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Patterns of SPARC expression and basement membrane intactness at the tumour–brain border of invasive meningiomas

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The matricellular glycoprotein SPARC (secreted protein, acidic and rich in cysteine), also termed osteonectin, has been found to regulate the invasive behaviour of several tumour types by interacting with basement membrane constituents. Brain invasive meningiomas are supposed to disrupt the pial-glial basement membrane. In the present study we aimed at determining the relationship of basement membrane intactness and SPARC protein expression at the meningioma–brain border. Sections of 51 brain-invasive meningiomas (31 meningothelial meningiomas WHO grade I, 11 atypical WHO grade II, and nine anaplastic WHO grade III tumours) were immunolabelled with antibodies against SPARC, epithelial membrane antigen (EMA), collagen IV and glial fibrillary acidic protein (GFAP). Twenty-two non-invasive WHO grade I meningothelial meningiomas were included in the study for comparison. At the tumour–brain border of invasive meningiomas, spindle-shaped tumour cells expressed SPARC. The number of tumours containing SPARC+ spindle cells did not differ significantly between WHO grades. By contrast, the number of WHO grade I tumours expressing collagen IV (15/31) was highly significantly elevated when compared with WHO grade II (1/11) and WHO grade III (0/9) (both $P < 0.0001$). There was an inverse relationship of the presence of SPARC+ spindle cells and basement membrane material. In conclusion, the destruction of the basement membrane is correlated with meningioma malignancy grade whereas the expression of SPARC protein at the tumour–brain border is not. Destruction of the basement membrane and appearance of SPARC+ spindle cells are not coincident during the course of brain invasion by meningiomas.

Keywords: SPARC, osteonectin, meningioma, brain invasion, plial-glial membrane, extracellular matrix

Introduction

SPARC (secreted protein, acidic and rich in cysteine), also termed osteonectin, is a member of the matricellular protein class. These proteins function as modulators of cellular interaction with the extracellular matrix. SPARC serves a multitude of functions. The protein inhibits cell adhesion and proliferation, regulates growth factor activity, and is associated with angiogenesis. As a result, it is highly expressed in areas of remodelling of extracellular matrix during embryogenesis, wound healing, inflammation, and tumour growth, invasion and metastasis (see [1–3] for reviews).

SPARC is expressed in numerous malignant tumours. However, the role of SPARC in tumorigenesis, progression towards malignancy, and metastasis appears to depend on the tumour type examined. Overexpression of SPARC in

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tumour samples compared with control tissue was found in oesophageal carcinoma [4] and gastric carcinomas [5]. Moreover, higher expression of SPARC was significantly associated with lymph node metastasis, lymphatic invasion and perineural invasion of gastrointestinal carcinoma, and the 3-year survival of patients with lower expression of SPARC was significantly better than those with a higher expression [5]. Increased amounts of SPARC have been shown in malignant, compared with benign, lesions of the breast [6]. SPARC protein was expressed to progressively higher levels in breast carcinomas as the tumour progressed from ductal carcinoma *in situ* to invasive carcinoma [7]. The molecule enhanced migration of a breast carcinoma cell line [8]. Suppression of SPARC expression by antisense RNA abrogated the tumorigenicity of human melanoma cells [9].

Other studies provided evidence for an inverse relationship of SPARC and higher malignancy. The relative levels of SPARC in the normal human colonic epithelium decreased with malignant transformation, as indicated by its decrease in immunohistochemical staining for SPARC [10]. Levels of SPARC in ovarian cancers were downregulated following malignant transformation [11], and downregulation of SPARC resulted in more aggressive ovarian cancer phenotypes [12]. The tumour growth of glioma cells overexpressing SPARC was delayed *in vivo* [13]. An inhibitory effect of SPARC has been found for proliferation and migration in breast and ovarian carcinoma cells [14]. Koblinski and colleagues infected MDA-231 breast carcinoma cells with osteonectin. They found that *in vitro* invasion of these cells through Matrigel was decreased [15]. The growth of Lewis lung carcinoma and B cell lymphoma was enhanced in mice lacking endogenous SPARC [16]. Taken together, the functions of SPARC require to be established for each tumour type individually.

Few data exist on the significance of SPARC expression in meningiomas. A microarray gene chip analysis revealed a higher expression of SPARC when compared with normal brain [17]. The grade of malignancy and the state of invasiveness of the tumours were not indicated. At the protein level, SPARC was present at the invading edge of 20 infiltrating meningiomas of all malignancy grades but not in non-invasive ones [18]. In the present study, we performed immunohistochemistry using anti-SPARC and anti-collagen IV antibodies to monitor SPARC expression in conjunction with the intactness of the pial-glial membrane.

Collectively, our results demonstrate that SPARC+ spindle cells are present at the tumour–brain border of a subset of invasive meningiomas (16/31 WHO grade I, 4/11 WHO grade II, 2/9 WHO grade III). There was no significant difference between tumour grades. By contrast, the number of cases showing basement membrane material at the tumour–brain interface was highly significantly elevated in WHO grade I meningiomas when compared with high-grade tumours ($P < 0.0001$ for both WHO grade II and III tumours). The appearance of SPARC+ spindle cells precluded the presence of basement membrane material at the tumour–brain border. Taken together, the intactness of the basement membrane (or lack of it) correlates with the malignancy grade of invasive meningiomas whereas the presence of SPARC+ spindle cells at the tumour–brain border does not correlate with the malignancy grade.

Materials and methods

Patients

Of a total number of 1081 patients who underwent surgery for meningiomas at the Department of Neurosurgery at Tübingen University between 1997 and 2005, 31 WHO grade I, 11 grade II, and five grade III were selected according to the WHO criteria, classified as infiltrating the brain parenchyma without intervening leptomeninges [19] or showing finger-like tumour projections into the adjacent brain parenchyma [20]. Four additional WHO grade III meningiomas meeting these criteria were included from the archives of Otto-von-Guericke-University, Magdeburg. Twenty-two randomly selected WHO grade I meningotheliomatous meningiomas, which were non-invasive according to the neurosurgeon's report and according to the histological findings, were also included in the study. The patients' average ages were 61 years (range: 29–85 years) for WHO grade I meningiomas, 64 years (range: 53–80 years) for WHO grade II, and 63 years (range: 39–84 years) for WHO grade III tumours.

Immunohistochemistry

Immunohistochemistry was performed using monoclonal antibodies against SPARC (Haematological Technologies Inc., Essex Junction, USA), collagen IV (DakoCytomation,

Glostrup, Denmark), glial fibrillary acidic protein (GFAP; DakoCytomation), and epithelial membrane antigen (EMA) (DakoCytomation) on 4-μm-thick paraffin sections. The sections were deparaffinized and immunostained using the Benchmark immunohistochemistry staining system (Ventana Medical Systems, Strasbourg, France). The automated protocol is based on an indirect biotin–avidin system. It was optimized for each antibody. Primary antibodies were diluted as follow: anti-SPARC 1 : 4000, anti-collagen IV 1 : 100, anti-EMA 1:5 and anti-GFAP 1 : 500, followed by a universal biotinylated immunoglobulin secondary antibody and diaminobenzidine substrate for visualization. The sections were eventually incubated with a copper enhancer (Ventana) and counterstained with haematoxylin. Negative control slides were processed in parallel with each batch of staining.

The presence of invasion was assessed histologically using haematoxylin-eosin preparations and confirmed by anti-GFAP immunolabelling of the brain parenchyma.

Statistical analysis

In order to test the null hypothesis of equal responses of SPARC and collagen IV (positive staining = 1; negative staining = 0), we performed contingency analysis for meningiomas of WHO grades I–III followed by Pearson χ^2 test. JMP IN (http://www.JMP.com) was used for statistical analysis.

Results

Tumour–brain border of WHO grade I meningiomas

SPARC was expressed by spindle-shaped cells (Figure 1**a**,**b**) at the tumour–brain border of 16/31 meningiomas (Table 1). These cells coexpressed EMA (Figure 1**c**), suggesting they were of arachnoidal origin. The spindle-shaped cells had long slender cytoplasmic processes which made contact with one another to form a single cell layer surrounding the circumference of a tumour tongue, or part of it (Figure 1).

Meningioma invasion is supposed to require disruption of the pial-glial membrane at some point of the infiltrative process. We assessed the intactness of the basement membrane by collagen IV labelling. The presence of collagen IV+ basement membrane material precluded the presence of spindle-shaped SPARC-expressing cells (Figure 2). Vice versa, collagen IV+ basement membrane material was absent from the tumour–brain border that showed SPARC-expressing spindle cells (Figure 3). There was, hence, an

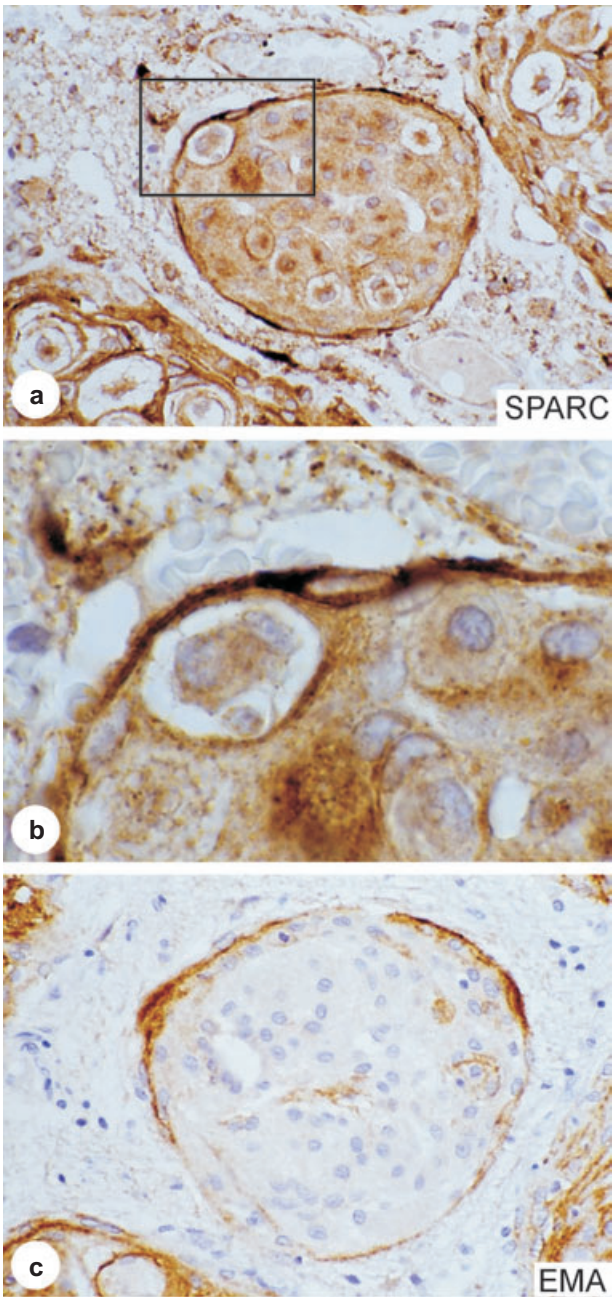

Figure 1. Invasive meningioma WHO grade I. (**a**) Strong immunoreactivity of SPARC in spindle-shaped cells at the tumour–brain border. (**b**) Higher magnification. (**c**) Epithelial membrane antigen labelling to confirm the arachnoidal origin of the spindle-shaped cells.

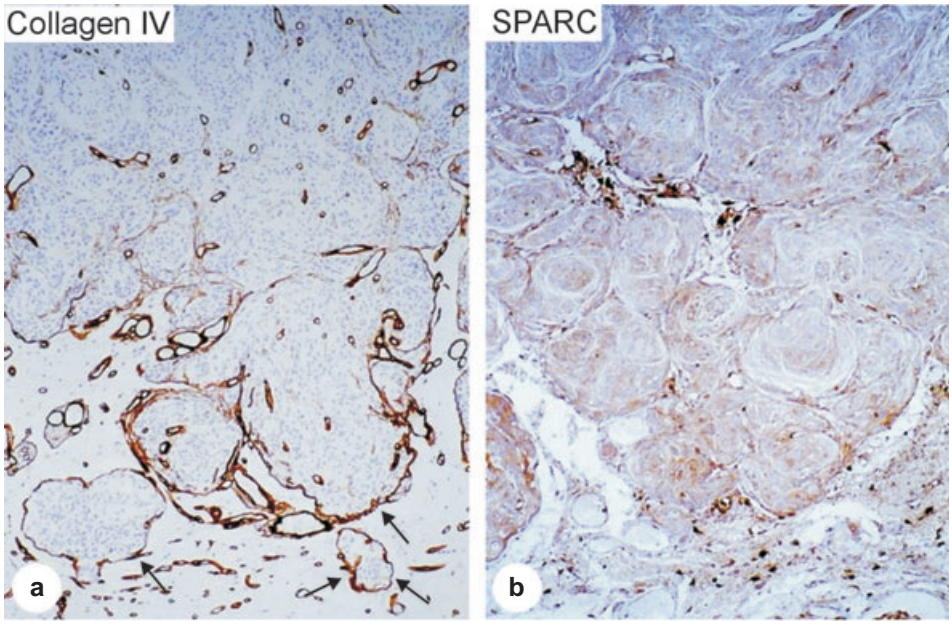

Figure 2. Invasive meningioma WHO grade I. (**a**) Abundant basement membrane material separates tumour from brain (arrows). (**b**) There is no layer of SPARC+ spindle cells at the tumour–brain border. Reactive astrocytes in the brain parenchyma display strong SPARC expression.

Table 1 Number of cases showing SPARC+ spindle cells and basement membrane material (collagen IV+) at the tumour–brain border

	WHO grade		
	I	*II*	*III*
Total	31	11	9
SPARC+	16*	4	2
Collagen IV+	0	0	0
Collagen IV–	16	4	2
SPARC–	15	7	7
Collagen IV+	15†	1	0
Collagen IV–	0‡	6	7

*The number of WHO grade I meningiomas showing SPARC+ spindle cells was not significantly different from the number of WHO grade II and WHO grade III tumours ($P = 0.26$).
†The number of WHO grade I meningiomas separated from the brain by basement membrane material was highly significantly different from the number of WHO grade II and WHO grade III tumours ($P < 0.0001$ for both WHO grade II and III tumours).
‡There was a highly significant difference between the number of WHO grade I meningiomas lacking both SPARC+ spindle cells and basement membrane material at the tumour–brain border when compared with WHO grade II and WHO grade III tumours ($P < 0.0001$ for both WHO grade II and III tumours).

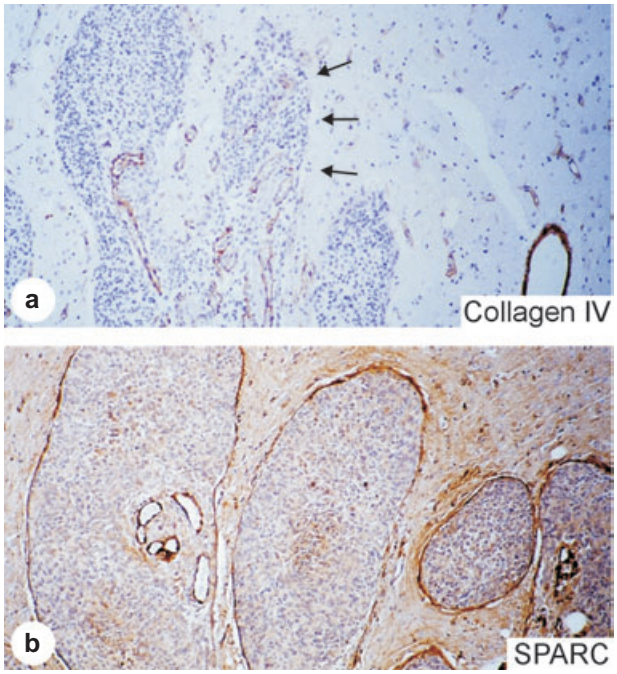

Figure 3. Invasive WHO grade I meningioma. (**a**) Lack of basement membrane between tumour and brain (arrows). (**b**) By contrast, SPARC is upregulated at the tumour–brain border by spindle cells forming a continuous layer.

inverse relationship of the presence of SPARC+ spindle cells and the presence of basement membrane material (Table 1).

Tumour–brain border of high-grade meningiomas

Spindle cells expressing SPARC were found at the tumour–brain border of 4/11 WHO grade II meningiomas and 2/9 WHO grade III tumours (Table 1). The number of meningiomas of WHO grade II and WHO grade III showing SPARC+ spindle cells at the tumour–brain border was not significantly different when compared with WHO grade I meningiomas. The SPARC+ spindle cells were characterized by short cytoplasmic processes that did not make contact with one another (Figure 4). This contrasts with the SPARC+ spindle cells of WHO grade I meningiomas which had long slender processes (Figures 1 and 2). Basement membrane material was present at the tumour–brain border of only 1/11 WHO grade II meningioma (Figure 4) and none of the WHO grade III tumours (0/9) (Table 1). Taken together, there was a complementary pattern of SPARC+ spindle cells and the presence of basement membrane, as in WHO grade I meningiomas. However, the number of cases of WHO grade II and WHO grade III meningiomas showing basement membrane deposits at the tumour–brain border was reduced when compared with WHO grade I meningiomas ($P < 0.0001$ for both WHO grades II and III).

Tumour mass

Endothelial cells of all tumours were SPARC+ regardless of whether tumour cells expressed the protein. The tumour mass of 19/31 WHO grade I meningiomas, 8/11 WHO grade II and 9/9 WHO grade III tumours showed cytoplasmic SPARC labelling (Figure 5). SPARC was present in whorl-forming cells (Figure 5**a**), in perinecrotic tumour

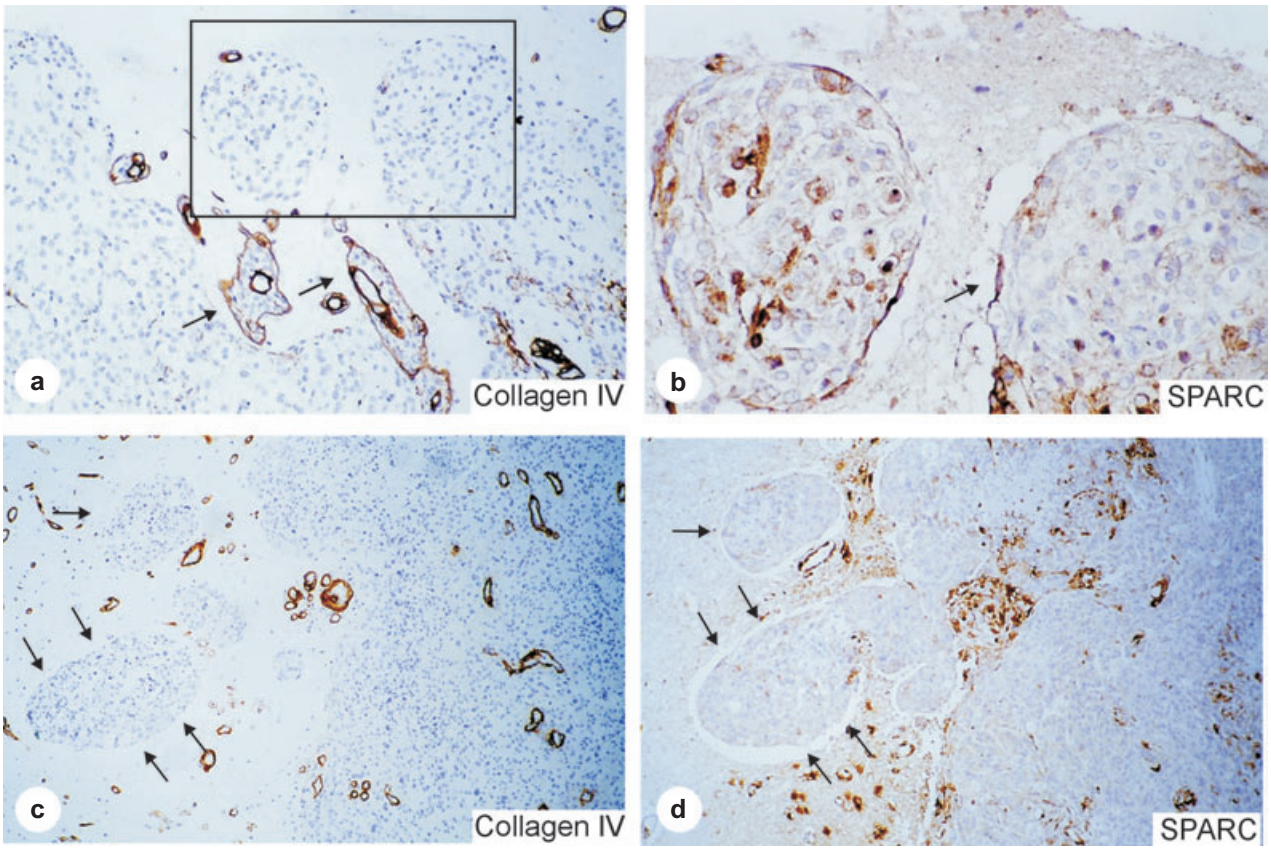

Figure 4. Expression patterns of SPARC and collagen IV in two invasive atypical meningiomas grade II WHO. (**a, b**) The first tumour shows patchy basement membrane material (**a**, arrows) and SPARC+ spindle cells (**b**, arrow) at the tumour–brain border. SPARC and collagen IV are not coexpressed. (**c, d**) The second tumour lacks a basement membrane (**c**) and SPARC-expressing cells (**d**) at the tumour–brain border (arrows).

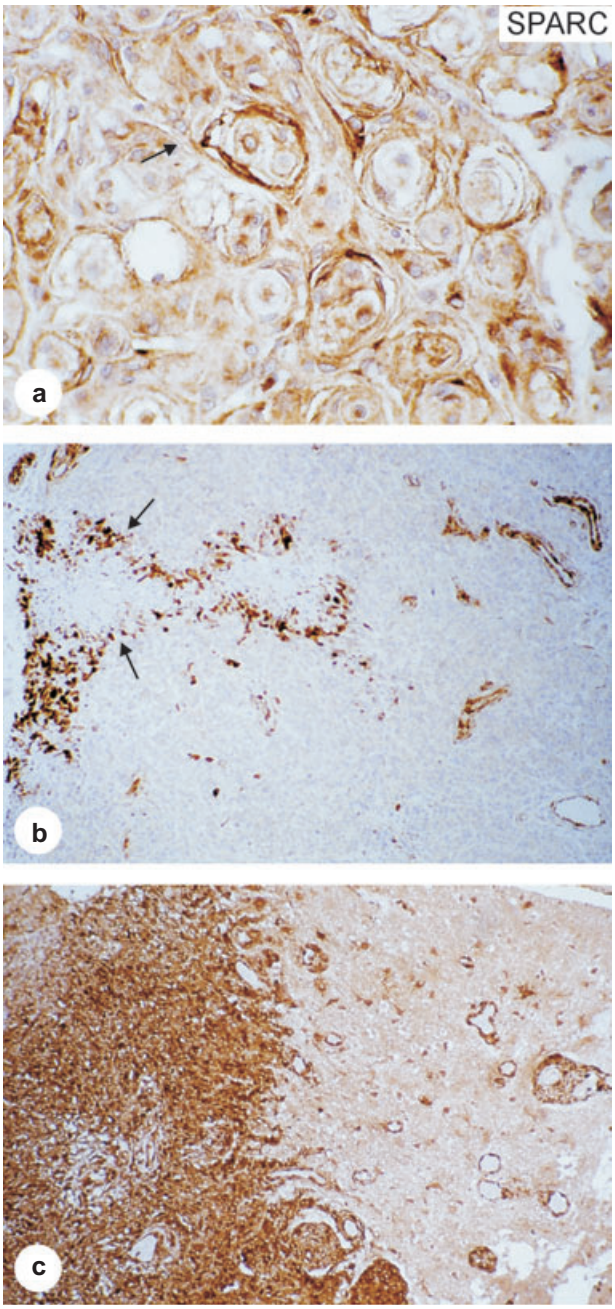

Figure 5. Cytoplasmic expression of SPARC in the tumour mass. (**a**) WHO grade I meningioma. SPARC is expressed in whorl-forming cells (arrow). (**b**) Anaplastic WHO grade III meningioma. SPARC expression is restricted to perinecrotic cells (arrows). (**c**) Anaplastic WHO grade III meningioma. There is strong cytoplasmatic expression of SPARC all over the tumour.

cells (Figure 5**b**), or it showed diffuse cytoplasmic labelling (Figure 5**c**).

The tumour mass of 18/22 non-invasive tumours showed SPARC labelling (not shown).

Discussion

This study was undertaken to examine whether the presence of SPARC at the invading edge of brain-invasive meningiomas is linked to the intactness of the pial-glial membrane and whether there is a relationship with meningioma malignancy.

We found distinct patterns of SPARC+ spindle cells and collagen IV+ basement membrane material at the tumour–brain interface of invasive meningiomas. The expression patterns of SPARC and collagen IV were mutually exclusive in our series of meningiomas; the presence of abundant basement membrane material at the tumour–brain border was paralleled by the absence of SPARC+ spindle cells, and vice versa (Figures 2 and 3). Taken together, basement membrane destruction and upregulation of SPARC are not coincident at the invading edge of brain-invasive meningiomas.

The role of SPARC in tumour invasion appears to be dependent on the tumour type. Increased amounts of SPARC have been shown in some types of malignant tumours when compared with their benign counterparts, whereas other studies provided evidence for an inverse relationship of SPARC and higher malignancy [4–16]. Our study failed to show a significant difference when the number of cases of WHO grade I meningiomas was compared with the number of high-grade tumours (Table 1). This does not lend support to the notion that the expression of SPARC at the meningioma–brain border is associated with meningioma malignancy.

Disruption of the pial-glial membrane is part of the invasive action of meningiomas. We have recently found that brain invasion precedes basement membrane disruption in most instances in grade I meningiomas. Major parts of the basement membrane remained intact at the surface of these meningiomas after interdigitation of tumour and brain had taken place. This growth pattern was deemed distinct from aggressive cancer growth [21]. The present study demonstrates that the number of cases showing this growth pattern is highly significantly elevated in WHO grade I meningiomas when compared with high-grade tumours, which showed a lack of basement membrane material in most WHO grade II meningiomas (10/11) and in all of the WHO grade III meningiomas (9/9) (Table 1, $P < 0.0001$ for both WHO grade II and III tumours). The lack of basement membrane material suggests that the invasive growth pattern of high-grade meningiomas resembles the growth pattern of infiltrating carcinomas.

SPARC expression was not restricted to the tumour–brain border. Diffuse cytoplasmic labelling of the tumour mass was found along with the staining of perinecrotic cells and of whorl-forming cells (Figure 5). These labelling patterns occurred both in invasive and non-invasive tumours. This does not favour the notion that SPARC is a universal marker that distinguishes brain-invasive meningiomas from non-invasive ones. By contrast, destruction of the basement membrane seems to correlate highly with increasing malignancy of brain-invasive meningiomas.

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