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Adhesion cell molecules as potential markers of aggressiveness in meningiomas

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Abstract

Routine histopathological criteria are poor predictors of the outcome in meningiomas. The present study tried to determine if some adhesion cell molecules could be helpful in assessing the histological aggressiveness in meningiomas of all grades. Our series comprised 113 cases, *WHO* grade I (*n*=53), *WHO* grade II (*n*=47) and *WHO* grade III (*n*=13). Three cases of meningeal non-meningothelial tumors (hemangiopericytoma, hemangioblastoma and fibrosarcoma) were also studied as control tissue. Immunohistochemistry for CD44, CD54, E-cadherin, progesterone receptor (PGR) and Ki67 was performed. CD54 was for the first time systematically assessed in meningiomas of all three grades of malignancy. CD44 and CD54 were expressed in 58.4 and 31.72% of cases, respectively. CD54 expression showed a direct correlation with the degree of histological anaplasia and Ki67 values. More than half of cases (58.11%) were negative for E-cadherin, mostly the anaplastic ones, which also showed less positive areas for this marker. Cell adhesion molecules were not significantly related to a particular histological type and proliferating potential of meningiomas. Overall, CD54 as well as E-cadherin could be used as additional aggressiveness indicators aside the classical ones. On the other way, Ki67 once again confirmed its significant role in the assessment of meningioma aggressiveness.

Keywords: meningioma, grading, histology, adhesion molecules, aggressiveness.

Introduction

Meningiomas are tumors with variable evolution and prognosis. Several factors seem involved in their behavior, as the cell adhesion molecules. Among these, CD44 is supposed to be related to tumor invasiveness and metastatic potential. It was studied on various tumors types, including meningiomas [1]. CD44 is a cell surface receptor for the extracellular matrix molecule hyaluronan, being involved in cell-matrix adhesion and matrix-mediated cell signaling [2–4]. It may be considered a marker of stem cells, together with CD133, OKT4, NANOG, and ALDH1 [5], and also seems to promote tumor cell proliferation and migration [6]. Its expression seems to be more intense in atypical *vs.* low-grade meningiomas, where it appears in variable degrees [7]. CD54 antigen (ICAM-1) is an integral membrane glycoprotein involved in intercellular adhesion, also involved in recruiting of macrophages and granulocytes [8]. Until the present study, its expression in meningiomas, as well as its possible correlations with the overall aggression degree of these tumors was only rarely studied, and mostly related to local angiogenesis [9]. E-cadherin is a transmembrane glycoprotein that mediates epithelial cell adhesion, maintaining cell and tissue structure, being also involved in morphogenesis [10, 11]. It appears to be expressed in the majority of meningiomas, being possibly related to tumorigenesis [12] and even to be considered as a valuable prognostic marker, in combination with clinical data [13].

Since all these molecules appear as possibly related to some behavioral characteristics of meningiomas, we tried to assess whether correlations exist between their immunohistochemical expression and histological subtypes, other prognostic molecules (*i.e.*, Ki67, and progesterone receptors – PGR), or age and gender, as well as with the overall outcome.

Materials and Methods

The study was done on paraffin-archived material from consecutive cases of meningioma from the archives of the National Institute of Neurology and Neurovascular Diseases, Bucharest, Romania. Only first interventions were taken into account, avoiding recurrent tumors at first presentation. Also, the cases were selected from a remote period of time (2000–2001 years) in order to have enough follow-up periods in each case. All patients were followed annually in the Clinic of Neurosurgery where they have been operated, with physical examination and CT scans made available to us. The series comprised 113 cases, *WHO* grade I (*n*=53, 31 females, 22 males, median age 52.69 years), *WHO* grade II (*n*=47, 31 females, 16 males, median age 54.21 years) and *WHO* grade III (*n*=13, eight females, five males, median age 54.61 years). In the benign category, the tumor subtypes included the following ones: fibrous – 14, transitional – 10, meningothelial – 21, microcystic – 4, psammomatous – 1, angiomatous – 2 and sclerous [14, 15] – 1. The *WHO* grade II group comprised 45 atypical cases and two chordoid ones. Among the high-grade, malignant cases, eight were anaplastic and five papillary. As control cases, we took into account: a meningeal hemangiopericytoma in a 60-year-old female, because of its possible histological resemblance with a patternless malignant meningioma, a meningeal hemangioblastoma in a 49-year-old woman,

possibly resembling histologically a microcystic or a clear-cell meningioma, as well as a meningeal fibrosarcoma in a 41-year-old woman, potentially mistaken for a fibrous meningioma.

Immunohistochemistry was performed on the paraffin-embedded material using the EnVision+ Dual Link System Peroxidase kit (Dako, Carpinteria, CA, USA), according to the manufacturer's instructions. Primary antibodies against the following antigens were used: CD44 (1:50), CD54 (1:50), PGR (1:100) (Novocastra, Newcastle Upon Tyne, UK), E-cadherin (1:50), EMA (1:75), Ki67 (1:50) (Dako, Glostrup, Denmark,), vimentin (1:100) (Biogenex, San Ramon, CA, USA). EMA and vimentin were used only to confirm the meningothelial nature of the tumor and are not further discussed in the article. The positivity for CD44, CD54, E-cadherin and PGR was considered on the entire tumor sample and expressed roughly as a proportion of all cell population. The Ki67 labeling index was manually counted in 100 nuclei in the most positive areas of the tumor and expressed as a percentage.

Subsequently, the values for cell adhesion molecules expression were compared among them and with those of Ki67 and PGR, as well as with the histological degree, subtype and localization of the tumor, also taking into account the age, gender and possibly the recurrence interval. Logistic regression was used for these correlations, a $p<0.05$ being considered as statistically significant. The statistical analysis was performed using Excel and StatView 5 software.

Results

CD44 was positive in 66 cases of the whole series (58.4%). On separate categories, it appeared expressed in 30 cases of the *WHO* I (benign – B) series (56.6%), in 30 cases of the *WHO* II series (atypical – AT) (63.88%) and in six cases of the *WHO* III series (anaplastic – AN) (46.15%).

The expression pattern was mostly patchy (Figure 1), sometimes very obviously limited to the cell membrane (Figure 2). The median values of positive tumor cells were 22.66% (B), 14.44% (AT) and 17% (AN).

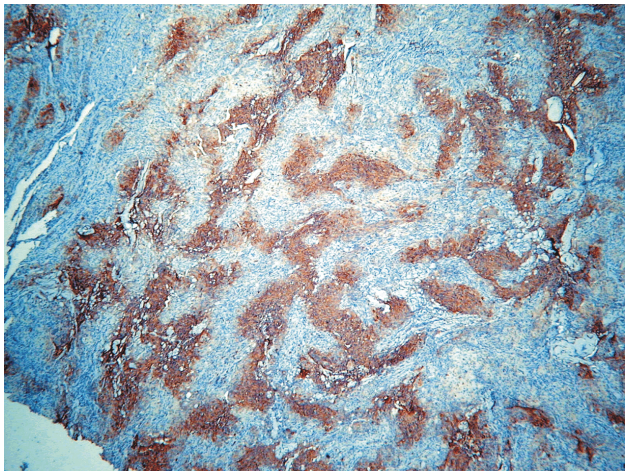

Figure 1 – ***Patchy expression of CD44 in an atypical meningioma. Immunohistochemistry, ×40.***

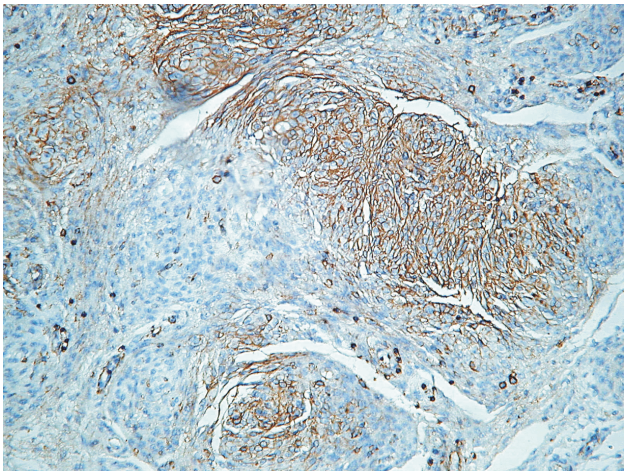

Figure 2 – ***CD44 expression in another case of atypical meningioma. The positivity is strictly confined to the cell membranes. Immunohistochemistry, ×200.***

A specific association with a particular histological pattern in the benign category was not found (*i.e.*, fibrous type cases showed either 100% positivity or a total absence of it).

CD54 was expressed globally in 37 cases of the whole series (32.74%) of cases. Within the three groups, we found positivity in 13 (24.5%) cases (B), 17 (36.17%) cases (AT), and seven (53.84%) cases (AN), respectively. The expression followed also a zonal pattern (Figure 3). The median values of positive tumor cells were 18.85% (B), 22.17% (AT) and 37.69% (AN), respectively. The results are depicted in Figure 4.

In our series, the cases positive for E-cadherin were as following: 24 (45.28%) (B), 21 (44.68%) (AT) and four (30.76%) (AN). Its expression had various degrees of intensity, within the same tumor and in separate cases. Overall, the median expression on the whole surface of the tumor was 44.45% for B, 27.82% for AT and 27.22% for AN. Some tumors exhibited strong focal positivity (Figure 5). The Ki67 labeling index was 3.8% (B), 13.42% (AT) and 18% (AN), respectively. Within the benign group, we found statistically significant correlations between the CD44 and CD54 expressions values ($p=0.0011$; $r=0.436$) (Figure 6) and CD44 with E-cadherin ($p=0.046$; $r=0.274$). A tendency toward parallel increase in the CD44 values with Ki67 expression was also obvious, even though not statistically valid ($p=0.057$; $r=0.261$).

In the *WHO* grade II series, only a parallel increase in CD44 and CD54 values was also statistically significant ($p=0.0113$; $r=0.366$). In the malignant category, however, the same parallel increase of the two adhesion molecules was not present. Here, the only association with statistical meaning was that of Ki67 decreasing in parallel with a greater expression of progesterone receptors (negative correlation) ($p=0.05$; $r=0.55$).

The progesterone receptors (PGR) were positive in 74 cases of the whole series (65.48%), with no gender differences. These comprised 32 (60.7%) benign, 35 (74.4%) atypical and seven (53.84%) malignant cases. The mean values of positive cells decreased with the histological grade: 24.9% (B), 22.83% (AT), and 15.38% (AN). On the other hand, no correlation appeared in relation with its expression and that of cell adhesion molecules. Remarkably, 30% of the hemangiopericytoma

cells and 20% of the fibrosarcoma cells were also positive for PGR.

It is to note that no gender differences were present for any of the studied markers, including progesterone receptors.

From the clinical point of view, from 53 benign meningiomas, only one (1.88%) recurred at four years interval after surgery. No particular expression of the studied molecules was noted in this patient. Of the 47 atypical patients, five (10.63%) recurred at two years interval after surgery. Of 13 high-grade cases, four (30.76%) recurred at one year interval (Figure 7).

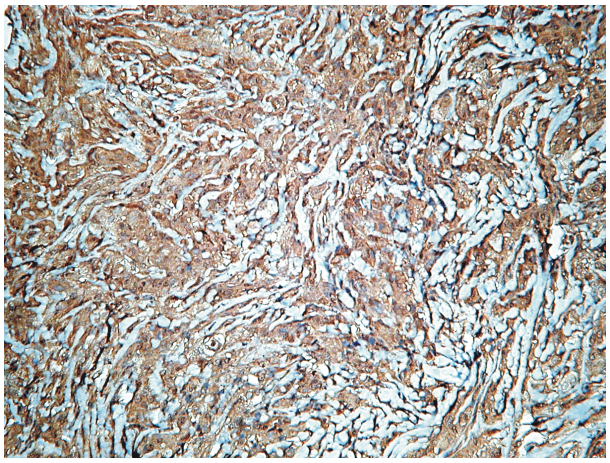

Figure 3 – ***CD54 is diffusely expressed in all the cells of this atypical meningioma. Immunohistochemistry, ×200.***

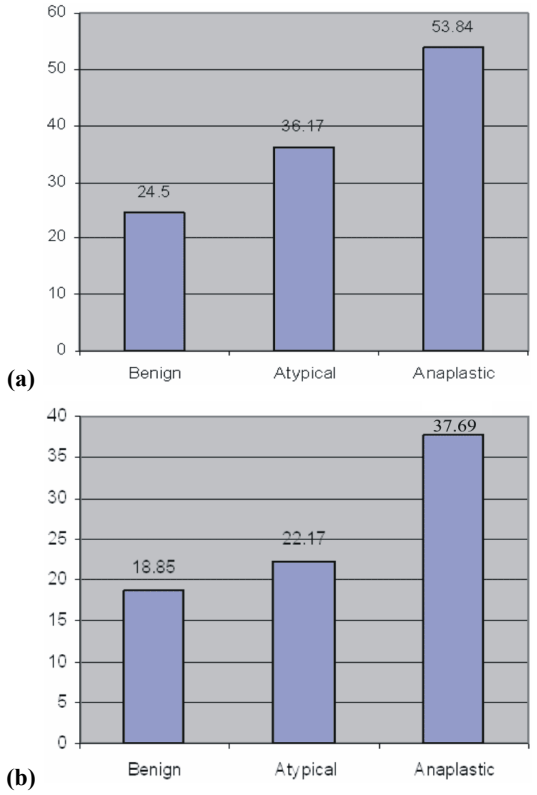

Figure 4 – ***The first diagram (a) shows the percentage of patients positive for CD54 in the three groups. The second one (b) represents the median surface area positive for CD54 in the three series and shows an obvious increase in values paralleling the higher WHO grade.***

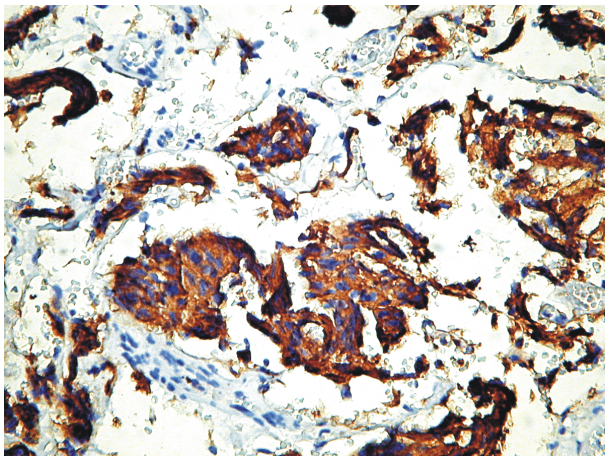

Figure 5 – ***E-cadherin shows strong positivity of meningothelial islands in a case of secretory meningioma. Immunohistochemistry, ×400.***

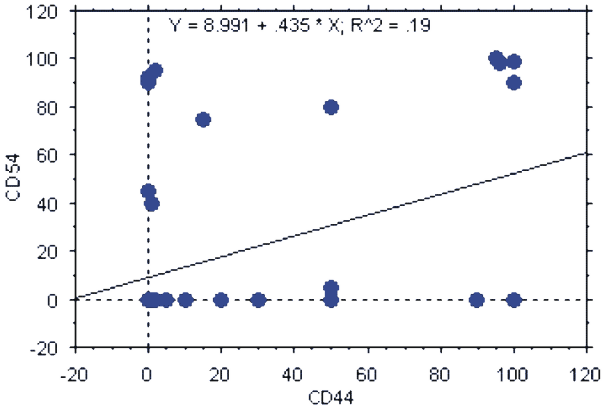

Figure 6 – ***The diagram shows a parallel increase in the CD44 and CD54 values in the benign series of meningioma.***

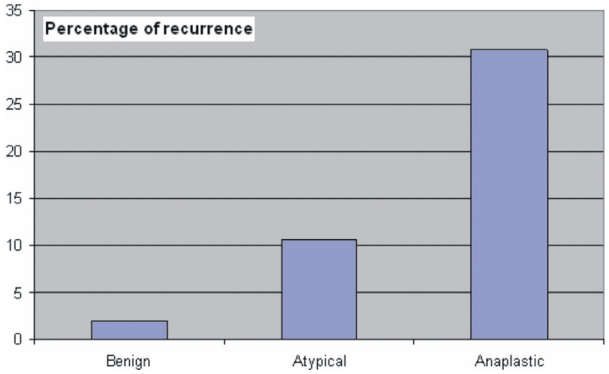

Figure 7 – ***Diagram showing the percentage of recurrent meningioma cases for the three grades of malignancy.***

It is noteworthy to emphasize that great variability exists between various regions of the same tumor regarding the expression of any of the studied markers. Large regions could be totally negative, while some remote ones are strongly positive for a given staining. Therefore, the results must be taken carefully as approximate ones, at least for meningiomas.

Discussion

Even if CD44 is considered as a marker for so-called tumor initiating cells in meningioma, which could promotes

tumorigenesis in this category [16], our findings did not find a parallel expression neither for the number of cases nor for the intensity of its staining related to the tumor grade.

Since CD54 was here for the first time assessed in meningiomas, we cannot make comparison with other similar findings. However, based on our data, the overall CD54 expression seems a seriously reliable diagnostic tool for predicting the overall clinical aggressiveness of the tumor. Its positivity showed massive increase regarding the number of cases paralleling the increase in tumor grade (53.84% in malignant *versus* only 24.5% in *WHO* I cases), as well as regarding the percentage of positive tumor cells (37.69% *versus* only 18.85%) for the same categories.

Some studies affirm a decline in the E-cadherin expression in meningioma parallel with an increase of anaplastic changes [17]. More recent studies either confirm this statement [18] while others invalidate it [19]. Nevertheless, recent studies affirm a total lack of correlation between the E-cadherin expression and the tumor grade [20]. Our findings tend to support mostly the first hypothesis, since both the number of cases positive for E-cadherin and also the stained tumor area decreased from *WHO* I category to *WHO* III one (45.28% to 44.68%, and 44.68% to 30.76%, respectively). Its stronger expression in low-grade tumors could explain their more benign behavior, despite the well-known tendency of meningiomas to invade adjacent structures regardless the tumor grade. The low percentage of E-cadherin expression in high-grade meningiomas of our series could be, however, a consequence, at least in part, of the smaller number of cases (n=13).

Our study found a significant negative correlation between progesterone receptors and Ki67 expression only in the malignant series of cases. This could not confirm other studies stating a negative correlation between PGR expression and the clinical aggressiveness in meningiomas [21]. The overall series showed only a discrete decrease in the PGR values, from 24.9% in benign cases to 15.38 in the anaplastic ones, without statistical significance. This is mostly different from recent studies, which found a drastic decrease between the same classes, from 96.8% to 0% [22]. The lack of differences between male and female patients in our series is in accord with other studies [23]. Even though lower values of PGR are recognized in higher-grade tumors [24], in our series only relatively small differences were noted as stated above. This could be explained by the relatively small cohort of high-grade patients in our study.

As Ki67 is considered an important factor in establishing the prognosis and recurrence of meningiomas [25], its determination appears as essential. Numerous studies are in accord with the relative major importance of determining Ki67 labeling index for meningiomas related to the clinical outcome [13, 26–28]. However, its accuracy is doubtful, since differences exist between laboratories, regarding the sample processing and even interpretation methods of the stained slides [29]. Due to this variability, a cutoff value should be established in each laboratory for the diverse meningioma categories [30].

Since great differences were obvious among various areas of the same tumor in the majority of cases for all the studied markers, both pathologists and neurosurgeons must be aware of this reality. The most accurate sampling and panel of antibodies used as possible for the former, as well as the most complete removal of the tumor for the latter must be the central goals in current practice. Additional markers should be added in order to obtain predictable data regarding the meningioma outcome [31].

Conclusions

The initial histological tumor grade was strongly associated with the recurrence-free interval since benign tumors recurred later and in a non-significant percentage, while the high-grade tumors recurred in a 15-fold percentage and in a much shorter interval. Additional markers to assess the overall clinical behavior proved to be Ki67 and progesterone receptors. CD54 expression however, with values increasing in parallel with increased anaplasia and histological grade, could be also thought as a valuable prognostic tool, but only in conjunction with Ki67 expression. E-cadherin expression was lower in higher-grade tumors and could be used as an additional prognostic marker. Further studies on larger series and targeting more adhesion molecules are needed for accurately predicting meningioma outcome.

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References

[1] 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(5):855–860.

[2] Naor D, Nedvetzki S, Golan I, Melnik L, Faitelson Y, *CD44 in cancer*, Crit Rev Clin Lab Sci, 2002, 39(6):527–579.

[3] Ponta H, Sherman L, Herrlich PA, *CD44: from adhesion molecules to signaling regulators*, Nat Rev Mol Cell Biol, 2003, 4(1):33–45.

[4] Naruse M, Shibasaki K, Yokoyama S, Kurachi M, Ishizaki Y, *Dynamic changes of CD44 expression from progenitors to subpopulations of astrocytes and neurons in developing cerebellum*, PLoS One, 2013, 8(1):e53109.

[5] Lun SW, Cheung ST, Cheung PF, To KF, Woo JK, Choy KW, Chow C, Cheung CC, Chung GT, Cheng AS, Ko CW, Tsao SW, Busson P, Ng MH, Lo KW, CD44+ cancer stem-like cells in EBV-associated nasopharyngeal carcinoma, PLoS One, 2012, 7(12):e52426.

[6] Perez A, Neskey DM, Wen J, Pereira L, Reategui EP, Goodwin WJ, Carraway KL, Franzmann EJ, *CD44 interacts with EGFR and promotes head and neck squamous cell carcinoma initiation and progression*, Oral Oncol, 2013, 49(4): 306–313.

[7] Lewy-Trenda I, Omulecka A, Janczukowicz J, Papierz W, *CD44 expression in human meningiomas: an immunohisto-chemical analysis*, Pol J Pathol, 2004, 55(1):33–37.

[8] Etienne-Manneville S, Chaverot N, Strosberg AD, Couraud PO, *ICAM-1-coupled signaling pathways in astrocytes converge to cyclic AMP response element-binding protein phosphor-ylation and TNF-alpha secretion*, J Immunol, 1999, 163(2): 668–674.

[9] Lossinsky AS, Mossakowski MJ, Pluta R, Wisniewski HM, *Intercellular adhesion molecule-1 (ICAM-1) upregulation in human brain tumors as an expression of increased blood-brain barrier permeability*, Brain Pathol, 1995, 5(4):339–344.
[10] Yap AS, Kovacs EM, *Direct cadherin-activated cell signaling: a view from the plasma membrane*, J Cell Biol, 2003, 160(1):11–16.
[11] Gumbiner BM, *Regulation of cadherin-mediated adhesion in morphogenesis*, Nat Rev Mol Cell Biol, 2005, 6(8):622–634.
[12] Howng SL, Wu CH, Cheng TS, Sy WD, Lin PC, Wang C, Hong YR, *Differential expression of Wnt genes, beta-catenin and E-cadherin in human brain tumors*, Cancer Lett, 2002, 183(1):95–101.
[13] Roser F, Samii M, Ostertag H, Bellinzona M, *The Ki-67 proliferation antigen in meningiomas. Experience in 600 cases*, Acta Neurochir (Wien), 2004, 146(1):37–44.
[14] Haberler C, Jarius C, Lang S, Rössler K, Gruber A, Hainfellner JA, Budka H, *Fibrous meningeal tumours with extensive non-calcifying collagenous whorls and glial fibrillary acidic protein expression: the whorling-sclerosing variant of meningioma*, Neuropathol Appl Neurobiol, 2002, 28(1):42–47.
[15] Kim NR, Im SH, Chung CK, Suh YL, Choe G, Chi JG, *Sclerosing meningioma: immunohistochemical analysis of five cases*, Neuropathol Appl Neurobiol, 2004, 30(2):126–135.
[16] Rath P, Miller DC, Litofsky NS, Anthony DC, Feng Q, Franklin C, Pei L, Free A, Liu J, Ren M, Kirk MD, Shi H, *Isolation and characterization of a population of stem-like progenitor cells from an atypical meningioma*, Exp Mol Pathol, 2011, 90(2):179–188.
[17] Schwechheimer K, Zhou L, Birchmeier W, *E-cadherin in human brain tumours: loss of immunoreactivity in malignant meningiomas*, Virchows Arch, 1998, 432(2):163–167.
[18] Utsuki S, Oka H, Sato Y, Kawano N, Tsuchiya B, Kobayashi I, Fujii K, *Invasive meningioma is associated with a low expression of E-cadherin and beta-catenin*, Clin Neuropathol, 2005, 24(1):8–12.
[19] Shimada S, Ishizawa K, Hirose T, *Expression of E-cadherin and catenins in meningioma: ubiquitous expression and its irrelevance to malignancy*, Pathol Int, 2005, 55(1):1–7.
[20] Brunner EC, Romeike BFM, Jung M, Comtesse N, Meese E, *Altered expression of beta-catenin/E-cadherin in meningiomas*, Histopathology, 2006, 49(2):178–187.
[21] Mukherjee S, Ghosh SN, Chatterjee U, Chatterjee S, *Detection of progesterone receptor and the correlation with Ki-67 labeling index in meningiomas*, Neurol India, 2011, 59(6):817–822.
[22] Shayanfar N, Mashayekh M, Mohammadpour M, *Expression of progestrone receptor and proliferative marker Ki67 in various grades of meningioma*, Acta Med Iran, 2010, 48(3): 142–147.
[23] Roser F, Nakamura M, Bellinzona M, Rosahl SK, Ostertag H, Samii M, *The prognostic value of progesterone receptor status in meningiomas*, J Clin Pathol, 2004, 57(10):1033–1037.
[24] Loussouarn D, Brunon J, Avet-Loiseau H, Campone M, Mosnier JF, *Prognostic value of HER2 expression in meningiomas: an immunohistochemical and fluorescence in situ hybridization study*, Hum Pathol, 2006, 37(4):415–421.
[25] Torp SH, Lindboe CF, Grønberg BH, Lydersen S, Sundstrøm S, *Prognostic significance of Ki-67/MIB-1 proliferation index in meningiomas*, Clin Neuropathol, 2005, 24(4):170–174.
[26] Nakasu S, Li DH, Okabe H, Nakajima M, Matsuda M, *Significance of MIB-1 staining indices in meningiomas: comparison of two counting methods*, Am J Surg Pathol, 2001, 25(4):472–478.
[27] Modha A, Gutin PH, *Diagnosis and treatment of atypical and anaplastic meningiomas: a review*, Neurosurgery, 2005, 57(3): 538–550; discussion 538–550.
[28] Bruna J, Brell M, Ferrer I, Gimenez-Bonafe P, Tortosa A, *Ki-67 proliferative index predicts clinical outcome in patients with atypical or anaplastic meningioma*, Neuropathology, 2007, 27(2):114–120.
[29] Willis J, Smith C, Ironside JW, Erridge S, Whittle IR, Everington D, *The accuracy of meningioma grading: a 10-year retrospective audit*, Neuropathol Appl Neurobiol, 2005, 31(2):141–149.
[30] Ho DM, Hsu CY, Ting LT, Chiang H, *Histopathology and MIB-1 labeling index predicted recurrence of meningiomas: a proposal of diagnostic criteria for patients with atypical meningioma*, Cancer, 2002, 94(5):1538–1547.
[31] Commins DL, Atkinson RD, Burnett ME, *Review of meningioma histopathology*, Neurosurg Focus, 2007, 23(4):E3.

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