

Differentiation of meningiomas from histologic mimics via the use of claudin-1

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Abstract

Background: Meningiomas have variable courses and it is important to distinguish these. Similarly they may be mimicked by other brain tumours from a purely pathological point of view e.g. schwannomas and meningeal haemangiopericytoma. Claudin-1 is a tight junction-associated protein recently shown to be expressed in anaplastic malignant meningiomas. It is important for neurosurgeons to find these patients.

The purpose of this study was to compare claudin-1 staining with other markers commonly used to differentiate meningiomas.

Material and methods: This study aimed at assessment whether immunohistochemical staining for claudin-1 could help distinguish meningiomas from histologic mimics, compared with commonly used markers. Tissue sections from 20 meningothelial meningiomas, 40 fibrous meningiomas, 20 atypical meningiomas, (including 14 with predominantly spindled morphologic features), 14 solitary fibrous tumours of the meninges, 10 meningeal haemangiopericytomas and 14 vestibular schwannomas were stained immunohistochemically for claudin-1, epithelial membrane antigen, S-100 protein, CD34, and glial fibrillary acidic protein. (p10-14)

Key words: Nervous system tumours and histochemical immunostaining

Introduction

The designation of meningioma has been extended through the years to diverse neoplasms sharing only a tendency to arise within the histogenetically complex tissues of the leptomeninges or dura mater. Thus, such dissimilar entities as meningeal haemangiopericytoma (HPC) currently accorded separate nosologic status among tumours of the central nervous system (CNS) and its coverings were once labeled together under the regrettable term of “angioplastic” meningioma (AM) and widely assumed to derive from a common progenitor. Distinguishing between meningiomas and other tumours arising in the meninges can occasionally be difficult. In particular, fibrous meningiomas (FM) are morphologically similar to other spindle cell tumours of the

CNS including HPC, solitary fibrous tumour (SFT) of the meninges, and vestibular schwannoma (VS). However, because of differences in prognosis and patient management, proper diagnosis is critical. For example meningeal HPC has a much higher propensity to recur and metastasize than does FM.¹

Although immunohistochemical analysis is helpful for differentiating among meningeal tumours, there is considerable overlap in their immunoprofiles. Perineurinomas and meningiomas contain numerous cell junctional complexes.¹⁰ Claudin-1 is a tight junction-associated protein that recently has been shown to be expressed in perineurinomas and anaplastic meningiomas.^{6,13} However, the distribution of claudin-1 in low grade meningiomas and in other dural-based spindle cell tumours has not been elucidated fully.

The purpose of this study was to determine whether immunohistochemical staining for claudin-1 could help distinguish meningiomas from potential histologic mimics, in comparison with markers commonly used in this differential diagnosis.

Material

Dural-based tumours were retrieved from the pathology files of Damanhour National Medical Institute Hospital. Representative H&E stained slides from each case were

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reviewed by two pathologists on staff to reach consensus diagnoses, which were based on standard and widely accepted criteria.⁵

In total, 20 meningothelial meningiomas (MM), 40 FM, 20 atypical meningiomas (World Health Organization Grade II), 14 SFT of the meninges, 10 HPC and 14 VS were selected for study.

Methods

Immunohistochemical studies were performed on 4-µm-thick, formalin-fixed, paraffin-embedded tissue sections. The antibodies, dilutions, pretreatment conditions, and sources are listed in Table 1. The envision plus detection system (DAKO, Carpinteria, CA) was used for all antibodies. Appropriate positive and negative control samples were used throughout.

Table 1 - Panel of antibodies used in the study

Antigen	Dilution	Antigen retrieval	Source
Claudin-1	1:50	None	Zymed
EMA	1:200	None	Dako cytomation
S-100	1:3000	None	Dako cytomation
CD 34	1:400	None	Dako cytomation
GFAP	1:8500	Microwave	Dako cytomation

Abbreviations: EMA = epithelial membrane antigen; GFAP = glial fibrillary acidic protein

Immunoreactivity was graded semiquantitatively as follows: 0, fewer than 5% tumour cells reactive; 1+, 5 to 25% tumour cells reactive; 2+, more than 25 to 50% tumour cells reactive; and 3+, more than 50% tumour cells reactive. For claudin-1, the intensity of staining was also graded as weak, moderate, or strong, with immunoreactivity of perineurial cells in normal peripheral nerve tissue as a reference for strong staining.

The Fisher exact test or X² analysis was used, as appropriate, to compare the different groups. A p value of less than 0.05 was considered statistically significant.

Results

The results of the study are summarised in Table 2.

Claudin-1 was positive in 42 (53%) meningiomas, generally with a granular staining pattern (Figs. 1 and 2).

Of the MMs (Fig. 1), 14 (70%) were positive for claudin-1. (with 10 cases showing 3+ staining and 2 cases each showing 2+ and 2+ staining (12 with moderate and 2 with

weak staining). Of 40 FM, 16 (40%) were positive for claudin-1 (Fig. 2), with 4 cases showing 3+ staining, 4 showing 2+ staining, and 8 showing 1+ staining (8 each with moderate and weak staining). Of the atypical meningiomas, 12 (60%) of 20 were positive for claudin-1, with 4 cases each showing 3+, 2+, and 1+ staining (8 with moderate and 4 with weak staining).

In contrast, no immunoreactivity for claudin-1 was seen in any of the other tumour types. Positive staining for claudin-1 was significantly more frequent in meningiomas than in the other neoplasms examined (p < 0.05). Claudin-1 was expressed in the (perineurial) capsule of a schwannoma and was negative in smooth muscle, normal brain tissue, and fibroblasts.

The tumours were also stained with other markers commonly used in the differential diagnosis of dural-based neoplasms. There was significant overlap in the immunoprofiles of these tumours (Table 2). Although 18 (90%) of 20 MM, all 40 FM (100%), and 18 (90%) of 20 atypical meningiomas expressed EMA (Fig. 2), staining for EMA was also detected in 4 (29%) of 14 SFT (Fig. 2) and in 2 (20%) of 10 HPC. Of note, both the MM and the atypical meningiomas that failed to stain for EMA were positive for claudin-1 (3+ and 2+, respectively). Immunoreactivity for S-100 protein was observed in 14 (100%) of 14 VS, but also was detected in 36 (90%) of 40 FM examined (22 cases with 3+, 10 with 2+, and 2 with 1+ staining). All 14 SFT (100%) were positive for CD34 (5 showing 3 + and 2 showing 1+ staining (Fig. 2), as were 6 (60%) of 10 HPC (each 3+). However, 16 (40%) of 40 FM (6 cases with 2+ and 10 with 1+ staining) and 12 (60%) of 20 atypical meningiomas (6 cases each with 3+ and 1+ staining) also showed immunoreactivity for CD34 (Fig. 2).

Table 2 - Results of immunohistochemical staining*

Antigen	MM (n=20)	FM (n=20)	AM (n=20)	SFT (n=14)	MHP (n=10)	VS (n=14)
Claudin-1	14(70)	16(40)	12(60)	0(0)	0(0)	0(0)
EMA	18(90)	40(100)	18(90)	4(29)	2(20)	0(0)
S-100	0(0)	36(90)	0(0)	0(0)	0(0)	14(100)
CD34	0(0)	16(40)	12(60)	14(100)	6(60)	0(0)
GFAP	0(0)	0(0)	0(0)	0(0)	0(0)	8(57)

Abbreviations: MM = meningothelial meningioma; FM = fibrous meningioma; SFT = solitary fibrous tumour; MHP = meningeal haemangiopericytoma, VS = vestibular schwannoma; EMA = epithelial membrane antigen; GFAP = glial fibrillary acidic protein

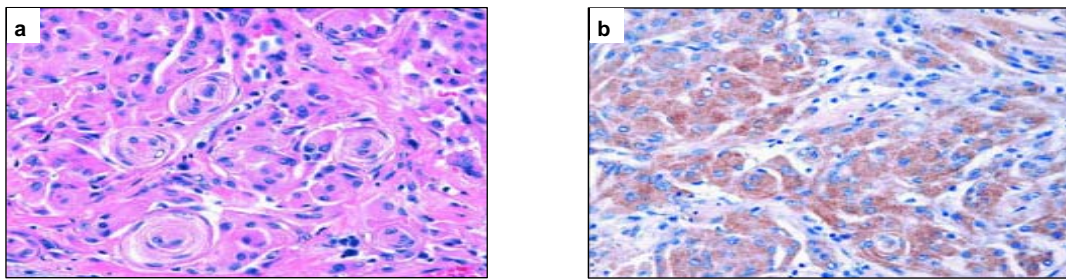


Figure 1 - Claudin-1 expression in a meningothelial meningioma. **a)** MM showing prominent whorls and nuclear pseudoinclusions (H&E x 400). **b)** Immunostaining for claudin-1 (x 400).

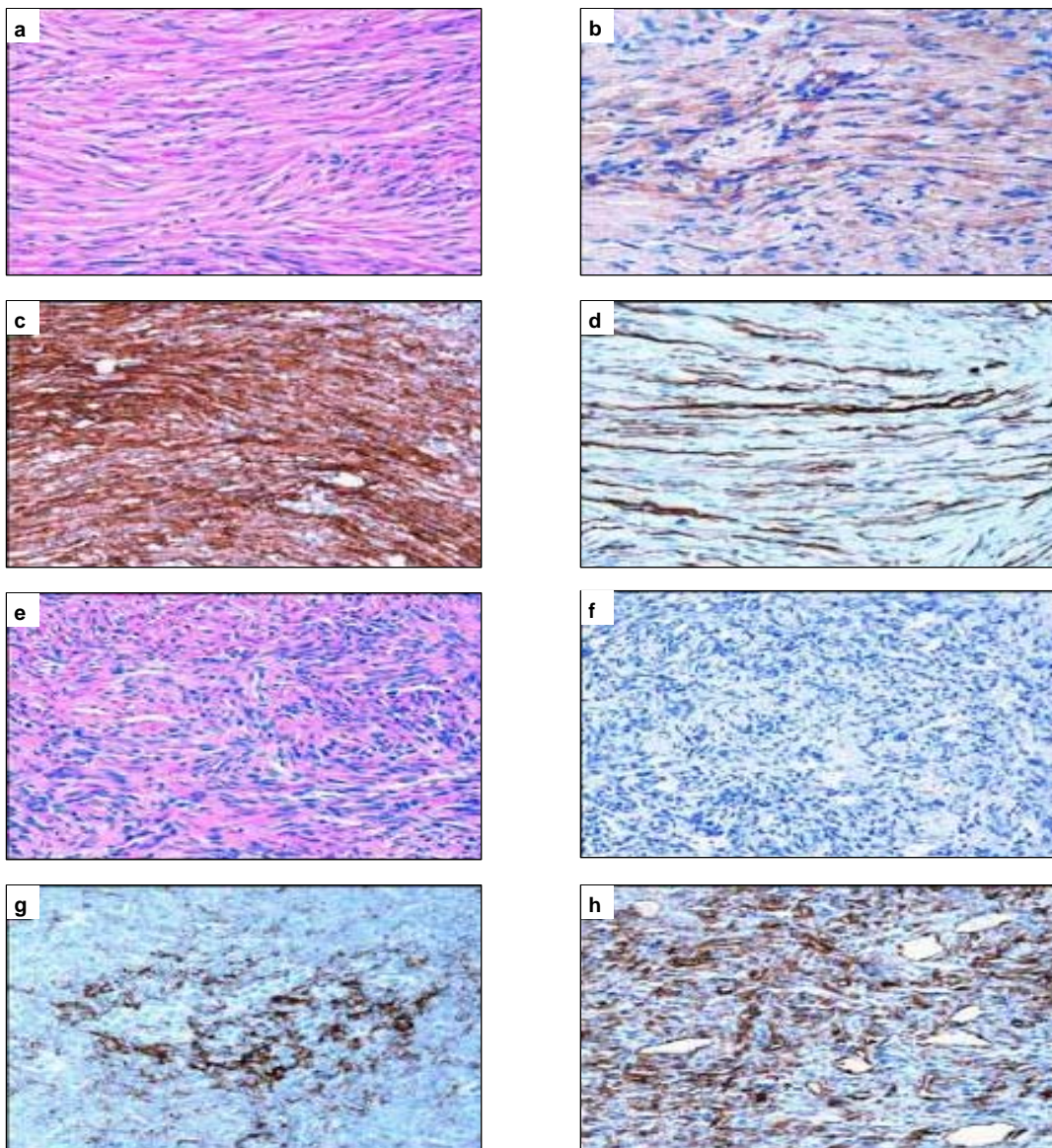


Figure 2 - Comparison between FM (**a-d**) and SFT of the meninges (**e-h**). The histologic appearances of FM (**a**, H&E x 400) and SFT (**e**, H&E x 400) are similar. Claudin-1 is expressed in FM (**b**, x 400), but not in SFT (**f**, x 400). In contrast, other markers are positive in both tumour types. Immunoreactivity for epithelial membrane antigen is seen in both FM (**c**, x 400) and SFT (**g**, x 400). Positive staining for CD-34 is seen in FM (**d**, x 400) and SFT (**h**, x 400).

Discussion

Meningiomas, in particular the fibrous variant, can be difficult to distinguish from meningeal HPC, SFT, and VS. Although a panel of immunohistochemical markers can be used to differentiate among these entities, there is significant overlap in their immunoprofiles. Claudin-1 is a tight junction-associated protein that has recently been shown to be expressed in AM. In accordance with the results of previous studies, we found EMA was positive in 95% of meningiomas and in 29% and 20% of meningeal SFT and HPC, respectively. Similarly, immunoreactivity for CD34 was observed in all SFT and 60% of HPC, as well as in 40% of FM and 60% of atypical meningiomas. S-100 protein was detected not only in VS, but also in 90% of FM, often with diffuse staining. In contrast, claudin-1 expression was specific for meningiomas in this context. Claudin-1 was detected in 53% of meningiomas but not in any of the other tumour types.

Epithelial membrane antigen (EMA), a marker commonly used to support the diagnosis of meningioma, is positive in approximately 30% of meningeal HPC and in a similar fraction of SFT. CD34, a marker that usually is positive in SFT, is expressed in up to 60% of FM. Finally, S-100 protein, although uniformly strongly positive in schwannomas, can be detected in as many as 80% of FM as well.

Briefly, atypical meningiomas (World Health Organization Grade II) are defined as meningiomas that have 4 or more mitoses per 10 high-power fields or 3 or more of the following histologic features: increased cellularity, small cells with high nuclear/cytoplasmic ratios, prominent nucleoli, sheet-like growth, and foci of geographic necrosis. There is significant overlap in the histologic appearances of spindle cell tumours arising in the meninges. For example, FM and meningeal SFT are composed of bundles of spindle cells with prominent background collagen fibres. Particularly in cases in which the diagnosis must be made based on the evaluation of small tumour fragments, immunohistochemical analysis can be very helpful. Unfortunately, the markers widely used in clinical practice are not tumour-specific. For example, EMA is positive in 50 to 100% of meningiomas and in normal meninges. However, approximately 30% of meningeal HPC and SFT and occasional schwannomas also are immunoreactive for EMA. Although diffuse S-100 protein staining can be used to support the diagnosis of schwannoma, FM also frequently strongly express this antigen. Finally, there is also significant overlap in the distribution of CD34 expression in SFT, meningeal HPC, and FM. Although SFT of the meninges and meningeal HPCs often show strong positivity for CD34, more than 50% of FM also express CD34.

As an aside, because HPC of soft tissue are morphologically indistinguishable from and show similar immunophenotypic features to the cellular areas of SFT, these two tumour types are now widely regarded to be closely related. The findings in the present study provide further evidence for the similar antigenic expression patterns in meningeal examples of these tumours. Similar to their soft tissue counterparts, meningeal HPC indeed may also represent uniformly cellular examples of SFT.

In striking similarity to our results, Rajaram, et al detected immunoreactivity for claudin-1 in 7 (54%) of 13 AM (grade III, whereas we found that 50% of grade I meningiomas and 60% of grade II meningiomas were positive for claudin-1. In contrast, Rajaram, et al reported that 2 (13%) of 15 meningeal HPC were focally positive for claudin-1, whereas all cases of meningeal HPC were negative for this marker in our study.² The discrepancy between these results may be due to differences in immunohistochemical staining techniques. Specifically, in contrast with the methods used by Rajaram, et al, we did not use antigen retrieval in our study.

Bhattacharjee, et al also reported immunoreactivity for claudin-1 in 17 (85%) of 20 meningiomas. However, our study also examined claudin-1 expression in SFT of the meninges, which can be difficult to distinguish from meningiomas with spindled morphologic features; all cases were negative.

Meningiomas are thought to originate from the cell clusters capping the arachnoid villi. Claudins are understood to be components of tight junctions. So how can claudin-1 expression in meningiomas be explained? There is increasing evidence that tumour cells can show aberrant expression or abnormal cellular localisation of claudin-1. Despite the fact that tight junctions are not identified by electron microscopy in the spindle cell component of biphasic and monophasic synovial sarcomas, claudin-1 and other tight junction proteins have recently been shown to be expressed in these areas. Schuetz, et al recently demonstrated staining for claudin-1 and other tight junction-associated antigens in many cases of Ewing's sarcoma/primitive neuroectodermal tumour, although well-formed tight junctions are not detected ultrastructurally in these tumours.

Conclusion

In conclusion, the present findings indicate that claudin-1 seems to be a specific marker for meningiomas in comparison with other spindle cell tumours arising in the meninges. Although its sensitivity is relatively low, claudin-1 may be helpful when used in a panel of immunostains to distinguish meningiomas from histologic mimics.

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GENTLE REMINDER

Effect of contrast injection on CT in common brain lesions

Enhancement after contrast	No contrast enhancement	Ring enhancement with contrast
Recent infarct Tumours, esp. metastases, gliomas, meningiomas Inflammatory foci Granulomas Angioma, aneurysm	Old infarct Old haemorrhage Demyelination Leucoencephalopathy Astrocytoma Behcet's disease	Glioma Brain abscess Metastases Subacute intracerebral haematoma Parasitoma