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Organoid Technology in Cancer Research

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Abstract

Organoids are generated from the stem cells through *in-vitro* three-dimensional culture that can imitate the structure as well as the functions of any particular tissue or organ *in-vivo*. This modern technology has sprung up during the past decades and has become one of the most popular technologies among researchers. Of late, there are several different kinds of 3-D organoid culture approaches available, each having specific benefits and challenges. Tumor organoids are specially cultivated for cancer treatment because they can preserve features of the original tumors. Though it is not well understood about the genesis of organoids and how they have been used in cancer treatment, but recently a number of researchers have confirmed that organoids have tremendous potential in the field of clinical research for drug development, drug screening, precision therapy, etc. through their studies. In this review article, we precisely reviewed the brief history of organoids, the cultivation processes of different tumor organoids, their potential applications, current limitations, and future prospects. Recent studies have demonstrated that the combination of organoids and some other technologies (Artificial Intelligence, gene editing tools, etc.) would be able to imitate the tumor microenvironment, which could develop new horizons in the field of cancer research in the future.

Keywords: Cancer, Drug Screening, Organoid, Organoid Cultivation, Tumor.

Introduction

Cancer is a global health concern and one of the most important causes of death. According to reports of WHO (World Health Organization), by 2040, global cancer rate could reach 29 million cases annually, with one out of every six deaths (1). Study of development of tumor, technologies to detect at early stage, and improvement of treatment efficacy are directions of cancer research, currently (2). To investigate molecular and cellular characteristics of cancer, controllable *in-vitro* models are necessary. During the past decades, basic topics in cancer biology have been addressed using the two-dimensional (2D) cultivation of cancer cells. Though traditional 2-D cell line cultures and PDTXs (patient-derived tumor xenografts) have contributed tremendously in cancer research, there are few drawbacks that hamper research. Poor tumor heterogeneity is the main drawback; another one is new mutations which are produced during cell culture process (3). For these, poor efficacy of anti-tumor drugs was observed during multiple clinical trials. PDTX models replicate growth of tumor and preserve the heterogeneity in the tumor microenvironment; however, success rate of establishment of PDTX models were low with huge cost (4).

Of late, organoid technology has evolved as an independent research tool. Organoids (Figure 1) are 3-D (three-dimensional) constructs which could be cultured or developed from ESCs (Embryonic Stem Cells), iPSCs (induced Pluripotent Stem Cells), Somatic Stem Cells, and Cancer cells (and Cancer Stem Cells) in specific 3-D cultures. As organoid models contain 3-D culture (with multiple cell compositions) environments, they can reflect structural as well as functional characteristics of any original tissue *in-vitro* in a better way; so, during the long-term experiments, they maintain phenotypic and genetic stability (5). In 2-D cell culture processes, stability of genetic amplification is deficient and it can be overcome by organoid models. Organoid models are also superior to PDTX models because of cost-effectiveness and rapidness in cultivation (cultivated *in-vitro*). By this modern technology, communications and interactions between cells

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and peri-cellular matrix are well preserved, which significantly shows the accurate tissue growth (6). Currently, organoid models have integrated in several aspects of molecular medicines and have been playing significant role in cancer therapy and research. In this review article, we tried to explain brief history and implementations of organoids in several cancer researches, discussed potential applications and limitations of organoid models in cancer therapies.

In Figure 1, to start the cultivation of organoids, ESCs (Embryonic Stem Cells) from human embryonic tissues and IPSCs (Induced Pluripotent Stem Cells) from human adult tissues undergo directed differentiation first, produce floating spheroids, and are then planted on ECM (Extracellular Matrix) in culture medium. Primary tissues from patients may be separated into functional units that include somatic SCs. These SCs are cultivated and enhanced in three-dimensions to create organoids. Tumor cells (isolated from cancer tissues) may also develop into tumoroids in a well-defined 3-D culture.

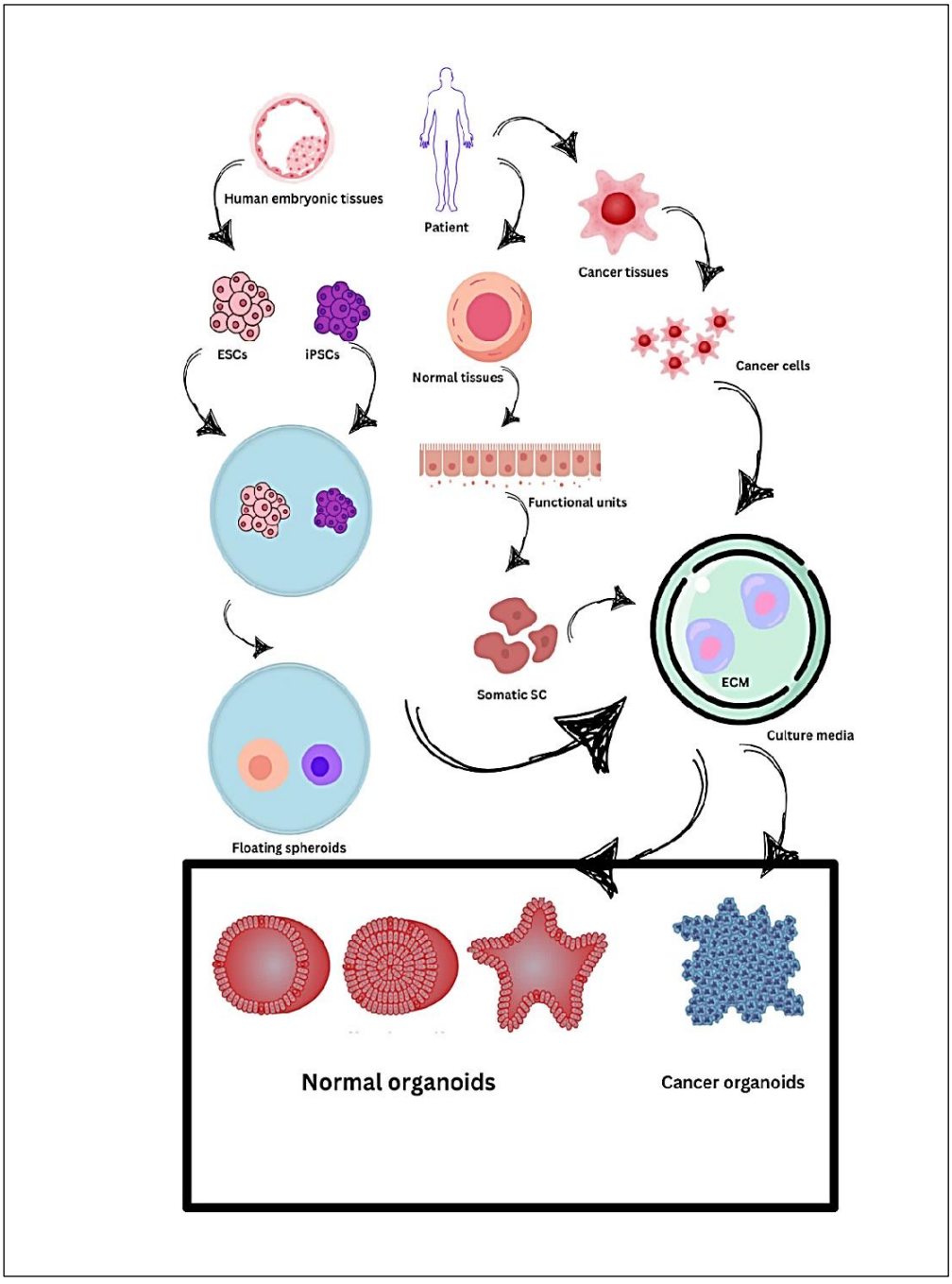

Figure 1: Establishment of Organoid from SCs (Stem Cells) and Cancer Cells

Organoids and its Brief History

In 1907, H.V. Wilson, a researcher of Baker Raleigh University, had discovered, under 3-D culture, sponge cells which are separated mechanically, could self-organize and reassemble into functional sponge organisms. Over the previous decades, the term 'Organoid' had several meanings; in 1950s, intracellular structures, mostly organelles, were referred. In 1960s, tumors and abnormal cellular growths were meant (7). Now, we define 'Organoids' as multi-cellular units which have been isolated from specific tissue samples. It is basically a 3-D culture approach that can also be studied after passaging.

In 2002, under the condition of EGF (Epidermal Growth Factor) and HGF (Hepatocyte Growth Factor) addition, scientists had observed that hepatocytes could be sucessfully transformed into BECs (Biliary Epithelial Cells) spontaneously (8). This particular discovery unveiled the key of rapid organoid development. In the year of 2008, another study, which laid the foundation for development of organoid, showed LGR5+ (Leucine-rich repeat containing G-protein coupled receptor 5) intestinal SCs could form cortical tissue spontaneously under specific conditions, and exogenous signals could regulate it (9). After many successful and landmark researches in next couple of years, in 2013, from human PSCs (Pluripotent Stem Cells) retinal organoids had been developed (10). Additionally, it was also observed that, in Matrigel, human PSCs (Pluripotent stem cells) could spontaneously develop into brain organoids (11). Since then, liver organoids, human prostate organoids, lung organoids, and bile duct organoids had been cultivated successfully (12-14). From circulating tumor cells and biopsy tissue, pancreatic cancer organoids and prostate cancer organoids were successfully cultivated by scientists (15, 16). Colorectal cancer organoids, glioblastomas organoids, breast cancer organoids, and gastric cancer organoids had also been successfully cultivated in recent years (17). Co-culture system for tumor organoids had been cultivated to understand detailed interactions between tumor organoids, tumor-associated microenvironment, and immune response of the tumors (18). Recently, lymphocytes have been co-cultured with the tumor organoids, by researchers, to generate tumor-specific T-cells, and it has great potential for tumor immunotherapy (19). APCs (Antigen Presenting Cells) were stimulated by tumor-specific antigens in a recent study; they were then co-cultured with CD8+ T-cells to promote cytolysis and proliferation before being co-cultured with organoids obtained from gastric cancer patients. This particular co-culture method helped to enhance the efficacy of gastric cancer treatment precisely (20). In the Table 1, we discussed the first reporting times of different tumor organoids.

Table 1: First Report Time of Different Types of Tumor Organoids

Tumor Organoid Source	First Report Time	Tumor Organoid Source	First Report Time
Colon	2011	Prostate	2014
Pancreas	2015	Brain	2016
Liver	2017	Endometrium	2017
Stomach	2018	Breast	2018
Bladder	2018	Esophagus	2018
Lung	2019	Rectal	2019
Kidney	2019	Biliary Tract	2019
Ovary	2019		

With the increasing interest and development of this technology, it is anticipated that much more organoids will be cultivated successfully, which will aid researchers in better understanding the pathophysiology of the disease and will provide authentic and accurate research models to overcome the limitations.

Discussion

Cultivation for Cancer Organoids and Applications

Cancer is a major threat to the human health and it is one of the most discussed topics in the field of research. Since establishment of small intestine organoid, this technology has been developed

vastly in clinical research and biomedicine. *In-vitro* models can mimic the human cancer heterogeneity which is advantageous to researchers to understand tumor pathogenesis and therapeutic responses, as well as adverse reactions. Though tumor organoids are not easy to cultivate, with basal medium, different cytokines are needed to establish different tumor organoids. Major organoid cultivation procedures for different cancer types have been shown in the Figure 2. It has been proved that 3-D organoids have massive potential for human cancer modeling (21-23). Three-dimensional system to cultivate organoids consists of basement membrane extract as extracellular matrix substitutes or Matrigel and culture medium. In organoid culture mediums, most important components are ADMEM (Advanced Dulbecco's Modified Eagle's Medium) / F12, primocin, HEPES, B27, penicillin/streptomyc -in, GlutaMAX, EGF, N2, FGF7, FGF10, HGF (Hepatocyte Growth Factor), Wnt3A, R-spondin-1, Noggin, gastrin, prostaglandin E2, neuregulin 1, nicotinamide, N-acetylcysteine, Y27632, A-83-01, and SB202190 (15,24-27) (as shown in Table 2 and Table 3).

Colon Cancer Organoids

First effectively grown tumor organoids were from colon cancers, which were described by Professor Hans Clevers, in 2011 (28). After six years, in 2017, researcher Toshiro Sato was able to confirm through his work that LGR5+ tumor cells exhibit the self-renewal and differentiation properties similar to tumor stem cells (29). Tumor regression was temporarily possible by knocking out LGR5+, but KRT20+ tumor cells rapidly differentiated into LGR5+ and promoted tumor growth. By using the colorectal cancer organoids, Mark Schmitt has demonstrated the direct effects of dying cancer cells on nearby epithelial cells (30). Additionally, his research detailed the molecular processes that lead to treatment resistance via paracrine actions. Dmitrieva-Posocco O's study using the colorectal cancer organoids has described that BHB (β-hydroxybutyrate) could increase the expression of HOPX and resist the proliferation of patient-derived cancer organoids (31). This study has great clinical value for treating colorectal cancer.

Prostate Cancer Organoids

Prostate cancer is another type of malignant tumors and its incidence is quite high in older men. Prostate cancer organoids have been grown successfully in laboratory by multiple researchers since Gao D's landmark research work in 2014. Scientists generated 6 cancer organoids (Prostate cancer) and 1 organoid cultivated from circulating tumor cells. Significant similarities were observed between tissue structures of metastatic samples (originated by them) and prostate cancer organoids (16). Hans Clevers, in 2016, demonstrated protocol to generate 3-D prostate cancer organoids within two weeks, and this protocol is used widely in drug screening and molecular mechanism researches (32). Organoids that are cultivated by using this protocol, imitate characteristics of prostate tumors *in-vivo*. Zhang Z, through his studies, discovered that anti-androgen resistance in prostate organoids is promoted by CAFs (Cancer-associated Fibroblasts). When NRG1/HER3 (NeuReGulin-1/ Human Epidermal Growth Factor Receptor-3) axis was blocked by using antibodies, tumors (prostate) were re-sensitive to anti-androgen therapy (33).

Table 2: Growth Factors Applied in Organoid Cultures

Growth Factors	Function
EGF	● Important growth factor for the epithelial tissues. ● Enhances tumor growth through stimulating the cancer cell proliferation. ● EGF bind to EGF receptors and induce hyperplasic changes.
FGF 7	● FGF7/FGFR 2 signaling could promote growth, invasion, and migration of tumor.
FGF 10	● FGF10/FGFR 2IIIb axis is vital for organ (liver, breast, stomach, prostate, etc.) development. ● Promotes growth, migration, and invasion of pancreatic cancer cells and enhances breast cancer tumorigenesis.
HGF	● HGF/Met signaling promotes oncogenesis, angiogenesis, and invasion of several tumor types.

Wnt	● Master regulator in regulations of cell proliferation, differentiation, development, adhesion, and polarity. ● Abnormal activation drives carcinogenesis and progression of cancer.
R-spondin-1	● Ligand of Lgr5 and a niche factor that is important for self-renewal activity of SCs and activates Wnt signaling. ● It could promote growth of cancer cells as well as facilitates metastasis.
Noggin	● Inhibitor of BMPs (Bone Morphogenetic Proteins) which regulates cell differentiation, proliferation, as well as death (by apoptosis). ● Facilitates bone metastasis of many cancers and connected with tumorigenesis of primary bone malignancies.
Nicotinamide	● Vitamin PP is a vital nutrient which is necessary for successful and long-term organoid culture.
Gastrin	● Stimulates growth of tumor through promoting proliferation; suppresses cell death (by apoptosis) of cancer cells.
Prostaglandin E_2	● Facilitates angiogenesis in gastric cancer patients by upregulating vascular endothelial growth factor.
Neuregulin 1	● Ligand of human EGF RTK (Receptor Tyrosine Kinases) -3 and -4. ● Associated with mammary development as well as tumorigenesis.

Table 3: Small Molecule Inhibitors Applied in Organoid Cultures

Molecule Inhibitors	**Functions**
Y27632	● Inhibitor of Rho kinase; anoikis of dissociated stem cells are reduced by this effectively. ● Important to improve culture media, promotes tumor epithelial cells growth and proliferation *in-vitro* for long-term.
SB202190	● Inhibitor of p38 which reduces proliferation as well as migration of cancer cells. ● High concentration of it results with a relatively reduced establishing efficiency for breast tumoroids.
A-83-01	● Inhibitor of TGF-β (Transforming growth factor β). ● Suppressor of organoid proliferation.

Pancreatic Cancer Organoids

A few tumors have shown worst treatment effects and Pancreatic cancer is one of them. Professor Boj SF and his team first cultivated pancreatic cancer organoid successfully, in 2015 (16). Barbara T. Grünwald, in 2021, cultivated pancreatic ductal carcinoma organoids to coculture them with CAFs (34). This study used pancreatic cancer organoids and demonstrated roles of mesenchymal subtypes (reactive, intermediate, and deserted) in development of tumors and drug therapy responses. Results of the study demonstrated tumor cell proliferation could be promoted by reactive tumor stroma which shortens the disease-free survival (34). To understand the characteristics of tumor microenvironment, in 2021, Jorgensen, C. published a work to describe significant role of laminin-integrin α3/α6 signaling pathway in cultivation and survival of pancreatic cancer organoids (35).

Liver Cancer Organoids

Over the past few decades, one of the most discussed malignant tumors is liver cancer. Scientists cultivated human primary liver cancer organoid successfully for the first time and tested 29 cancer drugs by using it (25). CC (cholangiocarcinoma), HCC (hepatocellular carcinoma), CHC (combined hepatocellular CC) are the major types of primary liver cancer organoids. For drug sensitivity testing, Nuciforo S cultivated liver cancer organoids from patient biopsy tissue, in 2018 (36). To discover role of specific gene mutation in tumor formation, researchers used gene editing tools (CRISPR-Cas9) and altered normal human liver organoid genes, in 2019 (37). They found mutations of BAP1 disturbed normal traits of the organoid and stimulated growth and fusion which imitates aggressive malignancies (37). In a recent study of 2021, Bin Li used liver

cancer organoids to screen more than 400 FDA-approved drugs (38).

Through the results, it was confirmed that loratadine has the capability to inhibit aggressive growth of liver cancer, desloratadine could be used as anticancer drug. It was also found that NMT1 could be a biomarker, as well as therapeutic target for the liver cancer (38).

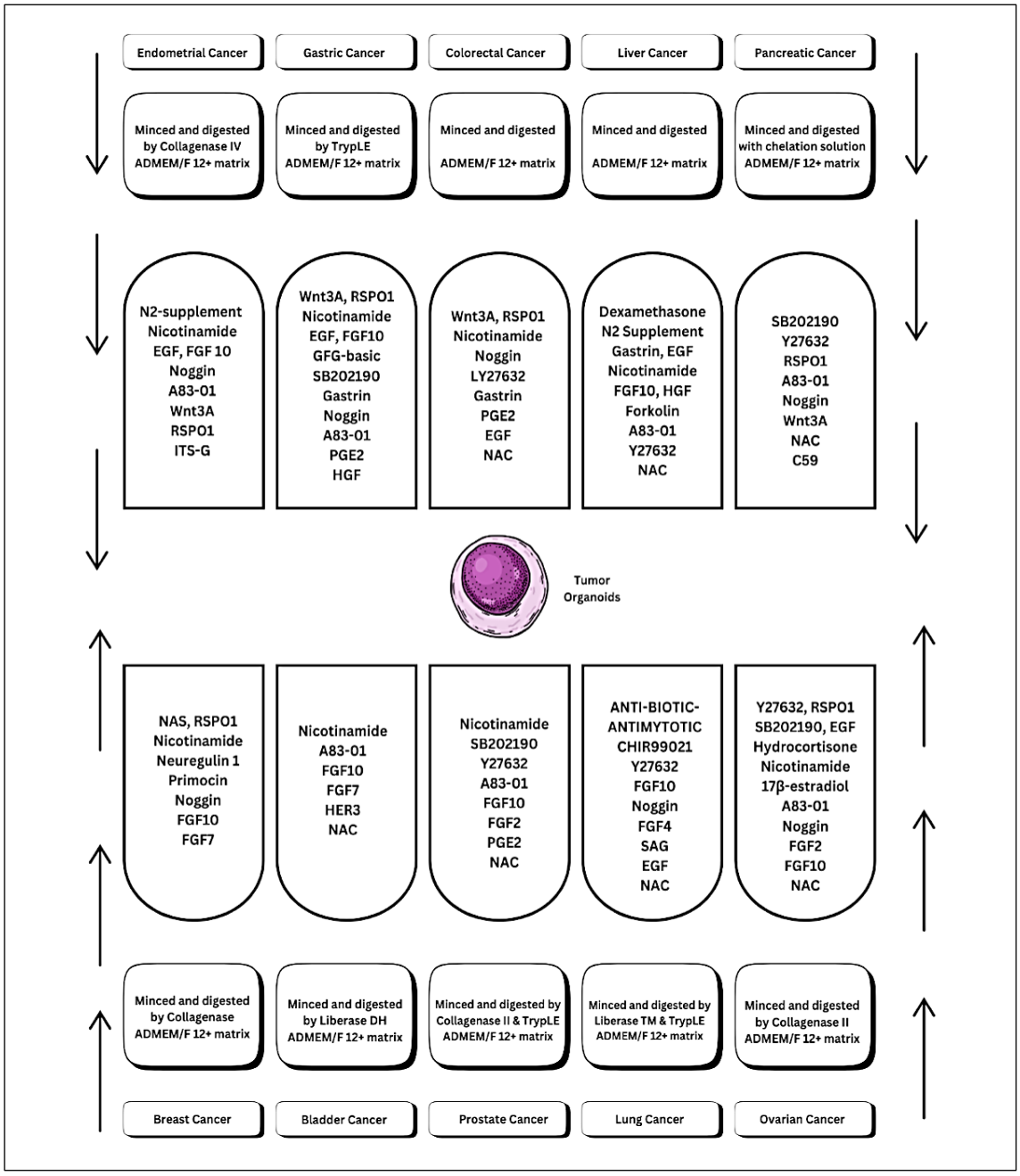

Figure 2: Required Cytokines for Cultivation of Different Tumor Organoids

Brain Cancer Organoids

One of the least survivable human tumors is brain tumors because of its aggressiveness. For the first time, in 2016, brain tumor organoids were grown from glioblastoma by team of Rich, JN (17). To understand occurrence of brain tumors, Shan Bian cultured brain cancer organoid model in 2018. This model was used for drug screening and this study screened 18 single gene mutations, as well as 15 mutation combinations (39). Further studies and other researchers demonstrated role of DHFR (dihydrofolate reductase) in brain cancers and found it could be inhibited by methotrexate which reduces the stemness properties of BTIC (brain tumor initiating cells) (40).

Stomach Cancer Organoids

Another common type of malignant tumor occurs in stomach cancer. In 2018, Vlachogiannis G.

successfully cultivated first gastric cancer organoid (24). Growth rates of gastric cancer organoids are much higher than *in-vitro* normal controls. To study molecular mechanisms and drug testing and screening, Seidlitz T. team cultivated gastric cancer organoids. Result of this study demonstrated that palbociclib could be used to target tumor organoids that carry deletion mutations of CDKN2A, trastuzumab could be used to target tumor organoids that carry mutations of HER2 (41). Drug resistance, efficacy, side effects and therapeutic effects can be observed in details through these studies. Recently, gene editing tools were integrated with gastric cancer organoids to study different characteristics of different mutation types. Yuan Hung Lo established first genetic model by using this technology to reveal characteristic phenotypes of a specific mutation (ARID1A mutations) of gastric cancers, in 2021 (42). Observations of this study found that mutation of ARID1A was the reason of mucinous metaplasia, inhibition of Wnt/β-Catenin signaling pathway; additionally, it promoted tumorigenesis (42). Tumor organoid technology, integrated with gene editing mechanisms, provides a great platform to study further.

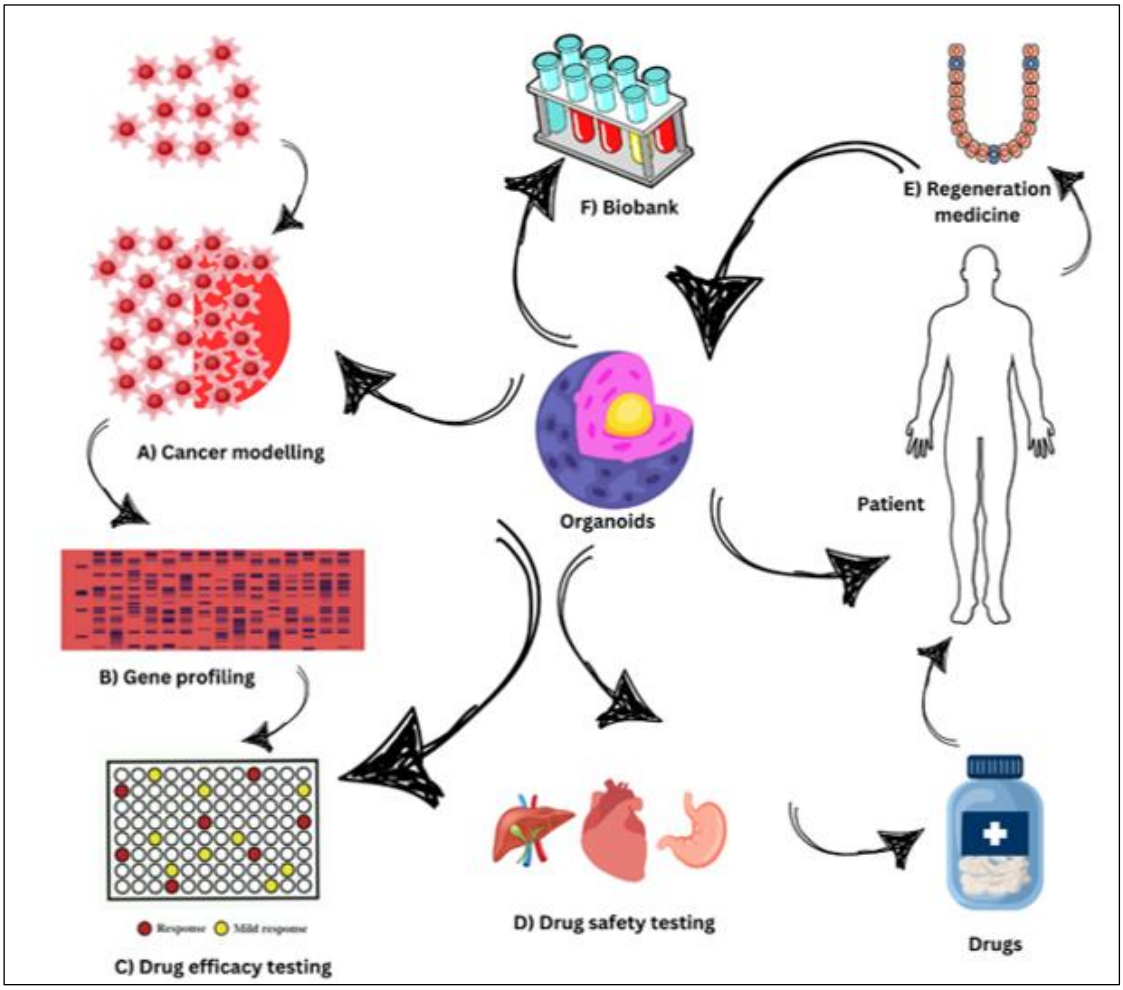

Figure 3: Applications of Organoids to Model Tumors, Drug Development as Well as Efficiency Testing, and Regeneration Medicine. Organoids can be Used to (A) Model Human Cancer, (B) Profile Genes, (C) Test Drug Efficacy, (D) Test Drug Safety, (E) Study or Develop Regenerative Medicine, (F) Establish Biobank for Further Studies

Breast Cancer Organoids

Breast cancer is one of the most common type of cancers and to study breast carcinoma several breast tumor organoids have been cultured in past few years. First ever breast tumor organoids were successfully grown by Hans Clevers, in 2018. Later, further studies demonstrated mutations of specific tumor suppressor genes (P53 and BRCA1/2) could cause breast cancer (26). To explore detailed molecular mechanisms of MTSS1 (metastasis suppressor 1), breast cancer organoids were cultivated by Cong, M (43). By using organoids

derived from patients, Xiaolong Wang demonstrated that targeting the circRNA-CREIT could act as a potential therapeutic technique for chemo-resistant breast cancers (triple-negative) (44). Later, breast cancer organoids biobank has been developed to contain tumor tissues of different stages of breast cancers and their molecular subtypes by Chen Ping (45).

Lung Cancer Organoids

Recently, lung cancer is one of the most concerning health problems. In 2019, first ever successful lung cancer organoid was cultured by Kim, M. (46). Lung cancer organoids are very much consistent in primary culture, so screening of drugs with gene sequencing data are mostly accurate (47). In 2023, largest research related to lung cancer organoids was published by Yilong Wu. Researchers developed more than 200 organoids from 107 patients, where success rate was 81.5% (48). This study was done to observe the efficacy of metastatic or locally advanced lung cancers (48). Further studies have been conducted for drug screening.

Bladder Cancer Organoids

Another significant malignant tumor type is bladder cancer and it originates from the urothelium of bladder. In 2018, Professor Michael M. Shen and his team cultured bladder cancer organoid successfully for the first time. In recent times, usage of bladder cancer organoids is increasing for researches and drug screening (49, 50). A study of Tan P. found that SIRT1 (Sirturin1) activator SRT1720 could inhibit the prolifertion of human bladder cancer organoids significantly (51). Another team co-cultured bladder cancer organoids with MUC1 (mucin1)-CAR-T cells to study CAR-T-cell mediated cytotoxicity. Expression levels of TNF-α (tumor necrosis factor-α), Interleukin-2 (IL-2), and IFN-γ (Interferon-γ) were increased. Observations of this study showed the application value of these patient-derived bladder cancer organoids to detect killing effects of CAR-T cells (52).

Other Cancer Organoids

Kidney, oesophageal, endometrial, ovarian cancer organoids have also been developed in recent times by several researchers (53-56). But, the applications of the organoids are quite similar. In the recent past, in the field of cancer therapy, molecular mechanisms of tumorigenesis, drug screening, and tumor precision therapies were three most important directions and challenges. With the rapid development of tumor organoids, scientists have been getting the chances to overcome difficulties.

Modern Strategies and Future Prospects

Modern technologies (such as gene-editing tools, Artificial Intelligence) have been implemented by several researchers in the vast field of organoids. In case of gene-editing, CRISPR-Cas9 system is the mostly used technique; isogenic models can be produced using CRISPR-Cas9-mediated genome engineering to study the onset, progression, and management of cancers. This tool could be implemented for efficacy and safety by studying gene repair *ex-vivo* in adult-stem-cell-derived tumor organoids, facilitating CRISPR–Cas9 clinical translation. Cancer patients may be benefitted from the transplantation of *ex-vivo* corrected adult stem cell-derived organoids (57).

In addition, integration of Artificial Intelligence with organoids is revolutionizing research by creating more accurate models for different tumors, with significant implications for drug testing, diagnosis, and treatment. The focus is on five key areas: optimizing construction strategies, efficiently extracting multiscale image features (machine learning guided), analyzing multi-omics data, conducting precise preclinical evaluations, and developing practical implementation methods. These advancements would accelerate organoid research and increase their clinical applications, maximizing the potential of AI-enabled organoids in both experimental and real-world settings (58, 59).

Current Challenges

Though there are lots of potential applications and advantages of tumor organoids (Figure 3), many challenges have been addressed by researchers. Success rate of tumor organoid construction is comparatively higher than PDTX models, but tumor organoids are uneven and dependent on different types of cancers. Additionally, cultivations of tumor organoids are highly susceptible to contamination (19). Another important problem is the lack of standard protocols to cultivate different types of tumor organoids, little differences in culture protocols established by different research groups result in different success rates. Moreover, tumor organoids take very little time to be constructed; in many

cases of drug screening and other data analysis, there was not enough time to wait to get results for appropriate drug matches (60). Special medium and different cytokines and inhibitors are needed to culture different kinds of tumor organoids, which are very costly. Moreover, some of these are signaling pathway inhibitors (for example: P38 inhibitor is SB202190, ALK inhibitor is A-83-010) which could affect the drug screening processes (54, 61). Immune cells and their responses, fibroblasts, nerves play significant role in growth of tumors, but lack of these important elements have been observed in case of tumor organoids (61, 62). For example, for malignant and aggressive progression of tumors, M2 macrophages play important role, TAFs (tumor-associated fibroblasts) could have the ability to promote aberrant cell growth (for tumors) by secreting specific cytokines (63, 64). Cultivated tumor organoids are still not able to replicate tumor microenvironment efficiently and it is a major problem for immunotherapy drug screening (65). Another problem is supporting matrix to culture organoids. Matrix is necessary and cultured mostly by using animal (or patient) derived mechanisms or collagen; several unwanted (or unknown) growth factors could be present in these matrices, in addition to collagen IV, entactin, laminin (66, 67).

Conclusion

In spite of a few challenges, there are many ways by which organoid technology is able to transform cancer-related research in upcoming years. This promising and extraordinary technology has tremendous potential to model different types of tumors accurately. With the expansion of this technology, it is expected to culture tumor organoids that would retain the characteristics of tumor microenvironment efficiently and that would also be manipulable. Organoid responses to specific drugs are correlated with clinical data, and it provides great model for cancer therapy. In the near future, organoid cultivations, integrated with some other technologies, would be able to open up new horizons for research, drug development, drug screening, and precision therapy.

Abbreviations

3-D: Three-dimensional, ADMEM: Advanced Dulbecco's Modified Eagle's Medium, APCs: Antigen Presenting Cells, BECs: Biliary Epithelial Cells, CAFs: Cancer-associated fibroblasts, Cas9: CRISPR-associated protein 9, ECM: Extracellular matrix, SC: Stem cells, EGF: Epidermal Growth Factor, ESCs: Embryonic stem cells, HGF: Hepatocyte Growth Factor, iPSCs: Induced pluripotent stem cells, LGR5+: Leucine-rich repeat containing G-protein-coupled receptor 5, PDTOs: Patient-derived tumor organoids, PDTX: Patient-derived tumor xenografts.

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Author Contributions

Rhitoban Ghosh: Wrote the whole manuscript, Designed the Figure 1 and Figure 2, Sangita Dan: Designed the Figure 1 and Figure 2, Soma Sett: Designed the Figure 3 and tables, Palash Dhara: Designed the Figure 3 and tables, Chandan Mandal: Revised the full manuscript, Subhranshu Mandal: Revised the full manuscript. All authors reviewed the full manuscript before submission.

Conflict of Interest

The authors declare they have no competing interests.

Ethics Approval

Not applicable.

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