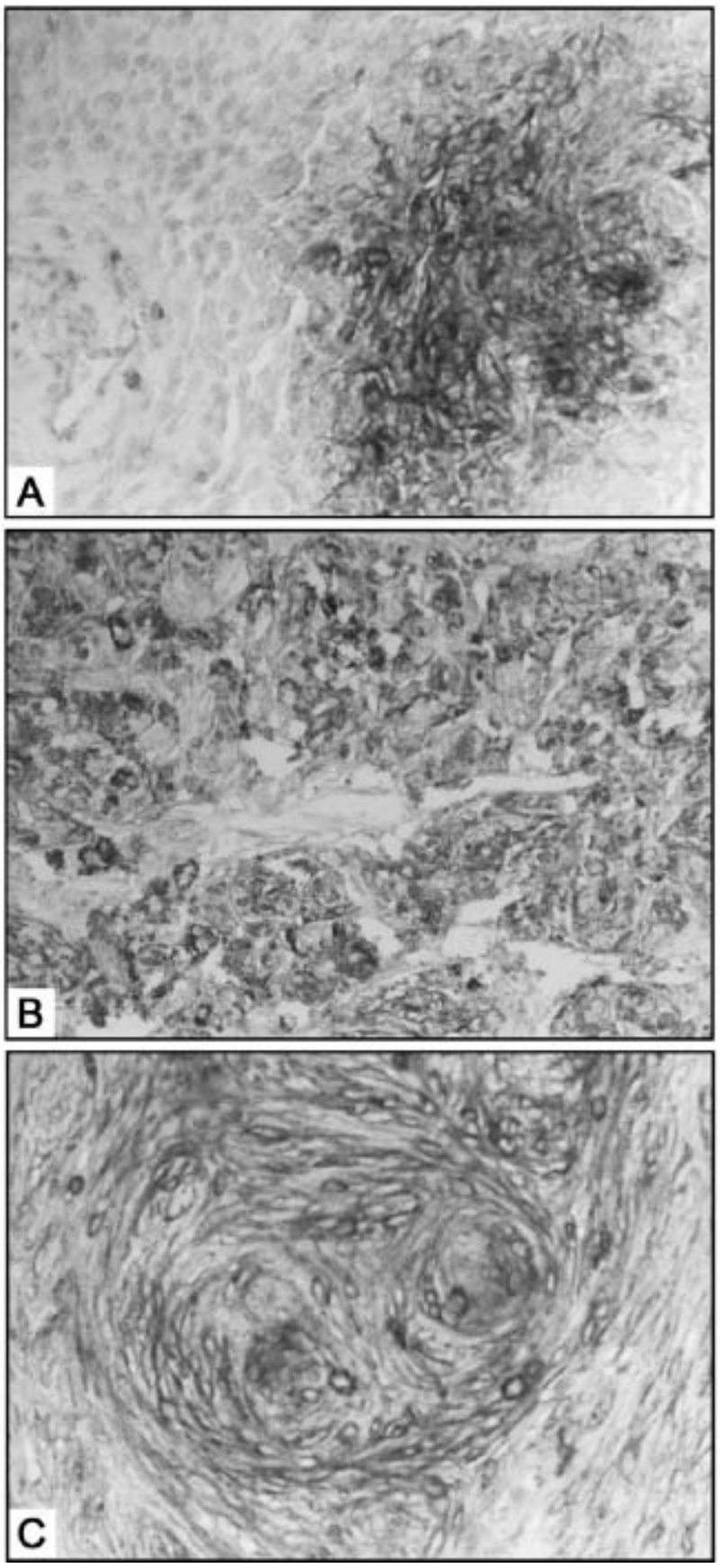

Fig. 1A. Expression of CD44 in meningothelial meningioma. Magn. 200x. Fig. 1B. Atypical meningioma. Expression of CD44 - intense membrane staining of tumor cells. Magn. 200x. Fig. 1C. CD44 expression in transitional meningioma. Magn. 200x.

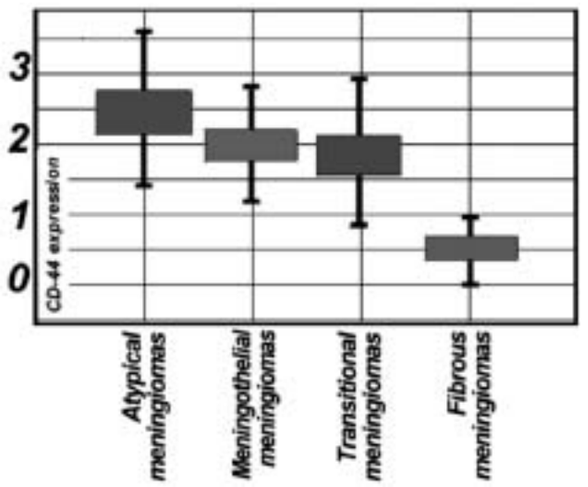

Fig. 2. Expression of CD 44 in meningiomas (box - and - whiskers).

ronate. It is encoded by a gene occupying 60 - 80 kb located at the chromosome 11p13 and consisting of at least 21 exons. The CD44 molecule has three core epitopes encoded by ten exons with alternative mRNA splicing of the remaining exons generating multiple isoforms (CD44v). The standard form of CD44 (CD44s) is expressed on almost all cell types and is heavily glycosylated. Variant isoforms are expressed in a cell- and tissue-specific manner [13, 15, 22].

Other authors have also reported varied expression of CD44 within neoplastic tissue: in lymphoma cells [24], prostate cancer [17], bladder cancer [23], melanocytic lesions [8], gastric cancer [9], rhabdomyosarcoma [11], ovarian neoplasms and uterine cervical tumors [20, 21].

Modified forms of CD44 were found in many types of malignant neoplasms [8, 9, 11, 17, 20, 21, 23, 24].

Several studies have confirmed the potential importance of CD44 as a prognostic factor for neuroblastoma [2, 6, 25, 26].

In squamous cell carcinomas, CD44s are downregulated in poorly differentiated tumors, whereas these molecules are uniformly expressed in the normal squamous epithelium, in proliferative skin diseases, and in non-malignant tumors [14].

In this study we have investigated immunohistochemical expression of CD44 in the most common types of G1 and G2 meningiomas.

Our studies suggest that atypical meningiomas, meningothelial meningiomas and transitional meningiomas are predominantly positive for CD44. The expression of CD44 was multifocal and diffuse. On the contrary, the fibroplastic meningiomas are CD44-negative (<10% of positive tumor cells). The strong expression was observed in atypical meningiomas, no expression in fibroplastic meningiomas and moderate expression in the other subtypes of benign meningiomas.

In 1997 Figarella-Branger et al. reported that 44 of the meningiomas studied expressed CD44. Strong CD44s expression characterized meningothelial and transitional meningiomas and was lower in the fibroplastic one. No correlation was found between CD44 expression and clinical data [3].

In our series of meningiomas, we also observed that tumors are predominantly CD44-positive. This small study appears to indicate that the expression of CD44 correlates with poor outcome; these results must be interpreted with caution because of the small sample size and possible confusing influence of subset analysis.

Statistically significant differences were revealed between the expression of CD44 in atypical meningiomas (G2) and benign meningiomas (G1). The results of this study support the thesis on the role of mutated CD44 molecule in the invasive growth potential of neoplastic cells.

Conclusions

- The expression of CD44 in meningiomas was membranous and multifocal, and diffuse with exception for negative reaction in fibroplastic meningiomas.
- The strong expression was observed in atypical meningiomas (G2), no expression in fibroplastic meningiomas and moderate in the other subtypes of benign meningiomas.
- Statistically significant differences were revealed between the expression of CD44 in G2 and G1 tumors.
- The results of this study support the thesis on the role of CD44 molecule in the invasive growth potential of neoplastic cells.

References

1. *Ariza A, Lopez D, Mate JL, Isamat M, Musulen E, Pujol M, Ley A, Navas-Palacios JJ:* Role of CD44 in the invasiveness of glioblastoma multiforme and the non-invasiveness of meningioma: an immunohistochemistry study. Hum Pathol 1995, 26, 1144-1147.
2. *Favort MC, Combaret V, Lasset C:* CD44 - a new prognostic marker for neuroblastoma. N Engl J Med 1993, 329, 1965.
3. *Figarella-Branger D, Roche PH, Daniel L, Dufour H, Bianco N, Pellissier JF:* Cell-adhesion molecules in human meningiomas: correlation with clinical and morphological data. Neuropath Appl Neurobiol 1997, 23, 113-122.
4. *Fox S, Fawcett J, Jackson D et al:* Normal human tissues, in addition to some tumors, express multiple different CD44 isoforms. Cancer Res 1994, 54, 4539-4546.
5. *Fox S, Gatter K, Jacson D et al:* CD44 and cancer screening. Lancet 1993, 342, 548-549.
6. *Gross N, Beck D, Beretta C, Jackson D, Peruisseau G:* CD44 expression and modulation on human neuroblastoma tumors and cell lines. Eur J Cancer 1996, 31A, 471-475.

7. *Gross N, Beretta C, Peruisseau G, Jackson D, Simmons D, Beck D:* CD44H expression by human neuroblastoma cells: reaction to mycN amplification and lineage differentiation. Cancer Res 1994, 54, 4238-4242.
8. *Harwood CA, Green MA, Cook MG:* CD44 expression in melanotic lesions: a marker of malignant progression? Br J Dermatol 1996, 135, 876-882.
9. *Heider KH, Dammrich J, Skroch-Angel P, Muller-Hermelink HK, Vollmers P, Herrlich P, Ponta H:* Differentiated expression of CD44 splice variants in intestinal- and diffuse-type human gastric carcinomas and normal gastric mucosa. Cancer Res 1993, 53, 4197-4203.
10. *Hsieh H F, Yu JC, Ho LI, Chiu SC, Harn HJ:* Molecular studies into the role of CD44 variants in metastasis in gastric cancer. J Clin Pathol: Mol Pathol 1999, 52, 25-28.
11. *Humphrey G, Hazel DL, MacLennan K, Lewis I:* Expression of CD44 by rhabdomyosarcoma: a new prognostic marker? Brit J Cancer 1999, 80, 918-921.
12. *Kahara N, Ozaki T, Doi T, Nishids K, Kawai A, Shibahara M, Inoue H:* CD44 expression in soft tissue sarcomas. Virchows Archiv 2000, 436, 574-578.
13. *Lesley J, Hyman R, Kincade PW:* CD44 and its interaction with extracellular matrix. Adv Immunol 1993, 54, 271-335.
14. *Lipponen P, Aaltoma V, Kosma VM, Ala-Opas M, Eskelnen M:* Expression of CD44 standard and variant-v6 proteins in transitional cell bladder tumors and their relation to prognosis during a long-term follow-up. J Pathol 1998, 186, 157-164.
15. *Mackay C, Terpe HJ, Stauder R et al:* Expression and modulation of CD44 variant isoforms in humans. J Cell Biol 1994, 124, 71-82.
16. *Mayer B, Jauch KW, Gunthert U, Figdor CG, Schilberg FW, Funke I, Johnson JP:* De novo expression of CD44 and survival in gastric cancer. Lancet 1993, 342, 1019-1022.
17. *Nagabhushan M, Ptetlow TG, Guo Y-J, Amini SB, Pretlow T, Sy M-S:* Altered expression of CD44 in human prostate cancer during progression. Am J Clin Pathol 1996, 106, 647-651.
18. *Regauer S, Ott A, Berghold A, Beham A:* CD44 expression in sinonasal melanomas: is loss of isoform expression associated with advanced tumor stage? J Pathol 1999, 187, 184-190.
19. *Rooprai HK, Liyanage K, King A, Davies D, Martin K, Pilkington GJ:* CD44 expression in human meningiomas: An immunocytochemical, immunohistochemical and flow cytometric analysis. Int J Oncol 1999, 14, 855-860.
20. *Saegusa M, Hashimura M, Machida D, Okayasu I:* Down-regulation of CD44 standard and variant isoforms during the development and progression of uterine cervical tumors. J Pathol 1999, 187, 173-183.
21. *Saegusa M, Machida D, Hashimura M, Okayasu I:* CD44 expression in benign, premalignant, and malignant ovarian neoplasms: relation to tumor development and progression. J Pathol 1999, 189, 326-337.
22. *Sneath RJS, Mangham DC:* The normal structure and function of CD44 and its role in neoplasia. J Clin Pathol: Mol Pathol 1998, 51, 191-200.
23. *Soughagate J, Tredojdosiewicz LK, Smith B, Selby PJ:* Patterns of splice variant and CD44 expression by normal human urothelium in situ and in vitro and by bladder-carcinoma cell lines. Int J Cancer 1995, 62, 449-456.
24. *Sy MS, Guo YJ, Stamenkovic I:* Distinct effects of two CD44 isoforms on tumor growth in vivo. J Exp Med 1991, 174, 859-866.
25. *Terpe HJ, Christiansen H, Gonzalez M, Berthold F, Lampert F:* Differentiation and prognosis of neuroblastoma in correlation to the expression of CD44s. Eur J Cancer 1995, 31A, 549-552.
26. *Terpe HJ, Zimmer C, Gunthert U, Korf B:* CD44 expression in human brain tumors. Clin Neuropathol 1993, 12, 271-272.

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