Epithelial-glial transition in an atypical meningioma – a case report

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Abstract

Atypical meningiomas with a mixed glial-epithelial phenotype are rare reports, and here we described an aggressive case on which double immunofluorescence ascertained the co-expression of epithelial membrane antigen (EMA) with glial fibrillary acidic protein (GFAP) in the same tumor cells. A 62-year-old female presented with acute intracranial hypertension symptoms occurred over the last 24 hours, muscle weakness on the right side, cerebellar ataxia, and diffuse base. Magnetic resonance imaging (MRI) examination showed a right cerebellar hemisphere non-homogeneous tumor, with intense gadophylia, diffuse contours, and necrotic inner areas. There were also scar-like areas at the level of the left cerebellar hemisphere, and the patient recalled a previous surgical intervention at the age of 6 years old without further diagnostic data. The patient suffered an ischemic event in the brain stem and died shortly after the surgical removal of the tumor. Histopathology revealed an epithelial-like tumor with moderately pleomorphic and elongated cells arranged in fascicles, rare necrotic areas, and a high Ki-67 proliferating index of around 40%. The case was deemed as an atypical meningioma, and interestingly, a double IHC for GFAP/EMA revealed a strong colocalization of the two markers in the tumor mass. Although extremely rare, the reports of meningiomas expressing a mixed epithelial/gliarial profile might be connected with their aggressive evolution. Double IHC might help in predicting the evolution of these cases and determine which patients should benefit from closer surveillance.

Keywords: atypical meningioma, mixed gliarial-epithelial phenotype, aggressive evolution, recurrence.

Introduction

Meningiomas are the most frequent primary tumors of the central nervous system, accounting for 13–26% of all primary benign tumors of brain [1].

Typical, most tumors are benign, usually well-circumscribed, with the base oriented against the dura, however grade III malignant forms infiltrate the brain parenchyma [1]. Grade II atypical forms involve mostly non-skull base areas, affect mostly the males, and show a recurrent evolution after surgery [2]. In grade I meningiomas, microscopy is characterized by a wide variety of histological patterns ranging from uniform epithelioid cells organized in parallel sheets, with lobular growth patterns and psammoma bodies, spindled cells with whorl formation and storiform disposition, various densities of blood vessels and microcystic spaces [3]. Atypical forms are invasive in the surrounding neuropil, have an increased cellularity, contain small cells with increased nuclear-to-cytoplasmic ratios, prominent nucleoli, loss of lobular architecture, necrosis and an increased number of mitoses.

Rare variants have been described to exhibit oncocytic, sclerosing, mucinous or glial fibrillary acidic protein (GFAP)-positive phenotypes.

The most specific immunohistochemical markers for meningioma are represented by antibodies raised against the epithelial membrane antigen (EMA) and for the SSTR2A somatostatin receptor; with all EMA negative meningiomas expressing SSTR2A and vice versa [4]. In the tumor cells, EMA shows a membranous pattern, and basically EMA/vimentin positivity and lack of GFAP expression distinguishes meningiomas from glial-lineage tumors [5]. Atypical meningiomas retain usually, to different extents, vimentin and EMA reactivity. Recurrence rates after surgery have been reported to variate between 7–40% for World Health Organization (WHO) grade II forms, and up to 90% for grade III tumors, but without a clear-cut correlation between the histopathology data and the aggressivity profile [6–9].

In the present study, we describe a patient with an aggressive and possible a long-time evolution, for whom along with the clinical and imaging data, we have performed a detailed immunohistochemical profiling of the tumor.

Case presentation

Clinical data and surgery

A 62-year-old woman was admitted to the Department of Neurosurgery (Emergency County Hospital, Craiova, Romania) with acute intracranial hypertension symptoms occurred over the last 24 hours, muscle weakness on the right side, cerebellar ataxia, and diffuse base. Magnetic resonance imaging (MRI) examination showed a right cerebellar hemisphere non-homogeneous tumor, with intense gadophylia, diffuse contours, and necrotic inner areas. There were also scar-like areas at the level of the left cerebellar hemisphere, and the patient recalled a previous surgical intervention at the age of 6 years old without further diagnostic data. The patient suffered an ischemic event in the brain stem and died shortly after the surgical removal of the tumor. Histopathology revealed an epithelial-like tumor with moderately pleomorphic and elongated cells arranged in fascicles, rare necrotic areas, and a high Ki-67 proliferating index of around 40%. The case was deemed as an atypical meningioma, and interestingly, a double IHC for GFAP/EMA revealed a strong colocalization of the two markers in the tumor mass. Although extremely rare, the reports of meningiomas expressing a mixed epithelial/gliarial profile might be connected with their aggressive evolution. Double IHC might help in predicting the evolution of these cases and determine which patients should benefit from closer surveillance.
Romania) with headache, vomiting and respiratory insufficiency, symptoms that had occurred in the previous 24 hours. It seems that the patient had suffered a previous surgical intervention at the level of the lower right occipital area (at the age of 6 years old), but without any available diagnostic data, except that she did not follow any treatment at that time. Clinical evaluation revealed a hypertensive patient with muscle weakness of the right limbs, cerebellar dysarthria, dysmetria for the right arm and foot, wide base gate, together with signs of intracranial hypertension.

A cranial magnetic resonance imaging (MRI) was next performed and revealed on T2 and T1 modes, a right cerebellar hemisphere irregular mass of 48.7×38.4×41.9 mm, non-homogenous T2 hyperintense, non-homogenous T1 hypointense, with non-homogenous and intense gadophylia (Figure 1). Tumor adhered to the tentorium cerebelli, which was also intensely gadophyl. Moreover, there were also two scar-like cystic areas at the level of the left cerebellar hemisphere, of 46.7×29.2 mm and respective of 25.4×14.6 mm, probably a result of the previous surgical intervention (Figure 1). Overall, moderate cerebral and cerebellar atrophy was noted.

A written informed consent was obtained from the patient regarding the presentation and publication of her pathology data.

Figure 1 – MRI data indicated a tumor in the right cerebellar hemisphere, non-homogenous T2 hyperintense (a), non-homogenous T1 hypointense (b–f), with intense gadophylia and involvement of the tentorium cerebelli (arrowheads). The tumor was infiltrative, with necrotic inner areas. Besides general telencephalic cortical atrophy, there were also two scar-like cystic areas in the left cerebellar hemisphere, probably following the previous surgical intervention (arrows).

Surgical removal was decided, and during the intervention, the proliferation proved to be adherent to the meninges, and without a clear demarcation edge towards the depth of the cerebellar brain matter.

Histopathology

All tissue blocks obtained after the extemporaneous examination and during the autopsy were fixed in neutral buffered formalin and processed for paraffin embedding, sectioning as 4 μm-thick sections, Hematoxylin–Eosin (HE) staining and enzymatic immunohistochemistry (IHC) in the Department of Pathology of the University of Medicine and Pharmacy of Craiova, Romania. Further on, double fluorescence IHC was performed in the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova.

For single IHC, Leica-Biosystems (Linford Wood, UK) [cluster of differentiation (CD) 31, CD68, epidermal growth factor receptor (EGFR), Ki-67, p53, vimentin – ready to use] and Dako (Glostrup, Denmark) [rabbit anti-GFAP, 1:30.000; mouse anti-EMA (E29), 1:100; mouse anti-cytokeratin (CK) AE1/AE3, 1:100; mouse anti-CK 8/18 (EP17/EP30), 1:50; mouse anti-progesterone receptor (PrgR), 1:50] diagnostic-grade antibodies together with the rabbit anti-oligodendrocyte transcription factor-2 (Olig-2) (Sigma Aldrich, 1:500) antibody were utilized together with the Leica Bond Polymer Refine Detection System, in a Leica Bond-Max automated immunostainer. For double immunofluorescence, the slides were manually processed for dewaxing, re-hydration, antigen retrieval (microwaving in 10 mmol/L citrate buffer, pH 6 for 21 minutes at 650 W), blocking of unspecific antigenic sites [30 minutes incubation in 5% skimmed milk (Bio-Rad, Watford, UK)], and overnight incubation (4°C) with both
tumoral neuropil, but also in some tumor cells. Ki-67 reactive glial cells. IHC for GFAP, however, revealed cytoplasm of the tumor cells (Figure 2). Vimentin was tumor cells, with mostly a diffuse granular pattern in the tumor mass, almost all EMA/GFAP signal colocalized with EMA, but with the most of the tumor areas and the diffuse EMA/CK 8/18 positive, EGFR/Olig-2 negative immunophenotype, the tumor was considered an atypical meningioma with GFAP co-expression.

Five hours after the surgery, the patient died following an ischemic event at the level of the brainstem, most probably occurred as a consequence of atheromatosis and hypertension, combined with post-operatory stress, despite the anti-coagulant therapy.

Discussion

Atypical meningiomas are more aggressive and are progressing faster compared to typical meningiomas that have a relatively good prognosis; local recurrences in atypical meningioma occur frequently, despite optimal surgery. Predictive factors of tumor recurrence are represented by proliferative markers, age, histological grade, and extend of resection.

Meningiomas are generally considered as originating in the arachnoid cap cells of the arachnoid granulations, and they present an epithelial-mesenchymal phenotype, the tumor cells being typically positive for both EMA and vimentin [10]. Hemangiopericytomas are EMA-negative and this helps in differentiating them from meningiomas. Most meningiomas exhibit a membrane and diffuse cytoplasmic reactivity for EMA, for many of the meningothelial and transitional forms, while in less differentiated forms the staining seems to be decreased and shifted towards the cytoplasm, or even absent [10, 11]. A heterogeneous staining pattern was also identified in the present case, with most cells exhibiting a diffuse cytoplasmic staining, but with some cells still retaining the membrane staining. Other authors have identified EMA expression for their patients, without any significant difference between typical and atypical meningiomas, however its expression seems to be lower in tumors located in supratentorial regions compared to infratentorial tumors [12].

The double positivity of meningiomas and arachnoid cap cells to both epithelial (EMA, and sometimes CKs) and mesenchymal markers (vimentin) reflects most probably that there is a mixed mesenchymal-epithelial pattern of these tissue.

The present case did show positivity to CK 8/18, and we have also utilized deconvolution double IHC to show that in the tumor cells EMA is colocalized with GFAP. GFAP is an intermediate filament protein expressed especially by astrocytes, which increases in most of the specific and non-specific brain injuries, and which is also expressed by some other cells like fibroblasts, liver stellate cells, Schwann cells or enteric glia [13–15].

There are a few reported cases in the literature that postulated the existence of EMA/GFAP double positive cells in meningioma, but none have utilized colocalization techniques to ascertain it, and none of these cases was in an infratentorial area [15–17]. Some of these cases have been reported to express CKs [15], while some seemed to be negative [16].

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Primary antibodies (GFAP/EMA). Next day, after thorough washing in phosphate-buffered saline (PBS), the sections were incubated for 30 minutes with a mixture of goat-anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Fluor 596 secondary antibodies (1:300, Thermo Fisher Scientific, Waltham, United States). After washing, the slides were coveredslipped with a 4,6-diamidino-2-phenylindole (DAPI)-containing medium (Vectashield, Vector Laboratories Ltd., Peterborough, UK).

Light microscopy and fluorescent images were grabbed utilizing a Nikon Eclipse 90i motorized microscope (Nikon CEE GmbH, Vienna, Austria) equipped with a 16-megapixel Nikon DS-R2 complementary metal-oxide semiconductor (CMOS) camera, together with the Nikon NIS-Elements image analysis software. Fluorescence images were obtained by consecutive scanning of each channel with highly selective custom-made filters in order to eliminate the cross-bled of the fluorophores and to provide a reliable visualization for DAPI, Alexa 488, and Alexa 594 spectra (Chroma Technology Corp., Bellows Falls, USA). For colocalization analysis, all images were stored in Nikon’s proprietary format, then they were subjected to a blind deconvolution algorithm in NIS Elements software.

Histopathology described the tumor as being composed of areas of epithelial-like spindle cells with eosinophilic cytoplasm, and moderate-to-low pleomorphism, with only rare mitotic figures (Figure 2). These cells were arranged as fascicles, sometimes with a storiform pattern. A few necrotic areas could be identified, but without surrounding palisading tumor cells, and no psammoma bodies could be described either. A few proliferating-like vessels were present in the surrounding neuropil, with multiple layers of hyperplastic endothelial cells, but without glomeruloid features found in a classical glioblastoma.

A staining for EMA showed variable positivity in the tumor cells, with mostly a diffuse granular pattern in the cytoplasm of the tumor cells (Figure 2). Vimentin was intensely expressed by both the tumor cells and the reactive glial cells. IHC for GFAP, however, revealed intense staining in the reactive astrocytes from the peritumoral neuropil, but also in some tumor cells. Ki-67 was variable positive, with an average proliferative index at approximately 40%. The staining for CK AE1/AE3 was completely negative in all the tissue fragments, but interestingly some of the tumor cells exhibiting an epithelioid-looking pattern were labeled by the anti-CK 8/18 antibody. The p53 marker showed a weak expression in many of the tumor cells (~30%), with only rare cells being PrpR positive (~5%). EGFR was completely negative, the tumor cells did not pick up CD68 nor Olig-2, and a Periodic Acid–Schiff (PAS) staining revealed no PAS-positive granules in the cytoplasm of the cells.

Interestingly, a double immunofluorescence study for EMA/GFAP showed that in the tumor mass, almost all GFAP signal colocalized with EMA, but with the most intense EMA-stained cells being negative for GFAP (Figure 3).

As there was no typical necrosis with palisading tumor cells, no glomeruloid vascular proliferations, but instead an epithelioid pattern together with variate immunopositivity for GFAP, EMA, CK 8/18 and PrpR, a diagnostic of glioblastoma could not be supported. There was no genetic data available but the family did not recall any prior brain tumor pathology among their relatives. Given the fascicular-epithelioid pattern of most of the tumor areas and the diffuse EMA/CK 8/18 positive, EGFR/Olig-2 negative immunophenotype, the tumor was considered an atypical meningioma with GFAP co-expression.

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Figure 2 – Histopathology and immunohistochemistry data: (a) The tumor contained areas of necrosis without peripheral palisading (HE), with (b and c) moderate pleomorphism and epithelial-like spindle cells, and with few (d) proliferating-like vessels (HE), was variable positive for EMA (e and f), intensely positive for vimentin (g), with intra-tumor GFAP strong reactivity (h), high Ki-67 index (i), reduced p53 immunopositivity (j), with tumor cells moderately positive for CK 8/18 (k) but negative for CK AE1/AE3 (data not shown), with rare PrgR-positive cells (l), with Olig-2 expression only in the remnant interspersed oligodendrocytes (m), with no PAS+ inclusions in the tumor cells (n), with vascular lumens (CD34, o), with a proliferating profile (Ki-67, p) [(a) ×40; (b–d) and (f–p) ×400; (f1 and l1) insets, ×1200; (e) ×200]; Insets (f1 and l1) represent the enlarged region of interest. HE: Hematoxylin–Eosin; EMA: Epithelial membrane antigen; GFAP: Glial fibrillary acidic protein; CK: Cytokeratin; PrgR: Progesterone receptor; Olig-2: Oligodendrocyte transcription factor-2; PAS: Periodic Acid–Schiff; CD34: Cluster of differentiation 34.
Here we have found a moderate expression of CK 8/18, with a complete negativity for most of the rest of the CK spectrum. Furthermore, the complete lack of reactivity for CD10 and Olig-2 in the tumor cells helped in ruling out a metastatic brain carcinosarcoma or an oligodendrogial origin. Although present in a few cells only in our case, PgrR immunopositivity was also consistent with the diagnostic of meningioma. However, it was interesting to note that PgrR reactivity was restricted to a very small number of cells, and in some instances, it seemed to be both nuclear and cytoplasmic. As GFAP expression has been identified in non-glial compartments, it can also be conceived that complex mesodermal-ectodermal differentiation can occur at this level too. Moreover, some studies have reported contradictory data and variable CK expression in malignant meningiomas compared to benign variants [10, 18]. Considering the fact that tumor cells are highly dynamic, it can be that epithelial-to-mesenchymal transition could occur and play an important role in the evolution of meningiomas too, possibly inducing a more aggressive phenotype [19].

In typical meningiomas and even in reported atypical cases, EGFR and its ligands epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) seem to have high expression levels [20]. However, there have been reported benign, atypical and malignant meningioma cases that do not express EGFR, or at least not at the levels necessary for detecting them by IHC [21]. Furthermore, lack of EGFR expression has been linked with an aggressive behavior, and a negative prediction factor of survival in patients with atypical forms [22]. In the present case, EGFR was negative, and the average Ki-67 index and the positivity for p53 paralleled its aggressive behavior. On the other hand, the relatively low numbers of cells immunolabeled for p53 together with the CD10 negativity also helped in ruling out a gliosarcoma from the differential diagnostic panel.

Although no genetic data was available for this patient, except the lack of other known nervous tumor cases within the family, and although we did not have the diagnostic of the first surgical event, the late onset of this aggressive tumor phenotype still raises the question if this behavior could have occurred gradually after an initial less aggressive phenotype.

Conclusions

For patients with aggressive meningioma, it would be of great interest to assess the immunophenotypic profiles of these tumors, and to evaluate if increasing glial/epithelial co-expression might support/predict an increased aggressivity.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors’ contribution

Mihaela Carmen Pătruleasa & Otilia Clara Mărgăritescu have contributed equally to this work.

References


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