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Growth pattern and molecular biological analysis of primary meningioma cell cultures under different conditions

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Abstract. Cell culture is a common methodology used in tumor research. However, the manner in which initial tissue conditions, such as storage in the operating theatre, can affect proliferation has not been well-established. Additionally, there is little information about the behavior of primary cell cultures in meningiomas. Therefore, the present study analyzed the physiology of primary meningioma cultures under different tissue conditions. For the present study, 10 meningioma tissue samples were collected after surgery at Saarland University (Homburg, Germany) between June 2022 and December 2022. Primary cell cultures were separately established on the day of surgery and 1 day later, before the tissues were stored overnight in nutrient solutions. Cultures were split into two flasks, one was used for fluorescence *in situ* hybridization (FISH), whereas the other was frozen in liquid nitrogen. After 6 months, frozen cultures were thawed and recultivated. The proliferation rates were found to be ≥80% for cell cultures as a minimum. No significant difference was found between cultures established on the surgery day or postoperative day 1 in terms of proliferation rate and growth pattern. FISH revealed loss of the short arm of chromosome 1 (1p) in one meningioma, loss of the long arm of chromosome 22 (22q) in three samples, combined 1p and 22q loss in three samples, and diploid chromosome sets in three samples. In total, 16 out of 17 initially shock-frozen specimens were successfully recultivated after 6 months of cryopreservation. To conclude, meningioma cultures appeared to proliferate similarly regardless of whether the tissue was processed on the day of surgery or the following day, if viable cells were present. Frozen cultures could be revived after 6 months if cells remained viable. FISH provided evidence that primary meningioma cultures accurately reflected initial chromosomal aberrations of the tumor, even after freezing.

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Abbreviations: DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; FISH, fluorescence *in situ* hybridization; LOH, loss of heterozygosity; P0, passage 0; P1, passage 1; P2, passage 2; SSC, saline sodium citrate

Key words: human meningioma cells, meningioma model, growth pattern, FISH, LOH

Introduction

Studies on meningioma have been increasingly focused on understanding their biology at the cellular level, particularly through cell culture studies and animal models (1-6). These models have allowed for investigations into the characteristics, growth patterns and molecular mechanisms driving meningioma development (1,3,5-12).

Cell culture models are typically used to explore the genetic mutations and epigenetic modifications that contribute to meningioma pathogenesis and formation (4-6). Establishing primary meningioma cell cultures involves isolating tumor cells from surgical specimens and maintaining them *in vitro* under specific conditions that promote cell proliferation and survival. Typically, meningioma cell cultures are prepared using enzymatic digestion with various agents, such as collagenase or trypsin, to dissociate the tumor tissue, followed by cultivation in specialized media, including DMEM or RPMI, which are supplemented with FBS, antibiotics and growth factors (1,2,4,6,13). These cultures can be used to investigate cellular proliferation, genetic mutations and tumor responses to therapeutic agents. However, primary meningioma cultures frequently face challenges, such as limited lifespan, heterogeneity, selection of potent cells and difficulty in maintaining the original phenotype of the primary tumor over time. To overcome these limitations, immortalized meningioma cell lines have been developed through genetic modification techniques, providing consistent and long-term models for research (4-6,14,15). Additionally, three-dimensional culture systems and spheroid models are increasingly being utilized to optimally mimic the tumor microenvironment (16).

Meningioma cell culture is a key method in tumor research, allowing for the analysis of meningioma tumor cells *ex vivo*. To the best of our knowledge, the physiology of cells,

which is influenced by conditions in the operating theatre, transportation and storage from tissue removal to processing, is an important but poorly documented factor in the literature, especially regarding cells in primary cell culture.

Consequently, the present study used meningioma samples post-surgery to investigate how storage time and further cryopreservation treatment can affect the proliferative pattern of primary cell cultures. To assess cytogenetics in these cell cultures, fluorescence *in situ* hybridization (FISH) was performed on all cultures, the results of which were compared with those from native tumor tissue smears, as well as loss of heterozygosity (LOH) analysis, to determine whether cell cultures accurately represented the original tumor.

Materials and methods

Patient population and histology. From June 2022 to December 2022, 10 primary human meningiomas were collected after surgery at the Department of Neurosurgery at Saarland University (Homburg, Germany). Patients undergoing elective surgery for suspected or previously diagnosed meningioma were eligible for inclusion. Written informed consent was obtained from each patient as per the protocol approved by the Ethical Committee of the Medical Association of Saarland (approval no. 02/20; Saarbrücken, Germany). A total of 10 tumor samples from 9 patients (3 male patients; 6 female patients) were included in the present study. The mean age of patients at the time of surgery was 75.2±9.4 years (range, 62-86 years). All patients had a primary tumor and patients with recurrence were not included in the present study. All tumors were classified according to the actual 2021 World Health Organization (WHO) classification of tumors of the nervous system (17) by a neuropathologist.

Cell culture and preparation. From each meningioma, fragments of the tumor were placed by one individual in DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 1% penicillin/streptomycin directly after preparation in the surgical field by a neurosurgeon in the operating theatre at room temperature (22-28˚C) before being transferred to the neurosurgical laboratory with standard conditions as previously described (1). A segment of the tissue sample was prepared for culture on the same day as the surgery took place, whilst another was stored overnight in DMEM with 1% penicillin/streptomycin in a refrigerator (4-6˚C). The remainder of the tissue was cryopreserved in cryotubes in a freezer at -80˚C. For cell culture preparation, the tumor sample was minced using a scalpel and small scissors. Cells were suspended in DMEM containing 10% fetal calf serum (Thermo Fisher Scientific, Inc.), 1% non-essential amino acids and 1% penicillin/streptomycin, before subsequently being distributed into two different 25-cm^2 cell culture flasks for the next steps. The primary cultures were incubated at 37˚C with 5% CO_2 in air and the medium was changed twice per week.

Primary cell cultures at passage 0 (P0) were established on the day of surgery and 1 day postoperatively, and these were classified as culture groups A and B, respectively. These cells from group A and B were stored overnight in DMEM with 1% penicillin/streptomycin in a refrigerator (4-6˚C). Cultures from group A and B were split into two passage 1 (P1) cultures. The termination of the cell culture occurred when the cell culture occupied the flask space completely or at least 10 days had passed. One P1 culture was used for FISH, while the other culture was frozen in liquid nitrogen after a cell count. The latter was then thawed after 6-7 months and cultivated again at passage 2 (P2).

Cell counting, freezing and thawing. The second culture flask from the first passage was prepared for freezing in liquid nitrogen as aforementioned. The medium in the appropriate culture flasks was first transferred to a sterile round tube. The cell culture in each flask was then incubated with 2 ml 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) at 37˚C in the incubator for ≥2 mins. Proteolysis was halted by adding 2 ml of DMEM. The cell suspension was subsequently transferred to a centrifuge tube and centrifuged at 37.02 x g for 5 min at room temperature with a brake. The supernatant was removed and discarded, and the cell pellet was resuspended in 1 ml of fresh medium.

For cell counting, 10 μl 0.4% trypan blue solution was pipetted into a 1.5-ml tube. Cell suspension (10 μl) was added and both liquids were mixed well. From this mixture, 10 μl was placed onto a cell counting slide. The LUNA-II™ cell counter (Aligned Genetics, Inc.) was used to analyze the sample, and provided the total cell count, along with the number of live and dead cells, and the viability ratio.

A special freezing medium was used for freezing of the cells. This was prepared from 10 ml dimethyl sulfoxide (DMSO) and 90 ml DMEM, aliquoted into 5-ml portions in round tubes and stored at -20˚C. DMSO protects cells from mechanical damage due to ice crystals during freezing. The added medium was centrifuged at 37.02 x g for 5 min at room temperature with a brake. The supernatant was pipetted out and discarded. The pellets were then resuspended in 1 ml freezing medium and transferred to cryotubes, which were placed inside a freezing box. The freezing container filled with isopropanol allowed for controlled cooling of the cells in the cryotubes, achieving a cooling rate of approximately -1˚C per min. The container was frozen overnight at -80˚C. The following day, the cryotubes were removed and transferred to liquid nitrogen at -196˚C, where the cells were stored for subsequent analysis. After 6-7 months, the corresponding cryotubes were thawed and the cells were recultivated at P2. The cultures from P2 were then treated in the same manner as the primary cultures (as described for P1) except for the stage involving the splitting of the initial cultures into two separate cultures. Medium changes and termination of the cultures occurred as soon as a dense cell lawn was observed under the light microscope (Olympus CKX; Olympus Corporation) in daily observations.

Preparation of dabbed slides. In addition to cell culture, one tumor fragment from each sample at P1 and P2 was used for the preparation of dabbed slides. To achieve this, a fragment of the tumor was dabbed onto object slides coated with silane for 5 sec at room temperature, and was fixed with DeLauney fixative, comprising 1:1 acetone/ethanol with 0.05% trichloroacetic acid, at room temperature for 5 min. Samples were stored at -20˚C.

FISH. Primary tumor cells were dispersed with 0.05% trypsin-EDTA and subsequently suspended by centrifugation

at 30.85 x g for 8 min at room temperature. The supernatant was discarded, and the pellet was treated with 0.075 M KCl solution at room temperature for 5 min and resuspended. The cell suspension was centrifuged once more at 30.85 x g for 8 min at room temperature. The supernatant was once again removed and the cells were fixed with methanol/acetic acid (3:1) for 1 h at room temperature. Subsequently, the cell suspension was dropped onto the object dry slides. Additionally, dabbed slides were used to compare both methods as partially described previously (1).

The slides were treated with RNase A (Fisher Scientific; Thermo Fisher Scientific, Inc.) for 30 min at 37˚C and placed three times in 2X saline sodium citrate (SSC) for 5 min at room temperature. Cells were digested in 100 ml 0.01 M hydrogen chloride with 10 mg pepsin (SERVA Electrophoresis GmbH) at 0.7 mA for 1 min and 45 sec at 37˚C. Slides were dipped in 1X PBS for 5 min at room temperature, 4% paraformaldehyde/1X PBS for 10 min at room temperature for fixation and 1X PBS for 5 min at room temperature. Samples were subsequently dehydrated in 70, 80 and 95% ethanol and air-dried. Dual-probe hybridization was carried out using locus-specific probes for band 36 on the short arm of chromosome 1 (1p36; D6021-100-OG; MetaSystems) and band 11 on the long arm of chromosome 22 (22q11; D5117-100-OG; MetaSystems).

The probes were then pipetted onto the slides and denatured for 2 min at 75˚C. Afterwards, samples were incubated overnight at 37˚C in a humidified chamber. Stringency washes were performed in 0.4X SSC for 2 min at 72˚C and 2X SSC/0.05% Tween-20 for 30 sec at room temperature. Following this, slides were counterstained with DAPI antifade (Vectashield; Vector Laboratories, Inc.; Maravai LifeSciences).

In total, ≥200 non-overlapping nuclei per sample were evaluated according to the Hopman criteria (18) using an Olympus BX43 fluorescence microscope (Olympus Corporation). Cut-offs for alterations were determined by comparison with human lymphocytes as control samples at 10% for deletions of 1p36 and 22q11.

LOH analysis. PCR-based microsatellite analysis was performed on meningioma probes directly from the operating theatre (group A) in a manner similar to the protocols previously described for oligodendrogliomas, using different probes (18). For the PCR reaction 1.3 μl DNA, 1.0 μl primer mix (both forward and revers primer at a concentration of 20 μM; Eurofins Genomics), 10.2 μl water and 12.5 μl HotStarTaq Master Mix Kit (Qiagen GmbH) were mixed (25.0 μl total reaction volume). The PCR reaction was performed according to the protocol developed by Hartmann *et al* (19).

For the investigation of the short arm of chromosome 1 (1p), the probes D1S 1608, D1S 1161 and D1S 1184 (created by Qiagen GmbH for the Institute of Neuropathology, Saarland University, Homburg, Germany), and a probe for the gene locus of AT-rich interaction domain 1A were used. For the long arm of chromosome 22 (22q), the following probes were used: D22S 445, D22S 684, D22S 268 and D22S 258 (created by Qiagen GmbH for the Institute of Neuropathology, Saarland University). PCR products were visualized on high-resolution Spreadex® EL 800 Wide Mini gels (AL-Labortechnik & Diagnostik GmbH) using an Origins electrophoresis system (AL-Labortechnik & Diagnostik GmbH) and SYBR™-Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific, Inc.). Gel electrophoresis was conducted for 90 min at 120 V in TRIS-acetate-EDTA-buffer (cat. no. 42548.01; TAE buffer, SERVA Electrophoresis GmbH) at 54˚C. Subsequently, gels were washed once with distilled water, stained with staining solution for 30 min at room temperature [final buffer concentration, 0.8X TAE (cat. no. 42548.01; SERVA Electrophoresis GmbH), 0.8X Destaining Solution (cat. no. 3037.01; SERVA Electrophoresis GmbH), 2X SYBR™ Gold] and washed again with distilled water. The results were documented using the 'EOS Utility' program (Canon, Inc.). Blood samples for LOH analysis were obtained during clinical routine as a standard procedure for neuropathological diagnostics and were stored at room temperature in EDTA tubes for further analysis within 12-48 h.

Statistical analysis. The graphing and analysis of data were performed using SPSS (version 27.0; IBM Corp.) and Microsoft Excel (Microsoft 365; Microsoft Corporation). The variables were initially tested for normal distribution using the Shapiro-Wilk test. Normality was assumed if the P-value exceeded the significance level. Fisher's exact test was also used for calulations. Since some variables were not normally distributed, the P-values were calculated using the Mann-Whitney U test. Data are presented as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

General results. Primary cultures and native tissue samples from 10 meningiomas were established, derived from 3 male and 6 female patients. Notably, 1 patient, designated patient 7, had two tumor probes from different localizations (T9175 and T9177). These two different tumors were surgically treated using two separate surgical approaches and were treated as two separate tumor pathologies in the clinical and scientific setting. All tumors showed soft tumor tissue and could be cut for further preparation of tumor samples. The mean age of patients at the time of surgery was 75.2±9.4 years. Histopathological analysis revealed 8 meningiomas classified as WHO grade 1 and 2 meningiomas classified as WHO grade 2. The tumors were located at the sphenoid wing (n=3), convexity (n=2), parasagittal (n=3), posterior fossa (n=1) and anterior skull base (n=1). Further details are provided in Table I.

Proliferation rate of P0 cultures in cell culture. A total of 8 out of 10 meningioma samples showed signs of proliferation in both cultures tested from the day of surgery and from 1 day after surgery. In one case, only the sample in group A P0 showed proliferation, whereas in another case, no proliferation was observed in the group A P0 cell culture flask and in the group B P0 cell culture flask. Overall, 17 out of 20 primary cultures proliferated, whilst 3 did not show proliferation after 10 days of observation (Table II). The proliferation rate was calculated as 85% for P0.

Culture on the day of surgery. There was sufficient cell proliferation in approach A to transfer the P0 cultures into two P1

Table I. Overview of the parameters of the tumor samples.

T-no.	Patient no.	Sex	Age, years	WHO grade	Cytogenetic findings	FISH analysis	Localization
T9077	1	Female	82	1	Diploid set of chromosomes	Diploid set of chromosomes	Convexity, on the right, parasagittal
T9092	2	Female	65	1	Diploid set of chromosomes	Diploid set of chromosomes	Sphenoid wing, on the right
T9108	3	Female	74	1	22q-	22q-	Convexity, on the left, parietal
T9145	4	Female	84	1	1p-, 22q-	1p-, 22q-	Parasagittal, on the left, parietal
T9152	5	Female	86	1	Diploid set of chromosomes	Diploid set of chromosomes	Sphenoid wing, mediolateral
T9168	6	Male	75	1	1p-, 22q-	1p-, 22q-	Sphenoid wing, on the left
T9175	7	Male	62	1	22q-	22q-	Posterior cranial fossa, infratentorial
T9177	7	Male	62	2	Diploid set of chromosomes	22q-	Cerebello-pontine angle, on the left
T9181	8	Male	65	1	1p-, 22q-	1p-, 22q-	Falx cerebri/parasagittal, bifrontal
T9194	9	Female	84	2	1p-	1p-	Frontobasal, on the left

T-no., tumor number; WHO, World Health Organization; 1p-, loss of the short arm of chromosome 1; 22q-, loss of the long arm of chromosome 22; FISH, fluorescence *in situ* hybridization.

Table II. Average duration (days) of cell culture in groups A and B at passage 0 to splitting, passage 1, and passage 2.

Cell culture	Passage 0		Passage 1		Passage 2	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
A	7.78±3.27	9	11.56±2.78	9	7.67±1.65	9
B	8.75±2.91	8	12.38±3.66	8	8.25±1.75	8
Total	8.24±3.05	17	11.94±3.15	17	7.94±1.67	17

FISH, fluorescence *in situ* hybridization; N, number.

culture flasks, with 9 successful cultures among a total of 10 cultures (90%) in both. The splitting process led to faster cell proliferation, as seen in daily microscopic observations of the cell cultures, ensuring that the P1 flasks contained enough cells to use one culture for FISH and freeze the other in liquid nitrogen. Representative images of cell culture are shown in Fig. 1.

Culture on the first postoperative day. For approach B, widespread proliferation of meningioma cells was observed in 8 out of 10 cultures (80%) at P0, allowing for further processing. Cultures initiated on the first postoperative day exhibited lower proliferation rates compared with those established on the day of surgery. However, this difference was not statistically significant (P>0.35) (Fig. 2; Table II).

Average time to cell outgrowth in primary cultures. Cell outgrowth from tissue was observed after an average of 1.4±0.9 days. Cultures from group A showed initial cell proliferation after an average of 1.3±1.0 days, whereas cultures in group B exhibited proliferation after an average of 1.5±0.9 days (data not shown). The difference between these two groups was not statistically significant.

Splitting of P0 cultures. Splitting of cultures at P0 could be performed after 8.24±3.05 days on average. Culture group A was split after an average of 7.78±3.27 days, whilst culture group B was split after 8.75±2.91 days (Table II). This indicated that group B cultures were split nearly a day later on average; however, this difference was not statistically significant.

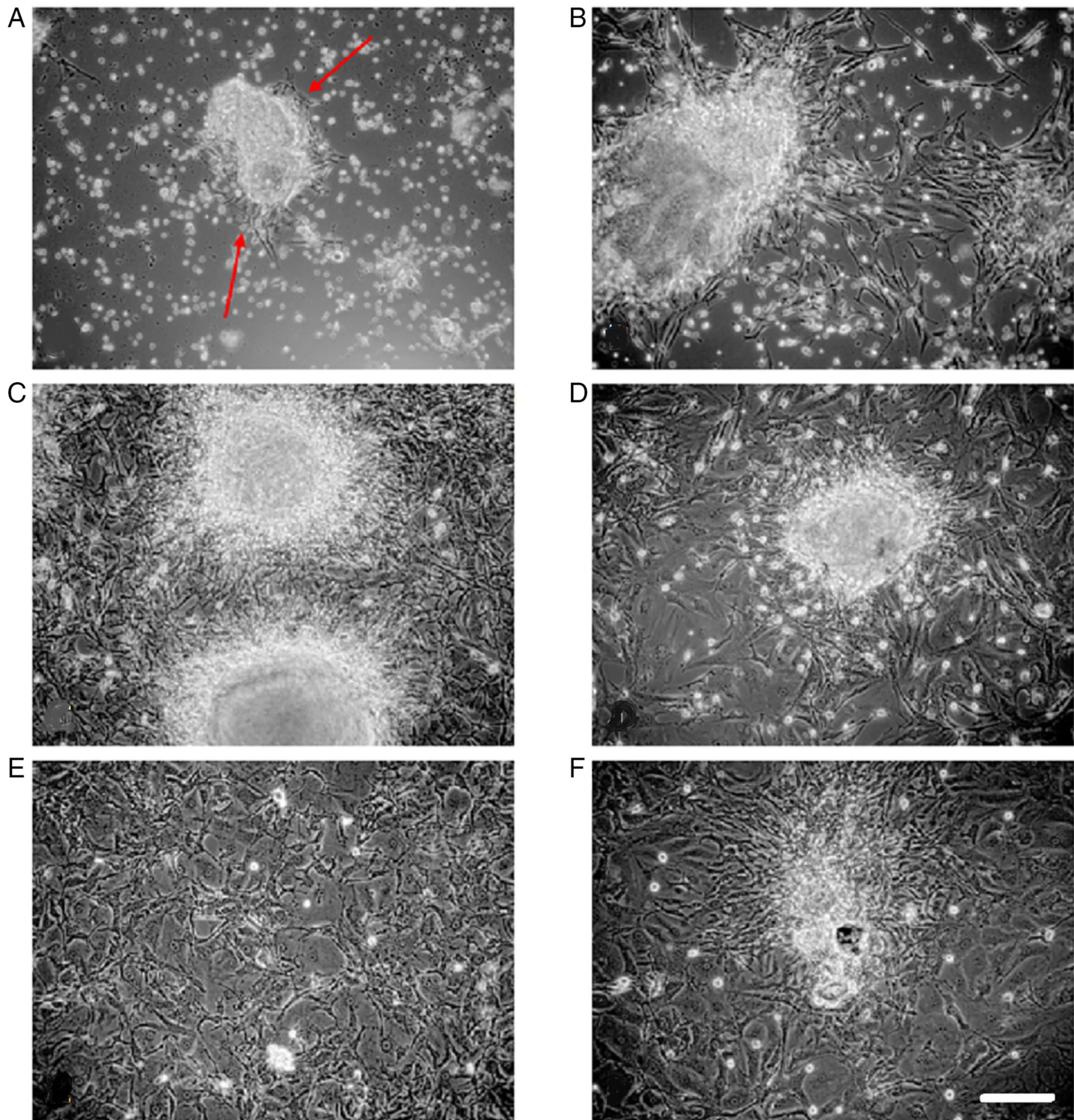

Figure 1. Representative images of cell culture from sample T9181 at passage 0 and P1 over time. (A) Days 1 and (B) 3 of cell culture. (C) Day 9 involved the splitting of samples into two P1 cultures. (D) Day 10 of cell culture. On day 15, the P1 cultures were terminated for (E) fluorescence *in situ* hybridization and (F) freezing. Red arrows indicate proliferating cells (magnification, x10; scale bar, 100 μm). P1, passage 1.

Duration until culture termination for FISH. The average duration before termination of primary (P0 and P1) cultures for FISH was 11.94±3.15 days. Cultures from group A were terminated after an average of 11.56±2.78 days, whereas group B cultures were terminated after 12.38±3.66 days. The difference was not statistically significant (Table II).

Cell viability of primary cultures. The mean cell viability across all primary cultures was 81.74±10.17%. The viability of culture group A was 79.14±11.27%, while the viability of group B was 84.77±8.70%. No significant differences were observed between the two groups (group A and B for P1; Fig. 3).

Growth rate of P2 cultures. In the secondary generation, 9 cultures from culture A and 8 from culture B were recultivated after freezing in liquid nitrogen for 6-7 months. Of the 17 thawed cultures, 17 were able to continue proliferating, resulting in a re-growth rate of 94.1% for P2. All cultures that proliferated again had established cell attachment within 24 h. Representative cell culture images are shown in Fig. 4.

Recultivation of culture B. When recultivating cultures that were initially established the day after surgery, 7 out of 8 cultures (87.5%) exhibited widespread proliferation on the culture surface in the secondary generation (Fig. 2).

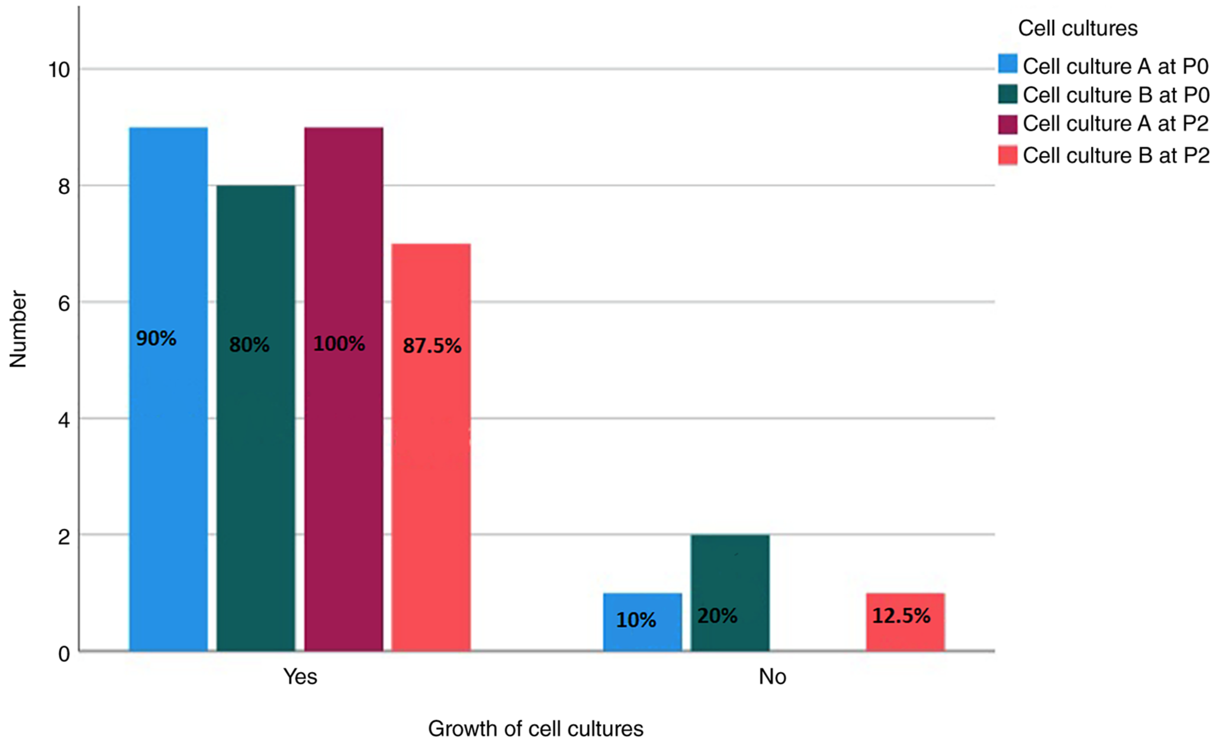

Figure 2. Comparison of the proliferative patterns of cell cultures A (prepared on the day of surgery) and B (prepared on the day after surgery) at P0 (n=10), as well as cultures A (n=9) and B at P2 (n=8). P0, passage 0; P2, passage 2.

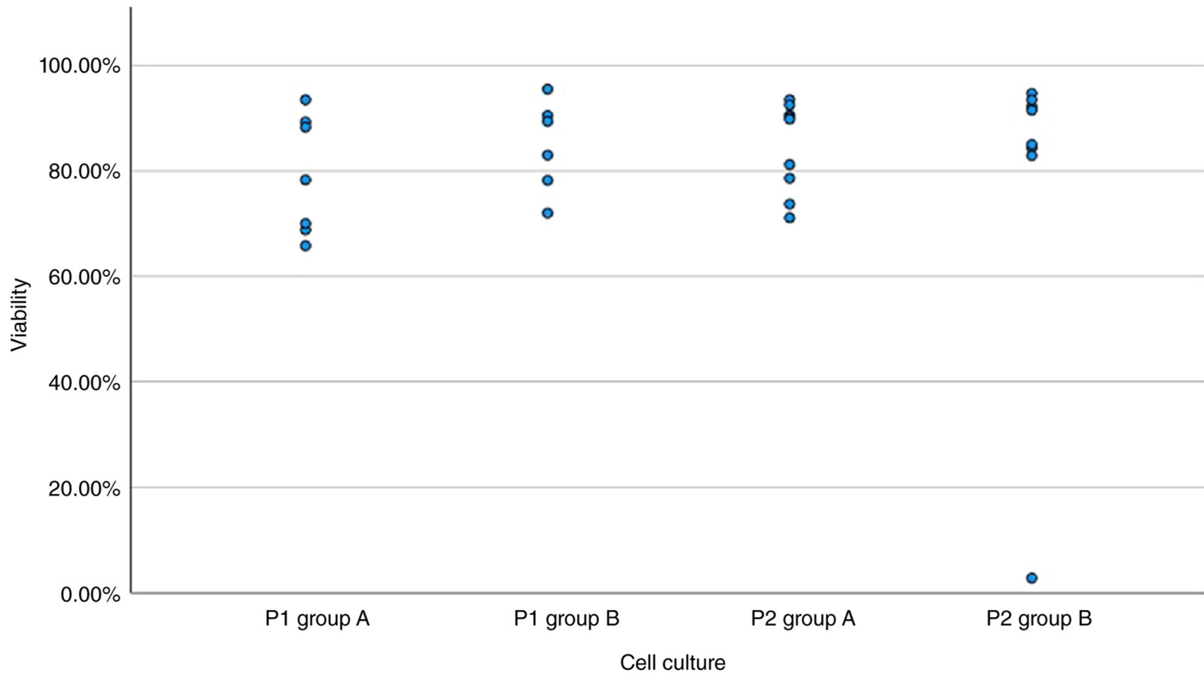

Figure 3. Comparison of the viabilities of cell cultures in groups A (prepared on the day of surgery) (n=7) and B (prepared on the day after surgery) at P1 (n=6), as well as culture groups A (n=9) and B at P2 (n=8). P1, passage 1; P2, passage 2.

Overall proliferative performance. Comparing the two generations of cell cultures, primary cultures showed an 85.0% cultivation success rate, whilst secondary cultures had a success rate of 94.1%. Out of the 37 cultures derived from vital tissue or cells, 33 exhibited widespread growth, resulting in an overall proliferative rate of 89.1% (Table II). Statistical comparison between generations was not feasible because primary cultures used tissue as the starting material, whereas secondary cultures involved pre-prepared cells.

Duration until culture termination in the secondary cell culture generation. The average time before culture

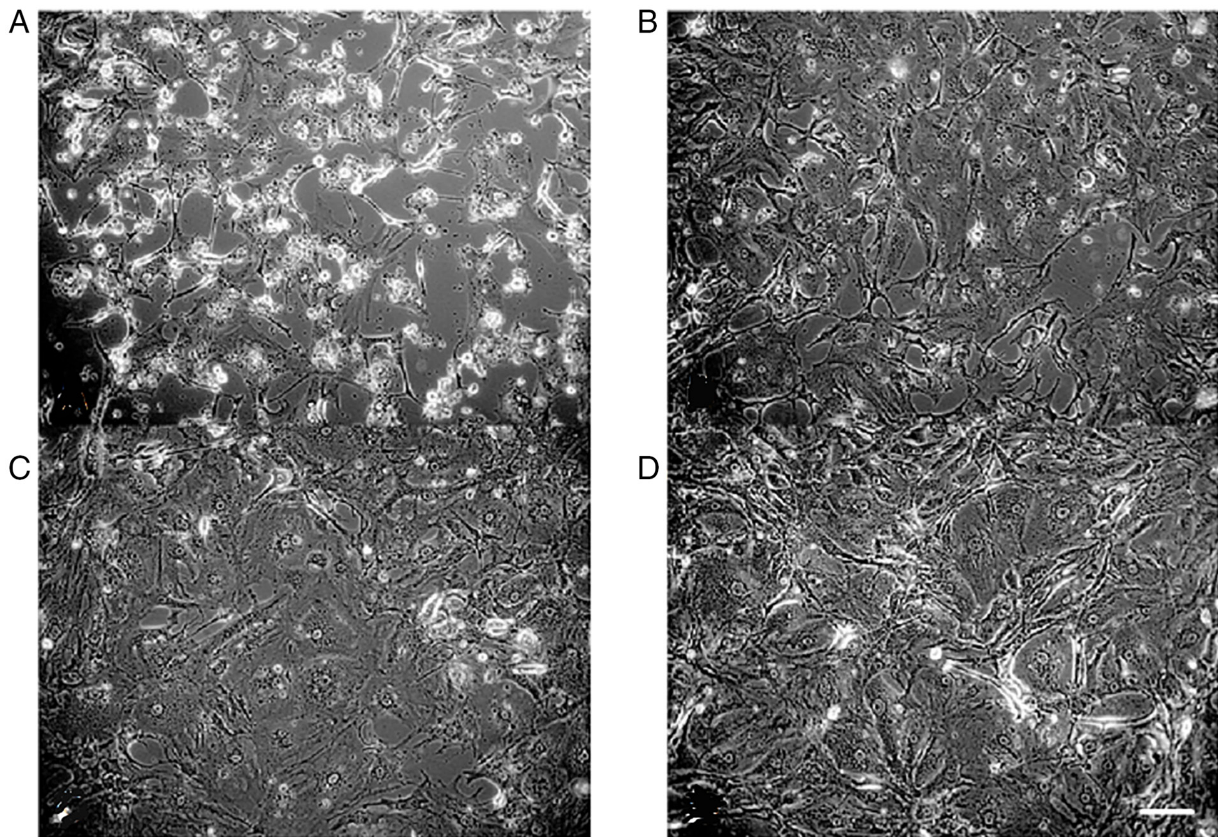

Figure 4. Representative images of cell culture from sample T9181 culture group A at passage 2 over time. Cell culture on days (A) 1, (B) 4, (C) 7 and (D) 10 after samples were recultivated. On day 10, the culture was terminated for fluorescence *in situ* hybridization (magnification, x10; scale bar, 100 μm).

termination for FISH was 7.94±1.67 days. Culture A was terminated after an average of 7.67±1.65 days, whereas culture B was terminated after 8.25±1.75 days. The difference was minor and not statistically significant.

Viability of secondary cultures. The average viability of all secondary cultures was 82±2%. Culture A had a viability of 85±9%, while culture B exhibited an average viability of 78±3%. The difference observed was not statistically significant (P=0.47; Mann-Whitney U test) (Fig. 3). There was no evaluable cell counting possible for culture groups A and B for samples T9077 and T9092 at P1 although there were cells detected due to technical reasons. Therefore, there were only 7 instead of 9 tumors for group A at P1 and 6 instead of 8 tumors for group B at P1.

LOH analysis and FISH. In four meningiomas, LOH analysis revealed no chromosomal aberrations (Fig. S1). A total of three tumors showed a combined loss of 1p and 22q. Additionally, an isolated 22q loss in two cases and an isolated 1p loss in one case were observed in meningiomas (Table I).

For each meningioma, a dabbed preparation was made, and for both culture approaches, drop preparations of both the P1 and P2 cell cultures were primed when cultures were established. In total, up to five preparations per meningioma were analyzed using FISH. Representative images of FISH analysis are shown in Fig. 5. The results from all preparations of a single meningioma, regardless of whether they were derived from P1, P2 or frozen tissue, were consistent. FISH analysis revealed an isolated loss of 22q (22q-) in three meningiomas, combined losses of both regions 1p and 22q in three meningiomas, and loss of 1p (1p-) in one meningioma. No chromosomal losses were detected in three tumors.

Comparison of LOH and FISH analysis results. FISH and LOH analysis yielded identical results in 9 out of 10 meningiomas. In 1 case, there were discrepancies: Sample T9177 exhibited a diploid chromosome set in LOH analysis. By contrast, FISH detected a notable loss of 22q in both drop preparations at P1 and P2, in addition to in the dabbed preparation.

Discussion

In the present study, meningioma tissue was intentionally subjected to various conditions before establishing cell cultures. The primary focus was to investigate the impact of variations in time until further processing of the tissue post-surgery. Other factors, such as preparation, temperature and nutrition solution, were standardized. The present study was performed using a well-established protocol for meningioma cell culturing (1,9,12,20). Specifically, the proliferative behavior of meningioma cells was observed to assess changes when the tissue was processed for cell culture on the day of surgery, rather than after 24-h storage of the tissue in nutrient solution at 4˚C, as was the case for the comparison cultures. Additionally, the proliferative patterns of secondary cultures were examined after the primary cultures were frozen for

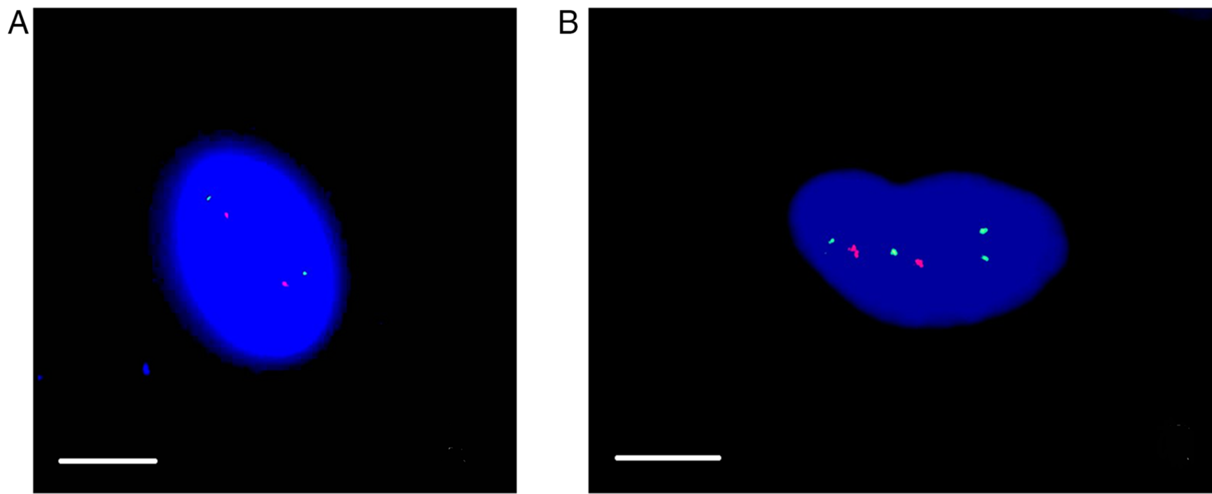

Figure 5. Representative images of fluorescence *in situ* hybridization. (A) Fluorescence image of sample T9152 within culture B (prepared on the day after surgery) at P1, showing a diploid chromosome set. (B) Fluorescence image of sample T9194 within culture A (prepared on the day of surgery) at P1, where the depicted nucleus of the droplet preparation shows tetraploidy concerning chromosome 22 (green) and a deletion of chromosome 1 (red). Scale bar, 10 μm. P1, passage 1.

6-7 months and subsequently thawed. FISH analysis was used to detect chromosomal aberrations in meningioma cells under different conditions and preparation steps. The results from various drop and dabbed preparations were compared with neuropathological findings from LOH analysis. This comparison allowed an assessment of whether the native tumor tissue was accurately represented by the cell cultures.

Demographic factors and histopathological grading did not influence the results (growth variability and growth rate) in this small sample size. It was demonstrated in previous studies (1,21-24) that sex, WHO grading, localization of the tumor or age of the patient did not influence meningioma cell cultures. Additionally, clonal origin of meningioma cells could be demonstrated in cell culture (25).

Meningioma cells were first cultured in 1978 (26). Little is known regarding their cell behavior in meningioma cultures, despite this being a common method in scientific research for studying tumors. The proliferation success rate of these cells in a study reported by Kredel (20) was 60%, and this was observed to be 65% in another study reported by Westphal *et al* (23). The proliferation rates in the present study showed superior results compared with the aforementioned studies. Therefore, based on these observations, an association can be retrospectively established between the consistency of the tumor material during sectioning for initiation and proliferative behavior in the present study. The cell cultures that exhibited proliferation were based on a tissue section that predominantly had soft components and could be cut well.

In the primary generation, a proliferation rate of 90% was observed in culture A. Culture B showed sufficient proliferation for further processing in 80% of the cultures. This difference was not statistically significant. It can therefore be concluded that the 24-h interval before initiating the cultures had no real impact on the proliferative behavior of the cells. Therefore, it is likely not necessary to process the tissue immediately after surgery; samples could be stored in the refrigerator and processed at a later time within the first 24 h. In the secondary generation, all cryopreserved cultures in group A proliferated. Of the eight frozen cultures in group B, seven were able to be recultivated. This resulted in a proliferation rate of 87.5%. Storing cells in liquid nitrogen therefore represents a potential method for keeping meningioma cells viable and capable of reproduction. A direct comparison of proliferative rates between the two cell generations was not possible due to their different starting conditions. Primary cultures were established from native tissue, which required cells to grow out first and then be split after sufficient proliferation. By contrast, secondary cultures were derived from thawed cells that only needed to proliferate in the culture flask and were not split further. The process of cell outgrowth from tissue, followed by proliferation and splitting at P0 and P1, took longer compared with the proliferation of already established cells at P2.

Another aspect to investigate would be how long frozen meningioma cells can be cultured for and whether their properties change during that time. Sugimoto *et al* (24) studied cell count, viability, differentiation ability and aging in mesenchymal stromal cells obtained from the pelvic bone of pediatric patients. These cells were cryopreserved at -80˚C in a commercial cryopreservation solution with 10% DMSO and serum for a period of 1-20 years. Cell proliferation occurred even after 20 years of cryopreservation. However, the viability of the cells was inversely proportional to the number of years cryopreserved; the longer the cells were frozen, the fewer viable cells were measured in the secondary culture. To determine possible aging of the cells, they were examined for the deposition of senescence-associated β-galactosidase. All cells that had been frozen for ≥5 years showed such deposition. However, the extent of this was independent of the number of years. This led to the conclusion that cryopreservation does not accelerate cell aging. Furthermore, the cells in the previous study retained their differentiation ability.

Despite largely consistent results between the LOH findings and FISH analysis in the present study, there were discrepancies in two tumors. These differences arose from the use of different methods to determine the molecular cytogenetic status of the tumor cells using PCR-based microsatellite

analysis for LOH detection or FISH. In the former, DNA was extracted from the tumor tissue provided by the Department of Neurosurgery (Saarland University, Hospital, Homburg, Germany), followed by PCR amplification using primers specific for various microsatellites. The PCR products were then separated through gel electrophoresis, where the tumor bands were compared with the blood bands of patients. For FISH, a different piece of tumor tissue was used to establish cell cultures, where chromosomal alterations from these cultured cells were analyzed using the FISH method.

To explain the different results observed in these meningioma samples, several factors must be considered. The tissue samples used for neuropathology and experimental neurosurgery were obtained from different regions of the tumor, which could have contained genetically distinct, mutated material (25). Chromosomal heterogeneity within different regions of a meningioma has been described in previous studies, including those by Urbschat *et al* (12) and Pfisterer *et al* (26). Additionally, the interpretation of both methods used in the present study is examiner-dependent: In LOH analysis, subjective judgment was involved in band evaluation and determining chromosomal loss, whilst in FISH, counting probe signals and determining the chromosomal number in cells also involved subjective assessments. Nevertheless, the results from FISH corresponded with those from LOH analysis in nine meningiomas when considering the specific probes used.

The predominantly consistent results between the neuropathological findings and FISH analysis led to another insight: The cells in the cell cultures accurately represented the original tumor tissue. Chromosomal aberrations did not change during cell outgrowth from the tumor tissue nor after splitting the cultures in the observed time period of the present study. This observation has also been reported in previous studies. Lerner *et al* (1) and Linsler *et al* (3) described FISH as a complement to molecular pathological examinations, enabling early therapy for higher-grade meningiomas. It was then noted that the results of FISH were as reliable as those of other cytogenetic methods already established in clinical routine, where this approach provided results in a more cost-effective and time-efficient manner (1). Similarly, another study by Uhlmann *et al* (6) used primary meningioma cell cultures and observed an association between the tumor tissue and cultured cells regarding cell morphology and growth characteristics. Although not observed on a cytogenetic level, Buckley and Eisenhardt (21) described in 1929 that meningioma cells in paraffin sections, cell cultures and vital preparations exhibited morphological similarities.

Whilst it is acknowledged that the sample size of the present study is limited, there are several factors to consider. Primarily, the sample size and preparation of samples were representative for meningiomas. Smaller sample sizes can also yield valuable qualitative insights that are important for understanding complex biological processes. These insights can guide future research directions and clinical applications. Furthermore, to the best of our knowledge, there is no literature available providing a profound description of primary meningioma cell culturing and growth patterns for comparison. Based on the present data, further studies of primary cell culturing under other conditions might be performed in the future.

In summary, the proliferative rates presented in the present study can be utilized both within and outside of clinical settings to estimate the success of meningioma cell culture establishment. The present study revealed that the growth pattern of meningioma cell cultures was independent of whether cultures were established on the same day as surgery or 1 day post-surgery if there were enough viable cells present in the tissue. Cell cultures, frozen for several months or years, can be successfully recultivated if the cells remain viable after freezing and thawing as shown in the present study. In addition, the results of FISH verified that cell cultures can accurately represent the tumor material even after freezing and recultivating.

Meningioma cell culture provided a means to accurately represent native tissue without altering the genome. This allowed cultures to be used as a basis for investigating tumor-specific gene mutations, similar to native tumor tissue. Additionally, viable cells from an individual tumor were able to be preserved for extended periods, enabling studies on tumor response to various therapies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SL and SU were responsible for conceptualizing the present study. SL, RK, SU, JO, WSS and ALM were responsible for the methodology. SL, WSS, JO and RK contributed towards the validation. SL, ALM and SU performed the formal analysis. SL and SU were responsible for investigation. SL and ALM were responsible for writing the original draft, while SL, RK, WSS, JO, ALM and SU all contributed towards reviewing and editing the final manuscript. SL and ALM were also responsible for visualization, while SU and JO provided supervision. SL, JO SU were also involved in project administration. SU and SL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all subjects involved in the present study. The present study was approved by the Ethical Committee of the Medical Association of Saarland (approval no. 02/20; Saarbrücken, Germany).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lerner C, Ketter R, Linsler S, Henn W, Oertel J and Urbschat S: Establishment of a molecular cytogenetic analysis for native tumor tissue of meningiomas-suitable for clinical application. Mol Cytogenet 7: 12, 2014.
2. Linsler S, Ketter R, Oertel J and Urbschat S: Fluorescence imaging of meningioma cells with somatostatin receptor ligands: An in vitro study. Acta Neurochir (Wien) 161: 1017-1024, 2019.
3. Linsler S, Kraemer D, Driess C, Oertel J, Kammers K, Rahnenführer J, Ketter R and Urbschat S: Molecular biological determinations of meningioma progression and recurrence. PLoS One 9: e94987, 2014.
4. Choudhury A and Raleigh DR: Preclinical models of meningioma: Cell culture and animal systems. Handb Clin Neurol 169: 131-136, 2020.
5. Ishikawa T, Matsuda M, Ishikawa H, Toyomura J, Ohyama A, Sakamoto N, Zaboronok A and Ishikawa E: Establishment of a novel benign meningioma cell line spontaneously immortalized under hypoxic conditions. Hum Cell 38: 22, 2024.
6. Uhlmann EJ, Rabinovsky R, Varma H, El Fatimy R, Kasper EM, Moore JM, Vega RA, Thomas AJ, Alterman RL, Stippler M, *et al*: Tumor-derived cell culture model for the investigation of meningioma biology. J Neuropathol Exp Neurol 80: 1117-1124, 2021.
7. Jellinger K and Slowik F: Histological subtypes and prognostic problems in meningiomas. J Neurol 208: 279-298, 1975.
8. Ketter R, Kim YJ, Storck S, Rahnenführer J, Romeike BF, Steudel WI, Zang KD and Henn W: Hyperdiploidy defines a distinct cytogenetic entity of meningiomas. J Neurooncol 83: 213-221, 2007.
9. Ketter R, Rahnenführer J, Henn W, Kim YJ, Feiden W, Steudel WI, Zang KD and Urbschat S: Correspondence of tumor localization with tumor recurrence and cytogenetic progression in meningiomas. Neurosurgery 62: 61-69; discussion 69-70, 2008.
10. Ketter R, Urbschat S, Henn W, Feiden W, Beerenwinkel N, Lengauer T, Steudel WI, Zang KD and Rahnenführer J: Application of oncogenetic trees mixtures as a biostatistical model of the clonal cytogenetic evolution of meningiomas. Int J Cancer 121: 1473-1480, 2007.
11. Sayagues JM, Tabernero MD, Maíllo A, Trelles O, Espinosa AB, Sarasquete ME, Merino M, Rasillo A, Vera JF, Santos-Briz A, *et al*: Microarray-based analysis of spinal versus intracranial meningiomas: Different clinical, biological, and genetic characteristics associated with distinct patterns of gene expression. J Neuropathol Exp Neurol 65: 445-454, 2006.
12. Urbschat S, Rahnenführer J, Henn W, Feiden W, Wemmert S, Linsler S, Zang KD, Oertel J and Ketter R: Clonal cytogenetic progression within intratumorally heterogeneous meningiomas predicts tumor recurrence. Int J Oncol 39: 1601-1608.
13. Linsler S, Müller SJ, Müller A, Senger S and Oertel JM: Fluorescence image-guided resection of intracranial meningioma: An experimental in vivo study on nude mice. Ann Anat 237: 151752, 2021.
14. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS III, Johnson BE and Skolnick MH: A cell cycle regulator potentially involved in genesis of many tumor types. Science 264: 436-440, 1994.
15. Trasaktaweesakul T, Asavaritikrai P, Talabnin K, Kongnawakul D, Tastub S, Jaturutthaweechot P, Naewwan N and Talabnin C: In vitro properties of four benign meningioma cells derived from WHO grade 1 meningiomas. Hum Cell 38: 84, 2025.
16. van de Weijer LL, Ercolano E, Zhang T, Shah M, Banton MC, Na J, Adams CL, Hilton D, Kurian KM and Hanemann CO: A novel patient-derived meningioma spheroid model as a tool to study and treat epithelial-to-mesenchymal transition (EMT) in meningiomas. Acta Neuropathol Commun 11: 198, 2023.
17. Smith HL, Wadhwani N and Horbinski C: Major features of the 2021 WHO Classification of CNS tumors. Neurotherapeutics 19: 1691-1704, 2022.
18. Hopman AH, Ramaekers FC, Raap AK, Beck JL, Devilee P, van der Ploeg M and Vooijs GP: In situ hybridization as a tool to study numerical chromosome aberrations in solid bladder tumors. Histochemistry 89: 307-316, 1988.
19. Hartmann C, Mueller W, Lass U, Kamel-Reid S and von Deimling A: Molecular genetic analysis of oligodendroglial tumors. J Neuropathol Exp Neurol 64: 10-14, 2005.
20. Kredel FE: Tissue culture of intracranial tumors with a note on the meningiomas. Am J Pathol 4: 337-340 3, 1928.
21. Buckley RC and Eisenhardt L: Study of a meningioma in supravital preparations, tissue culture and paraffin sectios. Am J Pathol 5: 659-664 3, 1929.
22. Kajikawa H, Kawamoto K, Herz F, Wolley RC, Hirano A and Koss LG: Flow-through cytometry of meningiomas and cultured meningioma cells. Acta Neuropathol 44: 183-187, 1978.
23. Westphal M, Hänsel M, Brunken M, König A, Köppen JA and Herrmann HD: Initiation of primary cell cultures from human intracranial tumors on extracellular matrix from bovine corneal endothelial cells. Exp Cell Biol 55: 152-163, 1987.
24. Sugimoto Y, Yamazaki Y, Moriyama K, Sugimoto T, Kumazawa K, Baba K, Sone Y and Takeda A: Differentiation and proliferation potencies of human bone tissue-derived mesenchymal stromal cells (hBT-MSCs) after long-term cryopreservation - Comparison among cells stored for 1, 5, 10, 15, and 20 years. Regen Ther 18: 363-371, 2021.
25. von Deimling A, Larson J, Wellenreuther R, Stangl AP, van Velthoven V, Warnick R, Tew J Jr, Balko G and Menon AG: Clonal origin of recurrent meningiomas. Brain Pathol 9: 645-650, 1999.
26. Pfisterer WK, Hank NC, Preul MC, Hendricks WP, Pueschel J, Coons SW and Scheck AC: Diagnostic and prognostic significance of genetic regional heterogeneity in meningiomas. Neuro Oncol 6: 290-299, 2004.

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