

Review

Modelling Meningioma Using Organoids: A Review of Methodologies and Applications

Clara Elena López Vásquez^{1,2}, Clint Gray¹, Claire Henry² and Matthew J. Munro^{1,*} 

¹ Gillies McIndoe Research Institute, Newtown, Wellington 6242, New Zealand; clara.lopez@gmri.org.nz (C.E.L.V.); clint.gray@gmri.org.nz (C.G.)

² Department of Surgery and Anaesthesia, University of Otago, Wellington 6264, New Zealand; claire.henry@otago.ac.nz

* Correspondence: mattymunro@gmri.org.nz

Abstract: Meningiomas are the most common tumours of the central nervous system. According to the World Health Organization (WHO), this disease is classified into three different grades: 80% of meningioma patients present with benign grade I tumours, while less than 2% present with malignant grade III meningiomas. Despite affecting thousands of people worldwide, much remains unknown about this disease, and the development of systemic treatments is still far behind in comparison to other types of tumours. Therefore, forming 3D structures (spheroids and organoids) could facilitate research on the mechanisms of formation, proliferation, migration, and invasion of these, for the most part, benign tumours, while also helping in the process of drug development. To date, there are three published methods for the formation of meningioma organoids primarily derived from patient tissue samples. Organoids offer many advantages in the development of treatments because they recapitulate the cellular complexity within tumours. These new methodological advances could open a substantial number of possibilities for the further characterisation and treatment of meningiomas. This review includes an overview of the disease and a description and comparison of established protocols for meningioma organoid formation.

Keywords: meningioma; organoids; spheroids; treatments; cancer



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1. Meningioma

Meningiomas are the most common tumours of the central nervous system [1]. The incidence rate varies from 1.3 to 7.8 cases per 100,000 worldwide [2]. According to the World Health Organization (WHO), this disease is classified into three different grades: grade I is considered a benign tumour, grade II is atypical, and grade III is malignant or anaplastic. Grade I meningiomas are the most common, comprising approximately 80% of all meningiomas diagnosed; ~17% are atypical, and less than 2% are considered malignant [3]. Each meningioma grade is further classified into histological subtypes. Meningothelial is the most common subtype of grade I meningioma (60%), followed by fibrous, transitional, psammomatous, secretory, angiomatous, lymphoplasmacyte-rich, metaplastic, and microcystic subtypes. Grade II meningiomas include three subtypes: atypical, chordoid, and clear cell. Grade III includes papillary, rhabdoid, and anaplastic subtypes [3–5].

Despite affecting thousands of people worldwide, much remains unknown about this disease, and the development of systemic treatments is still far behind in comparison to other types of tumours [3].

Meningiomas originate from the meninges [5]. Healthy meninges are composed of different types of cells, such as fibroblasts, which are the majority; arachnoid barrier cells with epithelial characteristics; immune cells; and endothelial cells [6]. The meninges comprise three layers that surround the brain and spinal cord: the dura mater, the arachnoid mater, and the pia mater [7]. These three layers play key roles in the stabilisation and

protection of the central nervous system. They also facilitate some immunological responses, including immunosurveillance [8]. One hypothesis establishes that meningiomas originate from precursor cells that are prostaglandin D2 synthase positive (PGDS+). These cells differentiate into arachnoid barrier cells (ABC) and dura border cells (DBC), each of which generates a different meningioma subtype [9,10]. DBC forms fibroblastic meningiomas, whereas ABC originates the meningothelial subtype [9]. Another hypothesis establishes that meningiomas that usually start forming in the arachnoid layer originate from arachnoidal cap cells [10,11]. These arachnoid cap cells, which are involved in the reabsorption of cerebrospinal fluid, have high metabolic activity and different junctions between them which are key to developing their function. Desmosomes and hemidesmosomes allow cells to adhere tightly to one another, whereas cadherin junctions facilitate their flexibility. This layer of the meninges is not well-vascularised; therefore, the cells also require gap junctions that allow the transport of different nutrients and metabolites between cells [12,13].

According to the tumour grade and subtype, meningiomas contain cells with different characteristics. Most cells possess mesenchymal features, such as intracellular aggregates of collagen and sometimes metaplastic changes, similar to cartilage and bone tissue. However, most meningioma tumour cells are identified as meningothelial-like cells, indicating that they are derived from the meningeal layers. These cells create a spherical structure that tends to mineralise to produce whorl formations and psammoma bodies [3,14]. Other properties of these tumours include bleached chromatin and cytoplasmic inclusions in the cell nuclei [3]. Since meningiomas can possess a wide array of molecular characteristics, their grades and subtypes are typically classified according to their histological appearance [10].

The difference in grading resides in the histology of the tumours. Grade I meningioma subtypes are characterised by cellular features. For example, the meningothelial subtype contains syncytial/meningothelial-like cells that have round nuclei and form whorls, the fibrous subtype contains cells with a spindle morphology, and the angiomatous subtype has a significant vascular component comprising endothelial cells. Grade II meningiomas typically present with an increase in mitotic cells, are more invasive (going further than the pial layer), have smaller cells, spontaneous necrosis, and an uninterrupted pattern with less growth or a larger nucleus. Grade III tumours present with a higher number of mitotic cells and show characteristics like melanoma, sarcoma, and carcinoma [15,16].

In grade I meningiomas, the meningothelial subtype presents as medium-sized cells with a high number of nuclei, and some with cytoplasmic inclusions. Cell limits are difficult to identify, and some groups of cells are surrounded by fibrous septa. The fibrous subtype is characterised by elongated spindle cells organised in parallel and surrounded by a matrix primarily composed of collagen [16,17]. Psammomatous meningiomas are identified by an abundance of psammoma bodies. This subtype is often observed in patients with spinal tumours. In contrast, the transitional subtype shows a combination of the previous subtypes described above. Some cells have psammoma bodies, some have spindled cells, and some are epithelioid [16].

As the name indicates, angiomatous lesions are identified by many blood vessels within the tumour. Cells often show a foamy-like cytoplasm and nuclear atypia. This group usually presents in combination with the microcystic subtype, which displays features like those of the arachnoid layer, where the cells are elongated with spaces between them [16].

Metaplastic meningioma is similar in appearance to classical meningioma histology, but sometimes acquires features of other tissues such as cartilage, bone, and connective tissue. The secretory subtype displays epithelial characteristics and gland-like structures filled with eosinophilic secretions. This is the only subtype that does not possess mutations in *NF2*, but they are characterised by abundant *KLF4* and *TRAF7* mutations. The lymphoplasmacyte-rich subtype is identified by inflammation, presenting with infiltrating immune cells such as macrophages, lymphocytes, and plasma cells [13,16,17].

Grade II meningiomas are divided into three subtypes. Chordoid tumours present with elongated cells, eosinophilic cytoplasm and a matrix with basophilic characteristics [16]. The clear cell subtype is characterised by abundant glycogen in the cytoplasm [18]. Among

other criteria, atypical tumours, which include all grade II tumours, are prone to brain invasion and have a mitotic index close to 4.

Grade III meningiomas include papillary tumours that possess papillary structures, a high proliferation rate, and necrosis with prolongation of the blood vessels; the rhabdoid subtype characterised by eosinophilic cytoplasm with cytoplasmic inclusions, whorl formations, and a mitotic index ≥ 4 ; and the anaplastic subtype, which has a mitotic index of ≥ 20 , occasional meningothelial whorls and psammoma bodies, and features resembling melanoma, sarcoma, or carcinoma [16,17].

Most meningiomas harbour somatic mutations in *NF2*, *SMO*, *AKT1*, *KLF4*, *POLR2A*, and/or *TRAF7* and germline mutations in *SUFU*, with certain mutations associated with each tumour subtype and location [11,19,20] (Table 1). Neurofibromin 2 (*NF2*) is a gene involved in the formation of the merlin protein that connects proteins from the membrane and cytoskeleton and is also considered a tumour suppressor gene. Mutations in this gene or deletions in the chromosome where it resides (22q) are the most common molecular characteristics for identifying meningiomas [10,21–25]. Smoothed (*SMO*) and suppressor of fused homolog (*SUFU*) participate in the Hedgehog signalling pathway implicated in the proliferation, growth, and migration of cells [19,20,26,27]. *ATK1* is a proto-oncogene that encodes a serine-threonine kinase that plays a key role in the *PI3K* pathway implicated in growth signalling [27–29]. Krüppel-like factor 4 (*KLF4*) is involved in cell proliferation, growth, and differentiation [27,30]. RNA polymerase II subunit A (*POLR2A*) is commonly mutated in meningothelial meningiomas [31–33]. This somatic mutation is also associated with meningioma development in the tuberculum sellae [32]. TNF receptor-activated factor 7 (*TRAF7*), which is mutated in >20% of meningiomas, encodes an E3 ubiquitin ligase that oversees the degradation of other proteins [27,34].

Table 1. Main mutations present in Meningioma.

Gene	Function	Mutation Types	Changes	References
<i>NF2</i>	Codes for the merlin protein that connects membrane proteins to the cytoskeleton	Point mutations, exon deletions, 22q LOH, monosomy 22	Occur at various sites, especially exons 2, 6, 7, 8, 11 & 13; point mutations predominantly C>T and can occur throughout the gene	[10,21–25]
<i>KLF4</i>	Cell proliferation, growth and differentiation	Point mutation	K409Q	[27,30]
<i>AKT1</i>	Proto-oncogene encoding a serine-threonine kinase	Point mutation	E17K	[27–29]
<i>SMO</i>	Member of Hedgehog signalling pathway	Point mutations	Various, including L412F & W535L	[26,27]
<i>TRAF7</i>	Encodes an E3 ubiquitin ligase	Point mutations	Numerous reported mutations, but many are located in the C-terminal WD40 domains	[27,34]
<i>SMARCB1</i>	Modifies chromatin structure to facilitate transcription	Frameshift, missense, 22q LOH, monosomy 22	P48L; R368H; numerous mutations in exons 4, 7, 8 & 9, and intron 5	[35,36]
<i>POLR2A</i>	RNA polymerase II subunit	Inframe codon loss	437-439 DLH/D	[31–33]
<i>SUFU</i>	Member of Hedgehog signalling pathway	Point mutation	R123C	[19,20]

Some meningiomas contain mutations in the *SMARCB1* (matrix-associated actin-dependent regulator of chromatin subfamily B member 1) gene, whose primary function is to help loosen the chromatin structure and facilitate transcription [35,36] (Table 1). Mutations in *HOX* genes cause alterations in transcription factors and processing of genetic information [37,38]. The mutations in the NR4 (nuclear receptor 4) family can be present

in meningiomas, and these genes regulate the signalling pathways involved in apoptosis, migration, and proliferation [39,40]. *FOSL2*, which is implicated in the regulation of cell differentiation, proliferation, and transformation, can also be mutated in certain meningiomas. *TCF8* encodes zinc finger homeobox protein 1, which is responsible for processing genetic information [38,41]. Ultimately, members of the DUSP (dual-specificity phosphatase) family dephosphorylate threonine, serine, and tyrosine residues, regulating the activity of other proteins such as MAP kinases [42,43]. Mutations also vary according to tumour location. Tumours appearing at the convexity of the brain typically present with mutations in *NF2* and *SMARCB1*, whereas those located at the skull base will usually present with mutations in *TRAF7*, *AKT1*, *KLF4*, *SMO*, and *PIK3CA* [38]. Finally, the tumours more commonly located in the spinal cord have mutations to *HOX*, *NR4*, *FOSL2*, *KLF4*, *TCF8*, and *DUSP* [38,44].

Meningioma most commonly affects people aged between 65 and 85 years [3,45]. The risk of meningioma increases with age and ionising radiation exposure and is influenced by ethnicity and sex [3,46]. A higher incidence has been reported in Asian and African populations than in European and Latin populations [3,47]. In addition, people with *NF2* mutations that cause the hereditary disease neurofibromatosis 2 are more likely to develop meningiomas. Other potential risk factors include a high body mass index (BMI), smoking, and high blood pressure [46,47].

Women are twice as likely to develop meningiomas as men (2:1), and this increases during female reproductive years (3:1) [46,48]. Some studies have shown that pregnant women with meningiomas experience increased tumour growth during the childbearing period [49,50]. It has been suggested that the difference in incidence is related to the different levels of hormones between males and females, because meningiomas are known to have progesterone and oestrogen receptors that can influence the response of the tumour to sex hormones [47,51,52]. Other studies have focused on the connection between breast cancer and meningioma in relation to hormone levels because breast cancer has well-established hormonal involvement and patients with meningiomas are more likely to develop breast cancer [53]. Other studies conducted in females who received cyproterone acetate (a synthetic progestin) to treat severe cases of hirsutism suggested that this hormonal therapy increases the incidence of meningioma [54]. These findings support the hypothesis that meningioma formation and proliferation is influenced by hormones.

However, there is still much to uncover regarding the molecular mechanisms implicated in the formation and progression of this disease, as well as the development of new, less invasive, and more effective treatments. In addition, the number of models that can accurately represent the disease is limited, making it difficult to understand the underlying mechanisms [55]. Studying meningioma with 3D cell models could enable breakthroughs in this field, making the research more accurate and representative of patient tumours.

2. Organoid Applications

Over the last 100 years, scientists have developed different methods to form organoids that resemble the different tissues and organs of the human body. All of them are derived from stem cells, and organoids have been successfully obtained from the intestines, colon, breast, liver, pancreas, and retina [56]. Brain organoids are among the most recent models developed [56] because of the complexity of the brain, making it challenging to suitably recapitulate its characteristics in vitro [57].

Organoids have helped in understanding diseases, especially cancer. These in vitro 3D structures contain different cell types and resemble organs in terms of complexity and organisation. The first organoid was formed in the early 1900s with the observation that sponges were able to regenerate from single cells, a feature attributed to stem cells [56]. This concept has been central to the development of organoid culture, whereby organ-specific stem cells, often derived from patients, are grown under tightly regulated conditions to differentiate and produce the cell types of the organ that self-organise to reproduce tissues, organs, tumours, or even organisms in their entirety [56].

Currently, the standard models used to study diseases such as cancer are cell lines and patient xenografts [55,58]. Organoids, an expanding research field, can maintain the genetic and phenotypic features of tumours, and are therefore a more accurate representation of the tumour. Furthermore, methods incorporating elements of the tumour microenvironment, including haematopoietic cells, blood vessels, and immune cells, will further increase the utility of these models [58]. Additionally, because cancer shows both intra- and inter-tumoural heterogeneity, which leads to different responses to the same treatment, the utilisation of organoids provides the possibility of personalised treatment options via analysis of patient-specific drug responses [59].

3. Organoid Culture Techniques

Organoids are complex 3D structures that have opened numerous possibilities in scientific research, especially in cancer. Although their formation requires precision and technique, they are considered the link between 2D cell lines and *in vivo* models [56,60]. Interest in the formation of structures that recapitulate organs and diseases, such as meningioma, has increased.

Organoids can be formed using various techniques, as explained and illustrated by Londoño-Berrio et al. [59]. The cells can be submerged in an animal-derived or synthetically engineered extracellular matrix-like substance, such as Matrigel or Cultrex, to provide a scaffold that facilitates the formation of a 3D structure. Alternatively, they can be cultured as an air–liquid interface by applying microfluidic systems, such as an organ-on-a-chip, or with a bioreactor, which allows the organoids to receive a constant flow of nutrients and oxygen [61]. The air–liquid interface consists of seeding cells in an extracellular matrix onto Transwell inserts. The organoids obtain oxygen from direct contact with the air and nutrients from beneath the insert where the matrix and the media are in contact [59]. The microfluidic technique consists of embedding cells in a culture device engineered from polydimethylsiloxane (PDMS), glass, or silicon, incorporating chambers for organoid growth with channels where the media can be pumped to maintain the organoid nutrient supply. This method could also be useful for representing the tumour microenvironment by perfusing channels with immune cells or facilitating the formation of blood vessels [59,62]. The last method is to cultivate organoids in bioreactors, which allows for precise control of different parameters, such as pH, concentration of nutrients and oxygen, and temperature. With this technique, the integrity of the organoid is maintained and the cell proliferation rate increases, but the extracellular matrix is not considered [59,61].

There are two predominant methods for the development of brain organoids. The first is the “guided method”, where specific factors are applied to pluripotent stem cells, often iPSCs, so that the cells can be guided to differentiate down specific lineages and form a cerebral organoid, or “mini-brain” [63]. The second method is an “unguided method”, in which the media is not manipulated, and cells spontaneously change their morphology and differentiate towards a particular lineage [57].

If brain organoids are maintained under adequate conditions, they can survive for more than a year [64]. With these long-lasting organoids, scientists can also recapitulate later stages of neural maturation [64,65].

In general, organoids must be studied to determine whether the genomic and transcriptomic characteristics, as well as morphology and function, vary over time in comparison to the original or parental tissue from which the cells were obtained [66]. In addition, the clonality of the organoids could be influenced by long-term culture in one media type, thereby reducing the inherent heterogeneity seen *in vivo* [66].

4. Differences between Organoids and Spheroids

Although the terms organoid and spheroid have often been used interchangeably, there are substantial differences between them. Spheroids are cell aggregates organised into three-dimensional structures formed from cell lines, digested tissues, multicellular mixtures, or primary cells [60]. However, most spheroids cultivated in suspension to form

these aggregates do not reflect the complex organisation of the organ or tumour as well as organoids. Spheroid formation is divided into three steps: formation of long-chain extracellular matrix fibres that facilitate the interaction between the integrins of adjacent cells, upregulating the expression of cadherins which principally function in cell–cell adhesion; accumulation of cadherins on the surface of the cell membrane; and binding between cadherins, forming tight cell–cell connections [59,60,67].

Two of the most common applications for spheroids are drug and biomarker discovery, whereas organoids have a higher number of applications because of their ability to reflect *in vivo* tissues more accurately [59,60]. Spheroids can be cultivated with or without growth factors, whereas organoids must be cultured with specific factors [60]. However, spheroids can be formed within 24 h, whereas organoids take longer to form, ranging from weeks to months [55,60,68,69].

5. Advantages and Limitations of Organoid Models

Organoids present advantages and limitations relative to other models (Table 2). The major benefit is that organoids reproduce the complexity of the organ or tumour from which they are derived almost entirely. This includes the recapitulation of cellular heterogeneity and organisation and maintenance of the 3D structure including cell–cell contacts [60,67,70]. Organoids derived from healthy tissue shed light on the formation, maintenance, metabolism, and signalling of cells within healthy organs, and can be utilised to reveal the implications of specific mutations when induced via gene editing techniques [71]. Tumour-derived organoids can facilitate our understanding of the mechanisms underlying tumour formation, progression, proliferation, and migration. These models can also help in the study of DNA, proteins, metabolites, and lipids using *omics* techniques (genomics, proteomics, metabolomics, and lipidomics), phylogenetics, and host–microbiota interactions [60,72].

Table 2. Advantages and limitations of organoid models.

Advantages	Limitations
Reproduce complex organisation of organs/tumours	No accurate reproduction of the microenvironment
Recapitulates cell heterogeneity	No standardised protocols
Maintain cell–cell contact	High use of resources
	Time-consuming
High number of applications: drug discovery, in-depth studies of diseases, biobank establishment, personalised treatments, among others	Use of animal-derived resources
	Low reproducibility

In addition, organoids simulate the gradient of nutrient availability seen in actual tumours, with limited access of drugs to the inside of the organoid making them a highly relevant model for drug development and screening [73]. Another advantage of using organoids in drug development is the possibility of forming biobanks for drug screening and facilitating the development of personalised treatments [60,74].

Organoids can recapitulate intra- and inter-tumoural heterogeneity, as well as intra- and inter-patient heterogeneity [75,76]. This heterogeneity can be identified by RNA-seq and exome sequencing to establish the genomic profiles of the cells forming the organoid [75]. In glioblastoma organoid studies, it was demonstrated that the best representation of the intra-tumoural heterogeneity is achieved when the organoid is derived through the cultivation of small sections of the tumour rather than from a single-cell suspension, so that the different cell populations and tumour clones are represented [75,77].

Organoid models are currently limited in their ability to simulate the microenvironment surrounding tumours, including the immune system, stroma, and blood vessels. This is an important research focus in organoid development [58,78].

The current lack of standardised and established protocols for some organoid types implies that the most efficient and effective method of cultivating organoids has not yet been achieved. Organoids require more time and resources than cell lines, a reflection of their complexity, and a cost that is balanced by the benefit of their greater similarity to *in vivo* biology (58). In addition, the use of animal-derived resources in the cultivation process, such as Matrigel or Cultrex, may interfere with drug screening [58]. The variability in animal-derived products and organoid culture protocols implies that the reproducibility of organoids is currently a significant challenge [60]. It is important to know the initial conditions in the development of organoids because every step can add variability, including the population of cells used to form organoids, the way the cells position themselves and self-organise, and the medium in which they are cultivated. Therefore, every step of the process needs to be monitored and performed precisely [59].

If there is a need to cultivate organoids for long periods of time, bioreactors must come into play to maintain nutrients and oxygen in continuous flow and at adequate levels because fluctuations in their concentration can limit growth, affect differentiation and selection, introduce variability, and influence the response to treatment [59,61].

6. Organoids to Model Meningioma

Cerebral organoids were first established in 2013 [56,63], but it was not until eight years later that the first meningioma organoids were described in the literature. To date, only three methods have been published for meningioma organoid formation (Table 3).

Table 3. Comparison of the three published meningioma organoid protocols.

Yamazaki et al. [55]	Chan et al. [73]	Huang et al. [68]
From IOMM-LEE cells and patient-derived tissue	From patient-derived tissue	From patient-derived tissue
Single cells	Single cells	Pieces of tissue 1 mm ³
Media: Neurobasal Medium, N-2, B-27, 50 ng/mL FGF and 50 ng/mL EGF	Media: DMEM, 10% FBS, 1% penicillin/streptomycin, 1× B-27, 1× N-2, 1% HEPES buffer, 1% glutamine, 20 ng/mL EGF, and 20 ng/mL FGF	Media: DMEM, 10% FBS, 1% Pen/Strep, 1× GlutaMax, 1× non-essential amino acids, and 0.25 uL/mL insulin
Cells seeded in Matrigel	Cells seeded in Matrigel	Pieces seeded in Media
11 Grade I, 4 Grade II and 1 Grade III	4 Grade I and 1 Grade II	12 Grade I and 4 Grade II.
Studies: RNA interference, RT-PCR, Cell proliferation assay.	Studies: Cell viability assays, immunostaining and haematoxylin and eosin staining.	Studies: sc-RNA-seq, immunostaining, murine orthotopic xenograft model. (SULT1E1 subpopulation detected)

Yamazaki et al. [55] developed a protocol to form organoids starting from a meningioma commercial cell line (IOMM-Lee), as well as from patient-derived cells, of which 66.66% were primary tumours and 33.34% were recurrent. Tissue samples were digested using collagenase IV and mechanical dissociation, and red blood cell lysis was performed using ACK Lysing Buffer (ThermoFisher Scientific, Waltham, MA, USA). Single cells were suspended in Neurobasal Medium supplemented with N-2 and B-27 (all ThermoFisher), 50 ng/mL FGF (fibroblast growth factor), and 50 ng/mL EGF (epidermal growth factor; both R&D Systems, Minneapolis, MN, USA), and then seeded in Matrigel (Corning, Corning, NY, USA) in a six-well plate. The medium was refreshed every few days to allow the cells to divide slowly and form organoids inside the matrix [55]. The similarity of the organoids to their parental tumour was investigated via IHC using antibodies against the meningioma diagnostic markers SSTR2A and Ki-67 and via whole-exome sequencing and structural variant analysis to confirm the conservation of *NF2* mutations and chromosome

22q deletions. The organoids formed from grades II and III showed a higher expression of Ki-67, associated with proliferation and STAT6, specifically in the cytoplasm of the meningioma cells. Furthermore, *FOXM1* has been identified as a contributor to meningioma progression by increasing proliferation, which was confirmed by RNA interference using two *FOXM1*-specific siRNAs [55]. This is the first published report of patient-derived meningioma organoids that successfully recapitulated the molecular and histological features of parental tissues from each patient, though the authors did not specify the cell types found in the meningioma organoids. A limitation in the process of meningioma organoid formation has been recognised—the need for organoids to be surrounded by normal tissue and to study the interactions between these cells to understand the behaviour, invasiveness, and proliferation of meningioma tumours [55].

The second method, by Chan et al. [73], utilised single cells obtained from the mechanical and enzymatic digestion of tissue samples collected from patients with meningioma. Two digestion methods were tested, one using 70 ng/mL collagenase IV and 124 ng/mL dispase, and the other using 0.05% trypsin, with the trypsin method leading to greater cell viability (89.8%) than the collagenase/dispase (86.5%). Once the cell suspension was obtained, the cells were strained through a 70 µm filter before being resuspended in Matrigel and dispensed cautiously into a culture dish in the form of 30 µL droplets. After the Matrigel had solidified, fresh media containing DMEM, 10% foetal bovine serum (FBS), 1% penicillin/streptomycin (antibiotics), 1× B-27, 1× N-2, 1% HEPES buffer, 1% glutamine, 20 ng/mL EGF, and 20 ng/mL FGF were added. Organoid morphology was studied under a microscope at 1, 7, and 14 days, and a progression from single ovaloid and spindle cells to meningioma cell aggregates with cell-to-cell interactions was observed. The authors reported distinct cell niches within the organoids but did not specify the cell types present in each [73]. To check whether the organoids recapitulated the characteristics of the parental meningioma tumours, haematoxylin and eosin (H&E) staining and immunostaining for epithelial membrane antigen (EMA/MUC1) were performed. All results demonstrated that the meningioma organoids obtained were similar to meningioma tissues, showing ovaloid and spindle cells, as seen in the parental tumours. In addition, the cells formed denser aggregates in the peripheral region of the extracellular matrix. With all the experiments conducted, the researchers demonstrated that during the organoid culture process, meningioma cell morphology changed over time [73].

The third protocol was published in early 2023 by Huang et al. [68]. Before establishing an organoid model, meningioma samples were studied using scRNA-seq, revealing eight distinct clusters of cells within the meningioma tumours, with most of them being part of the immune system: malignant cells, dendritic cells, macrophages, monocytes, neutrophils, NK cells, T cells, and tumour-infiltrating B lymphocytes. M2-like polarised macrophages were predominant in higher grades [68]. They then established a meningioma organoid protocol which differs from others by starting from small pieces of patient-derived tissue rather than a single-cell digest, allowing the retention of most cell types and their native cell–cell interactions. The tissues were minced carefully to achieve a size of ~1 mm³, washed with DPBS, treated with red blood cell lysis buffer, and then 20–30 pieces of tissue were transferred to an ultra-low attachment six-well plate (Corning) and grown in suspension. The plate was kept on a shaker in an incubator, and the organoid medium was changed every three days. The organoid medium contained DMEM, 10% FBS, 1% Pen/Strep, 1× GlutaMax, 1× non-essential amino acids (all ThermoFisher), and 0.25 µL/mL insulin (Sigma-Aldrich, St. Louis, MO, USA). H&E staining and immunostaining against SSTR2A (meningioma cells), CD31 (blood vessels), CD68, and CD3 (immune cells) were performed, as well as an extensive study utilising whole-exome sequencing and scRNA-seq to investigate the cellular heterogeneity within organoids. All these studies demonstrated that meningioma organoids recapitulated the gene expression, mutations, histological features, and cell populations (tumour cells, endothelial cells, tumour-infiltrating macrophages, and T lymphocytes) of the parental tumours [68]. They also identified a *SULT1E1*⁺ subpopulation within parental tumours, which was implicated in the progression of meningiomas

to a higher grade. This subpopulation was also identified in meningioma organoids. SULT1E1⁺ cells were confirmed to be invasive by using a murine orthotopic xenograft model. The authors completed their study by exploring possible targeted treatments for this cell population [68].

All three organoid-forming protocols were established mostly from female meningioma samples, reflecting the higher incidence in females. Huang et al. [68] reported successful organoid formation from 12 grade I meningiomas and 4 grade II meningiomas within a week from a total of 21 tumour samples (subtypes: meningothelial, angiomatous, fibrous, microcystic, and atypical). The brain regions of tumour development were not mentioned [68].

Similarly, Yamazaki et al. [55] formed organoids from 11 grade I meningiomas (meningothelial and secretory subtypes), 3 grade II meningiomas (atypical) and 1 grade III meningioma (anaplastic) within a week, with a 100% success rate [55]. The organoids were passaged at least ten times, yielding successful results [55]. Tumour locations were reported: four were from the falx, with others being from the convexity, middle cranial fossa, cavernous sinus, tuberculum sellae, parasagittal, olfactory groove, and petroclival (skull base) areas [55].

Finally, Chan et al. [73] established organoids from five of eight patient samples: four from grade I and one from grade II, all derived from different locations (two frontal lobe, two spheroid ridge, two convexity, one parietal lobe, one occipital lobe). The authors did not specify meningioma subtypes [73].

The largest difference between the protocols was the starting material. While Yamazaki et al. [55] and Chan et al. [73] digested the tissue completely to obtain single cells that were embedded in Matrigel, Huang et al. [68] used larger tissue pieces (1 mm³) which were suspended in media.

Current meningioma organoid formation protocols have limitations. Firstly, the variability in the media formulations reported to date could affect the reproducibility of organoid derivation and subsequent experiments. In some cases, the density of Matrigel used could also determine the success rate of organoid formation. The use of bioreactors seems to be uncommon but could help maintain meningioma organoids for longer periods of time. In our experience, foetal bovine serum (FBS) is necessary for the growth of grade I meningioma cells. Although it is an animal-derived product that may interfere with some organoid applications, FBS aids cell proliferation, viability, and growth [79]. Therefore, standardised protocols are needed for the reproducible formation of meningioma organoids, and scientists establishing new protocols should provide more detailed steps to allow for greater reproducibility and consistency.

Even though all meningioma organoids described above recapitulated the cell morphology and staining seen in the parental tumours, we think there should be more studies to further characterise the cell types present in meningioma tumours and to identify the stem cells present in the organoids, employing flow cytometry, transcriptomics, proteomics, and/or metabolomics. The cellular composition of the healthy meninges has been well studied, revealing the presence of arachnoid cells, fibroblasts, endothelial cells, and immune cell populations; however, a similar study in meningioma tumours is lacking. Another outstanding question in the development of meningioma organoids is to establish the period of time in which these organoids can be kept alive.

Huang et al. [68] used minced tissue as their starting material, thereby maintaining the cell type composition and spatial organisation. Could it be the case that the other two protocols are producing what could be considered meningioma spheroids rather than meningioma organoids? The organoid-forming cells reported by Yamazaki et al. [55] and Chan et al. [73] were derived from the digest of primary parental tissues, but it is unclear whether the cells self-organised to mimic the spatial relationships of the *in vivo* tumour or whether a heterogeneous cellular population survived and persisted within the organoid. These questions could be addressed by identifying the cell types present in both the tumour tissue and tissue-derived organoids by flow cytometry, and the cell types present and their

spatial arrangement within the organoids could be determined by embedding the organoid to perform H&E or IHC staining.

7. Future Perspectives

Meningioma is the most common brain tumour, and although 80% of cases are benign, these tumours can still have severe effects on patients depending on their location, including headaches, confusion, depression, and loss of vision. Furthermore, the high incidence of meningioma means that many surgeries are performed on these benign lesions, leading to postoperative morbidity in patients and requiring time and resources that could be better utilised in more urgent cases. Therefore, there is still a great deal of information that needs to be uncovered in our basic understanding of meningioma to find less invasive, more accessible, cheaper, and more practical treatments.

Advances in organoid culture and discoveries that have already been made through the utilisation of these models suggest a significant step in the development of methodologies to enable the consistent and reproducible culture of complex organoid models. Specifically, in meningiomas, the formation of organoids will provide a great advantage in the comprehension of the origin and molecular mechanisms of this disease, as well as in the development of new and repurposed treatments. However, protocols for producing meningioma organoids need to be further optimised and standardised, including the need to better characterise the cell types and spatial relationships within these models and within meningioma tumour tissues themselves.

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