

## Meningioma

# A comparison of the cell lines used in meningioma research

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### Abstract

**Background:** Immortal cell lines and cell lines derived from operative specimens transplanted into animal models are used in meningioma research. We address 2 criticisms of the mouse xenograft flank tumor model: Why are tumor induction rates derived from operative specimens low and inconsistent? Are flank tumors meningiomas?

**Methods:** Meningioma cell cultures were processed for Giemsa-band karyotyping and flow cytometry. Mouse flank tumors induced subcutaneously were analyzed microscopically, immunohistochemically, and ultrastructurally. Giemsa-band studies identified meningiomas with simple karyotype ( $\leq 1$  chromosomal abnormality) or complex karyotype (multiple chromosomal abnormalities).

**Results:** Cell cultures with complex karyotypes (IOMM-Lee, CH-157 MN, 2 operative specimens) grew rapidly in vitro and induced tumors in 49 (98%) of 50 animals. Meningioma cell cultures with simple karyotypes grew slowly in vitro and showed small, nongrowing tumors in mouse flanks (10/10). Meningioma flank tumors were vimentin-positive with ultrastructural features consistent with meningiomas. Cell cultures with complex karyotypes grew faster in cell culture and consistently induced flank tumors, unlike meningiomas with simple karyotypes.

**Conclusions:** Meningioma cell lines transplanted into flanks of nude mice exhibit microscopic, immunohistochemical, and ultrastructural features of meningiomas. The ease of monitoring tumor growth in the subcutaneous mouse flank model is its primary advantage, although we recognize an intracranial location is more biologically desirable.

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### Keywords:

CH-157 MN; IOMM-Lee; Meningioma; Xenograft mouse model

## 1. Introduction

Meningiomas are slow-growing, benign tumors that arise from the central nervous system meninges [2]. Specialized

*Abbreviations:* DMEM, Dulbecco Modified Eagle Medium; DPBS, Dulbecco phosphate-buffered saline; EMA, epithelial membrane antigen; FACS, fluorescent activated cell sorting; GFAP, glial fibrillary acidic protein; G1, gap 1; G2, gap 2; hpf, high-powered field; H&E, hematoxylin and eosin; IOMM-LEE, intraosseous malignant meningioma; IRB, Institutional Review Board; PBS, phosphate-buffered saline; S, synthesis; SD, standard deviation; TEM, transmission electron microscopy.

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meningothelial cells called arachnoid cap cells are the cells of origin of meningiomas. These cells are most common within the arachnoid villi but may be present throughout the craniospinal arachnoid space. Meningiomas account for approximately 20% of all primary adult intracranial tumors. They are more common in women (2:1) and generally occur in older patients. Meningiomas are graded as benign (approximately 91% of meningiomas), atypical (5%), and anaplastic/malignant (4%) [10,22]. The grading of meningiomas takes into account both the tumor subtypes known to have a higher rate of recurrence and specific histologic features suggesting a more aggressive biology [6,21]. Although most meningiomas can now be removed safely, their intrinsic biology and location are still the main determinants of the overall outcome of the patient [6,21,35].

Table 1  
Characteristics of the meningiomas used in this comparison study

Patient no./Sex/Age	Tumor grade <sup>a</sup> /Subtype	Location	Model number	Chromosomal Abnormalities
1. IOMM-Lee. male/61 [18]	WHO III	Intraosseous	48 (44-49)	X, -Y, -1, <i>add (2)(p11.2)</i> , <i>add (5)(p13)</i> , <i>add (6)(p13)</i> , <i>i(7)(p10)x2</i> , <i>add(9)(q21)</i> , <b><i>add(12)(q21)</i></b> , -17, <i>add(14)(q13)</i> , <i>add(19)</i> , <i>add(20)</i> , and +2-4 mar[p20]
2. CH-157 MN. female/55 [36,37]	Unknown	Unknown	73-82 <3n>	X, <i>add (X)(p11.2)</i> , -X, <i>add(1)(q21)</i> , <i>add(1)(p13)</i> , +2, +3, +5, -1, <i>i(8)(q10)x2</i> , +8, <i>add(11)(p11.2)</i> , +11, <b><i>+12</i></b> , <i>add(14)(p11.2)</i> , -15, <i>i(15)(q10)</i> , -16, +16, -17, -18, +20, -22, +4-9mar[cp10]
3. Male/71	WHO I/Meningothelial	Tentorial	47 (46-48)	<b>X</b> , <b><i>add(1)(p34)</i></b> , <b><i>add(4)(p12)</i></b> , <b><i>+del(5)(q31)</i></b> , <b><i>add(6)(q27)</i></b> , <b><i>+add(9)(p21)</i></b> , <b><i>add(12)(q21)</i></b> [cp20]
4. Male/55	WHO I/Transitional	Convexity	47 (47-48)	<b>X</b> , <b><i>add(1)(p34)</i></b> , <b><i>add(4)(p12)</i></b> , <b><i>+del(5)(q31)</i></b> , <b><i>add(6)(q27)</i></b> , <b><i>+add(9)(p21)</i></b> , <b><i>add(12)(q21)</i></b> , <i>add(12)(q23)</i> [cp20]
5. Female/71	WHO I/Transitional	Sphenoid wing	48 (47-51)	XX, None
6. Female/41	WHO I/Fibrous	Parasagittal	46	XX, -22
7. Female/63	WHO I/Transitional	Cerebellopontine angle	46	XX, None
8. Female/38	WHO I/Psammomatous	Olfactory groove	45	<b>X</b> , <b><i>add(4)(p12)</i></b> , +5, <b><i>del(5q)(q22q31)</i></b> , <b><i>add(6)(q23)</i></b> , +7, +9, <b><i>add(9)(q22)</i></b> , <b><i>add(12)(q13)</i></b> [cp10]
9. Female/49	WHO I/Transitional	Posterior fossa		
10. Female/38 (NYG)	WHO I/Transitional	Paraclinoid		
11. Female/47	WHO I/Transitional	Cerebellopontine angle		

+ indicates entire chromosome addition; -, entire chromosome deletion; add, partial chromosome addition; del, partial chromosome deletion.

Giemsa-band karyotyping was obtained on 8 meningiomas grown in vitro. Meningiomas studied exhibited a normal karyotype, a single chromosomal abnormality, or multiple chromosomal abnormalities. Text in boldface type indicates similar abnormalities between karyotyped meningiomas used in this study. Underlined text indicates meningioma chromosomal abnormalities reported in the literature. Italicized text indicates similarities between the immortal cell lines.

<sup>a</sup> Meningiomas were graded according to WHO [21].

The study of meningioma biology and possible treatment regimens involves in vitro and in vivo studies on immortal cell lines as well as cell lines derived from operative specimens [14,18,36,37]. Cell culture experiments are not necessarily a reflection of in vivo responses, so meningioma animal models were developed [25]. Transplantation of human meningiomas into guinea pig eyes was first described in 1945 [11]. Since that time, several xenograft animal transplantation models have been described to include the chorioallantoic membrane of the duck and chick, as well as the subcutis, subrenal capsule, flank, brain, and skull base of athymic mice [23-26,30,38,40]. Obviously, the intracranial model is the most biologically desirable, but it is limited by the difficulty in obtaining direct serial measurements. The subrenal capsule has high tumor induction rates, but transplantation to this location is technically challenging and does not allow for direct serial measurements. Flank models were initially limited by low tumor induction rates until Matrigel augmentation was first described by our laboratory in 1998 [13,15]. Initially, Matrigel augmentation was noted to induce tumors in 100% of mouse flanks, but this initial report was optimistic, and our experience over the last 8 years places tumor induction closer to 60% (R.L. Jensen, personal observation) [13]. Furthermore, other investigators have not been able to replicate our tumor induction rates (R.L. Jensen, personal observation).

The purpose of this article was to analyze our meningioma mouse flank tumor model to address 2 long-held criticisms: (1) Why are tumor induction rates so poor in

animals injected with cell lines derived from operative specimens? (2) Are mouse flank tumors meningiomas? We reviewed our laboratory experience over the past 3 years with 2 immortal meningioma cell lines and cells derived from operative specimens in an attempt to address these criticisms.

## 2. Materials and methods

### 2.1. Analysis of our experience with the meningioma mouse flank model

Over the last 3 years, our laboratory has used the mouse meningioma flank model to study the effects of various therapies on tumor growth (data not shown). The following report represents the control data from these in vitro and in vivo experiments. Consequently, not all operative specimens grown in vitro were processed for all experiments or implanted into the flanks of mice for in vivo studies.

### 2.2. Meningioma surgical specimens

Meningioma surgical specimens were obtained under a University of Utah IRB protocol and processed for immunohistochemistry and cell culture as described below. Tumors were graded according to World Health Organization (WHO) criteria [21]. Patient data for in vitro and in vivo studies are listed in Table 1. In this article, we will use the term "benign meningioma" to describe a WHO grade I meningioma and "primary cell line" to refer to

newly established cell lines established from operative samples [21].

### 2.3. Meningioma cell culture

Tumor specimens were obtained from patients harboring meningiomas and grown as monolayer as described previously [12,14]. Briefly, operative specimens were taken immediately from the operating room, digested in collagenase, and placed in DMEM (Sigma) supplemented with 10% fetal calf serum, L-glutamine (2  $\mu\text{mol/L}$ ), penicillin (50 IU/mL), and streptomycin (50 mg/mL). Cultured cells were maintained at 37°C in 7.5% carbon dioxide. Only passages 1 to 5 were used in experiments for this study.

The human immortal cell lines IOMM-Lee and CH-157 MN were also grown in DMEM supplemented with 10% fetal calf serum, L-glutamine (2  $\mu\text{mol/L}$ ), penicillin (50 IU/mL), and streptomycin (50 mg/mL) at 37°C in 7.5% carbon dioxide [18,36]. The IOMM-Lee and CH-157 MN cell lines were kind gifts from Dr Ian McCutcheon (University of Texas, M.D. Anderson Cancer Center, Houston, Tex) and Dr Yancey Gillespie (University of Alabama School of Medicine, Birmingham, Ala), respectively.

### 2.4. Karyotyping: Giemsa-band chromosome analysis

The meningioma cell lines were grown in T-175 flasks to confluence and processed by the cytogenetics core facility at the University of Utah for Giemsa-band chromosome analysis.

### 2.5. Cell counts by bright-line hemocytometer

Cell lines were plated into 6-well plates at a density of  $1 \times 10^4$  or  $1 \times 10^5$  per well. Cell counts were accomplished each day (days 1–14) by harvesting the cells with trypsin and counting them using a bright-line hemocytometer. Cells in 2 wells were counted for each time point, and 2 counts were done per well for a total of 4 counts per time point. Doubling times were calculated during logarithmic growth.

### 2.6. Fluorescent activated cell sorting for cell-cycle phase

IOMM-Lee, CH-157 MN, and meningioma cell lines derived from operative specimens were plated in T-175 flasks (immortal cell lines  $1 \times 10^6$  cells per flask and operative cell lines  $1 \times 10^7$  cells per flask) at 30% confluence and allowed to grow for 24 hours to ensure logarithmic growth. Cells were harvested using Accutase (MP Biomedicals, Irvine, Calif) and declumped by drawing them through a 26-gauge needle 5 times. Cells ( $1 \times 10^7$ ) were resuspended in 1 mL of ice-cold DPBS and fixed by adding 2 mL of ice-cold methanol, then stored at 4°C for at least 2 hours. Cells are then rehydrated for 5 minutes in DPBS on ice and stained in 1 mL of DPBS/propidium iodide solution (50  $\mu\text{g/mL}$  propidium iodide, 200  $\mu\text{g/mL}$  RNase A, 0.1% Triton X-100) for 30 minutes in the dark at room temperature. The cells were sorted within 5 hours of staining by FACScan Analyzer (Becton-Dickinson, Franklin

lakes, NJ), and results were analyzed using CellQuest (Becton-Dickinson).

### 2.7. Mouse xenograft flank model: transplanting and harvesting tumors

Cells were grown to confluence in T-175 flasks. All steps were carried out on ice. T-175 flasks were rinsed with PBS, trypsinized, counted, and pelleted at 1000 rpm for 5 minutes at 4°C. Excess medium was removed, and the pellet was resuspended in medium to obtain between  $5.0 \times 10^5$  and  $1.5 \times 10^6$  cells per milliliter for the IOMM-Lee cell line, between  $1.0 \times 10^6$  and  $1.25 \times 10^6$  cells per milliliter for the CH-157 MN cell line, and as many cells as were present in a T-175 flask for primary meningioma cell lines (cell counts for patients 3, 4, 5, and 6 are  $1.6 \times 10^7$ ,  $1.1 \times 10^7$ ,  $1.0 \times 10^5$ , and  $1.7 \times 10^6$  cells per flank, respectively). One-milliliter aliquots was placed in 2-mL microtubes and spun at 1000 rpm for 5 minutes at 4°C. Excess medium was removed, and the tumor cells were resuspended and aspirated into 1-mL tuberculin syringes. For the IOMM-Lee and CH-157 MN cell lines, 0.5 mL of media was used to resuspend the pellet before aspiration into the tuberculin syringe. For primary meningioma cell lines, 0.5 mL of Matrigel (B.D. Biosciences, Bedford, Mass) was used to resuspend the pellet before aspiration into the tuberculin syringe. Tumor cells were injected subcutaneously into the flanks of 3-week-old immunodeficient mice (CD1, Charles River Laboratories, Wilmington, Mass) using a 25-gauge needle. A total of 20 mice were injected with the IOMM-Lee cell line (5 mice  $\times$  4 experiments), and 10 mice with the CH-157 MN cell line (5 mice  $\times$  2 experiments). Five animals each were injected with cell lines derived from patients 3, 4, 5, and 6. Biweekly caliper measurements are started 10 to 14 days after injection to give time for the media or Matrigel to resorb completely.

Mice were killed with a lethal intraperitoneal injection of pentobarbital. Flank tumors were excised, cut into blocks, and placed in 10% formalin for paraffin blocks or in 2.5% paraformaldehyde and 1% glutaraldehyde for electron microscopy.

### 2.8. Survival analysis

Survival curves were graphed by analyzing the number of mice surviving until killing (Comparison of Survival Curves, GraphPad Prism 4.0, San Diego, Calif). Animals were killed because of large tumor size.

### 2.9. Immunohistochemical staining for vimentin, EMA, GFAP, and MIB-1

Immunohistochemical stainings were performed by ARUP Laboratories (Salt Lake City, Utah). Briefly, slides were cut at 4  $\mu\text{m}$ , then melted at 55°C to 60°C for 30 minutes, deparaffinized in xylene for 5 minutes, and rehydrated in graded alcohols (100%  $\times$  2, 95%  $\times$  2, 70%  $\times$  1) for 1 minute each. The following steps were performed on the Ventana ES (Ventana Medical Systems, Tucson, Ariz) at 40°C. Heat-

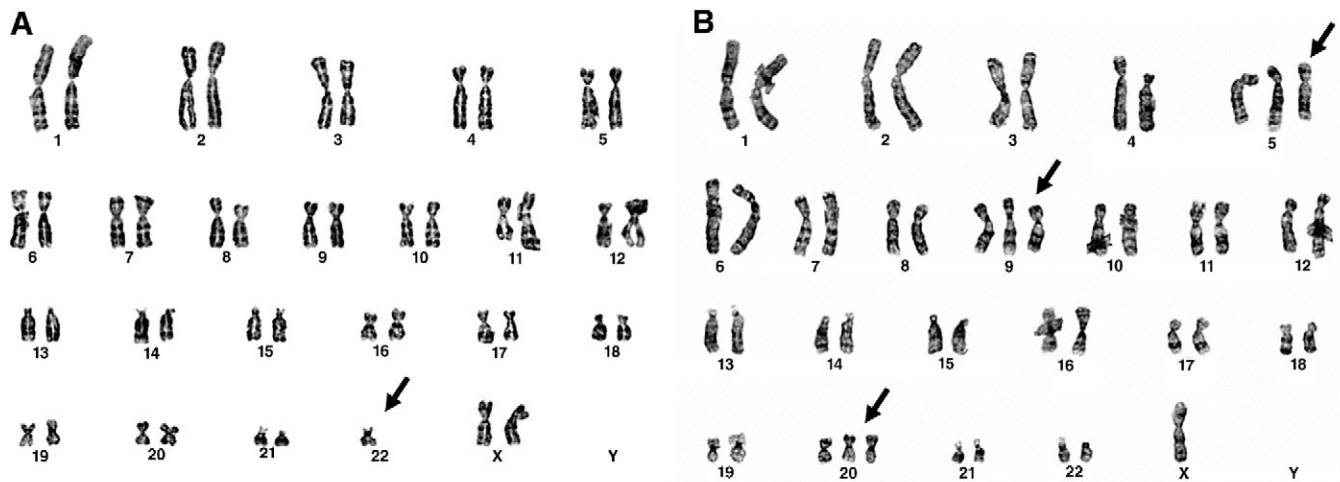


Fig. 1. Representative Giemsa-banded analysis of simple (A) and complex (B) karyotypes. A: Patient 6 showed a simple karyotype with monosomy 22 (arrow). B: Patient 4 exhibited a complex karyotype with multiple chromosomal abnormalities primarily involving chromosomes 1, 4, 5, 6, 9, and 12 (arrows).

induced epitope retrieval was applied in citrate buffer (pH 6.0) in a microwave oven for 15 minutes at half power and allowed to cool for 15 minutes for tissue stained for vimentin, EMA, and GFAP. For the MIB-1 immunohistochemical staining, heat-induced epitope retrieval was applied in citrate buffer (pH 6.0) in an electric pressure cooker for 3 minutes and allowed to cool for 27 minutes. The primary antibodies were applied for 32 minutes (Vimentin—1:300, mouse monoclonal Ab, Clone Vim 3B4, Dako Cytomation, Carpinteria, Calif; EMA—1:200, mouse monoclonal Ab, Clone E29, Dako Cytomation; GFAP—1:400, mouse monoclonal Ab, Clone 6F2, Dako Cytomation; Ki-67—1:100, mouse monoclonal Ab, Clone MIB-1, Dako Cytomation; Factor 8—1:1600, rabbit polyclonal Ab, Dako Cytomation) followed by the appropriate secondary antibody for 8 minutes (human tissue—goat antimouse/antirabbit [1:300, Rabbit Fab, Dako Cytomation]; mouse tissue—Mouse Fab [1:200, Mouse IgG, Dako Cytomation]). Detection was done using the IView DAB detection kit (Ventana), and the counterstain was done with hematoxylin (Ventana) for 4 minutes. Slides were then dehydrated through graded alcohols (70% × 1, 95% × 2, 100% × 2) for 30 seconds each, dipped in 4 changes of xylene, and covered with a coverslip. Positive controls consisted of normal uterus, normal pancreas, pancreas tumor, and normal tonsil for vimentin, EMA, MIB-1, and factor 8, respectively. Negative controls were accomplished by running the above positive control tissue without the primary antibody.

#### 2.10. MIB-1 immunohistochemical analysis

Analysis of MIB-1 staining was performed by taking 6 random pictures per slide at ×400 (10 ocular × 40 objective) using an Olympus Microfire camera. The images were analyzed using the Image-Pro Plus 5.0 graphic analysis program (Media Cybernetics, Silver Spring, Md). Results were reported as number of MIB-1 cells positive per ×40 hpf and represent the

mean (±SD) of 6 random fields per slide. Two slides were analyzed per original operative sample (ie, 12 total data points). For meningioma flank tumors, 1 slide was analyzed per animal, 3 animals per group (ie, 18 total data points).

#### 2.11. Transmission electron microscopy

Operative and mouse tumor samples were harvested, cut into 1-mm<sup>3</sup> blocks, and fixed in 2.5% paraformaldehyde with 1% glutaraldehyde. Cell culture meningiomas were grown to confluence in T-175 flasks, rinsed with PBS, scraped, pelleted, and fixed in 2.5% paraformaldehyde with 1% glutaraldehyde. After at least 24 hours of fixation, samples were rinsed twice for 10 minutes each in 0.1 mol/L sodium cacodylate buffer with sucrose and calcium chloride at pH 7.4. This was followed by postfixing in 2% osmium tetroxide in 0.1 mol/L sodium cacodylate buffer for 45 minutes at room temperature on a rotator. Samples were then rinsed in distilled water for 5 minutes and stained en bloc in aqueous uranyl acetate for 45 minutes at room temperature on a rotator. Samples were then dehydrated with increasing alcohol concentrations (50%–100%) at room temperature on a rotator. Cells were then infiltrated with and then embedded in Spurr's plastic. Thin sections were cut on a Leica UCT Ultramicrotome with a Diatome diamond knife, stained with uranyl acetate and Reynolds's lead citrate, and examined on a Hitachi H 7100 TEM at 75 kV. Photographs were taken on Kodak 4489 film.

### 3. Results

#### 3.1. Meningioma characteristics

Tumors were taken from 9 patients for establishment of primary cell cultures and the immortal cell lines IOMM-Lee and CH-157 for a total of 11 cell cultures. Of these 11 patients, 73% were women; the age range of the patients

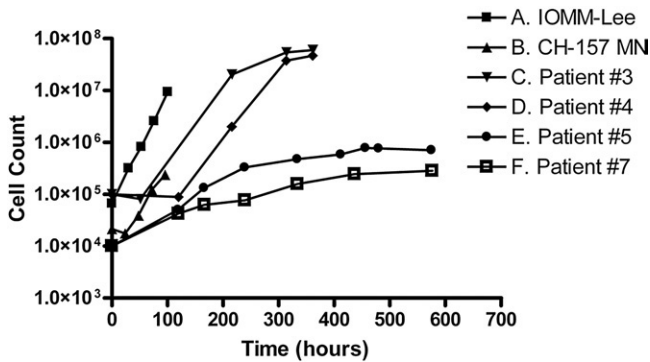


Fig. 2. Logarithmic graph (Log10) comparing the in vitro growth rates of immortal cell lines (A-B) and benign meningiomas cultivated from operative samples (C-F) (Table 1, patients 3, 4, 5, and 7). The IOMM-Lee (A) and CH-157 MN (B) malignant cell lines showed doubling times of ~20 and ~23 hours, respectively. Cultures obtained from operative specimens fell into fast- and slow-growing groups (C-F). Faster-growing cell lines were from patients 3 and 4 (C-D) with doubling times of ~40 and ~65 hours, respectively. Cell lines from patients 5 and 7 (E-F) showed slower growth with doubling times of ~120. Note: operative specimens exhibited contact inhibition with slowing of their growth rates as they reached confluence, unlike the immortal cell lines. Error bars represent  $\pm$ SD.

was from 38 to 71 years, with a median of 53 years (Table 1) [18,36]. Intracranial meningioma location in the patients varied, as did the meningioma subtypes (although 6 were benign transitional tumors) (Table 1).

### 3.2. Giemsa-band karyotyping

Giemsa-band karyotyping was achieved on both immortal cell lines and 6 primary meningioma cell lines (Table 1). Giemsa-band analysis exhibited 3 general karyotypes: normal, a single chromosomal abnormality, or multiple chromosomal abnormalities. We grouped these further into simple and complex chromosomal karyotypes. Simple karyotypes were defined as normal or only one chromosomal abnormality, whereas complex karyotypes were meningiomas exhibiting multiple chromosomal abnormalities (Fig. 1). Karyotyping of the IOMM-Lee cell line showed a complex karyotype with multiple chromosomal abnormalities fitting its initial description in 1990 (Table 1) [18]. The CH-157 MN cell line also exhibited a complex karyotype (Table 1). Both immortal cell lines showed additions to chromosomes 2, 5, 12, and 14 with loss of chromosome 17 (Table 1). Complex karyotypes were also seen in patients 3, 4, and 8, with similar chromosomal abnormalities noted on chromosomes 4, 5, 6, 9, and 12 (Table 1). Simple karyotypes were seen with patient 6 (monosomy 22) (Table 1) and patients 5 and 7 (normal karyotype) (Table 1).

### 3.3. In vitro growth rates

The in vitro growth rates of 6 cell lines were analyzed. Immortal meningioma cell lines showed the fastest in vitro growth rates, with doubling times of 20 and 23 hours for IOMM-Lee and CH-157 MN cell lines, respectively

(Fig. 2). Meningiomas cultivated from operative specimens were noted to fall into fast- and slow-growing groups (Fig. 2). Fast-growing cell lines were derived from patients 3 and 4, with doubling times of 40 and 65 hours, respectively (Fig. 2). Slower-growing cell lines were obtained from patients 5 and 7, with doubling times of approximately 120 hours (Fig. 2). Interestingly, faster-growing cell lines had complex karyotypes (IOMM-Lee, CH-157 MN, patient 3, and patient 4), whereas slower-growing cell lines were derived from cell cultures exhibiting a simple karyotype (ie, patients 5 and 7). Unlike the immortal cell lines, all cell lines cultivated from our operative specimens showed contact inhibition, slowing their growth rates as they reached confluence. Plating efficiency for the immortal cell lines was greater than 90%, whereas plating efficiency of cell lines obtained from operative specimens ranged from 40% to 90% and was dependent on trypsinization times; improved plating efficiency was seen with lower trypsin times.

### 3.4. Fluorescent activated cell sorting for cell-cycle phase

Flow cytometry for cell cycle characteristics was obtained on 7 cell lines growing logarithmically in vitro (IOMM-Lee, CH-157 MN, patients 4, 5, 8, 9, and 11) (Fig. 3). The mean ( $\pm$ SD) percentage of IOMM-Lee cells in G1, S, and G2 was  $40\% \pm 3\%$ ,  $37\% \pm 8\%$ , and  $23\% \pm 7\%$ , respectively. The CH-157 MN cell line exhibited  $58\% \pm 5\%$ ,  $38\% \pm 2\%$ , and  $4\% \pm 3\%$  of cells in G1, S, and G2 phase, respectively.

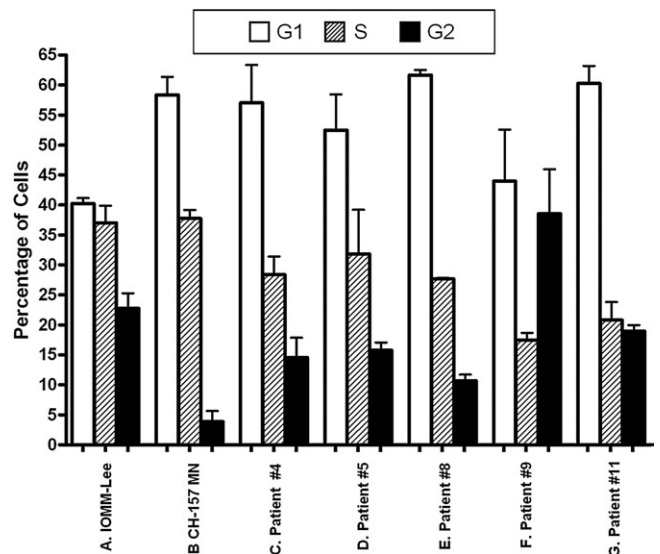


Fig. 3. Flow cytometry cell cycle characteristics of logarithmic growing meningioma immortal cell lines and cell lines from operative specimens (Table 1, patients 4, 5, 8, 9, and 11). The immortal cell lines IOMM-Lee (A) and CH-157 MN (B) showed a greater percentage of cells in S phase when compared with the 5 benign cell lines analyzed (C-G). Benign meningiomas exhibited variability in the percent of cells in G1, S, and G2. Error bars represent  $\pm$ SD.

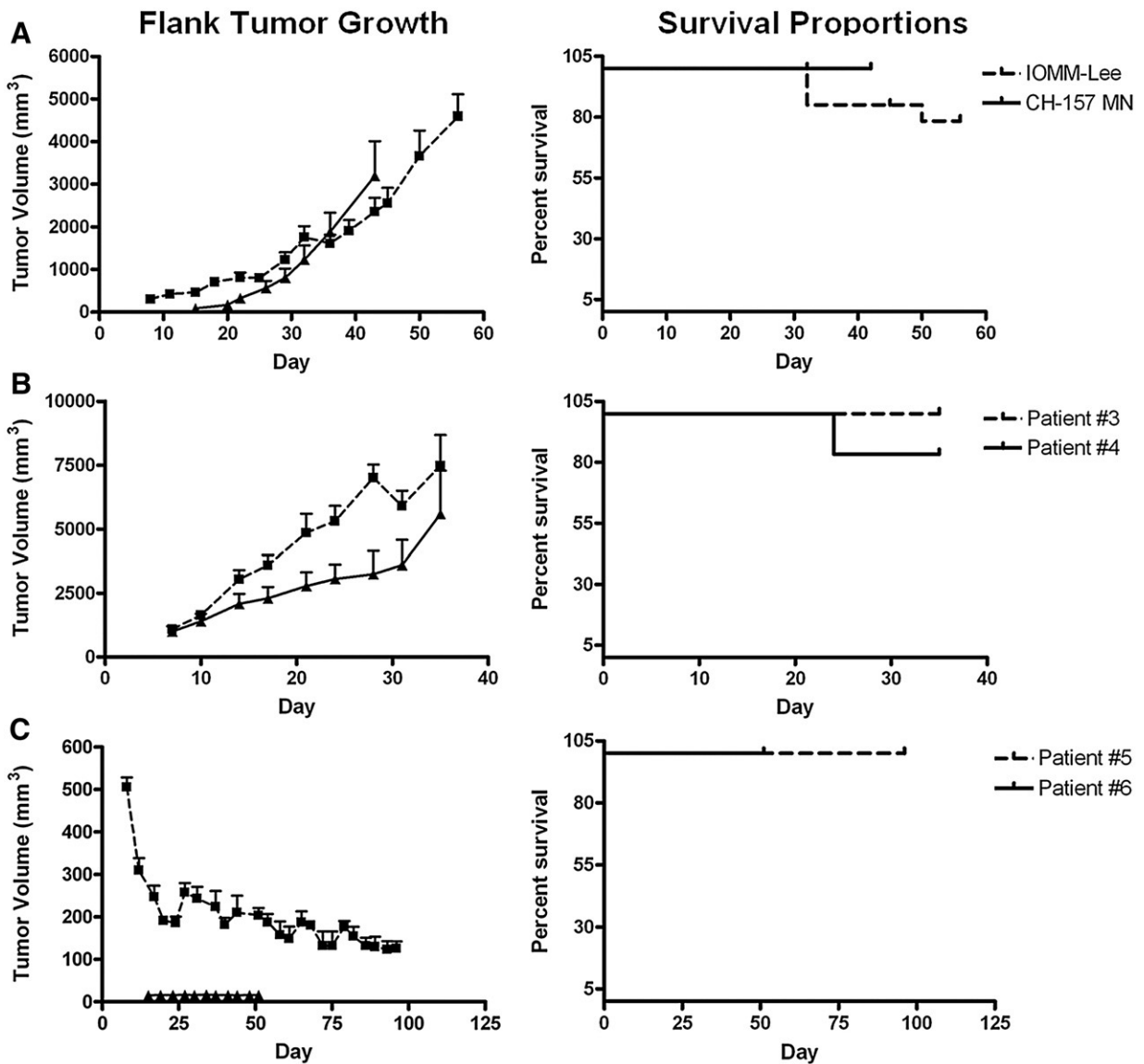


Fig. 4. Line graphs depicting the growth of meningioma subcutaneous flank tumors, as well as corresponding mouse survival proportions. A: The immortal cell lines induced flank tumors in 19 (95%) of 20 and 10 (100%) of 10 animals for the IOMM-Lee and CH-157 MN cell lines, respectively. Of 20 animals, 4 (20%) died in the IOMM-Lee group, whereas no animals died in the CH-157 MN group. Animals were killed after 43 days at varying times because of tumor size as per animal protocol. Error bars represent  $\pm$ SD. B: Two complex karyotype cell lines (patients 3 and 4) showed 100% (5/5) tumor induction. Of 5 animals with subcutaneous tumors induced from patient 4, 1 (20%) died. All animals were killed at 36 days because of tumor size as per animal protocol. Error bars represent  $\pm$ SD. C: Two simple karyotype cell lines incited tumors that did not grow (patients 5 and 6). No animals died. Error bars represent  $\pm$ SD.

The cell lines derived from operative specimens showed between 44% and 62% of cells in G1, between 17% and 32% of cells in S, and between 11% and 39% of cells in G2. Overall, the immortal cell lines IOMM-Lee and CH-157 MN showed greater than 35% of cells in S phase, whereas the percentage of cells in S phase of operative cell cultures was lower (Fig. 3).

### 3.5. Mouse flank tumor growth rates and survival proportions

A total of 6 cell lines were implanted into the flanks of nude mice, 4 cell lines with a complex karyotype (IOMM-

Lee, CH-157 MN, patient 3, and patient 4) and 2 cell lines with a simple karyotype (patients 5 and 6) (Fig. 4). The IOMM-Lee cell line induced flank tumors in 19 (95%) of 20 animals after subcutaneous injection of  $5.5 \times 10^5$  to  $1.4 \times 10^6$  cells per flank. Of 20 animals, 4 (20%) died of tumor involvement. At autopsy, all 4 animals showed severe abdominal ascites with tumors invading through the abdominal peritoneum. Large organ metastases (eg, lung, liver, kidney, and intestines) were not noted. Animal death was believed to be from compromise of the abdominal peritoneum with weeping of serous fluid into the abdominal cavity. All animals were killed at 56 days because of tumor

Table 2

Immunoreactivity of meningioma operative specimens and tumors grown in the mouse flanks

	IOMM-Lee	CH-157 MN	Pt. 3	Pt. 4	Pt. 5	Pt. 6	Pt. 7	Pt. 8
Operative								
GFAP	N/A	N/A	–	–	–	–	ND	ND
Vimentin	N/A	N/A	+++	+++	+++	++	+++	+++
EMA	N/A	N/A	+	+	+	+	–	+
MIB-1	N/A	N/A	5.7 ± 4.1	1.1 ± 1.1	1.9 ± 1.3	13.6 ± 6.8	10.2 ± 6.2	18.7 ± 4.8
Mouse								
GFAP	–	–	–	–	ND	–	N/A	N/A
Vimentin	+	+	+	+	ND	+	N/A	N/A
EMA	+	+	+	+	ND	–	N/A	N/A
MIB-1	211.0 ± 75.7	190.6 ± 23.8	243.9 ± 30	232.7 ± 60	ND	0 ± 0	N/A	N/A

N/A indicates not applicable; ND, not done; +++, strong; ++, moderate; +, weak; –, no immunohistochemical staining; Pt., patient. MIB-1 counts (mean ± SD).

size. Mean tumor volumes (±SD) at 15, 29, 43, and 56 days were 457 ± 347, 1228 ± 782, 2353 ± 1258, and 4593 ± 1377 mm<sup>3</sup>, respectively (Fig. 4).

The CH-157 MN cell line induced flank tumors in 10 (100%) of 10 animals after subcutaneous injection of 1.1 × 10<sup>6</sup> to 1.2 × 10<sup>6</sup> cells per flank. All animals survived to 43 days and were killed because of tumor size as per animal protocol. Mean tumor volumes (±SD) at 15, 29, and 43 days were 82 ± 46, 819 ± 654, and 3196 ± 2574 mm<sup>3</sup>, respectively (Fig. 4).

Two complex karyotype meningioma cell lines (patients 3 and 4) showed excellent flank tumor induction and growth. The cell line from patient 3 induced tumors in 5 (100%) of 5 animals after subcutaneous injection of 1.6 × 10<sup>7</sup> cells per animal flank, with Matrigel. All mice survived. Animals were killed at 35 days because of tumor size as per animal protocol. Mean tumor volumes (±SD) at 14, 24, and 35 days were 3038 ± 800, 5304 ± 1343, and 7473 ± 2694 mm<sup>3</sup>, respectively (Fig. 4). The cell line derived from patient 4 also induced tumors in 5 (100%) of 5 animals after subcutaneous injection of 1.1 × 10<sup>7</sup> cells per animal flank. One animal died at 28 days because of tumor involvement. At autopsy, significant abdominal ascites was noted with tumor invading through the abdominal peritoneum. No metastasis of large organs (eg, lung, liver, colon, or kidney) was noted. The cause of death was presumed to be similar to that in the IOMM-Lee animals, with serous exudate of the tumor into the abdominal cavity. Animals were killed at 35 days because of tumor size as per animal protocol. Mean tumor volumes (±SD) at 14, 24, and 35 days were 2080 ± 872, 3053 ± 1260, and 5588 ± 3405 mm<sup>3</sup>, respectively (Fig. 4).

Two simple karyotype meningioma cell lines (patients 5 and 6) were transplanted into mice and followed for a period of 2 to 3 months, during which the implanted tumors did not grow (ie, small tumors without growth). Flank tumors incited from simple karyotypes showed tumor take but no significant growth after injection of 1.0 × 10<sup>5</sup> and 1.7 × 10<sup>6</sup> cells per flank for patients 5 and 6, respectively. Histologic analysis of flank tumors from patient 6 showed

meningioma tumor cells (see below). All mice survived. Animals were killed at 96 and 51 days for lack of tumor growth from patients 5 and 6, respectively (Fig. 4).

### 3.6. Mouse flank tumor histology and immunohistochemical staining results

Mouse flank tumors were processed for H&E staining, as well as immunohistochemically for GFAP, vimentin, EMA, and MIB-1. Both immortal cell lines showed hypercellular sheets without whorl formation and stained positive for vimentin, negative for GFAP, and weakly positive for EMA (Table 2, Fig. 5). These flank tumors exhibited a high number of MIB-1–positive cells (Fig. 5). The IOMM-Lee tumor invaded soft tissue, whereas the CH-157 MN cell line did not, but both exhibited a central core of necrosis.

Flank tumors induced by meningiomas with complex karyotypes (patients 3 and 4) induced large tumors that showed lobular growth patterns with prominent nuclei, resembling their original operative specimens on H&E staining (Fig. 5). The tumor induced by meningiomas from patient 3 showed features of a meningothelial meningioma in both the operative and flank tumor specimen (data not shown). Immunohistochemically, the tumors induced from the meningiomas in patients 3 and 4 showed similar results when operative specimens were compared with their corresponding flank tumor: they were GFAP-negative, vimentin-positive, and with weak EMA staining (Table 2, Fig. 5). MIB-1 stainings of the operative samples showed significant proliferative rates.

Mouse flank tumors grown from meningioma cell cultures with a simple karyotype (patients 5 and 6) showed no significant tumor growth (ie, nongrowing tumors). Histologic analysis of the nongrowing tumors from patient 6 showed the characteristic whorls and cell rests of a transitional meningioma, unlike the original operative specimen, which exhibited fibrous meningioma features of parallel spindle cells with wide fascicles (Fig. 5). The operative MIB-1 staining showed many positive cells, whereas none were observed in the flank tumor sample.

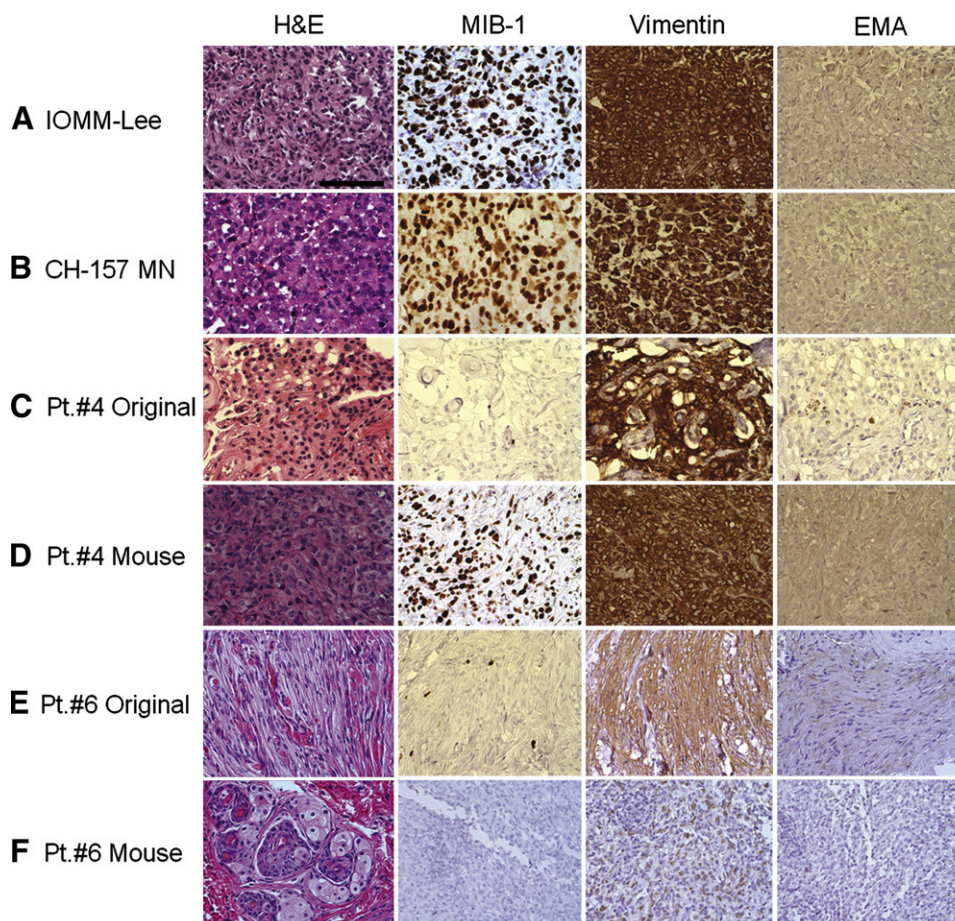


Fig. 5. Representative histology and immunohistochemical staining slides of meningioma operative specimens and subcutaneous mouse flank tumors ( $\times 40$  objective; bar =  $100 \mu\text{mol/L}$ ). A-B: Mouse flank tumors induced by the immortal cell lines IOMM-Lee (A) and CH 157-MN (B). C-F: Meningioma operative specimens and their corresponding subcutaneous mouse flank tumor. Representative tumors induced from a complex karyotype (C-D, patient 4) and a simple karyotype (E-F, patient 6) are shown. A-B: Both the IOMM-Lee (A) and CH-157 MN (B) flank tumors exhibited features of WHO III meningiomas of nuclear polymorphism, numerous mitosis, and high proliferative rates (MIB-1). CH-157 MN tumors resembled the WHO grade III rhabdoid meningioma subtype (eg, large cells with eccentric nuclei and abundant cytoplasm). Strong vimentin and weak EMA immunoreactivity were noted in both. C: The operative specimen from patient 4 showed H&E characteristics of a transitional meningioma (eg, lobular pattern with occasional whorl-like structure), with sparse MIB-1 staining. D: The mouse flank tumor induced from patient 4 exhibited H&E similarities with the original operative specimen. However, in comparison with the original specimen, MIB-1 immunohistochemistry showed a much higher proliferative rate. Strong vimentin and weak EMA immunoreactivity were noted in both the operative and flank tumor specimens. E: The operative specimen from patient 6 showed H&E characteristics of a fibrous meningioma (eg, parallel spindle cells with wide fascicles), with a few cells staining positive for MIB-1. F: Interestingly, the mouse tumor from patient 6 exhibited whorls, consistent with a transitional meningioma, unlike the original operative specimen. No cells stained positive for MIB-1. Moderate and weak vimentin immunostaining were noted in the operative and flank tumor specimen. Weak and no EMA immunoreactivity were observed in the operative and flank specimens, respectively.

Moderate and weak vimentin immunostaining was noted in the operative and flank tumor specimens.

### 3.7. MIB-1 counts

MIB-1 immunohistochemical staining data represent the mean number of positive cells per hpf  $\pm$ SD (Fig. 6). The original operative specimens for the immortal cell lines were unavailable for analysis. The 2 operative specimens that induced mouse flank tumors showed MIB-1 counts of  $5.7 \pm 4.1$  and  $1.1 \pm 1.1$  for patients 3 and 4, respectively. The 2 operative specimens that showed tumor regression showed MIB-1 counts of  $1.9 \pm 1.3$  and  $13.6 \pm 6.8$  for patients 5 and 6, respectively. Analysis of cell lines

inciting meningioma flank tumors showed MIB-1 counts of approximately 200/hpf ( $211.0 \pm 75.7$ ,  $190.6 \pm 23.8$ ,  $243.9 \pm 30$ ,  $232.7 \pm 60$  for IOMM-Lee, CH-157 MN, and cell lines from patients 3 and 5, respectively). Of the 2 primary cell lines from simple karyotypes, only one (patient 6) was processed for analysis. This cell line showed no cells positive for MIB-1 immunoreactivity. Interestingly, we observed that MIB-1 counts from operative specimens did not predict the ability to incite flank tumor growth. For example, patient 4 had the lowest MIB-1 mean but consistently incited flank tumors, whereas patient 5 had the highest MIB-1 ratio but incited nongrowing tumors (Fig. 6).



### 3.8. Transmission electron microscopy

Transmission electron microscopy was used to identify ultrastructural features consistent with the diagnosis of meningiomas, specifically desmosomes. Desmosomes are intercellular junctions that anchor vimentin filaments to the cellular membrane and are characteristic of meningiomas. The key features of desmosomes are opposing disc-shaped plaques located on the cytoplasmic membrane, with inserting cytoplasmic tonofilament bundles, and an amorphous material present within the intercellular space (Fig. 7). A

total of 4 cell lines, 3 flank tumors, and 1 operative sample were processed for TEM. Ultrastructural features of the IOMM-Lee cell line include interdigitating cells with prominent nucleoli and intermediate filaments. Microvilli were noted on the cellular surface. Tight junctions (ie, no intervening intercellular space) and junctions suggestive of desmosomes (ie, intercellular gap with shaggy appearance to lipid bilayer) were visualized. These features still fit its initial description in 1990 [18]. Ultrastructurally, primary meningiomas grown in cell culture (from patients 4, 8, and 11) exhibited interdigitating cell processes without intercellular junctions. Flank tumors induced by the IOMM-Lee, CH-157 MN, and patient 5 cell lines were processed for TEM. The IOMM-Lee and CH-157 MN mouse flank tumors exhibited junctional complexes similar in appearance to desmosomes but lacking clear cytoplasmic tonofilament insertion (Fig. 7). The mouse flank tumor induced from patient 5's cell line exhibited clear desmosomes (Fig. 7).

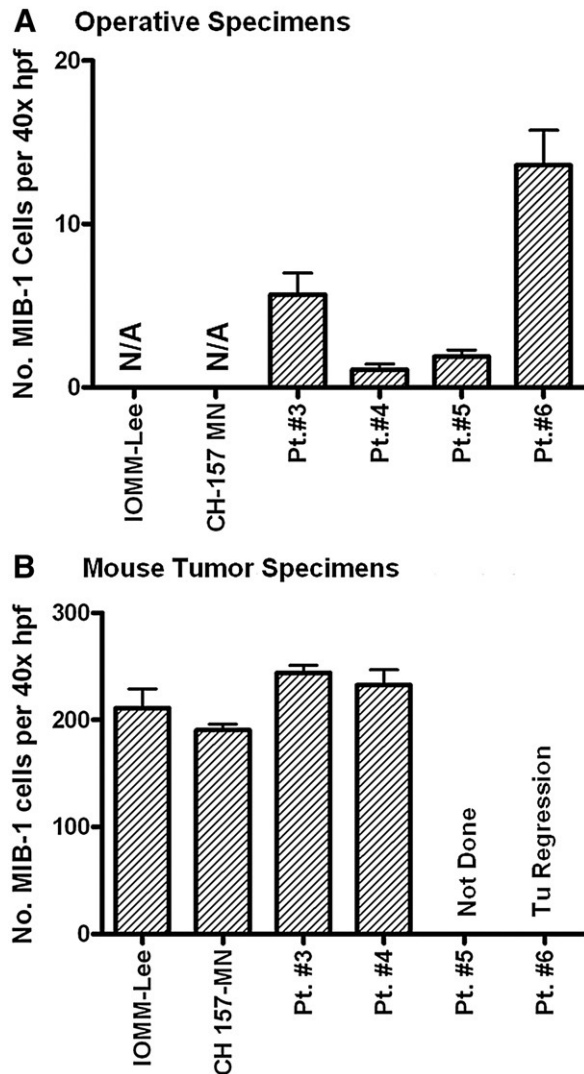


Fig. 6. A-B: Bar graphs depicting the mean number of cells staining positive for MIB-1 in  $\times 40$  hpf in the meningioma operative specimens (A) and mouse flank tumors (B). Error bars represent  $\pm$ SD. A: The original operative specimens for the immortal cell lines were unavailable for analysis. The 2 operative specimens that induced mouse flank tumors showed MIB-1 counts of  $5.7 \pm 4.1$  and  $1.1 \pm 1.1$  for patients 3 and 4, respectively. The 2 operative specimens that showed tumor regression showed MIB-1 counts of  $1.9 \pm 1.3$  and  $13.6 \pm 6.8$  for patients 5 and 6, respectively. B: Cell lines inciting mouse tumors showed MIB-1 counts of approximately 200 per hpf ( $211.0 \pm 75.7$ ,  $190.6 \pm 23.8$ ,  $243.9 \pm 30$ ,  $232.7 \pm 60$  for IOMM-Lee, CH-157 MN, and patients 3 and 5, respectively).

## 4. Discussion

This article addresses 2 long-held criticisms of the mouse meningioma flank model: (1) Why are tumor induction rates so poor in animals injected with cell lines derived from operative specimens? (2) Are mouse flank tumors meningiomas? The advantages to the mouse xenograft meningioma flank model are the ease with which tumors can be continually assessed during treatments and the relatively low cost of using mice as an animal model. Historically, the meningioma mouse flank model was limited by low tumor induction rates until Matrigel augmentation was first described in reports from our laboratory in 1998 [13,15]. Initially, Matrigel augmentation was noted to induce tumors in 100% of mouse flanks, but this initial report was optimistic and our experience over the last 8 years places tumor induction closer to 60% (R.L. Jensen, personal observation) [13]. Furthermore, other investigators have had little success in duplicating our model (R.L. Jensen personal observation). In this analysis, we identified that meningiomas grown in culture containing multiple chromosomal abnormalities consistently induced flank tumors, whereas normal cultures or those with a single chromosomal abnormality did not. Furthermore, flank tumors derived from immortal cell lines and operative specimens exhibit histologic, immunohistochemical, and ultrastructural features consistent with meningiomas.

### 4.1. A complex karyotype appears to predict the successful growth of meningiomas in mouse flanks

In this study, the chromosomal abnormalities exhibited are consistent with previously reported meningioma chromosomal abnormalities [29]. Cytogenetic and molecular studies have identified loss of heterozygosity of chromosome 22 in roughly 60% of all meningiomas, with the *NF2* tumor suppressor gene, located on chromosome 22q12.1, and its

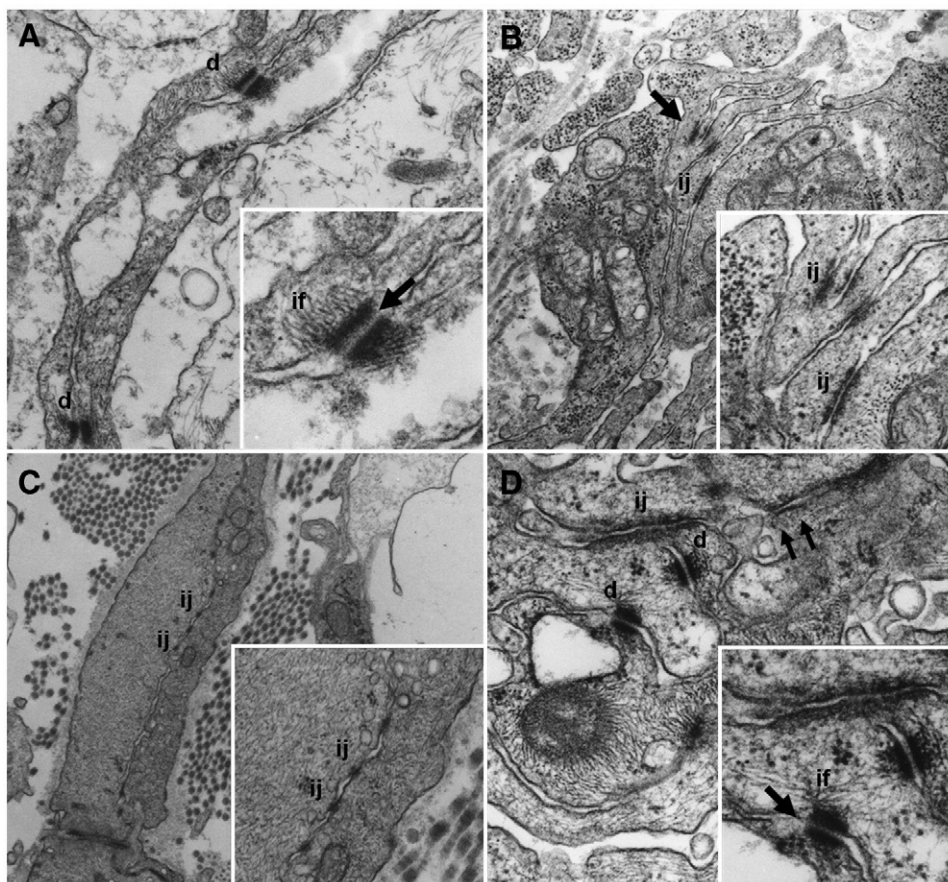


Fig. 7. Transmission electron microscopy pictures of a meningeoma operative specimen (A) and meningeoma mouse flank tumors (B-D). Three intercellular junction types have been described previously in meningiomas: desmosomes, intercellular junctions, and pentilaminar structures (double arrows). A: Patient 6, original operative specimen, exhibiting the key features of a desmosome: opposing disc-shaped plaques located on the cytoplasmic membrane, with inserting intermediate filaments and an amorphous material present within the intercellular space (arrow). Original magnification is  $\times 33\,782$  and  $\times 86\,004$  (inset). B-C: IOMM-Lee (B) and CH-157 MN (C) mouse flank tumors exhibited intercellular junctional complexes, which lack inserting intermediate filaments. IOMM-Lee original magnification,  $\times 25\,422$  and  $\times 52\,020$  (inset); CH-157 MN original magnification,  $\times 8\,286$  and  $\times 33\,782$  (inset). D: Patient 5, mouse flank tumor, exhibiting all 3 types of meningeoma junctions. Original magnification,  $\times 48\,600$  and  $\times 68\,040$  (inset). d indicates desmosomes; ij, intercellular junctions; if, intermediate filaments.

protein product (ie, schwannomin or Merlin) noted to be dysfunctional or lost in approximately one third of meningioma cases [4,9,19,20,29,33,42,43]. Many other cytogenetic alterations have been identified and associated with atypical or anaplastic histology, including the presence of dicentric or ring chromosomes and losses of chromosome arms 1p, 6q, 7, 9p, 10, 14q, 18q, 19, or 20, as well as gains or amplifications of 1q, 9q, 12q, 15q, 17q, or 20q [3,5,7,8,16,17,19,27,28,32,34,39,41]. The immortal cell lines IOMM-Lee and CH-157 MN showed chromosomal abnormalities consistent with cell lines passaged multiple times. Meningiomas from operative samples fell into 2 groups: those with complex karyotype and those with simple karyotype. The 3 meningiomas with a complex karyotype (patients 3, 4, and 8) had similar abnormalities despite being from 3 different meningioma subtypes (meningothelial, transitional, and psammomatous). All 3 exhibited additions to chromosomes 4, 6, 9, and 12 and deletions of chromosome 5. Meningiomas with a simple

karyotype exhibited either a normal chromosomal number (patients 5 and 7) or monosomy 22 (patient 6). Interestingly, cell lines with the complex karyotype grew quickly in culture and were able to induce mouse flank tumors.

Cell cultures derived from complex karyotypes exhibited faster growth rates in vitro and consistently induced fast-growing mouse flank tumors. It is unclear whether tumor induction was simply a function of the initial cell burden injected subcutaneously into the flanks of nude mice because meningiomas with multiple chromosomal abnormalities grew quickly in culture; a much greater cellular harvest was obtainable by the fifth passage, the arbitrary cutoff that we set for subcutaneous injection. It is the authors' belief that meningiomas with multiple chromosomal abnormalities act more aggressively because of unknown gene dysregulation that is not present in meningiomas with simple karyotypes.

Interestingly, we observed that MIB-1 counts from operative specimens did not predict the ability to incite flank tumor growth (eg, patient 4 had the lowest MIB-1

mean but consistently incited flank tumors, whereas patient 5 had the highest MIB-1 ratio but only incited nongrowing flank tumors). Induced mouse tumors showed significant MIB-1 counts with greater than 30% of cells stained positive, indicating high proliferative rates. Others have reported MIB-1 index of intracranial IOMM-Lee tumors to be 30% [40].

#### 4.2. *The meningioma mouse xenograft flank model recapitulates the histologic, immunohistochemical, and ultrastructural features of meningiomas*

All meningiomas grown in mouse flanks showed histologic, immunohistochemical, and ultrastructural features consistent with meningiomas. Previous work has characterized meningiomas grown in vitro on a microscopic, immunohistochemical, and ultrastructural level [12,14,31]. These studies conclude that cell cultures derived from meningioma operative samples appear to be of leptomeningeal origin. This conclusion is based on their polygonal shape, negative GFAP staining, and positive vimentin and EMA staining, as well as the exhibition of desmosomes by electron microscopy [12,31]. Low-passage cells (no greater than passage 5) were used for these experiments, making fibroblast overgrowth less likely. Finally, cell lines used for in vitro work were transplanted into the flanks of nude mice for in vivo experiments, and the xenograft tumors that developed resembled meningiomas histologically, immunohistochemically, and ultrastructurally.

Histologically, meningiomas grown in the mouse flank shared histologic features similar to that of the original operative specimen (eg, immortal cell lines exhibited cytoarchitecture of WHO grade III meningioma with nuclear polymorphism, and numerous mitosis and flank tumors from operative specimens showed lobular cellular patterns similar to their original operative specimens) (Fig. 5). Immunohistochemically, the hallmark of meningiomas is vimentin-positive staining and negative GFAP staining, with roughly 80% staining for EMA. Mouse flank tumors grown from meningioma cell culture showed 100% vimentin immunoreactivity. In this analysis, all flank tumors exhibited similar immunohistochemical stainings as the original operative specimen, with the exception of patient 6, which lost EMA reactivity in the flank tumor. Finally, ultrastructurally, meningiomas grown in mouse flanks exhibited desmosomes, as well as junctional complexes consistent with meningiomas.

#### 4.3. *Ultrastructural features of meningiomas*

Desmosomes are intercellular junctions characteristic of meningiomas and arachnoidal tissue [1]. Three molecular features are typical of meningiomas: vimentin, EMA, and desmosomes [1]. Desmosomes are unique to meningiomas and are not exhibited in other primary intracranial tumors [1]. Arachnoidal and meningioma desmosomes anchor vimentin filaments to the cytoplasmic membrane. Typical ultrastructural features of meningiomas include flat and often

interdigitating cell processes, desmosomal structures, and loosely arranged vimentin filaments, the amount of which strongly varies [1]. Cell processes and larger areas of cell bodies densely packed with intermediate filaments were adjacent to cells with only rare intermediate filament networks [1]. In addition to desmosomes, 2 other types of intercellular junctions have been described in meningiomas [1]. One is characterized by cytoplasmic plaque structures, smaller and less dense than those of desmosomes, and without intermediate filament association often formed by the approach of 3 cellular extensions. These intercellular junctions with diffuse cytoplasmic plaques and no associated intermediate filaments are frequently found in meningiomas, as well as intercellular structures with a pentilaminar appearance [1]. The other intercellular contact was formed by the direct attachment of neighborhood plasma membranes, forming a pentilaminar structure [1].

Doctor Wei-Hwa Lee first described his intraosseous malignant meningioma (IOMM-Lee) cell line in 1990, and electron microscopy shows ultrastructural features that still fit its initial description of prominent nucleoli, intermediate filaments, and sparse microvilli on the cellular surface [18]. When the IOMM-Lee cell line was grown in vivo and examined with electron microscopy, we identified intercellular junctional complexes consistent with meningiomas (Fig. 7). The CH-157 MN cell line grown in mouse flanks also exhibited similar junctional complexes. One operative specimen grown in vivo (patient 5) exhibited desmosomes.

#### 4.4. *Recommendations for inducing meningioma mouse flank tumors*

Induction of flank tumors from the immortal cell lines IOMM-Lee and CH-157 MN can be successfully accomplished by subcutaneously injecting  $1 \times 10^6$  cells suspended in 0.5 mL of media into the flanks of nude mice. One T-175 flask contains enough cells to inject approximately 30 mouse flanks. In our experience, therapies should be instituted 5 to 10 days after injection because these tumors appear to “take off” around 2 weeks. Waiting longer to institute therapies makes it difficult to separate growth curves.

Induction of flank tumors using primary meningioma cell lines should be undertaken with cell lines that are identified either to contain multiple chromosomal abnormalities or to grow quickly in cell culture with doubling times of less than 3 days (72 hours). The number of cells injected per flank should range from  $5 \times 10^6$  to  $1 \times 10^7$  and be suspended in 0.5 mL of Matrigel. Although higher cell numbers were injected in these experiments, these tumors grew too quickly, and lowering the initial cell burden would aid in decreasing the rapid growth. Treatment should be instituted between 10 and 14 days after injection to allow for Matrigel resorption. Slow-growing meningioma cell lines with doubling times greater than 3 days or simple karyotypes are not amenable for inducement of subcutaneous flank tumors.

## 5. Conclusions

Meningioma cell cultures with multiple chromosomal abnormalities consistently induce mouse flank tumors. The meningioma mouse xenograft model recapitulates the histologic, immunohistochemical, and ultrastructural features present in meningiomas.

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## Commentary

Ragel et al provide a review of their experience with meningioma research in both in vitro and in vivo models. Despite the characterization as “benign”, meningiomas remain a considerable clinical challenge to neurosurgeons. Many of these tumors are not safely resectable, or in cases where surgery is performed, the patients may be at risk for a number of neurological or nonneurological morbidities. Residual and recurrent tumors requiring further treatments including additional surgery and radiation are common. In light of these clinical challenges, further research is needed to characterize the biology of meningiomas in an effort to improve treatment options. At present, few laboratory models exist to study meningiomas. The author’s review of their laboratory investigations of meningiomas and characterization of the fine points of their techniques are a welcome addition to the limited methodologies currently available for the study of meningiomas. Improved

laboratory models will serve to advance treatments for patients with meningiomas.

Specifically, the authors have used 2 immortalized meningioma cell lines as well as meningiomas from 9 specimens harvested at the time of surgical resection. Techniques used include assessments of in vitro growth, Giemsa-band karyotyping for chromosomal aberrations, flow cytometry for cell-cycle markers, immunohistochemical analysis, and ultrastructural analysis with electron microscopy. In addition, implantations were performed in immunocompromised mice.

Using these techniques, the authors have shown that meningioma cells with complex karyotypes, including the 2 immortalized cell lines (IOMM-Lee and CH-157MN), grew more rapidly in vitro and induced tumors in immunocompromised mice more efficiently than did meningioma cells with minimal or no chromosomal aberrations as identified by karyotyping. Meningiomas induced after subcutaneous implantations demonstrated immunohistochemical findings consistent with meningioma including positivity for vimentin, epithelial membrane antigen, and negativity for glial fibrillary acidic protein. Ultrastructural findings characteristic of meningiomas as previously described in the literature, such as desmosomes, intercellular junctions, and pentilaminar structures, were identified in the meningiomas created in the subcutaneous implantation models. Many of the karyotype chromosomal abnormalities identified in their specimens were consistent with findings by other investigators. The FACS analysis for markers of cell cycle indicated a large percentage of cells in the S phase for the 2 immortalized cell cultures.

These investigations should serve as a model for future laboratory studies and should translate into advancements in treatment options for patients with meningiomas.

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