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Primary ectopic meningiomas: Report of 6 cases with emphasis on atypical morphology and exploratory immunohistochemistry

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Summary. Aims. To investigate the histological and immunohistochemical features of primary ectopic meningiomas (PEMs), especially those of primary ectopic atypical meningiomas (PEAMs).

Methods and results. We examined 6 cases of PEM, including 2 PEAM cases, which occurred separately in left nasal cavity, left lower lung, right neck, left orbit, right upper lung, and left upper lung by histological and immunohistochemical analysis. In general, of the 6 PEM cases analyzed, 4 cases exhibited morphology of Grade I, including 1 fibrous, 1 meningothelial, and 2 transitional variant. The remaining 2 cases shared similar atypical morphology of Grade II. The tumors were distributed in sheet-like patterns with loss of architecture of classic meningiomas. Significant hypercellularity, multi-focal necrosis, and thin-walled blood vessels were identified. The mitotic figures were estimated at 6 per 10 high-power fields in one case, and 8 mitotic figures in another. Immunohistochemically, the 6 PEM cases were all positive for Vimentin and EMA, while none showed immunostaining for CKpan, S-100, CD34, STAT6, SMA, Syn or Bcl-2. 4 PEM cases of Grade I were immunoreactive for PR but negative for P53, while the 2 PEAM cases displayed negative staining for PR but positivity for P53. As for Ki-67, the positive staining of 4 Grade I cases was no greater than 2%, while the positive rates of the 2 PEAM cases were 10% and 20%.

Conclusions. Our study has expanded cases of PEMs, especially the 2 PEAM cases in rare sites. Our study has also further summarized the pathological features of PEMs, focusing on the histological features of PEAMs, and the immunohistochemical features worthy of further investigations.

Key words: Meningiomas, Atypical mengiomas, Primary ectopic meningiomas, Immunohistochemistry

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Introduction

Meningiomas are one of the most common neoplasms of the central nervous system, accounting for approximately 18% of primary intracranial neoplasms, and 25% of intraspinal neoplasms. Meningiomas are generally benign and slow-proliferating with a 1.5:1 female/male ratio in intracranial sites, and a 10:1 female/male ratio in intraspinal sites (Foda et al., 2020).

In accordance with the descriptions by WHO 2016 standards (Wen and Huse, 2017), meningiomas are divided into three subtypes: benign (Grade I), atypical (Grade II), and malignant/anaplastic (Grade III). Grade I includes meningothelial, fibromatous, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmocytic, and metaplastic meningiomas with the absence of brain invasion and rare mitosis figures. Grade II, including atypical, clear cell, and chordoid meningiomas, represents an intermediate degree between Grade I and Grade III. Of Grade II, atypical meningiomas are defined when revealing 4 or more mitotic figures per 10 high-power fields, and/or 3 or more of the following clues: presence of increased cellularity, pattern-less sheet growth, prominent nucleoli, small cells and necrosis. Grade III includes rhabdoid, papillary, and anaplastic meningiomas, which display intense anaplasia and a high mitotic index (Salles et al., 2021). As described above, histopathological findings are particularly important for grading meningiomas. Indeed, immunohistochemistry has also been the routine practice for pathological diagnosis and differential diagnosis of meningiomas for decades. But the study of predictive immunological and genetic alterations,

especially manifesting neoplastic characteristics, behaviors and proliferative capacities, should be considered when assessing the diagnosis of Grade I, II and III. In the previous literature, it has been reported that the grade of primary intracranial or intraspinal meningioma may be positively correlated with P53 and Ki-67, and negatively correlated with progesterone receptor (PR) (Marcos et al., 2018; Küçükosmanoğlu et al., 2022). Yet it has not been systematically involved in primary ectopic meningiomas (PEMs).

Epidemiologically, PEMs are rare with the incidence ranging from 0.9% to 2.0% of all meningiomas, and also more frequently identified in females (Lu et al., 2015). These tumors mostly occur within the head and neck region, while other uncommon sites of involvement include lung, mediastinum, skin, retroperitoneum, muscle, etc (Aiyer et al., 2013; Yang et al., 2015; Kishore et al., 2017; Huang et al., 2018; Ohashi-Nakatani et al., 2019; Singh et al., 2022; Hu et al., 2022). The morphological and immunohistochemical features of PEMs are considered to be similar to those of primary intracranial or intraspinal meningiomas. PEMs with atypical morphology, that is, primary ectopic atypical meningiomas (PEAMs) are exceedingly rare. Up to now, there are only few case reports of PEAMs, or even primary ectopic malignant meningiomas (Mogi et al., 2012; Murakami et al., 2019; Anderson et al., 2022).

Therefore, in order to further understand the pathological features, we performed a systematic retrospective study on 6 PEMs with emphasis on atypical morphological features and investigative immunohistochemical characteristics.

Materials and methods

Cases

This is a retrospective study, in which 6 PEMs were retrieved from 619 meningioma cases which were diagnosed between January 2012 and January 2021 at the Department of Pathology in the First Affiliated Hospital of Soochow University. The slides stained with Hematoxylin & Eosin (H&E) were reviewed by two experienced investigators, and clinical histories were obtained whenever available. This study was approved by the Institute Research Ethics Committee of the First Affiliated Hospital of Soochow University.

Immunohistochemistry

Tumor tissues were fixed in 10% formalin, and embedded in paraffin. 3 μm thick sections were subjected to immunohistochemical analysis using antibodies as follows: Vimentin (V9), epithelial membrane antigen (EMA) (E29), progesterone receptor (PR) (MX009), cytokeratin (CKpan) (AE1/AE3), S-100 (4C4.9), CD34 (QBEnd/10), STAT6 (polyclonal); smooth muscle actin (SMA) (1A4), synaptophysin (Syn) (SP11), Bcl-2 (8C8), P53 (DO-7), and Ki-67 (MIB-1). The immunoreaction was performed using the labeled streptavidin-biotin method and overnight incubation. 3,3'-Diamino-benzidine was used as the chromogen. The antibodies were all purchased from Maixim Biotechnology Company of China.

The immunoreactivity was evaluated in a semiquantitative manner. For each antibody, a minimum of 100 tumor cells were examined, and the resulting score was calculated by multiplying the staining intensity (0=no staining; 1=mild staining; 2=moderate staining; 3=strong staining) by the percentage of immunoreactive tumor cells (0-100). Immunostaining was considered 0 or negative when the score was <25; 1+ or weak when 26-100; 2+ or moderate when 101-200; 3+ or strong when 201-300.

Results

Clinical features

The clinical characteristics are summarized in Table 1. There were 6 PEM affected individuals ranging in age

Table 1. Clinicopathologic characteristics of 6 PEMs.

Case	Age/Sex site	Tumor symptom	Clinical (cm)	Tumor size (month)	Follow-up
1	37/F	Left nasal cavity	Nasal bleeding	1.2	86, NED
2	49/M	Left lower lung	Asymptomatic	0.7	82, NED
3	38/F	Right neck	Neck mass	1.5	10, NED
4	76/M	Left orbit	Blurred vision	2.0	6, R
5	47/F	Right upper lung	Asymptomatic	0.4	32, NED
6	52/F	Left upper lung	Cough	0.6	8, NED

F, female; M, male; Neg, negative; NED, no evidence of disease; R, recurrence.

Table 2. Immunohistochemical features of 6 PEMs.

	Markers										
Case	Vimentin	EMA	CKpan	S-100	CD34	STAT6	SMA	Syn	Bcl-2	PR	P53
1	3+	2+	-	-	-	-	-	-	-	3+	-
2	2+	3+	-	-	-	-	-	-	-	2+	-
3	2+	3+	-	-	-	-	-	-	-	-	1+
4	3+	1+	-	-	-	-	-	-	-	-	2+
5	3+	1+	-	-	-	-	-	-	-	2+	-
6	3+	2+	-	-	-	-	-	-	-	2+	-

from 37 to 76y (mean, 49.8y; median, 48y), and female to male ratio was 2:1. Cases occurred separately in left nasal cavity, left lower lung, right neck, left orbit, right upper lung, and left upper lung. Due to differences in locations, these patients had different clinical manifestations, from asymptomatic to nasal bleeding, neck mass, blurred vision, and cough. Tumors in the nasal cavity, neck, and orbit (Cases 1, 3, 4) were detected by enhanced MRI, and those in lungs (Cases 2, 5, 6) were detected by enhanced CT. They were all confirmed by imaging examination to have no intracranial or intraspinal mass, and were treated with surgery in the initial treatment without any postoperative radiotherapy or chemotherapy. During the follow-up period, 5 PEM cases (Cases 1, 2, 3, 5, 6) were alive with no evidence of disease, while 1 case (Case 4) was alive with recurrence at the original site detected by enhanced MRI at a six-month follow-up after surgery. This patient underwent radiotherapy after the second operation, and his current physical condition was fair.

Morphological findings

Macroscopically, tumor sizes ranged from 0.4 to 2.0cm (mean, 1.1cm; median, 1.0cm) in diameter. Masses of 3 cases (Cases 1, 3, 4) were oval-shaped, and the cut surfaces were homogenously solid with yellowish-white color, while the remaining 3 (Cases 2, 5, 6) were mainly grayish-white irregular nodules in gross.

Histopathologically, 4 cases (Cases 1, 2, 5, 6) exhibited morphology of GradeI, including 1 fibrous (Case 1), 1 meningothelial (Case 5), and 2 transitional variant (Cases 2, 6). The tumor of fibrous variant was composed of spindle cells arranged in parallel or staggered bundles with abundant reticular and collagen fibers. Mineralizations could be focally identified (Fig. 1A). Tumors of meningothelial variant displayed whorl formations with meningeal endothelial-like cells. The

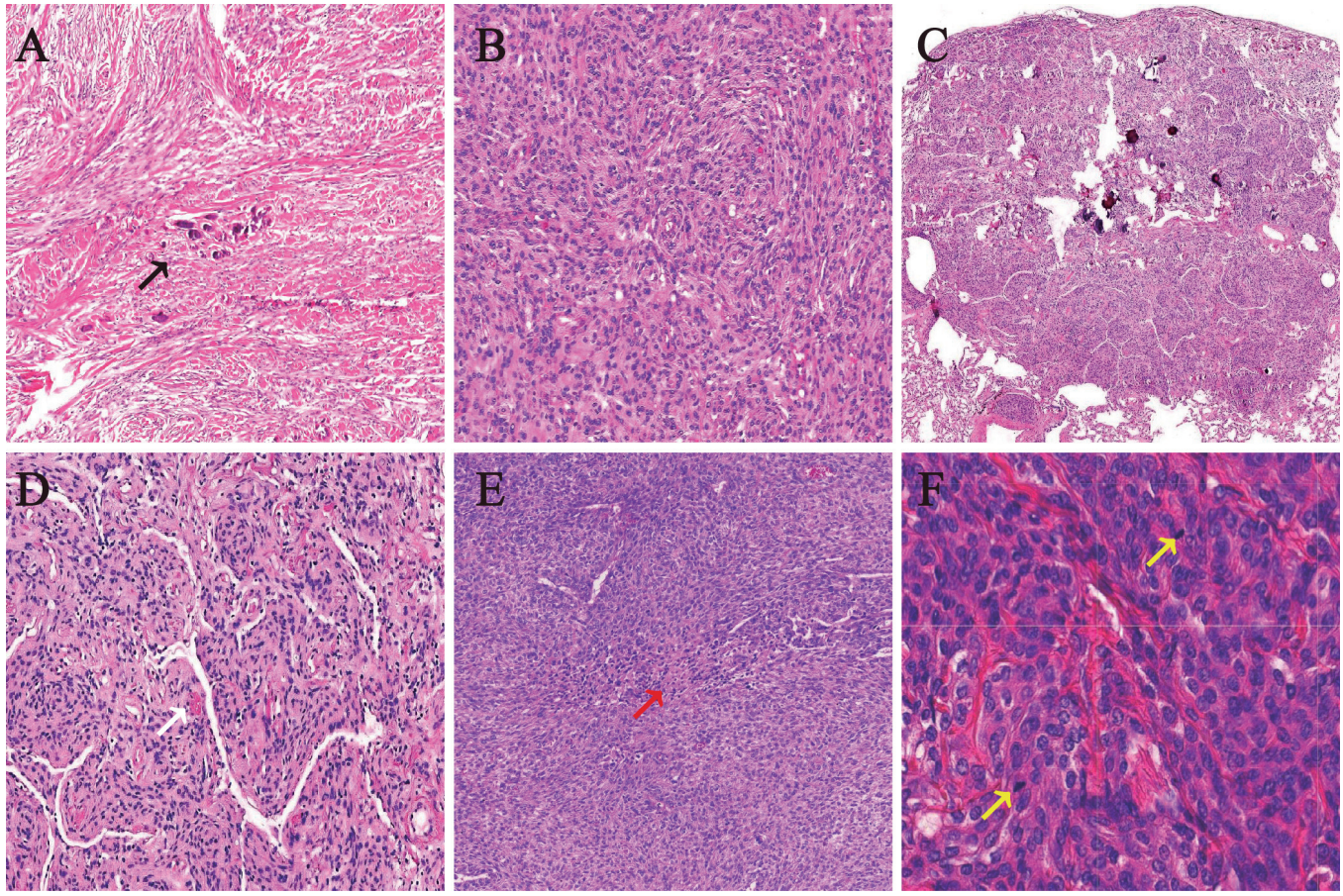

Fig. 1. A. The tumor of fibrous variant was composed of spindle cells arranged in parallel or staggered bundles with abundant reticular and collagen fibers. Mineralizations could be focally identified (black arrow). **B.** The tumor of meningothelial variant displayed whorl formations with meningeal endothelial-like cells. **C, D.** Tumors of transitional variant were composed of well-differentiated cells also arranged in whorl patterns, and distributed around the small central blood vessels (white arrow), mixed with lymphocyte infiltrates. **E, F.** the tumors of 2 PEAM cases were distributed in sheet-like patterns with loss of architectures of classic meningiomas. Significant hypercellularity, multi-focal necrosis (red arrow), and thin-walled blood vessels were identified. The epithelioid tumor cells with moderate atypia showed abundant cytoplasm, vacuolated nuclei, and prominent nucleoli. The mitotic figures were estimated at 6 and 8 per 10 high power fields, respectively (yellow arrow). H&E. A, B, D, E, × 100; C, × 40; F, × 400.

tumor cells were characterized by eosinophilic cytoplasm, and oval nuclei with inconspicuous nucleoli (Fig. 1B). Tumors of transitional variant both occurred in lungs with no capsule but well-defined borders. Focal mineralizations could be seen as well (Fig. 1C). The well-differentiated tumor cells were also arranged in whorl patterns, and distributed around the small central blood vessels, mixed with lymphocyte infiltrates (Fig. 1D). In addition, 2 cases (Cases 3, 4) shared similar atypical morphology of Grade II. The tumors were distributed in sheet-like patterns with loss of architectures of classic meningiomas. Significant hypercellularity, multi-focal necrosis, and thin-walled blood vessels were identified under a higher

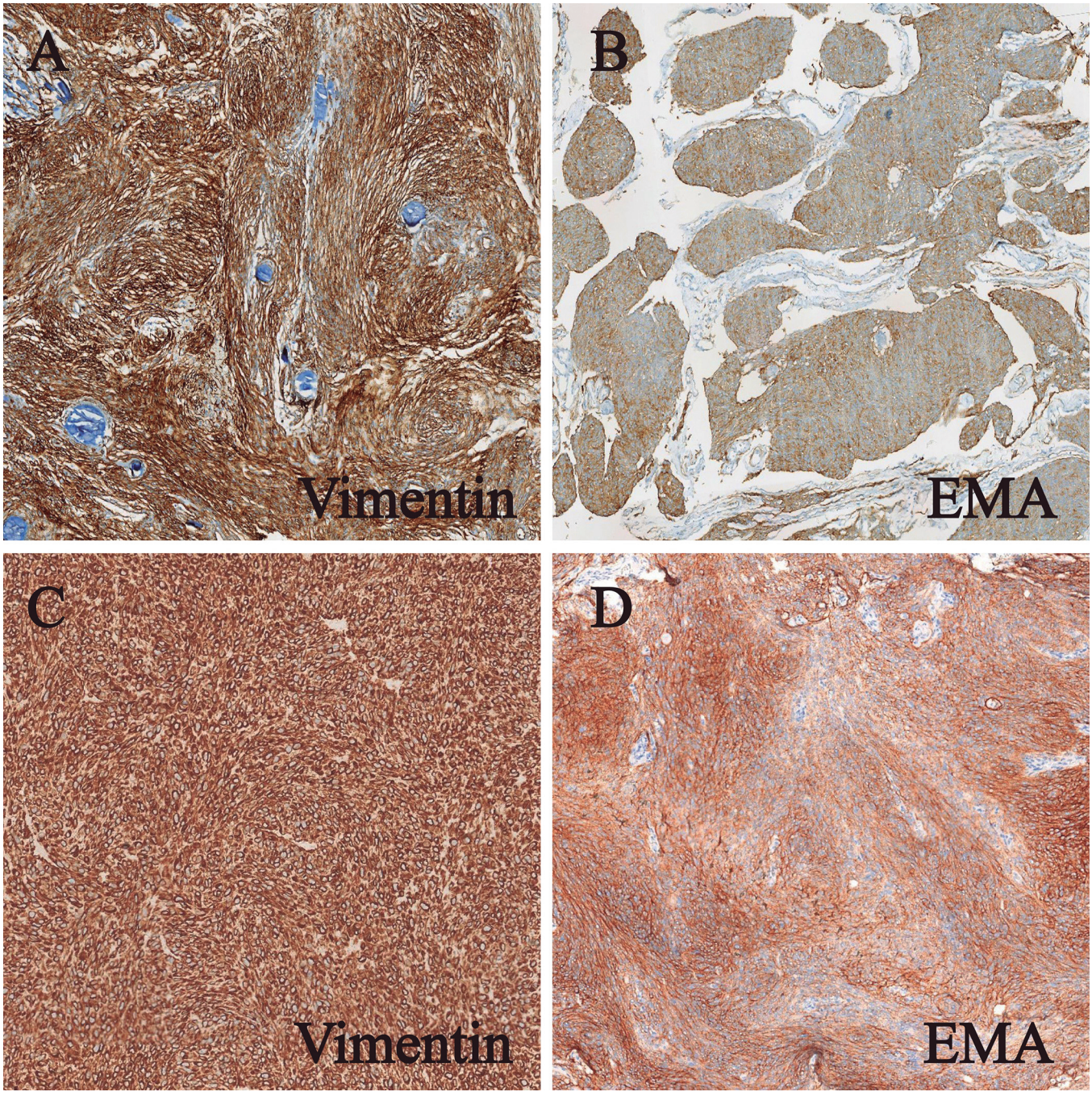

Fig. 2. A, B. 4 PEM cases of Grade I morphology were all immunoreactive for Vimentin, and EMA. **C, D.** Both PEAM cases were moderately (2+) to strongly (3+) positive for Vimentin, while EMA staining was strongly (3+) positive in one case and weakly (1+) positive in the other. × 100.

magnification (Fig. 1E). The epithelioid tumor cells with moderate atypia showed abundant cytoplasm, vacuolated nuclei, and prominent nucleoli. The mitotic figures were estimated at 6 per 10 high-power fields in one case (Case 3), and 8 mitotic figures in another (Case 4) (Fig. 1F).

Immunohistochemical analysis

The immunohistochemical features are shown in Table 2. The 6 PEM cases were all moderately (2+) to strongly (3+) immunoreactive for Vimentin (Fig. 2A,C). 4 of 6 PEMs were moderately (2+) to strongly (3+) positive for EMA (Fig. 2B,D), when the other 2 revealed weak (1+) positivity. Meanwhile, 4 cases of Grade I morphology were moderately (2+) to strongly (3+) positive for PR (Fig. 3A), but 2 PEAM cases displayed negative staining (Fig. 3D). Conversely, 4 cases of Grade I were all negative for P53 (Fig. 3B), while 1 was weakly (1+) positive and 1 moderately (2+) positive in 2 PEAM cases (Fig. 3E). In addition, no cases demonstrated immunoreactivity for CKpan, S-100, CD34, STAT6, SMA, Syn or Bcl-2.

As for Ki-67 staining, the positive rates of 2 PEAM cases were 10% and 20% (Fig. 3C), when the positive staining of the other 4 was no greater than 2% (Fig. 3F).

Discussion

Meningiomas are one of the commonest neoplasms of the central nervous system, derived from arachnoid cells of meninges. They are often clinically indolent and morphologically heterogeneous. Conversely, PEMs are rare tumor entities with an incidence from 0.9% to 2.0% of all meningiomas (Lu et al., 2015). The origin has been debated, but PEMs have been more understood as arising from ectopic meningothelial cells abnormally displaced during embryonal development, that is why the morphological and immunohistochemical characteristics of PEMs are similar to those of intracranial or intraspinal counterparts (Huang et al., 2018a,b; Xu et al., 2018; Cimini et al., 2019; Ohashi-Nakatani et al., 2019; Žulpaitė et al., 2019). When it comes to PEAMs, only few cases of scalp, mediastinum, and renal hilum have been reported so far through

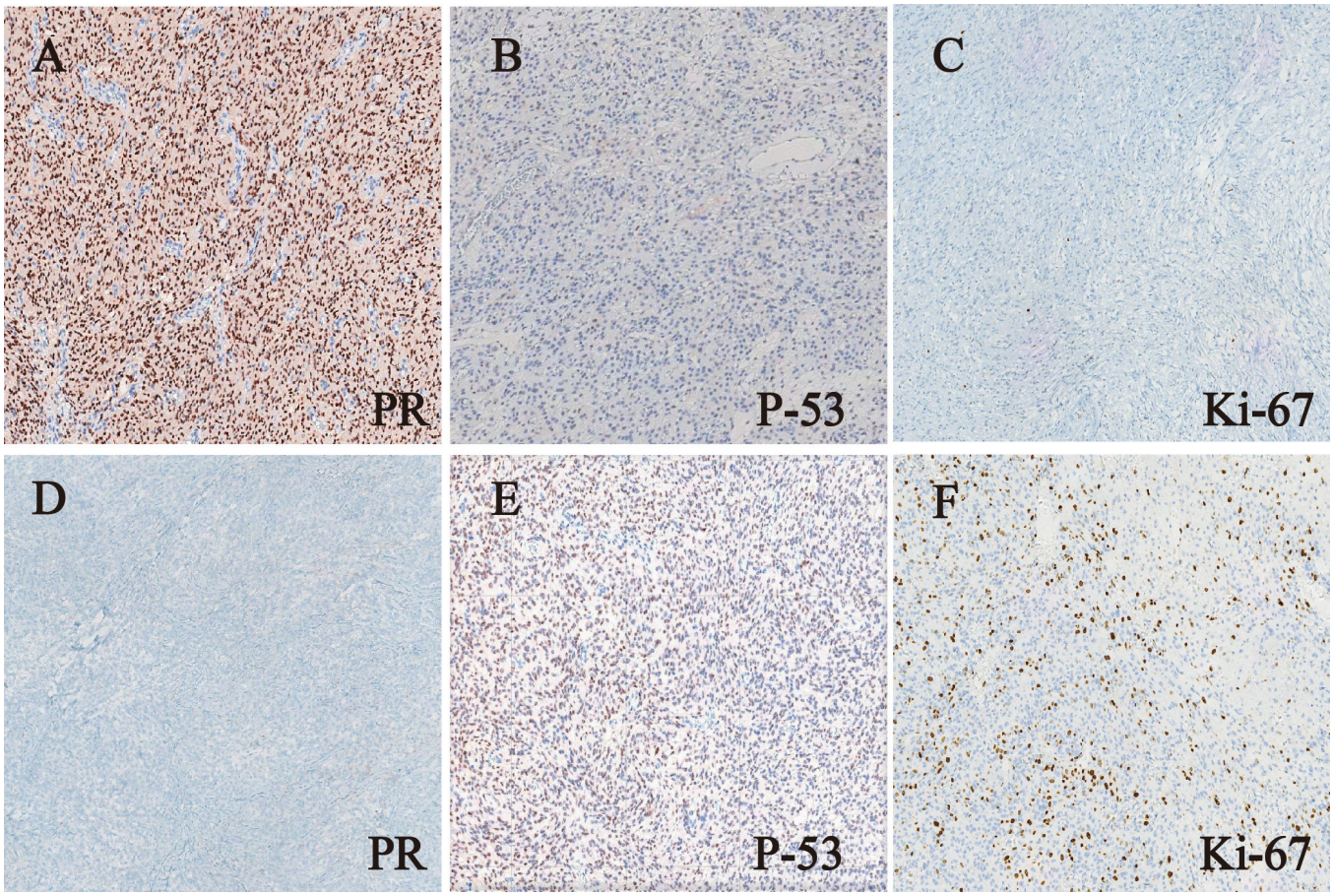

Fig. 3. A, B. 4 cases of Grade I morphology were moderately (2+) to strongly (3+) positive for PR, but negative for P53. **C.** The positive Ki-67 staining rates of 4 Grade I PEM cases were no greater than 2%. **D, E.** 2 PEAM cases were both negative for PR, but weakly (1+) to moderately (2+) positive for P53. **F.** As for Ki-67, the positive rates of 2 PEAM cases were 10% and 20%. × 100.

reviewing the published data (Mogi et al., 2012; Marcos et al., 2018; Murakami et al., 2019; Anderson et al., 2022).

Overall, the locations of 4 Grade I cases in our study, including left nasal cavity, left lower lung, right upper lung, and left upper lung, were generally consistent with previous reports in the literature. However, to date, the other 2 PEAM cases located in the right neck and left orbit have not been reported in the published data. Patients affected ranged from middle-aged to elderly, with a predominance of female, and clinical presentations also varied. The 6 cases in this study all underwent surgical resection and postoperative follow-up, and 1 recurrent case received radiotherapy after the second surgery. In fact, due to the rarity of PEMs, preoperative imaging diagnosis is usually difficult, and there is even no clear standard for surgical methods. Fortunately, an effective treatment modality, the hypofractionated gamma knife radiosurgery, has been shown to be effective and safe in the treatment of patients with benign and malignant intracranial tumors, especially meningiomas. Furthermore, 68Ga-DOTATOC-PET/CT has also been proved to be a valuable prognostic evaluation method for patients with meningioma after surgery (Barone et al., 2021; Inserra et al., 2022). This also provides us with effective ideas for the treatment and follow-up of PEMs, especially PEAM cases.

Despite the simple and clear diagnostic criteria, the diagnosis of this tumor remains challenging due to the rarity of PEMs and the uncertainty of their location. Depending on where they occur, PEMs need to be differentiated from a variety of tumors with similar morphologies that can occur at that site. When PEMs occur in the nasal cavity, they need to be differentiated from neurofibromas, perineuriomas, inflammatory myofibroblastic tumors, and sinus-type hemangiopericytoma; when PEMs occur in the neck, they need to be differentiated from nodular fasciitis, paraganglioma, synovial sarcoma, and metastatic malignant melanoma; PEMs also occur in the lung and need to be differentiated from solitary fibrous tumor, inflammatory myofibroblastic tumor, epithelioid hemangioendothelioma, thymoma, and many more. Therefore, we selected such an immunohistochemical combination, including Vimentin, EMA, CKpan, S-100, CD34, STAT6, SMA, Syn, Bcl-2, to differentiate PEMs from the above histologically similar tumors.

In addition, there is another morphological issue worth exploring. PEMs are sometimes morphologically indistinguishable from minute meningioma-like nodules, especially in the lungs. There is no standard for differential diagnosis of the two in the current WHO classifications. However, the observation of similar genetic abnormalities has led to a discussion of a possible relationship between them. Subsequently, published papers have concluded that the differential diagnosis is accordingly determined using the size of 3mm as a boundary (Gomez-Aracil et al., 2002; Ohashi-Nakatani et al., 2019).

As stated above, the grades of meningiomas have been reported to correlate with PR, P53, and Ki-67 expression, but not reported in PEMs. In this study, 4 Grade I cases were PR positive, P53 negative, and Ki-67 index was low, whereas 2 PEAM cases were PR negative, P53 positive, and Ki-67 was relatively high. In particular, the relapsed case showed more significant differences. To some extent, the above revealed similar conclusions with intracranial or intraspinal meningiomas, and further suggested that the immunohistochemical panel can also have some suggestive value for recurrence. Actually, to clarify its statistical value, further expansion of cases and further studies are still definitely required in our follow-up study due to the limited number of cases (Maiuri et al., 2021; Küçükosmanoğlu et al., 2022). Collectively, besides morphological detections, immunohistochemical assessment of PR, P53, and Ki-67 expressions should also be incorporated into the grading of PEMs.

Genetically, chromosomal loss of 1p36 is frequent in meningiomas (Ruiz et al., 2010). Francesco Peppe et al have reported that EGFR, ERBB2, FLT3, and BRAF were positively correlated with meningioma recurrence by next generation sequencing (Pepe et al., 2020). Meanwhile, mutations in the NF2 and TERT genes in meningiomas are also considered to be potential new therapeutic targets in the future (Salles et al., 2021). However, the above studies are all aimed at the primary intracranial meningioma, and the molecular pathological study of PEMs has not been reported so far.

In summary, our study has expanded cases of PEMs, especially 2 PEAM cases in rare sites. Our study has also further summarized the pathological features of PEMs, focusing on the histological features of PEAMs, and proposed the value of the immunohistochemical panel, including PR, P53, and Ki-67 in grading PEMs. In our further research, the study of molecular pathological features of PEMs, especially PEAMs, should be focused on as well.

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Competing interests. All the authors declared that they had no conflict of interest to this work.

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