Proliferation Behaviour of Meningiomas

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Although generally considered benign, the biological behaviour of meningiomas varies considerably. The microscopic histopathological classification of the World Health Organization (WHO) cannot entirely predict the clinical behaviour of these tumours [21]. Between 7 and 32 % of benign meningiomas recur after total resection, and between 19 and 50 % (or more) after subtotal removal [6, 17]. Quantifying the proliferative potential may help to predict the biological behaviour of individual tumours of comparable histology. The prognostic significance of various proliferative indices in meningiomas has already been assessed and it has been suggested that the tumour proliferative potential can predict the patient's clinical course [1, 35, 36, 38, 39]. The nuclear antigen Ki-67 expressed by proliferating cells has become available for routinely processed paraffin section [12]. The Mib-1 antibody detects an epitope on the Ki-67 antigen, a nuclear protein present only during active phases of the cell cycle (G1, S, G2 and M). The higher incidence of meningiomas among women, their behaviour during pregnancy and the reported epidemiological link between meningiomas and breast carcinomas have led to the assumption that sex steroid hormones may influence the growth of meningiomas [28, 34]. Supported by promising results in breast cancer therapy, new chemotherapeutic approaches based on hormone manipulation were tested in meningioma patients [25]. Meningiomas are highly vascularized tumours. Neoangiogenesis has been correlated to biological aggressiveness as it enables neoplasms

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to grow beyond the limits given by the host vessels [10]. Therefore, neoplasms able to produce angiogenetic factors are more prone to spread and recurrence. The quantity of neoangiogenesis is a prognostic parameter in several neoplasias [7, 49]. The prognostic value of angiogenesis also has been studied extensively in meningiomas using microvessel density or vascular endothelial growth factor (VEGF) [2, 3, 26, 42].

Decisions regarding patient management therefore rely on a variety of clinical, radiological and pathological prognosticators as the clinical behaviour of meningiomas with a tendency to recur led to the assumption that meningiomas cannot be classified as a benign entity despite their pathological classification.

3.1   Materials and Methods

Based on a database of 1,766 meningiomas treated on at the Neurosurgical Department, Klinikum Hannover Nordstadt (1979–2002), and 953 meningiomas at University Hospital Tübingen (2004–2012), all of these tumours were analysed according to the current WHO classification. Five hundred eighty-eight paraffin blocks of 554 patients with intracranial meningiomas, operated between 1990 and 2000, were randomly retrieved from the archives of the Department of Pathology for immunohistochemical analysis. All of these patients were followed for a median of 74 months or until death. The material included 45 patients with neurofibromatosis type 2 disease (for a total of 62 tumours). Later on in Tübingen, cell culture studies were performed to further analyse growth behaviour and influence thereof.

Meningioma subtypes were defined according to the new WHO classification. Immunohistochemistry was performed with paraffin sections using Anti-Mib-1 and antihuman progesterone receptor 1A6. For Ki-67, the area of densest staining ('hot spot') was searched for and counting was performed in 10 contiguous fields. The average of the results in these fields determined the proliferative index (LI). The progesterone receptor status was determined by a semi-quantitative scoring scale according to the immunoreactive score (IRS) of Remmele et al., with respect to staining intensity and percentage of positive tumour cells [44].

3.1.1   Immunofluorescence

An immunocytochemical analysis was performed on primary cell culture growing in a monolayer fashion in culture. The cells were plated in 4-well glass slides and allowed to remain in growth media for 24 hours (h). After removal of the growth media, the slides were rinsed with PBS for 5 minutes (min) followed by fixation in methanol for 15 min at – 20 °C. The slides were rinsed with PBS and cells were blocked with 10 % horse serum in PBS for 30 min at room temperature (RT). Slides were incubated with primary antibodies overnight at 4 °C. Staining was developed using the secondary fluorescence antibody Alexa Fluor 488 (1:200) for 1 h at RT in the dark. The slides were mounted with DAPI-containing fluorescence mounting medium (Vector Laboratories, Burlingame, CA) (Fig. 3.1).

3.1.2   Immunocytochemical Staining

An immunocytochemical analysis was performed on meningioma cells growing in a monolayer fashion in culture. The cells were plated in 4-well glass slides and allowed to remain in growth media for 24 h. After removal of the growth media, the cells were rinsed with PBS for 5 min followed by fixation with ice-cold methanol for 10 min at – 20 °C. The slides were rinsed with PBS and incubated with 3 % H_2O_2 for 10 min at RT. All cells were blocked with 10 % rabbit serum in PBS for 30 min at room temperature. Slides were incubated with primary antibodies overnight at 4 °C. Staining was developed using the VECTASTAIN Elite ABC system and Vector® NovaRED™ (Vector Laboratories, Burlingame, CA). Sections were counterstained with haematoxylin and examined by light microscopic studies (Fig. 3.2).
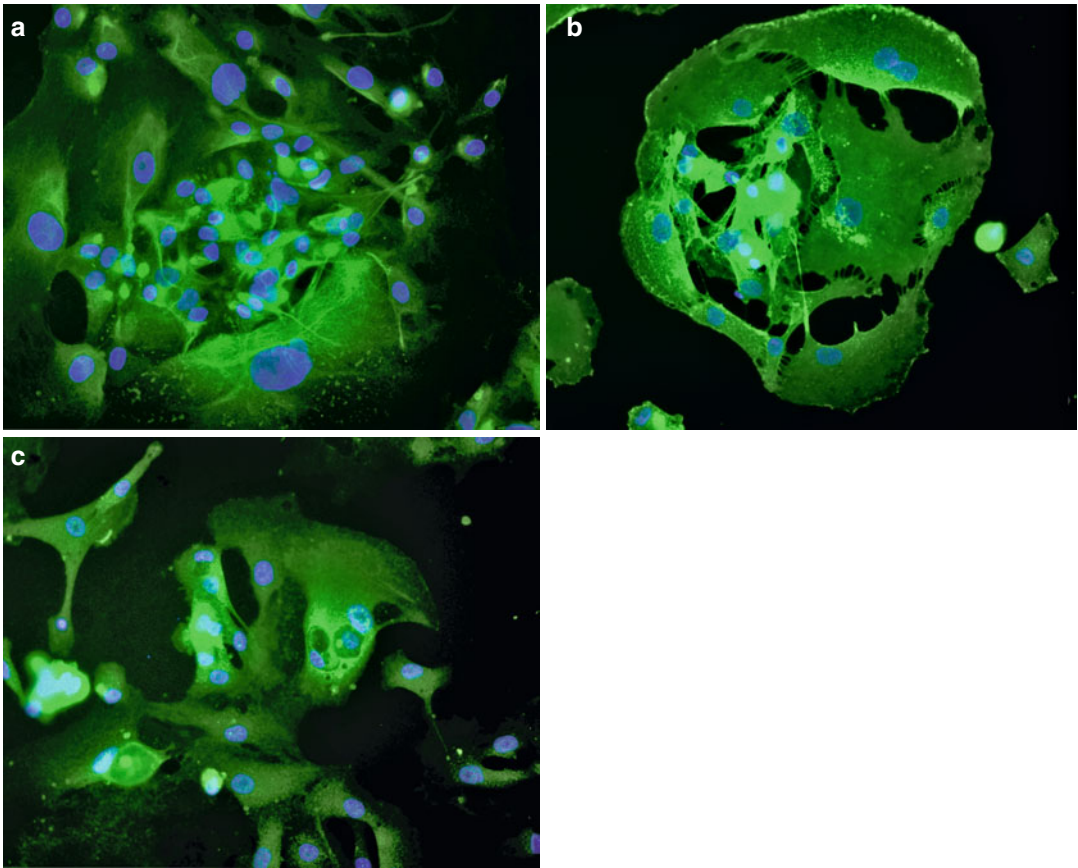
Fig. 3.1 Marker expression of primary meningioma cell cultures. Positive immunoreactivity appears as green staining (Alexa Fluor 488). (**a**) vimentin; (**b**) CD44; (**c**) EMA. Original magnification 20×
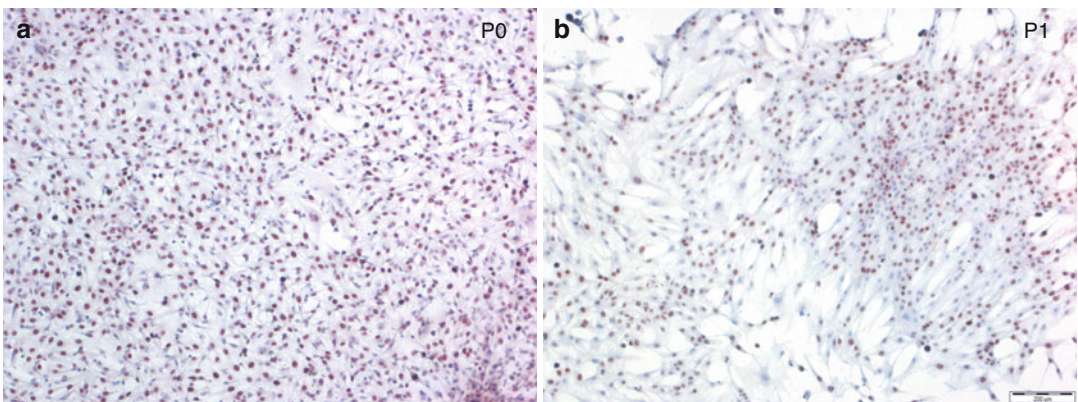

Fig. 3.2 Proliferation rate in primary meningioma cell cultures decreases with increasing passage (P). (**a–d**) Ki-67 nuclear staining in a representative meningioma. Positive immunoreactivity appears as red staining (Vector® NovaRED™). Original magnification 4×. (**a**) P0; (**b**) P1; (**c**) P2; and (**d**) P7
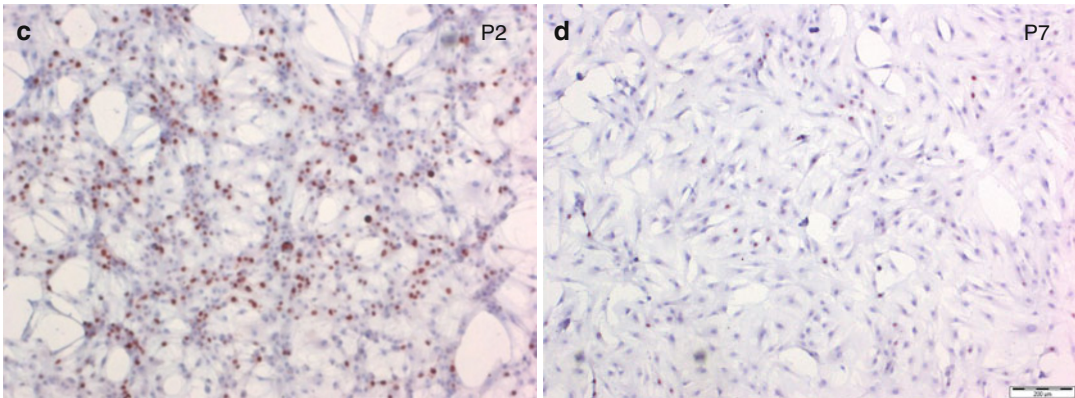
Fig. 3.2 (continued)

3.1.3 Senescence-Associated β-Galactosidase Staining

Expression of senescent-associated β-galactosidase activity was analysed in different passages of primary meningioma cell cultures using the Cellular Senescence Detection Kit (BioVision, Milpitas, CA) (Fig. 3.3).

3.2 Results

The mean Mib-1 SI in male patients was 5.8 %, whereas that of female patients was 3.9 %; patients with NF-II show significant higher mean Ki-67 LI (6.2 %). Ki-67 LI in WHO I tumours was significantly lower than in WHO II (atypical) and WHO III (anaplastic) ($p < 0.0001$), but not within the subclassifications of WHO grading (meningotheliomatous 3.3 %, fibrous 3.9 %, transitional 2.9 %, psammomatous 1.1 %, angiomatous 3.0 %, clear cell 0.7 % or microcystic meningiomas 4.4 %) (Table 3.1). First time treated meningiomas had a mean Ki-67 LI of 3.9 % versus 6.9 % in recurrent meningiomas ($P < 0.0001$). Ki-67 LI increases from the initial tumour operation to its second, third and in 5 cases to its fourth local recurrence. There was a transformation from benign to atypical meningioma from second to third local recurrence in 16 % of the cases, whereas no dedifferentiation from WHO I to WHO III or WHO II to WHO III has been seen. Significantly lower PR expression in WHO II (atypical) and III (anaplastic) meningiomas, compared to WHO I meningiomas, has also been observed.

Excluding WHO II and III meningiomas, as well as partially resected meningiomas for survival analysis, in WHO I and SI resected meningiomas, no significant difference in survival according to different mean Ki-67 LI or PR status has been found. Combining two factors of proposed prognostic significance in benign meningiomas – proliferation index (Ki-67 LI < 4 % vs. ≥ 4 %) and PR status (neg. vs. pos.) – a significant decreased recurrence-free survival could be shown for negative PR status and Ki-67 LI ≥ 4 % (Fig. 3.4).

For proliferation and PR status analysis, multiple age groups for each sex have been examined as well (<40, 41–55, 56–70 and >70), but no significance has been seen (Fig. 3.5). No relationship between the calcifications, meningioma subtype or proliferation index could be established. Although recurrent meningiomas exhibit higher proliferation rates in general, no age dependency has been seen.

3.3 Discussion

Meningiomas are mostly benign tumours that usually do not invade the brain parenchyma. The WHO grading system aiming to describe different types of tumour frequently fails to determine the clinical behaviour of meningiomas. Even in cases of complete removal according to the
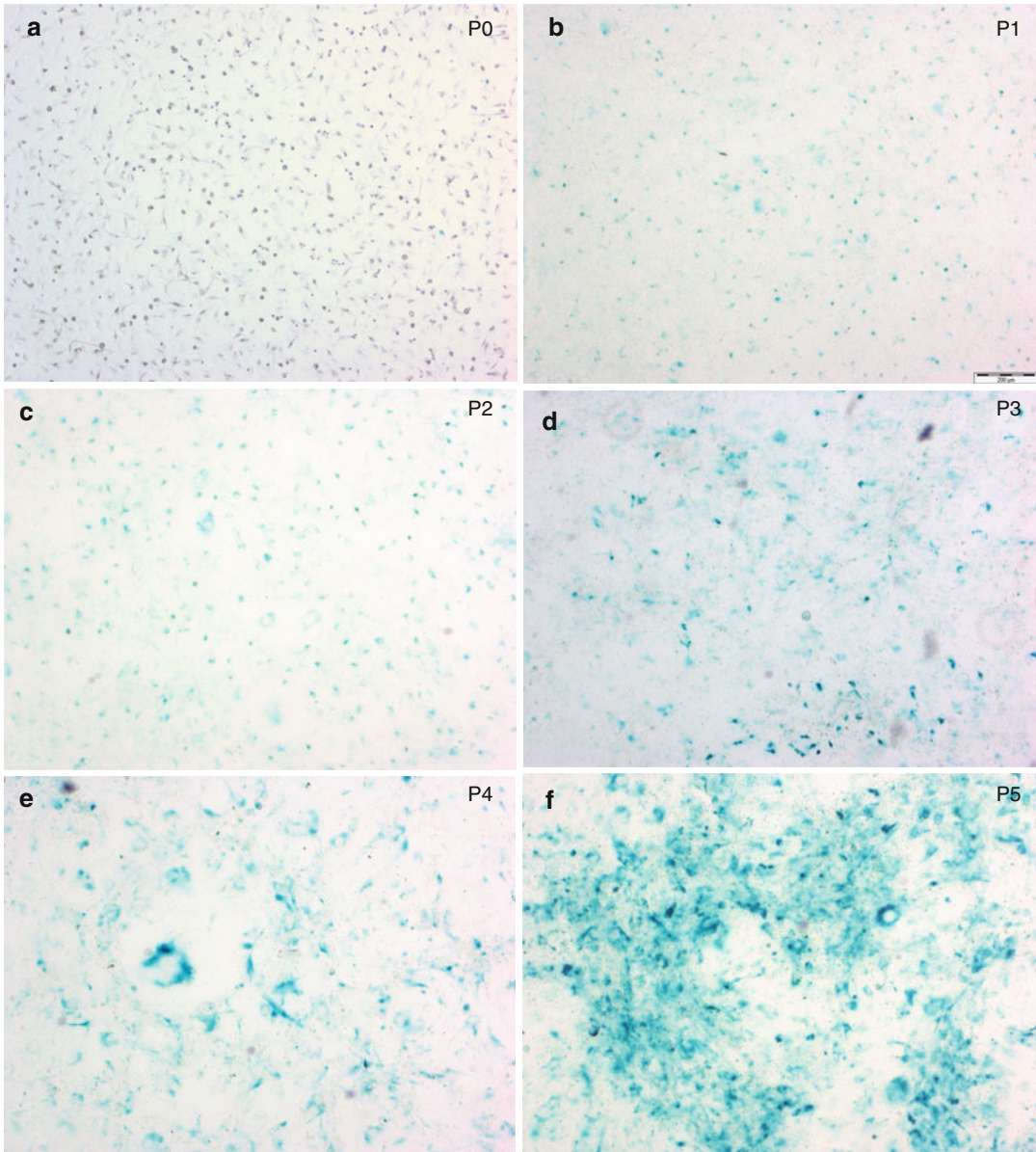
Fig. 3.3 Senescence development in primary meningioma cell cultures. Senescent cells express a senescent-associated β-galactosidase (X-gal). X-gal positive cells appear as blue-green staining. Original magnification 4×. (**a**) P0; (**b**) P1; (**c**) P2; (**d**) P3; (**e**) P4; and (**f**) P5

Simpson classification the chance of recurrence is high [52]. Pathological specimens in those cases do not show either atypical features or histopathological signs of increased biological activity. Proliferation markers like BrdU or biochemical markers like telomerase and topoisomerase IIα have been used to describe the clinical course in meningioma patients [8, 51]. In the case of BrdU Gudjonssona et al. a PET study showed that BrdU is not suitable as proliferation marker [14]. Whereas telomerase is a potential predictor for recurrence in meningiomas [18, 30], there are conflicting results for topoisomerase IIα as predictor for recurrence in meningiomas [23, 24].

Table 3.1 WHO grading in 1766 meningiomas operated from 1979 to 2002

WHO grading	Young (<70 years) n = 1,544		Aged (≥70 years) n = 222	
	Female n = 1,088	Male n = 456	Female n = 162	Male n = 60
I	92.2 % (1,424)		91.9 % (204)	
Meningotheliomatous	63.8 % (694)	58.6 % (267)	54.9 % (89)	58.3 % (35)
Fibrous	13.1 % (142)	11.2 % (51)	13.6 % (22)	8.3 % (5)
Transitional	11.0 % (120)	8.1 % (37)	11.1 % (18)	8.3 % (5)
Psammomatous	5.2 % (57)	2.6 % (12)	11.1 % (18)	10.0 % (6)
Other types	2.2 % (24)	4.4 % (20)	1.9 % (3)	5.0 % (3)
II	6.4 % (99)		7.2 % (16)	
Atypical (including clear cell, secretory)	4.0 % (43)	12.3 % (56)	6.8 % (11)	8.3 % (5)
III	1.4 % (21)		0.9 % (2)	
Anaplastic	0.7 % (8)	2.9 % (13)	0.6 % (1)	1.7 % (1)

Model1: PR-status negative or score <2
+Ki-67 LI > 4 %

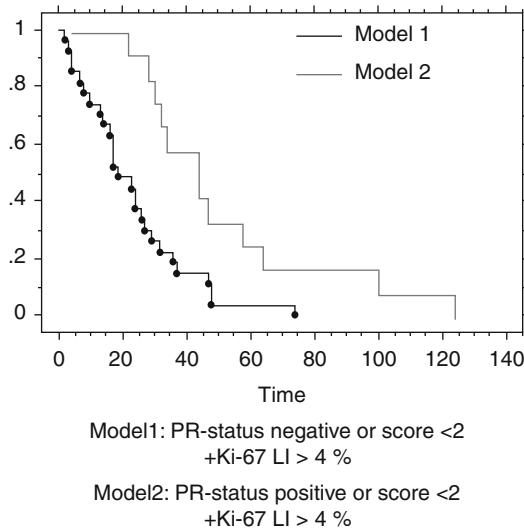Model2: PR-status positive or score <2
+Ki-67 LI > 4 %

Fig. 3.4 Recurrence-free survival II. Kaplan-Meyer Cum. Hazard plot for new recurrence. A model of two prognostic factors considering prognosis in total removed meningiomas (Simpson I+II). Time scale in month

Fig. 3.5 Statistical analysis of Ki-67 distribution in different age groups (1, <40; 2, 41–55; 3, 56–70; 4, >70) and separated for sex (*left*, females; *right*, males)

The Mib-1 monoclonal antibody, staining the Ki-67 antigen, has demonstrated its reliability in analysing meningioma growth and recurrence by several authors [1, 33, 36, 39, 47, 48].

Møller separated different resection grades and histology before survival analysis in 25 cases and could not give any significant prediction with Ki-67 immunohistochemistry as well [35]. Abramovich reported the absence of a significant difference in proliferation in
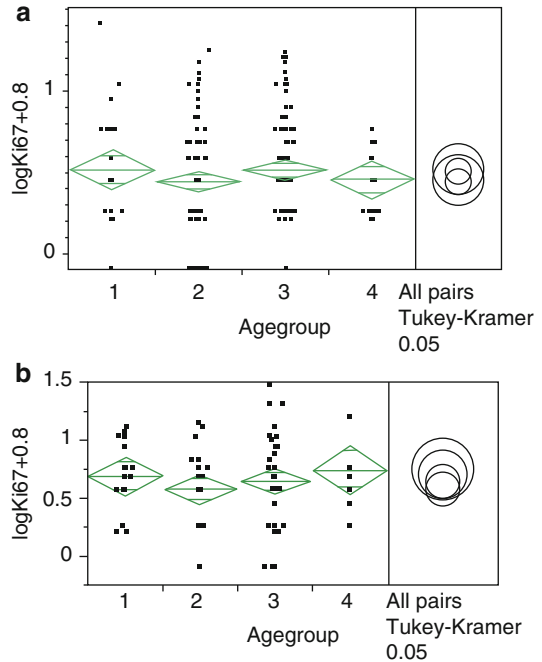
59 meningioma patients that had been radically operated, recognizing a clear overlap of the index range between the groups [1]. On the contrary, Ohta et al. showed a correlation with recurrence-free interval in 42 patients, but the follow-up time was 60 months and the statistics

were performed without dividing the meningiomas according to the WHO classification [38]. Perry et al. showed values for interpretation of borderline atypical meningiomas in 425 meningiomas through multivariate analysis [39]. Brain invasion, mitosis count > 3/10 and a mean Ki-67 LI > 4.2 % correlated with decreased recurrence-free survival. Matsuno et al. and Nakasu et al. reported a 3.2 and 3 % cut-off point for higher recurrence tendency [33, 36]. However, 20–50 % of the recurrent meningioma groups were atypical, and all nonrecurrent meningiomas were WHO I. Survival analysis was performed taking all patients together without dividing for surgical resection or histological grade. The small number of patients in each group and the uneven distribution of histological grades might have additionally influenced the mean LI and the survival analysis. Failing to separate different histopathological and resection grades exposes these studies to the criticism that Ki-67 may simply describe a well-known phenomenon, in other words that more aggressive histology and less aggressive regression correlate with shorter disease-free survival times.

Elevated proliferative activity in recurrent meningiomas throughout the histological grouping has been shown [1, 33, 35, 36, 38]. A decrease of the LI has only been reported by Madsen et al. [29]. Caution is recommended when using the proliferation index as the sole prognostic indicator or as a substitute for morphological diagnosis due to the overlap in each group (WHO I mean Ki-67 LI 0–30 %, WHO II mean Ki-67 LI 3–35 % or WHO III mean Ki-67 LI 5–58 %) [19, 22, 27]. This might be due to the heterogeneity of biological activity within the tumour tissue. Proliferating cells in all histological grades were found to be distributed heterogeneously throughout the tumour, especially in recurrences [38]. It is debatable that the focal accumulation of proliferation may affect tumour recurrence for the 'highest-area' counting method. The 'highest-area' counting method is preferred to minimize missing of focal accumulation of biological activity within the meningioma tissue, as it is recommended by Nakasu et al. [36]. This is an important issue when considering modern

neurosurgical techniques, where only small parts of tumour with unknown precise location of the tissue reach the pathologist's hands. Indeed, it is debatable whether recognized areas of high mitotic activity in meningiomas determined with either counting method reflect the proliferation status of the whole tumour.

A substantial number of patients inherit mean Ki-67 LI of 1 % with recurrence times ranging from 11 to 148 month. Therefore, a cut-off value over which a tumour becomes suspicious cannot be given. Precise values from different laboratories are not applicable to other institutions because of differences in methodology, counting procedures and interpretation of the results as reflected by the relatively wide range of initial and recurrent Ki-67 LI determined by several investigators.

The role of steroid hormones in the progression of meningiomas is still a matter of controversy. Several studies, which included meningiomas of all grades, suggested a more favourable prognosis for PR-positive meningiomas [15, 16] with high progesterone receptor status being associated with lower recurrence rates and vice versa [9, 46]. However, these studies frequently included meningiomas with different resection grades and histological subtypes. Hsu and colleagues suggested that only a combination of three proposed prognostic factors for survival, that is, WHO grade, proliferative index and PR status, should be used to predict meningioma recurrence [16]. However, their model was tested containing atypical and malignant meningiomas and therefore did not demonstrate the true influence of PR status on survival. The focus of interest should be benign borderline cases where additional information is needed for prognostic considerations. Maiuri et al. compared two groups of completely resected WHO I meningiomas, with and without recurrence [31]. Patient age, tumour location, consistency, vascularity, histological subtype, oestrogen receptor, vascular endothelial growth factor (VEGF), and epidermal growth factor receptor (EGFR) were not correlated with tumour recurrence. Higher mitotic index and Ki-67 LI and PR negativity were predictive factors of recurrence of benign completely resected

meningiomas. This study also showed a negative association between Bcl-2 and PR.

Data in the literature is pointing to a female preponderance in PR expression; however, these studies included a substantial number of atypical and malignant meningiomas [5, 9, 20]. These meningiomas are mostly devoid of PRs and more often found among male patients. After excluding atypical and malignant cases, no sex-related difference in PR expression could be found. Therefore, the previously reported sex-related difference might be due to particular selection criteria, which produced an inhomogeneous patient population.

Previous studies on the human endometrium have shown that progesterone plays a role in neovasculogenesis. High levels of progesterone receptors in meningiomas may indicate a similar situation. However, Fewings and Roser suggested that progesterone may inhibit angiogenesis, by enhancing the production of thrombospondin-1, an angiogenesis inhibitor [9, 46]. It is difficult to draw conclusions from these data, as the PRs in meningiomas are non-functional in the majority of cases [4]. Moreover, our observations come from a clinical and histopathological analysis and may well reflect a multifactorial situation with progesterone being only one variable in the process of tumour vascularization.

However, the PR status alone cannot be used to predict behaviour in benign meningiomas and should not influence the decision about follow-up intervals and therapeutic strategies. Moreover, Matsuda et al. demonstrated that the antitumoral effect of antiprogesterone agents on meningiomas, both in vitro and in vivo, exists regardless of the tumour's PR status, suggesting that the antitumoral effect is mediated by other pathways [32]. This was supported by Verheijen and coworkers, who suggested that PR-like proteins do not have a biological function and may lead to the overestimation of the PR status for clinical purposes [54].

Analysing Ki-67 LI and PR status in spinal versus intracranial meningiomas, the former was found to be higher in spinal disease (the latter being almost identical) [45]. These findings were compared with recurrence-free survival at follow-up to determine if a relationship between these two biological indicators and clinical behaviour of the tumours could be seen. However, recurrence-free survival after total tumour resection was found to be very similar in both patient groups. The data suggest that spinal meningiomas differ from their intracranial counterpart in terms of proliferation as measured by the Ki-67 LI; however, they display similar clinical behaviour and PR status. The discrepancy between the Ki-67 LI and recurrence rates cannot be fully explained on the basis of the present data.

Meningiomas are highly vascularized tumours. Several studies have demonstrated that VEGF has a significant influence in the proliferation and migration in meningiomas [26, 43]. Whereas some groups observed a correlation between the degree of VEGF expression and meningioma histological grade [26, 50], Barresi and Pfister showed that VEGF expression and WHO grade did not correlate in meningiomas [3, 40]. VEGFR2 is the predominant mediator of VEGF-stimulated endothelial cell migration, proliferation, survival and enhanced vascular permeability [13]. The presence of an autocrine loop for VEGF and VEGFR2 has been demonstrated in several tumour cell lines, which resulted in increased proliferation [11, 53]. Pfister et al. showed that KDR expression is mostly low or absent in meningiomas, which suggests that mechanisms besides VEGFA signalling through KDR influence angiogenesis in meningiomas [40]. In addition, this study demonstrated that meningiomas display a high PDGFRβ expression. The combination of high PDGFRβ levels and low KDR and PDGFB expression in meningiomas indicates that PDGFRβ has a significant function in tumorigenesis in meningiomas. When meningiomas were stimulated with exogenous VEGFA in vitro, they displayed a significant higher proliferation rate. This proliferative stimulus was abolished once PDGFRβ was inhibited before administration of exogenous VEGFA.

The variety and distribution of benign meningioma subtypes are age independent with the exception of a higher percentage of psammomatous meningiomas among older patients

[47]. Philippon et al. have suggested the higher percentage of calcified psammomatous meningiomas as a reason for lower recurrence rates in meningiomas [41]. Indeed, we found a significant higher percentage of psammomatous meningiomas in the elderly, but a relationship between psammomatous bodies and intratumoral calcification could not be established. Moreover, psammomatous meningiomas did not display lower proliferation rates than other benign subtypes. Proliferation rates and PR status, useful biological indicators of tumour activity, are age independent [47]. Survival measurements showed comparable outcome in both age groups within benign meningiomas. Different growth rates in different age groups cannot be explained by means of these two parameters alone.

Ongoing tissue research studies should focus on the growth behaviour of benign meningiomas to characterize specific alterations that could provide us with new diagnostic and prognostic criteria as a rational basis for treatment. The unsolved situation of clinical aggressive benign meningiomas (despite radiotherapy) should lead us to new concepts like chemotherapeutic agents which might play a role as radiosensitizers [37].

In histopathological borderline cases, with some but not convincing aspects of atypia, the Ki-67 LI, combined with the routine histopathological workup, can provide more insight in the behaviour of a meningioma, particularly in the presence of high vascularity, low PR status, subtotal resection and recurrence. PR status in combination with the proliferative index can be a useful prognostic tool for benign meningiomas. High scores of Ki-67 are worrisome and should lead to a closer follow-up for evidence of recurrent tumour, but the confidence in high LI should not interfere with the decisions for treatment plans as some authors recommend.

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