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Review Article

Use of Organoids for Anticancer Drug Development

Tripathi, K.[1*] & Lazarevic, M.[2]

[1]*Internal Medicine, SMT NHL Medical Municipal College, Gujarat, India*

[2]*Internal Medicine, Swedish hospital, Chicago, Illinois*

[*]**Correspondence to:** Dr. Tripathi, K., Internal Medicine, SMT NHL Medical Municipal College, Gujarat, India.

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Abstract

Much of organoid research has changed the way developmental neuroscience works, providing unprecedented access to human neurodevelopment and function [1-7]. Figure 1 illustrates the timeline in which the neural stem cells (NSC) were cultured [8]. Glioblastoma is a highly malignant brain tumour with significant intratumor heterogeneity, which could be ascribed to Glioblastoma Stem Cells (GSC) activity variations. Using a variety of experimental techniques including quantitatively evaluating lineage trace, clonal size, mutational marker evaluation, and single cell RNA sequencing showed a growing accretion in GBM and outlined the outcome of tumour cells is regulated by a neurogenesis developmental pathway [9-12].

List of Abbreviations

1. Neural stem cells (NSC) in line 7
2. Glioblastoma Stem Cells (GSC)
3. Cancer stem cell (CSC)
4. EGF (Epidermal Growth Factor)

5. FGF2 (Fibroblast Growth Factor)
6. NeoCor (neoplastic cerebral organoids)
7. CNS-PNET (Primitive Neuro-Ectodermal Tumor)
8. GBM (Glioblastoma Multiforme)
9. GS (Glioma spheroid)
10. BO (Brain organoid)
11. CAD (Computer assisted design)
12. HeLa (Henrietta Lacks)
13. head and neck squamous cell carcinoma (HNSCC)
14. Patient derived organoids (PDO)
15. PDX (patient derived xenografts
16. CNS (central nervous system)
17. 5FU (5 Fluorouracil)
18. PD-L1 (Programmed death-ligand 1)
19. CCC1 (Clear cell ovarian cancer)

Introduction

Inhibiting the stem cell could also limit tumour recurrence in another brain tumour that originated in the cerebellum [13]. With the application of the above-mentioned studies, the cancer stem cell (CSC) model displayed a structure for understanding tumour variation, predicting tumour progression, and potentially assisting in the development of new therapeutics. There exists a duality in GSC with it displaying tumor heterogeneity when replicating in culture or xenotransplantation, which makes it difficult to treat. While utilizing 2D monolayer cultures did not yield variation and 3D relative spatial organization displaying a lack of interaction with various the cancer extra cellular matrix and microenvironment. Furthermore, they may not accurately determine therapeutic effectiveness, as medications that first demonstrated promising results in cultured cell lines did not find their way into clinical trials. Hence more sophisticated model frameworks that can replicate complex cancer characteristics while enabling exhaustive examination are necessary. Particularly in light of the necessity to provide more precise forecasts for novel therapeutic benefits. Successful results in other cancer disciplines have incentivized several laboratories to generate organoid glioblastoma models consisting of 3D self-organizing structures, allowing particular cell-cell contacts leading to a generation of a specific microenvironment [14-18]. Consequently, Glioblastoma multiform organoids could exhibit complexity and variation in their proliferative capacity and response to treatment.

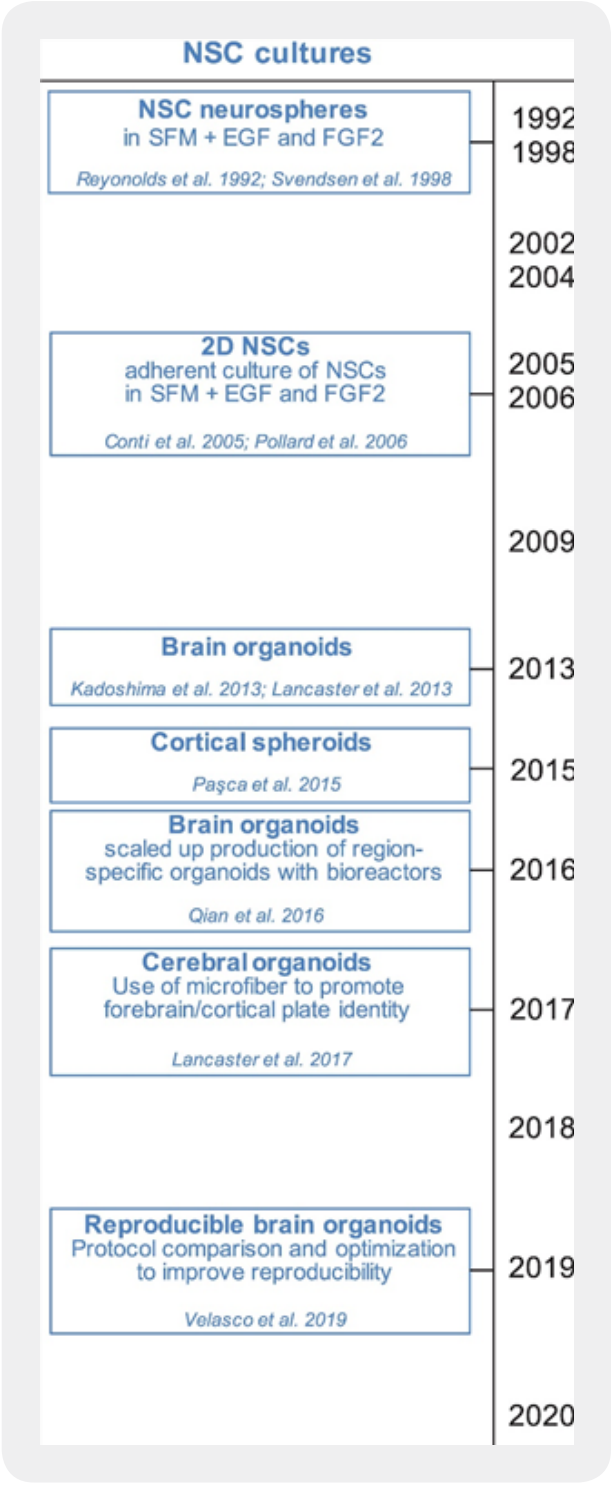

Figure 1: *Timeline of in vitro method to culture neural stem cells.*

Discussion

Glioblastoma stem cells are derived from tumor tissues and cultivated long durations in culture [19-27]. EGF (Epidermal Growth Factor) and FGF2 (Fibroblast Growth Factor) maintain GSC development *in vitro* [26,28,29]. GSC is produced in 2D or 3D cultures, regarded as a first "3D model" because the cells preserve polarisation and 3-dimensional spatial configuration [21,30]. Neurospheres, on the other hand, have a necrotic foundation and can grow to a peak value of roughly 300 m before requiring breakage and replating to thrive [31-33]. Furthermore, cells in neurospheres lack their contact with extracellular matrix proteins, hence do not closely resemble GSC behavior *in vivo*. Jürgen Knoblich and Inder Verma genetically modified organoids to grow malignancies lately [34,35]. Bian *et al.* (2018) looked for changes leading to cancer and called them NeoCor (neoplastic cerebral organoids): the authors used a transposase-based approach to overexpress recognised oncogenes and/or used CRIPSR Cas-9 to remove tumour suppressor gene activities. Organoid cells targeted with nucleofection at an early stage of development, and the cells containing the genetic modifications were labelled with green fluorescent protein, permitting cell proliferation and tumour development to be monitored precisely. MYC overexpression, as well as a few other genetic sequences induced proliferation. Transcriptome profiling revealed that tumors with MYC possess CNS-PNET-like identity (CNS-PNET: Primitive Neuro-Ectodermal Tumor), whereas different alternative tumors possess a GBM-like identity, implying that different anomalies can cause tumours that possess unique markers. Researchers have discovered that certain genetic alterations, such as HRasG12V activation and p53 disruption, can produce mesenchymal GBM in organoids. Although these studies suggest that some GBM subtyping can be cloned, it remains to be seen whether all GBM subtypes could be cloned with the technology and how much the GBM-derived organoids reflect patient-derived GBM cells [8].

Because they more precisely mimic the complexity and heterogeneity of a natural tumour, 3D *in vitro* models are promising for studying GBM biology and predicting treatment response. In fact, the majority of the models presented depict selected vulnerabilities to pharmaceuticals or radiation, which mimic tumour sensitivity *in vivo* [34,36,37]. Furthermore, the creation of a live GBM biobank has been facilitated by newer generation of 3D GBM organoids using a new and quicker methodology (one to two weeks). Some models discussed in this study (34,35,37-39) allow non/GBM brain cells to be combined together. This is particularly valuable for Investigating the interaction between tumor infiltration into normal tissue and tumor brain cells. It is possible to study the exact role of genes involved in cell-cell interaction, adhesion, guidance, and migration. This may lead to the discovery of new therapeutic targets which inhibit tumor infiltration. These 3D GBM organoid models advance the study of cancer stem cell variation by providing cancer cells with an environment in which they can maintain the coexistence of different stem and progenitor cells [40]. They will also allow researchers to investigate the developmental hierarchy of CSCs in malignancies, as well as the impact of other cell types or the microenvironment on CSC fate decisions. Cells such as microglia or other immune cells, can be incorporated into organoids [41-44]. The key problem is to replicate an environment with the vasculature and other cell types that can display inflammatory and immune described similarly to those seen in a healthy brain [45-47]. The presence of multiple cell types and greater diversity is probably the greatest strength and weakness. Because while they reflect the complexity of the primary tumor, they are also the source of the variations in a 3D environment [48-50]. Thus, depending on whether they want to undertake bulk analysis on a homogeneous cell population, investigators may have to select amongst growing cells in traditional 2D monolayer or spherical cultures or 3D organoids.

Some model kinds are as follows: Glioma spheroid (GS) is a kind of glioma (with serum) cancer stem cell growth is not especially encouraged in a dense conglomeration of cells grown in serum. Glioma tumouroid tumour organoids were created by culturing primary tumour material in suspension in the absence of serum under specific media conditions, with CSCS specifically promoting cellular heterogeneity and maintaining cellular heterogeneity. Brain organoid (BO) is a model organ derived from stem cells grown in particular medium and under specified growth circumstances to encourage tissue lineage development. It has some of the functions and physical characteristics of a natural organ. GS/BO and GT/BO are two different types of GS/BO. Single cells, spheroid, and tumoroids supported in liquid solution. Matrix-supported Single cells, spheroid, or organoid encased in a three-dimensional matrix. Below is a table describing models and their corresponding findings [51].

Model Type	Cell Origin	Culture Method	Findings
		Adult GBM	
Free tumouroid	Cerebral organoid generated from HESC cell line H1.	Oncogenesis transduced with oncogene and knockdown of p53.	Tumouroids can be generated from cerebral organoids via gene manipulation.
Free tumouroid	Dissociated GBM specimens.	Suspended in serum-free media.	Tumouroids recapitulated the morphology and expression profile of parent GBM tumours.
Free tumouroid co-culture	GA-MSCs and CSCs were isolated from surgical specimens of GBM stroma and GBM, respectively.	Dissociated and resuspended in liquid differentiation media.	Stromal GA-MSCs excrete exosomes that increased proliferation of GSC xenografts and decreased median survival of the host animals when pre-treated with stromal GA-MSCs-derived exosomes.
Free tumouroid/ spheroid	Patient-derived GSCs/ cell line U87	Non-adherent plates.	All patient-derived tumouroids from primary GSCs were Nestin and Sox2 positive. Chemotherapeutics were effective only on 3D U87 spheroids. Tumouroids from the one recurrent cell line were the most drug-resistant. TMZ efficacy was patient-specific.

Figure 2: *Organoid models and their findings.*

Paediatric GBM			
Ex-supported tumouroid (passaged in PDX models then extracted)	Specimens of PGBM	Xenografts of human PGBM patients with therapy-naive, recurrent and lethal disease were extracted, minced and enriched for CSCs.	An AUKRA inhibitor was most effective on therapy-naive tumouroids, followed by recurrent ex-xenografted tumouroids.
Free tumouroid	Tumour specimens from six PGBM patients	Stem cell population expanded via specialised media.	EGFR and PDGFRA amplification and deletion of RB1, CDKN2A/B & PTEN was observed.
Free tumouroid	Dissociated PGBM specimens from two patients	Suspended in serum-free media.	Stemness markers nestin, CD133, Sox2, melk, PSP and bmi-1 were expressed.
Free tumouroid	Dissociated PGBM specimens from 14 patients	Suspended in neural stem-cell media.	Stemness markers CD133 and Nestin were expressed and self-renewal was retained even when secondary tumouroids were formed from a single cell.

Figure 3: *Pediatric Organoid models and their findings*

Model Type	Cell Origin	Culture Method	Findings
Matrix -supported spheroid	GBM lines E98, E468 & U-251MG	Spheroids formed with hanging drop and implanted in nude rats, rat brain slices, rBM-based hydrogel layers or 3-layers of astrocytes. Hyaluronic acid was added to media.	Migration on brain slices was through blood vessels. Spheroids on rBM hydrogel and astrocyte layers recapitulated some migratory patterns seen in live rat brains. Higher HA concentration in media induced more rapid migration.
Matrix -supported spheroid	GBM cell line U251N	Hanging drop then embedded in collagen gel.	TMZ was effective in dose- and time-dependent manner
Matrix -supported spheroid	Patient-derived cell lines K301, GBM6, GS024 & GS025	Tumouroids were formed in suspension, dissociated, then transferred to HA-based hydrogel in a microfluidic chip.	Higher HA induced proliferation and drug resistance.
Matrix- supported tumouroid	Patient-derived CSCs.	Low-attachment plates and neurobasal media then encapsulation in HA/collagen hydrogel. Interstitial pressure was applied by deferentially filling a Millipore insert in a cell culture well.	Increased flow through the channel induced patient-specific increase in migration between 1.3 and 1.5-fold. With knockdown of CXR4, CXCL12 and CD44, a flow-induced increase in migration was neutralised.

Figure 4: *Matrix supported models and their findings.*

Brain organoid	HESC cell line H9	Differentiation media	Organoids were transduced to invoke oncogenesis. The number of modified, malignant cells surpassed healthy organoid cells over weeks.
Brain organoid	HESC cell lines H1, H6 or H9	Matrigel-coated plates & differentiation media	A primitive ventricular system and neural rosettes were formed & a proliferative zone of neural stem cells was present.
Brain organoid	IPSCs	Differentiation media & transfer to orbital shaker or millifluidic device	Millifluidic media exchange successfully reduced size of necrotic and hypoxic regions. No overall size difference was observed.
Brain organoid	HESCs	Low-attachment plates & differentiation media	Induction of common GBM genes with electroporation resulted in malignant cells overtaking healthy organoid cells within a month.

Figure 5: *Brain Organoid models and their findings*

Cancerous Constituent	Culture Method	Healthy Brain Constituent	Culture Method	Findings
Tumouroid	Dissociated primary CSCs cultured in low-attachment plates with differentiation media	Brain organoid	HESC cell line H1 cultured in low-attachment plates & differentiation media	Radial migration of tumouroid cells. Modification of ECM related expression similar to in-vivo.
Spheroid	SK2176 GBM cell-line cultured in low-attachment plates	Brain organoid	HESC cell line H1 cultured in differentiation media	Spontaneous attachment and invasion of tumour cells into cerebral organoid. 30% of organoid volume was invaded after 24 days. Degree of invasiveness in model correlated with lethality of orthotopically xenografted tumouroids.
GSC cell line in suspension	Co-culture	Brain organoid	HESC cell line H1, H6 or H9 culture in Matrigel-coated plates with differentiation media	Co-cultures were more resistant to chemo-therapeutic agents and radiation versus 2D cultures. EGFR levels of parent tissue were recapitulated in 3D co-cultures and absent in 2D analogues.
Transfection of 18 GBM-like gene mutations/ amplifications	Oncogenesis of organoid via electroporation	Cerebral organoid	Generated from EBs with differentiation media	GBM can be initiated by selective gene manipulation. Increased invasiveness, higher expression of invasion-related genes and lower expression of tumour-inhibitive genes were observed in gene-altered cells.

Figure 6: *Co-culture models and their findings*

Bioprinting is a type of matrix-supported cell culture in which cell-loaded bioink is applied to a print bed with a custom-made 3D printer. The two most common processes are extrusion (filament) and inkjet (droplet). Inkjet printing is a non-contact method that uses heat or electricity to eject bioink droplets from nozzles and can also be performed using a dedicated office printer. The extrusion pressure is either pneumatic or a piston that applies pressure to the bioink reservoir and pushes it into the nozzle. You can then use the CAD (computer assisted design) file to modify the nozzles to create a precisely controlled bio-ink pattern. Individual cells can be manipulated and placed individually using a laser-based printing technique known as laser-based direct writing. Bioinks are hydrogels with living single cells or cell aggregates (spheroids) or cancerous tumors. Using 3D printing for layered gel structures gives you unparalleled control over the distribution patterns of different cell / gel structures. The first bioprinted cell / hydrogel structure was made with HeLa (Henrietta Lacks) cells and gelatin / alginate / fibrin bioink. Below is a table that describes the bioprinted culture and its corresponding results [51].

Model Type	Cells Used	Gel Material and Organisation	Findings
Bioprinted matrix -supported co-culture	GBM cell line U87MG, GSC lines G166, G144 & G7 monocyte cell line MM6	RGD-alginate + <250 mg/L HA or collagen I. Central tumouroid was printed then surrounded by a stroma-like cell-laden gel construct.	Printed GBM cells remained viable (>90%) for months and CSCs retained stemness. Temozolomide IC50 doubled for printed spheroids compared to 2D co-cultures. GBM cells printed alongside fibroblasts were more resistant to TMZ.
Bioprinted matrix -supported co-culture	GBM cell line GL261 & macrophage cell line RAW264.7	GELMA was used as both GBM and stroma-like bioink to create a GBM tumour model enclosed by a macrophage-laden gel construct.	Shear-thinning GELMA decreased printing-related cell death. Macrophages migrated towards GBM cells in co-culture and GBM cells had 15-fold increases in GBM-specific markers compared to 3D and 2D mono-culture.

Figure 7: *Bio-printed models and their findings*

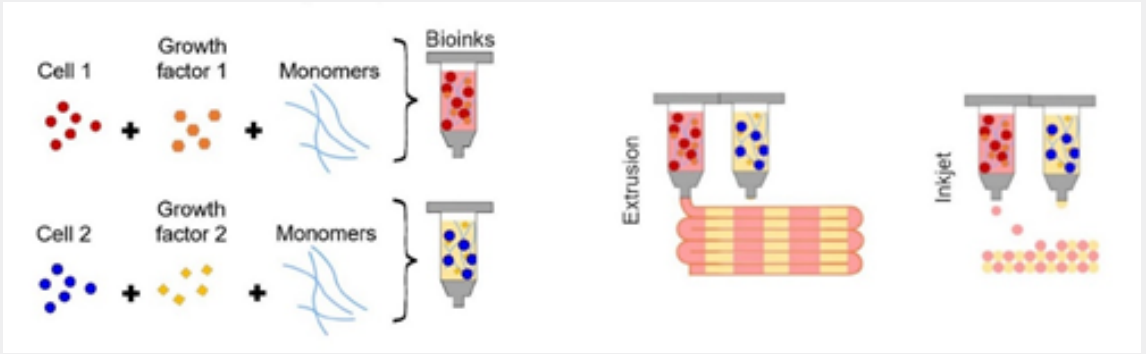

Figure 8: *Illustration of bioprinting technology and the two types of gels that are used*

Model Type	Gel Material and Layout	Findings
3D GBM- vascular niche with patient- derived CSCs co-cultured with HUVECs	A straight fluidic vascular channel was printed with collagen I and lined with HUVECs. CSCs were seeded adjacent to the microvessel.	At the highest concentration of laminin (100 μg/mL), CSCs migrated 1.5× further than in the gel containing 10 μg/mL of laminin.
GBM-on-a- chip with continuous cell line U-87 and patient- derived line co-cultured with HUVECs	A circular fluidic vascular channel was printed in collagen and a bioink developed from decellularised porcine brain ECM. GBM-laden hydrogel was printed in the centre of a ring of collagen gel containing HUVECs. This was surrounded again by a microchannel with an outer boundary printed in gas permeable silicone.	GBM cells grew in dense spheres with an anoxia-normoxia gradient and peripheral pseudopalisading cells. Cells in the intermediate region excreted factors leading to microvessel formation in the periphery. In porcine brain-derived gel, angiogenesis, proliferation and expression of pro-angiogenic genes and ECM remodelling proteins increased. All patient-derived cells in co-culture with HUVECs exhibited a dose-dependent response to TMZ but those on-chip recapitulated clinical therapy resistance, unlike the same cells cultured in 2D and 3D monoculture. Following multiple treatments, GBM cells extracted from patients with a longer survival exhibited decreased metabolic activity even after treatment ceased, whereas the metabolic activity increased after treatment ceased in the cells originating from patients with a shorter survival.

Figure 9: *Vasculature models and their findings*

Driehuis *et al*. created an organoid biobank (N = 31) from head and neck squamous cell carcinoma (HNSCC). Patient derived organoids (PDO) mimics the parental HNSCC and creates tumors when transplanted into immunocompromised mice. Various reactions were noticed by drugs used in a clinical setting. These drugs included cisplatin, carboplatin, cetuximab, and *in vitro* radiation therapy. In addition, drug screening demonstrates reactivity to pharmaceuticals not routinely used in clinics for patients with HNSCC. The results could individualize HNSCC treatment and expand the HNSCC drug portfolio. In another study, the authors reported that HNSCC-derived PDO are able to be utilized to study reactions to photodynamic therapy, while simultaneously testing it on corresponding normal tissue-derived organoids to ensure the safety of treatment [52,53].

On drug sensitivity and resistance testing, clear cell ovarian cancer (CCC1) organoids were resistant to paclitaxel, cisplatin and carboplatin in comparisoon to alternative organelles. This is due to the fact that clear cell ovarian cancer is resistant to platinum-based chemotherapy (response rate: 11.1% clear cell, 72.5% serous) [54]. CCC1 has a mutation in the SWItch/Sucrose NonFermentable (SWI/SNF)-associated gene; PBRM1 (p.P1460L) and ARID1A (p. P1995Lfs * 22, p. Q1098Rfs * 16) implying that blocking the immune checkpoint could be an approach. High grade serous cancer cells (HGSC) called HGSC1 and HGSC3 patients have CNV-like HRD, whereas HGSC2 are restricted with CNV. HGSC1 and HGSC3 exhibited sensitivity to paclitaxel treatment; However, HGSC2 was resistant. HGSC1 contains the variant BRCA1, hence it is sensitive to PARP inhibitors, olaparib and cisplatin relative to other organelles [54]. Both HSGC1 and HGSC2 are from FIGO stage IIIC tumors. The duration at which there was no disease after platinum treatment was lengthier in HGSC1 compared to HGSC2 [54]. Kopper *et al*. stated *in vitro* drug susceptibility was reproduced *in vivo* by xenotransplantation of ovarian cancer organoids [55].

Because drug responses are varied and better correlated with genomic changes in 3D culture than in 2D culture. Organoids are a suitable culture format for drug susceptibility analysis in translational studies [56]. Disadvantages of the organoid model include the absence of cancer stroma such as fibroblasts, blood vessels, and immune cells. However, recently there have been reports of gas-liquid interface methods that maintain the microenvironment of the tumor immune system [57].

Esophageal adenocarcinoma organoids have been treated with standard chemotherapy (5FU, epirubicin, and cisplatin). All patients except one had a meager reaction to chemotherapy, and the organoids of the patients who displayed a response were not obtainable for testing [58]. A common response in the organoid and tumor reactions of four patients to chemotherapeutic agents (cisplatin, paclitaxel, 5FU, epirubicin, and irinotecan) was noted in another report [59]. In a report, gastric cancer organoids obtained from patients prior to treatment were sensitive to standard chemotherapy (5fluorouracil (5FU), cisplatin, oxaliplatin, and irinotecan), despite the contribution of radiation therapy. It reproduced the patient's complete pathological response after chemotherapy, though the clinical response due to the radiotherapy had not been thoroughly examined [60]. An additional study found inconsistent outcomes with a combination of 5FU, oxaliplatin, and epirubicin with seven gastric cancer patients mentioned in this report, only two patients were found to correlate with drug reactions (5FU, oxaliplatin and epirubicin combination). A clinical response that corresponded with an organoid response was only recorded in one patient [61].

Gastric cancer organoids derived from ascites displayed a varied respond to chemotherapeutic agents (oxaliplatin, 5FU, cisplatin, docetaxel, Irinotecan, epirubicin, and paclitaxel) among patients, which also had synonymous heterogenous responses with peritoneal metastasis [62].

Colorectal cancer organoids were utilized in cases who would take advantage of the cross-sensitivity to olaparib and oxaliplatin. This induces PARP-dependent DNA damage repair. In two patients who reacted to oxaliplatin, the organoids responded well to olaparib and oxaliplatin. In a different case who responded, the organoid was resistant to therapeutics. The organoids in this patient reacted particularly strongly to panitumumab. Panitumumab was utilized in treatment and may be a major contributor to the clinical response, elucidating inconsistencies among organoids and reaction [63].

Organoids representing metastatic GI cancers had sensitive reactions with cetuximab (anti-EGFR monoclonal antibody), but possessed resistance in patients with EGFR amplification and KRAS wild-type [64].

Rectal cancer organoids underwent treatment with standard chemotherapeutics (5FU alone or FOLFOX (5FU, leucovortin and oxaliplatin) or radiation therapy (one dose, 08 Gy). An association of 5FU or FOLFOX ($r = 0.86$) was detected in relation to progression-free survival in seven patients. In the case of radiation therapy, the organoids that were resistant to radiation therapy originated from already irradiated tumors or tumors without or negligible responses. Radiosensitive organoids originated in cases which had a reduced tumor size by a minimum of 50% or those who displayed complete clinical remission after radiation therapy. In addition, organoids ($N = 80$) that underwent neoadjuvant chemoradiotherapy such as 5FU (5 Fluorouracil) and irinotecan indicated predictions of remission to treatment with chemotherapeutics (sensitivity 78.01%, specificity 91.97%) [65,66].

Everolimus was identified as a therapeutic candidate for patients which had GBM organoids obtained from them and elicited an incomplete reaction [67]. Cancer organoids in GBM patients had also been tested for susceptibility to standard chemotherapy temozolomide and agents directing towards mTOR, PI3K, or DNA damage responses. Varied reactions to monotherapy and the combination of temozolomide with targeted drugs has been detected between organoids in diverse cases [68].

A CNS carcinoma, chordoma organoid possessed PD1-positive CD8 T cells and was utilized to establish a connection with a nivolumab reaction (PDL1 blockade). Patients with PDL1-positive and PDL1-negative tumors had dose dependent reactions. This is consistent with observations that under expressed PDL1 may possess a response to PDL1 inhibition. It can be inferred that response to the therapy of cancer organoids may be foreseeable irrespective of PDL1 standing, but an association with distinct patient responses was not established. 24 patients with primary chordoma cases at the G. Pascal and G. Pini Foundations. Two monoclonal antibodies were utilized to stain surgical samples against PDL1, E1L3N, and 288 (Cell Marque) with the BenchMark XT kit and an automated immunostainer Ventana Medical Systems, as directed by the manufacturer. PDL1-positive cancer cells and lymphocytes were determined as the percentage of positive cells in all sections according to FDA guidelines. The median age in the study was sixty-five years with a range of fifty-five to seventy-nine. The average period for a follow up was usually six months or higher. Twelve patients had passed away and the survival period was at a median of fifty months. Immunotherapy aiming automatic cell death 1 receptor (PD1) and its ligand 1 (PDL1) produced remarkable outcomes in progressive cancers showing increased expression of PDL1,1,2. Displaying exemplary therapeutic effect but a lack of patients for trials do not assist in concretely proving therapeutic uses. This is especially significant for chordomas, rare malignancies that are located primarily on the spinal axis, have an elevated recurrence rate (43-85%). They are less prone to distant metastases. chondroma is a candidate for immunotherapy because it expresses more PD1 / PDL1 than healthy bone tissue though it is resistant to chemo and radiotherapy. Clinical trials investigated the efficacy of PDL1 targeted therapy with nivolumab only or with ipilimumab. Studies on the combination of nivolumab and stereotactic radiosurgery have also been conducted. From a clinical point of view, the selection of cancers / patients who are sensitive to anti-PDL1 blocking therapy based on PDL1 expression in tumor cells and lymphocytes that infiltrate tumors would be extremely useful. The antibodies reacting to PDL1 was evaluated the expression of all surgical specimens of tumor cells and tumor-infiltrating lymphocytes distinctly, comparing the results with clinical limits. As the potential benefits of organoids for cancer cell culture are increasingly recognized, organoids from patients are also produced, quantifying diameter, cell death and PDL1 presence to determine the dose-dependent effect of nivolumab. This helped establish a treatment response and method [69].

Several strains of organoid Retinoblastoma have been produced and underwent standard chemotherapy (melphalan, topotecan, and methotrexate) and display similar responses to tumor cells in cases with progressive disease. Though no direct correlation to patient reaction was ever established. Lasting or recurring retinoblastoma (RB) is related with vitreous and/or in subretinal metastasis in progressive RB and is a primary reason for treatment failure. This requires the improvement of new therapeutics and, therefore a cutting-edge RB model for testing therapies. The authors created a three-dimensional self-assembling organoid model derived from chemo sensitive tumors. They established and equated the response of organoids to drugs and associated the organoid model with advanced RB in terms of drug susceptibility.

Organoids show histological characteristics suggestive of retinal tumors and seeds, and have been found to retain changes in DNA copy count and gene and protein manifestation from the parent tissue. Cone signaling circuits (M / L + cells) and glial tumor microenvironments (Glial fibrillary acidic protein positive cells) were predominantly existing in organoids. Topotecan isolated or a combination of topotecan and melphalan successfully targeted the organoid cancer cones (RXRγ + Ki67 +) and blocked the invasion of mitosis after 24 hours of therapeutic exposure. In comparison, methotrexate was slightly effective in treating cancer cells. The reaction of organoids was constant with that of cancer cells in progressive disease. Organoids from patients lead to the production of models for use in exploring new therapies for use in retinoblastoma.

Drugs clinically used for intravitreal chemotherapy (melphalan, topotecan, and methotrexate) were treated to determine if the advanced RB drug response was reproduced in organoid cultures (RB688). In addition, comparisons have been made between combination drugs (melphalan and topotecan) and individual drug regimens, making systematic implementation in the clinic an issue. The drug concentration used in this study was comparable to the levels in the vitreous cavity. Since tumor organoids have a cell structure similar to that of tumor tissue. It has been shown that the accessibility and uptake of the active ingredient occurs in the deepest regions of the tumor organoid core. This is due to the increase. ΓH2AX lesion, which is a DNA damage reaction marker [70].

Copper's influence on brain development and function is documented. Unfortunately, minute information is available on how Copper works mechanically in certain central nervous system processes. Cultures are available to make brain organoids also known as cerebral globules that contain cell types that mimic multiple areas of the brain. As far as it is known, the neurobiology of Cu-based disorders in brain-derived organoids has not yet been investigated. Brain organoids were tested for Cu content using X-ray fluorescence microscopy technology. This showed low copper in the areas scrutinized [71].

When we are enhancing cancer treatment for an individual patient, it is crucial to advance the effect of the cancer treatment on the tumor tissue and to personalize it. The current main problems with current chemotherapeutic agents are the harmful side effects leading to the restricting their use over time and finding the right dose. In order to defeat the inadequate rate of new therapies that progress through the stages of the clinical trials, improved processes for establishing serious side effects in the preclinical stage are desirable. The use of non-transformed organoids obtained from tumor tissue enables the ability to test the specificity of therapeutic agents [72,73]. Moreover, organoids originating from tissues primarily disturbed by side effects, used to proactively identify and explore potential problems. The central nervous system organoids are utilized to evaluate neurotoxicity. In this regard, Liu *et al*. evaluated the toxic effects of vincristine on brain organoids. Dose-dependent toxicity to both neurons and astrocytes was revealed. In addition, Schielke et al. depicted the benefits of utilizing brain organoids to improve radiation therapy for CNS tumors [74-77].

Conclusion

There is a greater need to explore and study ideas the use of organoids, this will lead to a greater increase in life span and 5-year survival rates. Classic cancer cell lines and animal cancer models are physiologically

and clinically more established than patient-derived tumor organoids. Furthermore, as compared to traditional cancer cell lines and PDX (patient derived xenografts) models, patient-derived tumor organoids are more capable of capturing and retaining the molecular, cellular, genetic, and histological characteristics of the tumor of origin while also preserving patient-specific tumor heterogeneity. Despite the obstacles ahead, human organoids offer a lot of potential in cancer treatment. With the rapid advancement of other technologies, we believe that synergistic applications using organoids can help bridge the gap between *ex vivo* and *in vivo* organoids, paving the door for new cancer therapies. With the proper use of these extraordinary 3D cultures, a thorough development of high-throughput drug screening for improved prediction may be possible.

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