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Clinical Study

# An audit of immunohistochemical marker patterns in meningioma

David S. Baxter [a], Abiel Orrego [b], Jeffrey V. Rosenfeld [c], Tiit Mathiesen [a,*]

[a] Department of Clinical Neuroscience, Section of Neurosurgery, Karolinska Institute, R3:02 KS, Stockholm S-17176, Sweden
[b] Department of Clinical Pathology and Cytology, Karolinska University Hospital, Stockholm, Sweden
[c] Department of Neurosurgery, Alfred Hospital, Monash University, Melbourne, VIC, Australia

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ABSTRACT

Meningiomas may express a number of potentially growth-promoting receptors including receptors for progesterone, growth hormone and vascular endothelial growth factor (VEGF). These and other receptors are potential targets for chemotherapy. We have prospectively studied a panel of markers as a routine in order to obtain data of individual expression of markers that may provide targets for anti-receptor treatment. One hundred and seventy-five consecutive patients operated on for meningiomas between 2005 and 2008 were prospectively analysed with antibodies against receptors for growth hormone, insulin-like growth factor 1 (IGF-1), androgen receptors, progesterone receptors (PR) and antibodies against CD34, VEGF, Ki-67 and caspase-3. Expression of IGF-1 receptor (IGF-1r), epidermal growth factor receptor (EGFR) E30 and growth hormone receptor (GHr) was conserved across histological grades and found in 88% to 94% of meningiomas. PR were detected in 87%, but expression decreased in aggressive tumours. Angio-markers such as VEGF and CD34 were detected in 69% and 17% of meningiomas, respectively. Androgen receptors and caspase-3 were uncommon. The analyses of a panel were undertaken as a clinical routine in order to assess its feasibility and to provide data that can be utilised in a clinical setting. Three putative therapeutic receptor targets, IGF-1r, GHr and EGFR E30 were expressed in a large majority of tumours and in contrast to PR maintained expression despite increasing pathological grade of meningioma. Our data also suggest that anti-progesterone therapies and anti-angiogenic therapies could be targeted to subsets of meningioma patients who express PR or have CD34-positive tumours.



## 1. Introduction

Meningiomas account for up to 20% of all primary intracranial tumours [1]. The standard treatment is surgical [2]. In spite of removal, meningiomas are prone to recur. Recurrence rates of completely resected meningiomas approach 20% after 15 years [3] while subtotal resection results in a worse progression free survival rate: 61% at 5 years and 39% and 10 years [4]. Meningioma recurrence and progression are the main reasons for poor outcomes in meningioma patients [5].

Recurrent tumours may be surgically and radiosurgically inaccessible and adjuvant fractionated radiation therapy does not offer a cure [5]. There is a lack of effective pharmaceutical therapies. Several substances have improved outcomes in selected patients [6,7], but failed to show efficacy in prospective trials. We hypothesise that negative results for promising substances may reflect a lack of targeting to the relevant subgroups of recurrent meningiomas.

Immunohistochemistry (IHC) is an important tool to support diagnosis and prognosis in neurosurgical pathology [8]. Robustness is a prerequisite for the routine application of an antibody panel, limiting the analytic paradigm to standardised immunohistopathology of paraffin-embedded tissue. We presume that routine exploration of molecular meningioma markers allows a description of tumour vascularisation and receptor status, and that such a description is necessary for targeted therapies [9]. We have prospectively assessed a spectrum of IHC targets to enable a comprehensive description of meningiomas and to assess whether such a receptor signature may assist in understanding tumour behaviour and the selection of pharmacological therapies when needed. It is probable that the expression of a specific receptor is a prerequisite for a therapeutic effect of a drug that is targeted to the respective receptor. We sought a potential therapeutic target with conservation of target expression across all histopathological grades and with no negative correlation of expression with increasing proliferation indices.

## 2. Material and methods

Pathological diagnosis and IHC results of consecutive meningiomas surgically removed between 1 January 2005 and 30 April 2008 by one of the authors (T.M.) were prospectively collected

* Corresponding author. Tel.: +46 7 3966 1858.
*E-mail address:* tiit.mathiesen@karolinska.se (T. Mathiesen).

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from the Department of Pathology at the Karolinska University Hospital. All histopathology samples were diagnosed by an experienced neuropathologist and reviewed by one of the authors (A.O.).

Data on age, sex, primary, second primary, recurrence, World Health Organization (WHO) grading, histopathological subtype and IHC staining for vascular endothelial growth factor (VEGF)-A, epidermal growth factor receptor (EGFR) E30, insulin-like growth factor 1 receptor (IGF-1r), growth hormone receptor (GHr), androgen receptor (AR), CD34 and progesterone receptors (PR) were gathered along with IHC for caspase-3 and Ki67. Antibodies used were EGFR E30 (M 7239; Dako, Glostrup, Denmark), VEGF A20 (Sc 152; Santa Cruz Biotechnology, Dallas, TX, USA), IGF-1r (MAB1120; Chemicon/Millipore, Billerica, MA, USA), GHr (NCL-GHr; Novocastra/Leica, Heerbrugg, Switzerland), CD34 (M 7165; Dako), caspase-3 (NCL-CPP 32; Novocastra/Leica), AR (M 3562; Dako), PR (NCL-Pgr-312; Novocastra/Leica), and Ki67 (M 7240; Dako).

Analysis of frequency and variance was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) software for Microsoft Windows (Microsoft, Redmond, WA, USA). An assumption was made that the 191 tumours analysed represented a random sample of clinically significant meningioma. All hypotheses were formed prior to data collection and statistical testing in order to minimise the influence of mass-significance in multiple testing.

## 3. Results

One hundred and ninety cranial meningiomas in 182 patients were reviewed. Of these, 16 meningiomas were not operated upon by the author and were thus excluded. Of the remaining 175 tumours, 34 patients were male and 141 were female. There were 157 WHO grade 1 tumours, 17 WHO grade 2 and a single WHO grade 3 tumour. The age range spanned from 19 years to 88 years, with the highest incidence between the 5th and 7th decades of life (Fig. 1). There was no significant difference between the correlation of age at surgery and sex (n = 174, analysis of variance [ANOVA] $p = 0.89$), nor sex and WHO grade (n = 158, chi-squared $p = 0.653$).

Thirty-one patients had had at least one previous meningioma resection prior to the audit time frame. Of these previous resections, 23 patients had only one previous resection, five patients had two previous resections, two patients had three previous resections and one patient had six previous resections. When considering only the 175 tumour events included in the cohort, two patients had both the primary meningioma and a recurrence operated later and counted as separate events. One patient had the primary tumour and subsequently three resections for recurrences counted as four separate events. Twenty-three patients had at least one previous resection prior to the commencement of the audit. Of these, one patient's meningioma recurred twice within the dates of the audit and was thus counted as two separate tumour events. Although two patients' primary meningioma recurred during the study interval, the recurrence was not operated upon by the author and hence only the primary tumour was included in the study. The degree of PR expression was classified as documented in Table 1. Fifteen reports described specific "hot spots" of Ki67. The reported Ki67 value, not the hot spot value, was used.

A summary of the expression of cellular markers is shown in Table 2. Tumours from male patients had significantly higher Ki67 values than tumours from females (n = 171 [138 females, 34males] one-way ANOVA $p < 0.05$). Further, an ANOVA between Ki67 values and WHO grade demonstrated a significant positive relationship (n = 171, $p < 0.001$).

One hundred and seventy four tumours were analysed for both PR and Ki67/MIB1. Twenty-two meningiomas were PR negative, thus 87% were positive for PR expression (Table 2). A one-way ANOVA between Ki67 values and PR positivity demonstrated that Ki67 was higher in PR negative tumours (n = 170, $p = 0.001$). Further, a one-way ANOVA between Ki67 value and the percentage of tumour cells that were PR positive demonstrated a significant difference, with Ki67 values increasing as the percentage of PR positivity decreased (n = 162, $p = 0.002$). There was no statistical relationship found between EGFR E30, VEGF, GHr, AR or IGF-1r expression positivity and Ki67 value.

Additionally, there was no statistically significant relationship seen between caspase-3 expression and Ki67 value across all WHO grades. Examining the breakdown of tumours positive for caspase-3, of 147 grade I meningiomas 44 were positive, whereas five of 15 grade II tumours were positive.

Statistical chi-squared analysis to investigate the loss of expression of individual markers when compared with increasing WHO grade revealed no significant difference for EGFR E30 (n = 159), IGF-1r (n = 171), GHr (n = 169), CD34 (n = 103), caspase-3 (n = 162) or AR (n = 156). VEGF-A expression correlated negatively

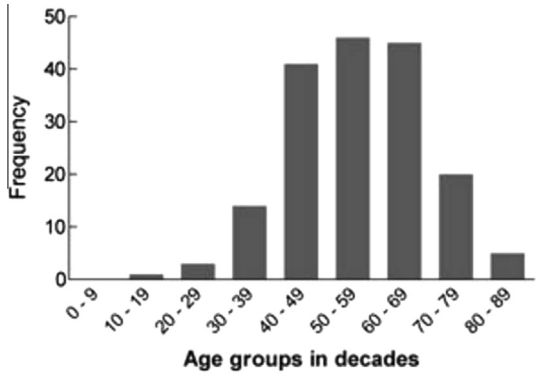


**Fig. 1.** Graph showing the frequency *versus* age at surgery for meningioma resection.

**Table 1**
Method of assigning percentage positive groups for descriptive reporting of progesterone receptor staining

| Group | Percentage positive | Descriptive equivalents |
|---|---|---|
| A | 0% | Absent |
| B | 1–20% | Occasional, focal, small, weak |
| C | 21–40% | |
| D | 41–60% | |
| E | 61–80% | Considerable |
| F | 81–100% | Majority, strong, bright |

**Table 2**
Summary of the percentage of all positive and negative receptor expression in meningiomas

| | Positive | Negative | Total | % positive |
|---|---|---|---|---|
| EGFR E30 | 144 | 15 | 159 | 91% |
| VEGF-A | 72 | 32 | 104 | 69% |
| IGF-1r | 161 | 10 | 171 | 94% |
| GHr | 148 | 21 | 169 | 88% |
| CD34 | 17 | 86 | 103 | 17% |
| Caspase-3 | 49 | 113 | 162 | 30% |
| Androgen | 10 | 146 | 156 | 6% |
| Progesterone | 152 | 22 | 174 | 87% |

EGFR = epidermal growth factor receptor, GHr = growth hormone receptor, IGF-1r = insulin-like growth factor 1 receptor, VEGF = vascular endothelial growth factor.