Emphasis on Organoids in Cancer Research

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Abstract Cancer has been one of the deadliest diseases for several decades and there is no precise and standard treatment option available up to date. Statistical data indicate that cancer has been one of the principal reasons for mortality worldwide. Although most of the novel techniques assist in the acceleration of cancer research, the available anticancer therapy does not exhibit expected success rates. This is due to a lack of understanding about the root cause of the disease, which can be accomplished by studying different types of tumors and the effects of various anti-cancer agents on the tumors. These studies require various in vitro study models which can mimic real, in vivo cancers. Conventional experimental models such as animal models, two-dimensional (2D) cell lines, patient-derived xenografts (PDX) are key models in cancer study but they have some shortcomings that are overcome by three-dimensional (3D), in-vitro tumor organoids derived from embryonic, induced pluripotent, or adult stem cells (ESCs, iPSCs, ASCs respectively). These organoid models closely recapitulate the original tumor present in vivo and thereby benefit in studying the development of cancer, efficacy, and safety of various anti-cancer agents, drug development, personalized therapy, low and high throughput screening. As a result, 3D organoids are becoming more successful experimental models over conventional 2D models. Therefore, this review emphasizes the effectiveness of organoid models in cancer study, their method of preparation, advantages and applications, drawbacks with solutions to address, followed by a brief outline on 4D organoids (assembloids),

and future perspectives.

Keywords Cancer, Tumor Organoids, 3D Model, Embryonic Stem Cells (ESCs), Induced Pluripotent Stem Cells (iPSCs), Adult Stem Cells (ASCs)

1. Introduction

Cancer is a very complicated disorder and the 2nd main cause of mortality globally [1]. There are around 200 different forms of cancer [2]. Cancer is comprised of a variety of heterogeneous and rapidly proliferating cells that emerge in the developing and adaptable microenvironment of the tumor. In recent decades, cancer research and cancer survival rates have greatly improved. Early detection or care, clinical strategies to control and monitor precancerous indications, improved understanding regarding smoking and other wellness behaviors, and therapeutically oriented tumor studies have all contributed to a 26% decrease in cancer mortality rates over the last two decades. Even after this advancement, cancer remains the leading reason for mortality. Additionally, it is predicted that cancer will remain the primary reason for mortality until 2035, with more than 2 lakh tumor fatalities anticipated in the UK. As we move closer to a cancer cure, it is becoming clear that evidence from several preclinical trials and research work doesn't

always work out in actual cases. Many conventional methods, such as two-dimensionally cultured cells, explants, organ-on-a-chip systems, and animal testing are critical for studying cancer biology but these models may fail to recapitulate the characteristics of the original tumor. Additionally, novel and creative model systems were found to increase preclinical study translational performance, and hence tumor-derived organoid cultures have emerged as a result, which provides an excellent methodology for designing and studying human cancer [3]. The detection of molecular biomarkers of drug sensitivity is a key step in the rapid production of multiple therapies with particular molecular targets. To find therapeutic biomarkers, tumor models must replicate the original tumor, predict the patient's in vivo treatment response, and be suitable for high-throughput screening [4]. Organoids are in vitro self-developing three-dimensional (3D) tissue recreations developed from embryonic stem cells, induced pluripotent stem cells, or tissue-resident progenitor cells; which mimic primary characteristics i.e., genetic, cellular, and pathophysiological features of the original tissue [5]. Organoids are developed by dissolving tumor biopsies into pieces and cells and incorporating them in an

extracellular 3D matrix scaffold (like Matrigel), cultivated in a blend of signaling and growth factors that are specific as well as optimum for each tumor form [6]. This review starts by explaining the pros and cons of the model systems currently being used in tumor research, thereby shifting onto the discussion of tumor cell-derived organoid models, along with their significance in cancer research, and emphasizing its benefits such as possible use in personalized medicinal therapy, drawbacks, and future prospects.

Cancer

Cancer is defined by the uncontrolled development of abnormal cells due to overproduction and malfunctioning of the body's cells and the acquisition of metastatic properties. In most cases, oncogenes activation and/or deactivation of tumor suppressor genes contribute to unregulated cell cycle development and deactivation of apoptosis pathways further contributing to cancer [7]. Cancer comprises more than 200 different diseases. If we consider the number of cases and death by organ site, disregarding other clinical as well as biological factors, then cancer falls under 3 classes:

Table 1. Classification of various cancers based on incidences & mortality by organ site [8]

Group 1: Cancer raised from Epithelia/ Carcinoma:	Cancers that are less prevalent than group 1:	Rare Cancers:
Lungs	Kidney	Soft tissues
Intestine/ Colorectal (more common in developed countries)	Pancreas	Brain
Breast (in females) (Common in both developed & Developing countries)	Bladder Stomach Liver	Testes
Prostate (in males)	Oesophagus	Bone
Skin (most prevalent; rarely lethal, except melanoma)	Cervix	Other organs
	Ovary (as frequent as leukemia & lymphoma)	

In the above table, group 1 indicates cancer arising from epithelia also known as carcinoma (cancer of cells which makes up skin or tissue lining) which includes tumors of some major organs like lungs, colorectal or intestinal, rarely lethal skin cancer (except melanoma), breast and prostate. Group 2 summarizes cancers that are less prevalent than group 1 cancers, these include- cancers of kidney, pancreas, stomach, liver, bladder, esophagus, cervix, and ovary. In group 2, ovarian cancer is as frequent as leukemia and lymphoma. Whereas, stomach, bladder, and liver cancers are found to be endemic in some countries with a greater number of young patients. 3rd group is of rare cancers like cancer of soft tissue, brain, testes, bone, and other organs.

Industrialization and smoking are responsible for the increased number of lung, kidney, prostate, testicular, and bladder cancer patients in recent years. Also, melanoma is increasing alarmingly in some areas. On other hand, awareness about hygiene and good food habits is leading to a reduction in the number of stomach cancer patients. Geographical and temporal differences have a large impact and hence it is a prerequisite to study cancer. E.g., prostate cancer in east Asia countries is 10-20 folds lower than in the USA [8]. As per the data obtained from THE

GLOBAL BURDEN OF DISEASE-2004 UPDATE, the death rate by cancer was 11.8% in females and 13.4% in males. Cancer was 3rd cause of death amongst all with more incidences and death due to cancers of the bladder, liver, pancreas, corpus uteri, mouth and oropharynx, leukemia, melanoma, and other skin cancers; in both males and females [9]. However, according to GLOBOCAN 2008 statistics, nearly 12.7 million cases of cancer and 7.6 million fatalities by cancer were registered in 2008, with developing countries responsible for 56 % of cases and 64 % of deaths. Breast cancer was the most common cancer in women whereas lung cancer was the more prevalent cancer in men [10]. By the end of 2012, almost 14.1 million new cancer cases were identified with 8.2 million deaths by cancer [11]. The data presented by GLOBOCAN 2020 indicate a rise in the incidence of cancer and deaths from 2020. Globally, an approximate 19.3 million new tumor cases and nearly 10.0 million fatalities by cancer occurred in 2020; in which 1.2 million cases were of melanoma, with 1 lakh deaths by the same. More cases of female breast cancer have been reported, exceeding lung cancer cases. Still, the mortality rate of lung cancer was higher.

Graph 1. Statistical representation of incidences & deaths by different types of cancers in 2020

The above graph shows incidences and mortality by various cancers of some major organs like liver, stomach, prostate, colorectal, lung, and breast cancer. The highest mortality rate and second-highest incidence rate were observed with lung cancer cases. The lowest incidence rate was of stomach cancer. In the case of breast cancer, though the incidence rate was highest, the mortality rate was found to be the lowest.

Owing to population changes the global cancer risk is projected to rise to 28.4 million new cases by 2040, hence the development of a robust framework for the dissemination of tumor risk reduction strategies as well as the provision of disease care is vital for global cancer prevention [12]. Surgical interventions, chemotherapy, immune therapy, site-directed therapy, stem cell therapy, radiation treatment, hormonal treatment, precision medicines, biomarker testing, oral anticancer medicines, and palliative care are some of the available cancer treatment options, according to NCI (National Cancer Institute) data [13]. The complicated biology of solid tumor growth is one of the main barriers to effective cancer treatment [14]. Cancer is characterized by unusual genetic activity and disturbed genetic expressional habits. According to research, produced genetic defects are linked to genetic mutations that trigger gene deregulation, leading to cancer [15]. Some cancer cells undergo metastasis and spread from the primary tumor site to other organs or throughout the body, leading to additional, unexpected health problems and intensifying complications. This increases patient dependency on drugs, potentially leading to serious side effects and many drugs shows resistance to treatment. Thus, although cancer therapy focuses mainly on the treatment of primary cancer, improving the quality of life with palliative care is equally important and beneficial. The success rate of surgery and radiotherapy is higher for tumors developed in organs. Whereas chemotherapy is usually preferred for advanced cancers, although the success rate is modest with more side effects. Testicular cancers are treated at a rate of more than 90% with a combination of chemotherapy and radiotherapy. In comparison to this, other cancers have less success rate as the underlying molecular or cellular variations are not apparent [9]. All these indicate that cancer remains one of the deadliest diseases in the world, since conventional anticancer treatments have several shortcomings, as cell biology is not understood well because of the unavailability of suitable experimental systems. Hence there is a need to establish new, universal, and promising anti-cancer strategies for cancer diagnosis and treatment [16].

2. Material and Methods

2.1. Experimental Models:

Most conventional models which are available to study

cancer development and the effect of novel anticancer drugs include; animal models, 2D cell lines, organ-on-a-chip, patient-derived xenografts (PDX), etc.

2.1.1. Animal model:

Preclinical animal models are crucial elements of cancer research in which the tumor is induced in genetically modified transgenic mice exposed to carcinogenic chemicals, viruses, or radiation. Despite being a conventional model, animal models are costly. They need substantial investment, yet several successful pre-clinical trials aren't tested actually in humans or aren't suitable for clinical use in drug production. Animals have distinct genomic, physiological, and neurological traits than humans. Often, face ethical constraints due to the use of a live animal.

2.1.2. 2D cell lines

These are either primary cell cultures (cultivated exclusively from the human or animal tumor) or well-established immortalized cell lines. These cell lines are simple to generate at a low cost and are highly reproducible but they grow slowly with a short life span. They lack a microenvironment and are difficult to relate to the original biological tumor. Do not exhibit heterogeneity in the cell and experience unnatural adhesion forces.

2.1.3. Organ-on-a-chip system

This is a multiple channeled, microfluidic, perfusion culture of live human tumor cells enclosed with either plastic, glass, or flexible polymers that allow for fairly precise modeling of the organ system's anatomy, but several times it requires cell lines as well. Moreover, this system shows some variation between chips and it is a non-standard protocol.

2.1.4. Patient-derived or Rodent xenografts

This approach involves implanting human tumor cell lines or tissues into humanized animals like mice, developing orthotopic (implanted at the location of a body wherein the tumor originated) or heterotopic (implanted somewhere else) xenografts. As the tumor is developed in mice, they lead to ethical constraints and the model works in a non-human environment with a different immune system. Additionally, it is an expensive technique that requires sufficient resources [3].

2.1.5. Spheroids

These are simple, in vitro 3D structures produced by a single cell type aggregate. They are difficult to manage for a longer period [17].

2.1.6. Organoids

All of the above-mentioned are conventional cancer models and almost all of them have some or other drawbacks that have surpassed by recently established in vitro 3D cell culture technique called "Organoids" that

resulted in the creation of the innovative and more functional model of normal as well as tumor tissues.

3. Organoids

After being incorporated in a 3D framework, adult stem cells developed from tissues may grow into self-organizing organotypic structures with high efficiency, called "organoids" that can recapitulate the tissue or organ of origin. In the year 2009, it was proven [18] that 3D epithelial cell organoids can be developed by intestinal stem cell with only one repeat that is rich in leucine and contains G protein-coupled receptor 5 (LGR5)+. [19] Although 3D tissue culture has been around for decades, the term organoid is now more widely utilized to define stem cell-derived models wherein cells can be pluripotent stem cells (PSC), such as embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC), or can be adult stem cells (ASC) from specific organs [20].

3.1. Method of Preparation of 3D and 4D Organoids

Figure 1. The process of preparing 3D organoids and 4D assembloids. [21, 22]

Matrigel is a gelatinous protein mixture originating

from mouse cancer cells that are used as a basement membrane matrix of stem cells to keep them undifferentiated [23]. Stem cells proliferate further to form ectoderm, mesoderm, and endoderm, which results in the development of organ-specific organoids. Drugs can be tested directly on these organoids, or organoids can be transplanted into mice to check the effectiveness of anti-cancer drugs. Fusion of two or more 3D organoids forms 4D organoids/ assembloids. The interesting part is that co-culturing of brain organoids with any other organ-derived organoids leads to the formation of assembloids in which derived organs may receive signals from brain organoids formed *in vitro*.

The diagram represents the process of preparation of 3D and 4D organoids *in vitro*. Embryonic, induced, or adult stem cells are taken from cancer patients and cultivated in Matrigel with specific growth promoters.

3.2. Cultural Components Required for Growth of Various Tumor Organoids:

3.2.1. Extracellular matrix (ECM):

Matrigel is used for pancreatic and bladder cancer. Matrigel (reduced growth factor) is used for stomach, intestine, and prostate cancer. Basement membrane extract for liver and Basement membrane extract (reduced growth factor) for breast cancer.

3.2.2. Molecular inhibitors:

A-83-01 used for all above cancers (except bladder cancer). Y27632 is used for all the above cancers (except pancreatic and prostate cancer). SB202190 is used for stomach, intestine, and prostate cancer.

3.2.3. Growth factors:

ADMEM/F12, Noggin, R-spondin-1, and penicillin/streptomycin are used for all cancer cultures. Gastrin and Wnt3A present in all (except breast and prostate organoids). N-acetylcysteine present in all cancer organoids (except stomach, intestine), and nicotinamide present in all cancers (except pancreatic). GlutaMAX present in all (except stomach and intestine). Prostaglandin E2 present in organoids of the stomach, intestine, and prostate. L- glutamine and bovine serum albumin used for the stomach and intestine. B27 and FGF are used in all (except bladder cancer). N2 is used for stomach, intestine, and liver organoids. EGF is used for all (except breast cancer). HEPES used in liver, pancreatic, and breast cancer. HGF and forskolin are used only for the intestine. Primocin for breast, bladder, and prostate. Testosterone, FBS, and neuregulin1 are used for prostate, bladder, and breast cancers respectively. Isolation medium definite for hepatic tumor organoid includes standard human hepatic organoid isolation media with the incorporation of dexamethasone and the exclusion of R-spondin-1, Wnt3A, and Noggin. Standard human

hepatic organoid isolation media with the exclusion of Y27632, Wnt3A, and Noggin for normal human hepatic tumor organoids [44]. Some techniques to develop pluripotent stem cell-derived organoids provide nominal differentiation information to the cells (without supplying sufficient growth promoters and nutrients), permitting inherent self-organization. E.g., Matrigel trapped neuroectodermal embryoid bodies from hPSC + no other precise conditions provided to the cells = generates brain organoids with several brain areas, including cortex, but also generates some endodermal and mesodermal ancestries. Whereas, in another method, cells are structured into more complex regions of the CNS, which usually depends on prior knowledge of the signals that regulate growth, with additional contributions from self-organizing methods. E.g., Matrigel trapped hPSC + dorsal or ventral forebrain structures = formation of region-specific brain organoids. Development factors for adult stem cell-derived organoids are normally similar to signals that regulate tissue recovery after injury or steady-state tissue maintenance in the body. As a result, agonists of Wnt signaling are required to maintain the cells and produce an in vivo-like complement of cell types in adult stem cells-derived epithelial cell organoids in the multiple organs in GIT [20].

4. Discussion and Result

4.1. Examples of Available Organoid Models:

To examine the impact of anti-cancer as well as other drugs, various 3D, self-originating organoids are obtained from all the organs mentioned in Figure 1. Such as—
mESC-derived Thyroid organoid [24]

Adult mouse's lung stem cells derived bronchioalveolar systems [25]

Organoids of the pancreas derived from embryonic pancreatic progenitor cells in mouse [26]

Hepatic organoids derived from adult stem cells [27];
liver buds derived from human iPSCs [28]

Adult stem cell-derived gut organoids [29, 30]

Human PSC-derived intestinal organoids [31]

hESC-derived cardiovascular patch [32]

A three-dimensional matrix obtained from myoblast progenitors anchors contracting skeletal muscle [33]

Human osteogenic cell-derived osteo spheroids [34]

Organoids of the kidney derived from mice and human PSCs include the uterine bud as well as metanephric mesenchyme [35-37]

Human and mice PSCs' derived optic cups organoids [38, 39]

Human and mouse PSCs derived Organoids of the brain [40]

Organoids of the adenohypophysis derived from mice PSCs [5]

Matrigel-embedded 3D breast epithelia [41]
mESC-derived inner ear organoids [42]
mESC-derived keratinocyte-derived stratified epidermal layer [43]

4.2. The Success Rate of Cancer-Derived Organoids

The graph shows the success rate or efficiency of available 3D organoids models in different types of cancer studies, with the number of organoids varying depending on the type of cancer being studied. Severe tumor indicates metastatic, circulating, combined hepatocellular-cholangiocarcinoma, high-grade serous carcinoma of the ovary, mixed carcinomas such as metastatic colorectal and gastrointestinal carcinoma in multiple cancer forms, whereas primary tumor indicates basic tumors. The success rate was found to be >80% for ovary, primary colorectal, metastatic liver, and rectal organoids. Whereas, lowest (<20%) success rate was observed in prostate organoids.

Graph 2: The success rate or efficiency of available 3D organoids models in different types of cancer studies. [27, 45-60]

4.3. Comparison of Organoids with Traditional Cancer Study Models

Graph 3: The success rates of various cancer study models [61-64]

The graph explains the success rates of various cancer study models namely; 2D cell lines, patient-derived xenografts (PDX), and organoids in four major cancer types namely- breast, colorectal, pancreatic, and prostate cancer. The graph indicates that the success rate of organoids is greater than that of conventional study models used for cancer research like 2D cell lines or PDX and hence it's becoming a more reliable cancer study model over other traditional study models

Comparison between 2D experimental models with the novel 3D organoid approach: The table compares traditional 2D experimental models such as 2D/ 3D cell lines and animal models such as a genetically engineered mouse (GEM) and patient-derived xenografts (PDX) with a novel 3D organoid approach such as cancer stem-like cell (CSC) derived organoids and cancer tissue-originated spheroids (CTOS), in terms of accessibility, feasibility, inter-tumor heterogeneity, intra-tumor heterogeneity, physiological features and application to high throughput screening (HTS).

Advantages

Adult stem cells can be studied by cultivating in organoids, and unique tissue lineages can be developed with limited input from other cell types in good purity (e.g., endothelial cells and fibroblasts).

Can also be generated for years without causing genetic changes.

Allows for the use of a broad range of proven methodological approaches.

Possible to extract with several initial sources, including adult as well as fetal tissues, ESCs, and iPSCs.

Organoids covering a wide spectrum of tissues can be produced.

Restricted quantities of precursor materials may be extended for several applications.

Patient-derived organoids can be used to study human disorders which are hard to analyze in animals, also avoid extensive animal studies.

It is possible to generate isogenic adult tissue for use in regenerative operations [66].

Table 2. Comparison between 2D experimental models with novel 3D organoid approach [65]

Model Name		Accessibility	Feasibility	Inter-tumor Heterogeneity	Intra-tumor Heterogeneity	Physiological Features	Applicability to HTS
Cell lines	2D	Good	Good	Can compare between cell lines	Low	Lost to the great extent	Good
	3D	Good	Complicated for some systems having biomaterials	Can compare between cell lines	Low	Partially re-established	Tough with some cell lines
Animal models	GEM	Relatively good once established	Intricate in case of double/triple GEMs	Can compare partially	High	Well established & includes immune system & micro-environment	Unfit for HTS
	PDX	Access to tissue network or hospital is desired	Good once generated	Can compare between multiple cases	High	Good, including micro-environment	Unfit for HTS
Organoids	CSC-derived organoid	Access to tissue network or hospital is desired	Skills required, may lead to the low recovery rate	Can compare between multiple cases	High	Well established	Possible but costly
	CTOS	Access to tissue network or hospital is desired	Skills are necessary	Can compare between multiple cases	High	Well established	Good applicability as an ex vivo setting

Drawbacks and Solutions to overcome some drawbacks:

More complex than 2D cell lines, thereby likely to show more variations which can occur between different initiating cell lines or genotypes, but also between batches of organoids from the same initial components, between multiple organoids within the same culture, or even between different sites of a single organoid. Organoids also vary significantly in their capability to segregate in specific categories of cells.

An absence of vasculature and immune system due to lack of nutrients limits their growth without cell death, and they cannot be utilized in studies involving vasculature and the immune system. Vascularization can be resolved by the use of spinning bioreactors for better nutrient exchange or by co-culturing with endothelial cells as well. The introduction of hybrid cultures in organoids is also possible to form a more reliable organoid culture with various cell types [67]. Also, limited stromal and immune components hamper the use of organoids in the study of inflammation and drug penetration [66, 68].

The absence of an original microenvironment as *in vivo* limits the interactions of stem cells with their niches, immune cells, etc. Also, the Prediction of inflammatory reactions to infection or therapeutic agents is difficult due to a lack of immune system. This can be prevented by the use of organotypic cultures, or co-culturing with other cell types like stromal cells [69] or immune cells.

The Matrigel matrix was insufficient to replicate *in vivo* growth factor gradients. So, we can apply a microfluidic technique that will create concentration gradients thereby overcoming this issue.

The inability to reproduce actual biomechanical conditions encountered by cells in the body can be recovered by the use of novel substrates and ECM factors to generate such interactions *in vitro* [70-72].

The utilization of organoids in drug evaluations can be hampered if the ECM is comparatively rigid. It is difficult to grow organoids from tissues whose niche factors are unknown (such as, the ovary). In such cases, we need to alter the physical properties of ECM such as composition, porousness, and rigidity or we can screen for small-molecule modulators of major signaling pathways and specific hormones as potential components in culture.

Organoids in a similar culture vary in respect of viability, shape, and size, making phenotype screening difficult, that's why live or time-lapse imaging is used to track organoids individually.

Organoid cultures rely on Matrigel originating from mouse sarcomas, which may show variations after implantation of organoids into humans. To compensate for this, better-defined ECMs which will promote organoid development are being used.

Applications:

Organoids have made it possible to reproduce human tumors *in vitro* in a way that has never been achieved

before. Organoids obtained from various animal or human cancers are now extensively used in the research of various cancers. Various organ-specific organoids are established in recent decades such as; a) Organoids of CRC (Colorectal Cancer) from various anatomical locations with changing sensitivity to Wnt3a and R-spondin proteins. b) Hepatic cancer organoids were extracted from patients through comprehensive medium refining to extend the 3 main subcategories: liver cell carcinoma, cholangiocarcinoma, and combined liver cell-cholangiocarcinoma. c) Enduring preservation and enhancement of pancreatic ductal adenocarcinoma (PDAC) organoids derived from human and animal prime cells maintaining the primary tumor's architecture and phenotypic heterogeneity. d) Primary breast cancer organoids closely resemble their original tumors, in terms of anatomy, pathology, hormonal receptor interaction, and mutation site. e) Some more tumor organoids, such as those for gastric, prostate, ovarian, brain, bladder, kidney, lung, and oesophageal cancers are also developed.

Human cancer can also be designed by introducing pathological mutations to wild-type organoids through gene editing techniques like transmission of genes, CRISPR-Cas9, or RNA interferences. For example, colorectal adenoma-carcinoma sequences can be reconstructed by introducing driver mutations (APC, KRAS, TP53, SMAD4, and PIK3CA) into stable wild-type organoids, which then develop into invasive cancer upon implantation. After further examination of various APC truncating mutations in intestinal organoids, the critical functional area for pathological Wnt stimulation in CRCs was discovered.

Seino and colleagues used CRISPR-targeting to develop cancer driver genes SMAD4, CDKN2A, TP53, and KRAS, in PDAC organoids, revealing an unforeseen Wnt niche adaptive behavior triggered by TP53 mutations.

Cancer-derived organoids usually sustain tumor heterogeneity, making them perfect for research on tumor growth. Organoids originating from predominant colorectal tumors and metastatic tumors isolated from the same patient expressed similar common sources and driver mutation, implying that the driver mutations happen before metastatic spread. For evaluation of diversities within the tumor, researchers generated clonal organoids from many individual cells from 3 CRCs as well as adjacent natural intestinal crypts. The global mutational landscape had been utilized for building phylogenetic trees, revealing that CRC cells have substantial mutational diversification and that the majority of mutations occurred during cancer's final predominant clonal expansion. When these three-dimensional organoids are integrated, they provide ground-breaking *in vitro* methods of development of cancer models, phylogenetic analysis, and clinical research in new drug development [73].

Future prospects:

In a large number of COVID-19 patients, researchers investigated SARS-CoV-2 neurotropism in single-layer neuronal cells and human pluripotent stem cells (hPSC) developed brain organoids, finding that this virus infects only the choroid plexus, causing apoptosis and transcriptional up-regulation of inflammatory genes, as well as functional impairment in the neuronal cells [74]. Several unresolved issues can be answered by hPSC-based models, like the accurate action of this virus on nerve cells, the actual mechanism behind differential viral vulnerability (such as activation of particular coreceptors in virus by dopaminergic neurons against cortical neurons) if COVID-19 infection can cause long-term nerve damage, as well as the recognition of the virus. The first research with primary organoids developed from human cells was already released to recognize therapeutic agents which may relieve other diseases caused following COVID-19 infection [75, 76], and it will be interesting to know if impacts found in this research can be transferred to a clinical surrounding [77].

The latest evidence has shown that cerebral organoids based on human neural stem cells can be successfully transplanted into mice brains and display improved integration, differentiation, and maturation [78, 79]. The discovery of synaptic communication between the graft and host neuron by the dissemination of axonal projections of humans in the brain of a mouse was an unexpected result. The question is whether or not these mice have been "humanized," and if it is true then can "human" thinking capacity and feelings will emerge in those mice?

From the above observations, it is clear that if 3D or 4D brain organoids begin experiencing conscious behavioral symptoms using current medical concepts, it would be important to develop forward-thinking ideas and guidance in the future.

5. Conclusions

This review emphasizes the utilization of 3D organoids as an experimental model of cancer research. According to statistics, cancer is now a non-curable disease that affects millions of people worldwide, with a substantial rise in morbidity and mortality. The data show that traditional anti-cancer treatments have failed due to a lack of information about the underlying etiology of cancer and the rapid growth rate of tumor cells. Conventional anti-cancer treatments are still used around the world, but they cause more severe side effects rather than curing cancer, so cancer treatment focuses more on palliative care. Though palliative care is useful, it cannot be used as a primary treatment or as a long-term cure for cancer, so standard therapy for all different cancers is needed. This is only possible after thorough research into cancer models including the study of growth, anatomy, and pathology of

cancer cells, apoptosis, the effect of various anti-cancer agents on tumor cells, treatment resistance, and so on. Traditional 2D models are useful for carrying out all of these experiments, but they have certain drawbacks that 3D organoid models overcome. Organoids are in vitro, 3D models that precisely replicate the original human tumor; therefore, all experiments with a higher success rate on organoid systems can be applied to original tumor cells in vivo. These 3D models are made from embryonic or induced pluripotent stem cells (ESCs or iPSCs) as well as adult stem cells (ASCs). Matrigel and specific growth factors are used to build stem cell cultures, which then proliferate to produce organ-specific tissue and, as a result, organ-specific organoids. Organoids are complex models with a higher success rate than other 2D models, and they are well suited for low and high throughput screening [80]. Organ-specific organoids are developed which have the significant ability to develop in vitro and mimic real tumors. They have several applications, including examining the effectiveness and safety of new or existing anticancer and other drugs, developing precision medicine [81], to build 4D organoids in vitro by fusion of two or more 3D organoids [82]. A link between two separate organ-derived organoids can be established using this process. Despite some disadvantages due to complex modeling, organoids remain the most effective model of all and serve as a boon in cancer research.

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