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ORIGINAL ARTICLE

Solitary and Multiple Meningiomas: an Immunohistochemical Comparison

Baiba Vikmane, Arvids Jakovlevs, Andrejs Vanags, Ilze Strumfa
Riga Stradins University, Riga, Latvia

SUMMARY

Introduction. Meningiomas are common primary tumors of brain meninges. These neoplasms develop from arachnoid cap cells and are multiple in 1 – 10% cases. Occasionally, significantly higher multiplicity rates have been reported, at least partially due to the increased application of computed tomography and magnetic resonance imaging in the diagnostics of intracranial pathologies. Meningiomas generally express progesterone receptors, but only few studies have focused on sex hormone receptor differences between solitary and multiple meningiomas. Similarly, there is limited information on cell proliferation and adhesion factors in solitary and multiple meningiomas.

Aim of the study. Was to evaluate the immunohistochemical differences in sex hormone receptor expression, cell proliferation and adhesion within solitary and multiple meningiomas.

Material and methods. In a retrospective study, 11 consecutive multiple meningiomas and 20 grade-matched solitary meningiomas were assessed by immunohistochemistry (IHC) to detect estrogen receptors (ER), progesterone receptors (PR), Ki-67 and neural cell adhesion molecule (NCAM). IHC was followed by quantitative microscopic evaluation. Descriptive and inferential statistics was applied including confidence interval (CI) analysis, Mann-Whitney U test and Spearmen correlation by IBM SPSS Statistics 22.0 software; p values less than 0.05 were regarded as statistically significant.

Results. Although PR were found in all samples, the mean expression was significantly lower in multiple meningiomas ($p = 0.03$): 30.0% (95% CI: 10.4 – 49.8) *versus* 70.6% (95% CI: 56.6 – 84.7) in solitary meningiomas. ER were invariably absent in both groups. The proliferation index did not differ in solitary and multiple tumors. There was a trend ($p = 0.07$) to higher mean expression of NCAM in multiple meningiomas than in control group: 48.3% (95% CI: 25.8 – 70.8) versus 24.6% (95% CI: 13.2 – 36.0), respectively. The multiple meningiomas showed diverse histological types and immunophenotypes in up to 33.3% patients.

Conclusions. Multiple meningiomas are characterized by significant down-regulation of PR and up-regulation of NCAM. The last finding can indicate neural differentiation and/ or peculiarities of cell adhesion and signaling that facilitate multifocal proliferation. Diverse histological types as well as PR and NCAM expression in separate meningiomas within same patient indicate independent multicentric origin.

Key words: meningioma, multiple meningiomas, proliferation index, progesterone receptors, NCAM

INTRODUCTION

Meningiomas are common primary tumors of brain meninges. In general, meningiomas comprise 36.1% of all primary tumors found in the central nervous system (CNS) and are the predominant primary CNS tumors after the age of 35 years (18). These neoplasms develop from arachnoid cap cells. Meningiomas are mostly benign corresponding to World Health Organization (WHO) grade I (17). The rate of multiplicity is reported to range between 1 – 10% (7; 15) and is rising with the increased application of computed tomography and/ or magnetic resonance imaging (15). Occasionally, estimates as high as 49% are published questioning if solitary meningiomas exist at all. However, regional multiplicity has been included in these data (4). The prognosis of multiple meningiomas does not differ from solitary meningiomas (11; 15). Although it is well known, that meningiomas express sex hormone receptors, only few studies have focused on estrogen receptor (ER) and progesterone receptor (PR) expression differences between solitary and multiple meningiomas. Neural cell adhesion molecule (NCAM) is a cell surface glycoprotein that exists in different isoforms and acts through several pathways, providing multiple functions (12). Some authors have described impact of NCAM on tumor progression and dissemination (16; 24; 25). NCAM expression has been found in solitary meningiomas (8; 10; 19), but expression in multiple meningiomas has not been studied before.

AIM OF THE STUDY

The aim of the study was to evaluate the differences in sex hormone receptor levels, cell proliferation and adhesion in solitary and multiple meningiomas by implementation of immunohistochemical analysis to detect ER, PR, Ki-67 and NCAM expression.

MATERIAL AND METHODS

In a retrospective study, archived formalin-fixed, paraplast-embedded tissues of 11 multiple meningiomas and 20 solitary meningiomas were analyzed by immunohistochemistry (IHC). The cases were identified by archive search of all consecutive patients (2011 – 2013) who were subjected to neurosurgical treatment by routine indications in a single university hospital. The diagnosis and grade of meningiomas was verified by 2 pathologists in accordance to the WHO classification (17). The basic clinical data were retrieved including patient's age, gender and radiological findings on the tumor number. Meningiomas were considered

multiple if several tumors have been simultaneously resected from different locations or if multifocal growth was evident radiologically in clearly separated spatial locations. Tumor recurrence in the site of previous operation was not considered as an evidence of multifocality. After the multiple meningiomas have been identified, grade-, age- and gender-matched control group consisting of 20 solitary meningiomas was created for the immunohistochemical part of the study. The meningioma samples were tested for Ki-67 antigen, ER, PR and NCAM expression. Immunohistochemical visualization was applied for this purpose, using heat-induced antigen retrieval in basic (pH 9.0) buffer. The characteristics of primary antibodies are listed in Table 1. The bound antibodies were detected by polymeric visualization system EnVision. All reagents for the immunohistochemistry were provided by Dako, Glostrup, Denmark. Positive and negative controls were performed and reacted appropriately. According to manufacturer's instructions, the following positive controls were employed: normal endometrium for estrogen and progesterone receptors and Schwann cells within neural fibers for NCAM. Normal endothelium was used as the internal negative control for NCAM, ER and PR. Tonsillar tissue was applied to control Ki-67.

Table 1. The characteristics and evaluation of immunohistochemical panel

Antigen	Antibody	Clone	Dilution	Pattern
Progesterone receptor	MMAH	PgR 636	1:1	Nuclear
Estrogen receptor alpha	MMAH	1D5	1:1	Nuclear
Ki-67 protein	MMAH	MIB-1	1:100	Nuclear
NCAM	MMAH	123C3	1:100	Membranous

Abbreviations in the Table: NCAM, neural cell adhesion molecule; MMAH, monoclonal mouse antibody against human antigen

The proliferation index by Ki-67 was assessed by counting positively stained nuclei within 100 neoplastic cells in a high-power resolution (HPR), using magnification 400x. The "hotspot" areas of the highest antigen expression were investigated. PR and ER were evaluated by counting positive nuclei in 3 "hotspot" HPR fields. NCAM expression was measured by the relative amount (%) of positive cells in the entire sample on low-power magnification (100x). Cells were considered positive for NCAM if an unequivocal positive reaction was observed in the cell membrane. All immunohistochemical data were expressed quantitatively as the relative number of positive neoplastic cells (%).

Data were analyzed using IBM SPSS Statistics 22.0 software. Descriptive statistical analysis was performed including calculation of 95% confidence interval (CI). The normality was assessed by Shapiro-Wilk test. Differences between groups were determined by the non-parametric Mann-Whitney U test. The non-parametric Spearman's rank correlation coefficient was used to identify the statistical dependence between two variables. The differences were considered statistically significant if $p < 0.05$.

RESULTS

The archive search for all consecutive surgically treated patients affected by meningiomas yielded 378 cases. Multiple meningiomas were found in 7 (1.9%; 95% CI: 0.8 – 3.9) patients, including six females and one male. The mean patient's age at the time of operation was 55.7 years (95% CI: 43.1 – 68.3). All tumors were grade I according to WHO classification. Three patients had more than one meningioma removed. Among them one had diverse tumors of fibrous and transitional histological types. The remaining two patients had transitional meningiomas. Three patients with single surgically removed meningioma had transitional meningioma, one patient – fibrous meningioma (Figure 1).

In the general group of solitary meningiomas, females constituted 77.6% (95% CI: 73.1 – 81.6) of patients. Solitary meningiomas were operated at the mean age of 60.3 years (95% CI: 58.9 – 61.7). For immunohistochemical evaluation, twenty grade I tumors were selected, including four meningothelial, six fibrous and ten transitional type meningiomas.

Proliferation index

There was no significant difference between solitary and multiple meningiomas, regarding Ki-67 expression. The mean values were 6.2% (95% CI: 4.9 – 7.5) in solitary meningiomas and 6.0% (95% CI: 4.5 – 7.5) in multiple meningiomas (Table 2). Both multiple and solitary meningiomas lacked any correlation between Ki-67 and NCAM or between Ki-67 and PR expression ($p > 0.05$).

Table 2. The immunohistochemical characteristics of solitary and multiple meningiomas

Parameters		Expression in solitary meningiomas	Expression in multiple meningiomas
Estrogen receptor,%	Range	0.0	0.0
Progesteron receptor, %	Range	1 – 96.3	2.0 – 93.0
	Mean	70.6	30.1
	95% CI	56.6 – 84.7	10.4 – 49.8
Ki-67,%	Range	3.0 – 15.0	2.0 – 10.0
	Mean	6.2	6.0
	95% CI	4.9 – 7.5	4.5 – 7.5
NCAM, %	Range	0.0 – 90.0	5.0 – 98.0
	Mean	24.6	48.3
	95% CI	13.2 – 36.0	25.8 – 70.8

Abbreviations in the Table: NCAM, neural cell adhesion molecule; CI, confidence interval for the mean

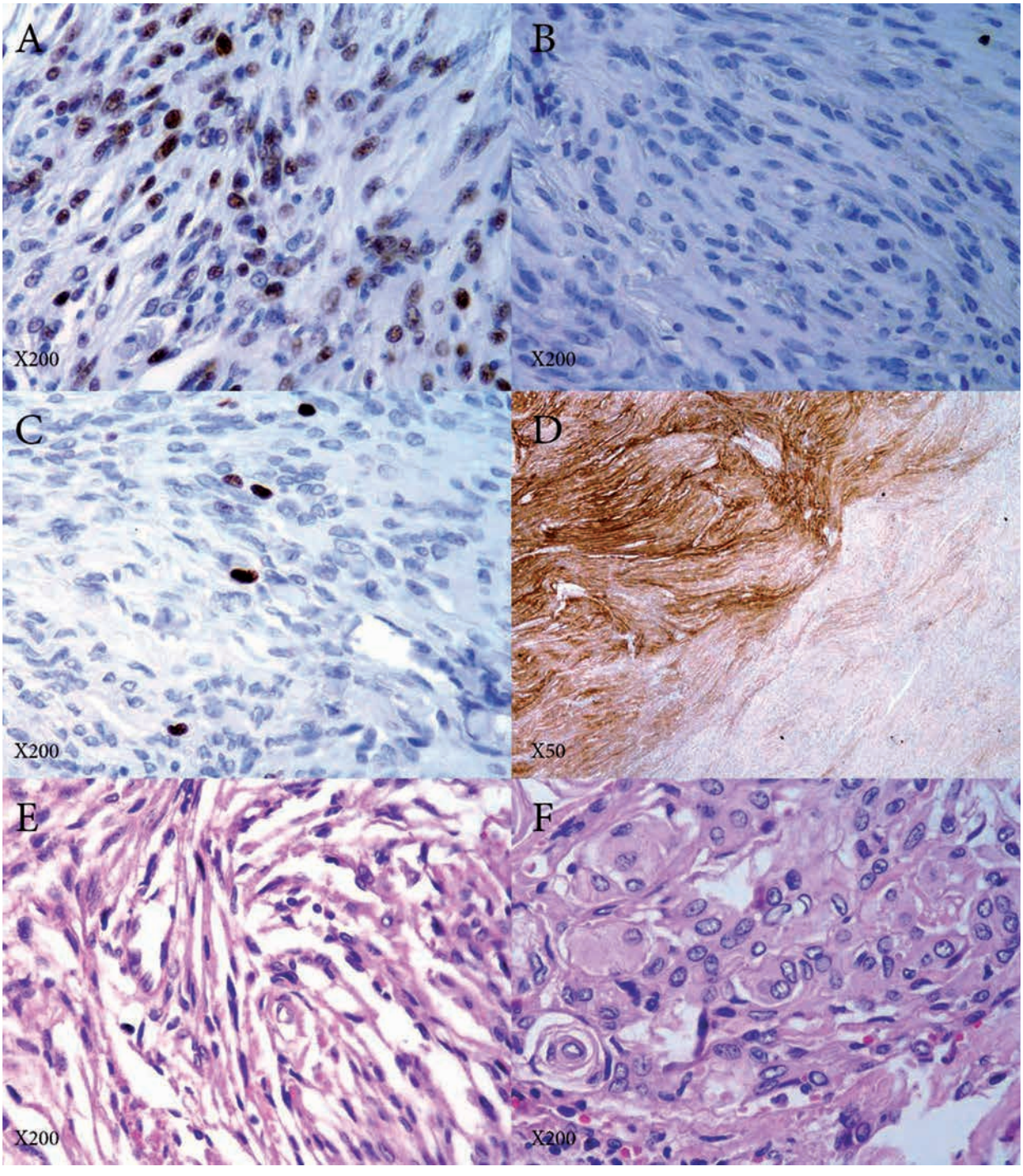

Fig.1. Immunohistochemical and morphological characteristics of meningiomas architectur (HE,OM x200) **1A**, Expression of progesterone receptors in a multiple meningioma. Immunoperoxidase (IP), anti-progesterone receptors, original magnification (OM) 200x. **1B**, Lack of estrogen receptors in meningioma. IP, anti-estrogen receptors, (OM) 200x. **1C**, Proliferation activity by Ki-67. IP, anti-Ki-67, (OM) 200x. **1D.** Expression of neural cell adhesion molecule (NCAM) in a multiple meningioma. Note the heterogeneity. IP, anti-NCAM, (OM) 50x. **1E.** Classic fibrous meningioma with spindled cells. Haematoxylin-eosin (HE), (OM) 200x. **1F**. Meningothelial meningioma. Note the characteristic whorled architecture. (HE, OM 200x)

Sex hormone receptors

PR expression was found in all the analyzed samples. The expression was heterogeneous and confined to tumor cell nuclei. PR expression was statistically significantly higher in solitary meningiomas ($p = 0.03$), showing the mean expression 70.6% (95% CI: 56.6 – 84.7). In contrast, the mean PR level in multiple meningiomas reached only 30.0% (95% CI: 10.4 – 49.8). Widespread PR expression, exceeding 50% of tumor cells, was observed in 85.0% (95% CI: 63.1 – 95.6) of solitary meningiomas but only in 27.3% (95% CI: 9.2 – 57.1) of multiple meningiomas. No statistically significant correlations were observed ($p > 0.05$) between PR expression and the other IHC variables, patient's age or gender. Estrogen receptors were absent from all analyzed cases.

NCAM

Presence of NCAM was found in all multiple meningiomas, ranging from 5.0 to 98.0% of cells, with the mean level 48.0% (95% CI: 25.8 – 70.8). Expression usually showed heterogeneous patchy pattern both in multiple and solitary meningiomas (Figure 1). NCAM was invariably detected only in tumor cells showing intense membranous and cytoplasmic staining. Normal endothelium did not stain with NCAM and was used as internal negative control to exclude any possible background staining, especially in cases with intense diffuse reactivity. Five of multiple and four of solitary meningiomas showed strong NCAM positivity in more than 50% of tumor cells. Among solitary tumors, NCAM was found in 16 cases (80.0%) with the highest expression reaching 90.0% and the mean level of 27.0% (95% CI: 13.2 – 36.0). Both in solitary and multiple meningiomas, there were no correlations between NCAM and the other immunohistochemically detected proteins ($p > 0.05$).

DISCUSSION

Meningiomas were first described by Cushing and Eisenhardt as early as on 1938. Already then, multiple meningiomas were recognized and defined as spatially separated tumors (15). The incidence of multiple meningiomas was estimated as 1 – 2% in the early reports while higher incidence has been observed with the increasing application of the computed tomography of brain. Magnetic resonance imaging yielded even higher numbers of multiple meningiomas due to the possibility to detect small tumors and meningiomas in the posterior fossa, skull base and vertex (15). In our study, we defined multiple meningiomas as spatially separated lesions, and the frequency of multifocality in our study is in line with the early reports (7). Thus, multiple meningiomas were rare in our study as only 11 cases have been enrolled despite the significant number of consecutive surgically treated meningiomas. Although the small sample size may affect statistical analysis, we succeeded to subject the entire scope of identified multiple meningiomas to the immunohistochemical study.

The indications for surgical treatment in multiple meningiomas are not different from those in solitary tumors: meningioma should be removed if it is symptomatic, larger than 3 cm or expanding. Multiplicity alone is not an indication for complete removal of all tumors (15).

So far only Huang et al. (14) have reported on the differences of PR expression in large groups of multiple and solitary meningiomas, including 45 multiple and 31 solitary tumors. They revealed PR expression in all meningiomas, with higher levels in multiple tumors. The authors explained their results by a hypothesis suggesting possible PR role in facilitating desquamation and dissemination of tumor cells. However, the expression of PR has been described as a favorable prognostic factor in most studies. Lack of PRs and/ or presence of ERs in meningioma are associated with higher possibility of genetic mutations, tumor recurrence and rapid progression, on the contrast higher grade meningiomas lack PRs (13; 20; 22; 23). Here we present our data evidencing significantly lower PR expression in multiple meningiomas. Appropriately matched control group, as was designed in our study, could be necessary to find and justify the true differences between solitary and multiple tumors.

An expression of ERs in meningiomas is rare and usually is associated with higher grade meningiomas. The scarcity of its expression in grade I tumors in this study concurs the reports of other authors (13; 20).

The mean values of proliferation activity by Ki-67 were not different in solitary and multiple meningiomas. This finding is in agreement with the grade consistency in both groups. Thus, multifocal growth of meningioma is not associated with higher cell proliferation and does not evidence aggressive tumor course in accordance with the literature data on the clinical course of multiple meningiomas (15). The mean value of Ki-67 albeit low exceeds the reported mean values for benign meningiomas: 3.54% in Abry *et al.* (1) study of 526 meningiomas and 3.28% in Roser *et al.* (21) investigation of 546 meningiomas. The differences can be explained by the study design. To account for the tumor heterogeneity and reveal the possible differences despite this heterogeneity, we counted the proliferation index in the areas with the highest antigen expression. Higher Ki-67 index is also a characteristic of meningiomas associated with *NF2* gene mutations (2) – a factor that was not assessed when analyzing samples.

Neural cell adhesion molecule is a cell surface glycoprotein molecule. Initially, the mechanism of NCAM action was described as a homophilic zipper-like binding between two NCAM molecules either on the same or different type of cells. In subsequent studies, heterophilic interaction with other molecules as fibroblast growth factor receptor FGFR, SRC family kinase FYN and glial-cell-line derived neurotrophic factor GDNF was found (5; 12; 25).

Due to alternative RNA splicing NCAM exists in multiple isoforms. 140 and 180 kDa NCAM isoforms are more

characteristic during the embryonal development while 120 kDa molecule is considered an adult form. However, 140 kDa NCAM is often dominating in tumors (5; 6; 8). The different isoforms, posttranslational changes and wide range of molecular interaction provide basis for the multiple functions of NCAM. Among them, tumorogenic properties have been described (16; 24; 25).

Posttranslational addition of polysialic acid provides polysialiated NCAM forms. Polysialiation diminishes the adhesive properties of NCAM, providing higher plasticity that is essential in neural system development. In adults it engages in regeneration and in memory development. In oncogenesis, the polysialiation facilitates cell dissemination (9).

Some authors have studied NCAM expression in meningiomas. Garin-Chesa *et al*. analyzed four meningioma samples where one sample showed homogenous expression, two samples – heterogeneous expression, but one was NCAM negative (10). Figarella-Branger *et al*. studied 47 meningiomas that all expressed 140 kDa NCAM. They also found NCAM expression in normal cap cells in arachnoidea that are considered the origin cells of meningioma (8).

This is the first study assessing NCAM in multiple meningiomas. The results show a trend to higher expression of NCAM in MMs; this might be one of mechanisms that facilitate cell dissemination. However, diverse NCAM expression as well as different PR expression and histological types in separate meningiomas within same patient indicate multicentric origin of multiple meningiomas.

CONCLUSIONS

Solitary and multiple meningiomas are biologically different. Multiple meningiomas are characterized by significant down-regulation of PR expression – marker that has been previously associated with clinical course. The lack of PR can lead to lower efficacy of antiprogesterone therapy in a fraction of patients affected by multiple meningiomas.

The up-regulation of NCAM in multiple meningiomas can indicate neural differentiation and/ or peculiarities of cell adhesion and signaling that facilitate proliferation in multiple foci. Diverse histological types as well as PR and NCAM expression in separate meningiomas within same patient indicate multicentric origin.

Conflict of interest: None

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Address:
Arvids Jakovlevs
Department of Pathology, Riga Stradins University
Dzirciema Street 16, Riga, LV-1007, Latvia
e-mail: Arvids.Jakovlevs@rsu.lv