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Opportunities and Challenges in Using Cancer Organoids Derived from Patients in Personalized Medicine

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Abstract:

A model system for precision medicine has been suggested using tumor organoids. Tumor organoids are unique for cancer research on a patient-by-patient basis because they are able to preserve properties of the original tumor. As a result, it is alluring to consider using tumor organoids to improve patient outcomes during clinical decision-making. Patient outcomes have a good correlation with tumor organoid responses to a variety of medicines in vitro. Before application in clinical cancer care can be considered, however, there are still significant obstacles to be overcome and large cohort prospective trials are desperately needed. Tumor organoids offer a lot of potential in preclinical research due to their unique traits and direct connection to patient data. Here, we have evaluated the most recent developments in the development and use of cancer organoids grown from patients for cancer biology research and customized treatment. We have concentrated on the potential of organoids as a platform for the discovery and creation of innovative targeted therapies for the most intractable malignancy, pancreatobiliary cancer.

INTRODUCTION

Human health is seriously threatened by cancer. The genetic makeup of many tumors has increasingly become known with the advancement of next-generation sequencing technologies. On the basis of the biological profile of malignancies, scientists throughout the world have been trying to develop molecular targeted treatments (1, 2). Nevertheless, only a few number of medications have received FDA approval following clinical studies (2). Treatment for cancer patients has changed over the past ten years, moving from treatment based on tumor type to treatment based on molecular features of a tumor or its microenvironment. This strategy, also known as precision medicine or individualized treatment, has improved the prognosis for many patients with advanced tumors and is being used in (neo-) adjuvant research (3).

Precision medicine has been driven by extensive tumor sequencing studies that resulted in the identification of various therapeutic targets. Although several breakthroughs based on DNA sequencing have been recorded, it is also evident that the majority of cancer patients still have an urgent demand for effective therapies (5). The emphasis on genetic abnormalities in a small number of coding areas has substantial drawbacks and fails to recognize or convey the disease's complexity. DNA sequencing has so far provided information on the factors that cause cancer that are rather well kept throughout the course of the disease, but it does not include additional modulators such epigenetic modifications or the impact of non-coding areas. These modulators are far more dynamic, and it is difficult to understand how they are relevant (6). Although cancer is a hereditary disorder, understanding the underlying malignant pathways at all cellular levels is important for developing effective therapies (6). It is obvious that there is a considerable need for a dynamic, adaptable model system that enables in-depth investigation of various aspects of tumor biology and accurately mimics the behavior of the primary tumor in patients. Ex vivo tumors that are alive and developed from individual patient have inspired a lot of interest in developing precision medicine. It is now feasible to grow tumors in three-dimensional (3D) structures on an individual basis according to the development of new technology. These so-called "organoids" are multicellular in vitro structures made from adult or embryonic stem cells that exhibit self-organization and self-renewal, as well as characteristics that match those of their origin tissue (7). In comparison to traditional 2D cell lines or patient-derived xenografts (PDXs), patient-derived cancer organoids (PDCOs) may be more appropriate for cancer research. In this article, we discuss PDCOs' present situation and potential for the future (8). We paid special attention to pancreatobiliary cancer organoids and their usage in personalized medicine. Our goal was to demonstrate their potential as a cancer model to better understand cancer biology and evaluate possible treatments in vitro, which would eventually lead to clinical applications and more

translational research.

Dimensional culture and organoid structure

An organoid is a 3D cell culture model that is similar to the source organs in the body. Additionally, an organoid is a population of cells that are specialized to an organ that are created from adult stem cells (AdSCs) or pluripotent stem cells (PSCs) (9). They can self-form similarly to the body by cell sorting and spatially constrained lineage differentiation. Currently, research including drug testing, disease modeling, and tissue regeneration and repair frequently employ animals as models; however, these models do not fully represent the physiological properties of the human body (10). The novel in vitro model organoid fills the gap between human beings and animal models by painstakingly recreating the cellular make-up and activity of a typical organism to mimic the physiological makeup of human organs (11). Organoid building benefits from personalization, rapid modeling, high-throughput genetic or pharmacological screening, and the potential for genetic modifications. In the fields of basic biology research, drug testing, and molecular medicine, organoids have emerged as a contemporary research hotspot with significant theoretical implications and promising future development (12). However, there are some disadvantages to organoid fabrication, including the inability to accurately replicate the in vivo microenvironment, insufficient vascularization, the slightly different size of organoid self-organization from that of normal organs, the lack of precise spatial ordering, and the lack of a recognized co-culture system with other cell types. Some of these drawbacks have been addressed by recent bioprinting methods (12).

Culture of organoids

Organoids are divided into two groups based on the features of their stem cells. Organoids made from pluripotent stem cells are the first, while organoids made from adult stem cells are the second. Both separate adult stem cells and organ-specific tissue pieces can be used to create organoids (13, 14). Cells may multiply in culture for a very long period while retaining their genetic stability and loyalty to their original tissue provided the right circumstances are met (15). The adult functioning tissues and somatic mutational processes in these cultures can be used as models for researching stem cell biology. Organoids are three-dimensional structures containing differentiated epithelial cells that have the capacity to self-organize and show important activities as mini organs when growth factors and suppressors are given to the media. With the identification of various niche factors that maintain specific microenvironments, it has become possible to culture organoids derived from various organs (7). Wnt, extracellular antagonist of BMP proteins (Noggin), inhibitor of TGF- type I receptor (A83-01), fibroblast growth factor, epidermal growth factor (EGF), epidermal growth factor (EGF), prostaglandin E2, nicotinamide, R-spondin, gastrin-I, and N-acetylcysteine amide are some of these niche factors. Organoids generated from a variety of organs, including the breast gland, bone, small intestine, stomach, colon, lung, liver, pancreas, prostate, fallopian tube, salivary gland, and tongue, have been described. Particularly, organoid technologies are adaptable (7). Theoretically, tiny tissue samples, which are normally taken by biopsy or from surgical specimens, can be used to start organoid cultures (7).

Cancer Organoid

Organoids offer enormous promise for use in many different contexts, including models for cancer progression, immunotherapy, and genetic illnesses. Among these, the available research suggests that organoids are preferable to 2D cell lines or PDXs as cancer models (16, 17). PDXs and cancer 2D cell lines, which are frequently employed as human cancer models, have major drawbacks despite having made substantial contributions to the field of cancer research. First, generating cell lines often has poor success rates, making the creation of cancer cell lines from primary tissue relatively ineffective (18). This is mostly because it can be difficult for cells to adapt to in vitro 2D growing circumstances. Additionally, because only a few clones are expected to persist and proliferate, the cultures occasionally fail to accurately recreate the genetic diversity of the original tumor. In order to examine the driver mutations necessary for tumor start and development, 2D cell lines may not be superior than organoids (19).

Fresh tissue from a patient is implanted into the subcutis or another orthotopic location in immunodeficient mice to produce PDXs. In contrast to 2D cultures, PDXs can accurately recreate the tumor microenvironment in a 3D structure, including the interaction of cancer cells with the stroma and the development of blood vessels [20]. However, PDX is a labor-intensive and expensive approach that has trouble engrafting and needs considerable resource inputs for its upkeep, making it unsuitable for high-throughput drug screening or genetic editing. Additionally, this method takes time and could need a tumor adaption particular to the host (the mouse) (20).

A number of cancer organoid cultures originated from humans have been created recently, and some information exploiting the characteristics of organoids has been presented (21-23). Depending on the kind of tumor, PDCO cultures have varying degrees of success. Organoid lines for colon, breast, lung, and liver malignancies were reported to be established with 90%, >80%, 70%, and 100% success rates, respectively. However, several tumor types or early-stage cancers, such as biliary tumors, intraductal papillary mucinous neoplasms of the pancreas, and ovarian tumors, are still challenging to culture and have poor chances of success. As described in more depth in following parts, cancer organoids have developed into a tool for research that integrates genetic editing methods with drug susceptibility testing, immunotherapy, and tumor microenvironmental investigations (22, 24, 25).

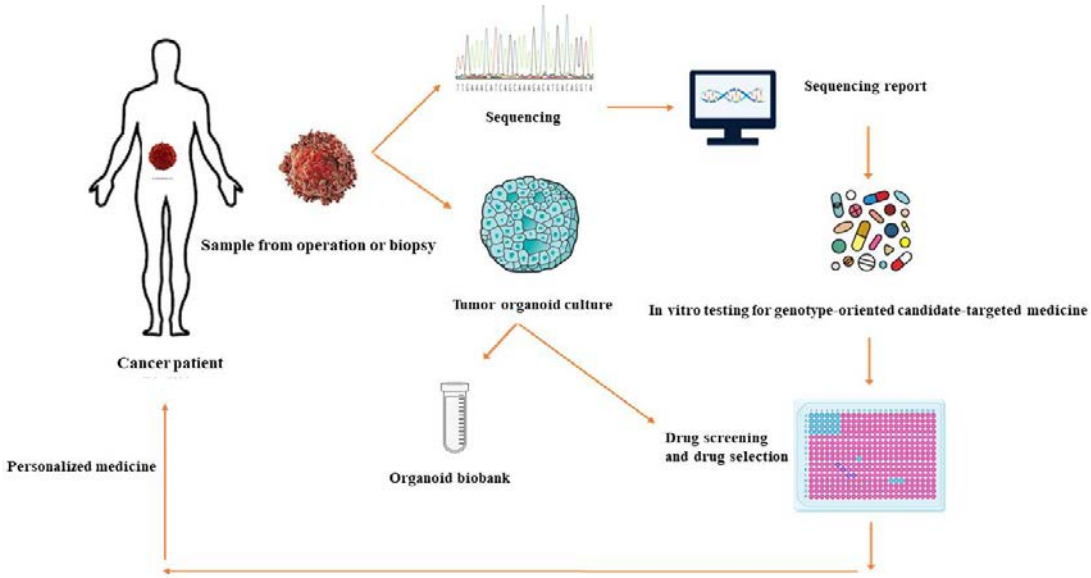

Fig 1. Organoid culturing and thorough genotyping are both components of the genotype-oriented, personalized medicine paradigm that we promote. Exome sequencing and organoid culture were combined in our model, which made it possible to find genotype-oriented targets for personalized medicine and evaluate the effectiveness of potential targeted medications in organoids.

Tumor organoid implementation in clinical decision making

Recent successes and future difficulties

In contrast to static sequencing data, tumor organoids keep the characteristics of the original tumor and provide the opportunity to investigate specific tumors as dynamic systems. MThe necessity for such a dynamic system is highlighted by the persistently high failure rate of medications evaluated in clinical trials, with success rates for cancer therapies in phase I–III clinical trials being as low as 3.4% (26). Tumor organoids may facilitate the transition from the bench to the bedside by providing a more flexible and individualized perspective on cancer biology and therapy response. Many scientists across the world have employed tumor organoids to address their scientific issues and generated a lot of data since the creation of the first organoid cultures made from stem cells (27). Long-term tumor organoid cultures may now be produced from a variety of human epithelial tissues, including the colon, liver, lung, pancreas, prostate, ovaries, bladder, breast, endometrial, esophagus, and others (27). In recent years, sizable live biobanks of tumor organoids have been developed, and this work has been widely described and debated by others. Along with such successes, further research on long-term organoid cultures indicates that organoids retain the phenotypic, genetic diversity, and mutational fingerprints of the original tumor sample. Furthermore, compared to other model systems, tumor organoids have better qualities. They fall between cell lines and patient-derived xenograft (PDX) models due to their biology. Transforming human malignancies into cell lines is far more difficult than doing it with organoids (28, 29).

The creation of a cancer cell monoculture from patient tissue has a reported success rate of about 26% for various cancer types. On the other hand, tumor organoids can be more easily established, less expensive to maintain, and do not require the use of experimental animals, in line with ethical goals regarding animal welfare (30). Tumor PDX models, however, continue to be the only system to capture individualized tumor growth in vivo, surrounded by and in crosstalk with the tumor microenvironment (TME) and parts of the immune system. All of the aforementioned benefits are crucial for clinical application and enable individualized tumor biology analysis by researchers (30).

The idea of taking a tumor specimen from a patient, producing and growing organoids, subjecting the patient-derived tumor organoids to a variety of medications, and then treating the patient with the best drug or combination of treatments appears to be within reach at this point. This would genuinely represent the best bench-to-bedside approach to cancer treatment (31). This strategy has been used by several organizations, including ours, and associations between organoid cultures before and after treatment and susceptibility to a certain medicine have been discovered. The hurdles that need to be overcome in order to properly harness the potential utility of tumor organoids for clinical decision making, however, have also been highlighted by humble experiences (32). The success rate of growing organoids varies between tumor types, allowing therapeutic translation to a minority of cancer patients even though it is higher than that of two-dimensional (2D) cultures. Resection vs biopsy, tumor cellularity, and the beginning material are all crucial elements in influencing the success of a culture, according to our experiences and those of others (33).

These variables differ significantly across different tumor types and are unquestionably a rate-limiting stage in the therapeutic use of organoids. Once a culture has been formed, the growth rate varies depending on

the tumor type, the intra- and inter-patient samples, and the culture itself. Slow-growing samples might cause a delay in making prompt decisions (34). It is not yet known whether they accurately reflect the features of the original tumor or if they could provide unintended biases in in vitro drug testing that rely on cellular growth. Additionally, cultivating organoids is time-consuming, and the necessity for a particular culture medium with a variety of growth factors is pricey and requires careful consideration based on the experimental outcome (35). ALK inhibitor A83-01 and p38 inhibitor SB202190 are two typical additives to organoid culture media that may interact with medications that target the same signaling pathway. Also a concern in prostate and non-small-cell lung cancer, contamination of normal epithelial cells can be damaging to the purity of organoid cultures. In addition to methodological difficulties, tumor organoids fail to include the TME, such as fibroblasts or immune cells, for instance (36). This has been somewhat addressed by the development of co-cultures, which are detailed below, but it still presents a challenge, particularly with regard to immunotherapy. It will be essential to find solutions to the existing issues if tumor organoids are to be used clinically.

View of pancreatobiliary cancer

PBC (pancreatobiliary carcinoma) is an incurable condition with a dismal prognosis. The lowest 5-year survival rate of all solid malignancies is found in those with pancreatic cancer, which is only 8%. Biliary cancer has a 5-year survival rate of about 25%, which is much lower than that for other gastrointestinal malignancies. Over the past few decades, PBC incidence and death rates have risen around the globe. To extend survival, curative resection with a negative surgical margin is required. The best therapeutic option is still surgical resection, however this has a major impact on the course of the disease. However, many patients with PBC have unresectable tumors when they are diagnosed, and thus have few chemotherapeutic alternatives available to them. Therefore, the creation of potent therapeutic medicines is critical (36, 37, 38). The emergence of next-generation sequencing technology has shed light on the genetic origins of PBC. Several molecularly targeted medications have been created recently, some of which have been administered to PBC patients. For instance, ivosidenib, an oral inhibitor of isocitrate dehydrogenase type 1, dramatically increased progression-free survival in patients with biliary cancer (IDH1) (39). Additionally, pre- and post-effectiveness chemotherapy's for PBC has been evaluated. Adjuvant chemotherapy is now the new standard of care for pancreatic cancer patients who have had resection; however, neoadjuvant chemotherapy may also be a normal procedure. In the past, prolonged surgery was used to treat PBC; today, multimodal therapy is used instead (40).

PBCs have distinctive mutational profiles compared with other cancers. TP53, SMAD4, KRAS, CDKN2A, and other gene alterations are often seen in pancreatic tumors. In particular, KRAS mutations are present in over 90% of pancreatic ductal adenocarcinomas, demonstrating the critical role aberrations in the RAS signaling pathway play in the development of pancreatic cancer (41). Additionally, in pancreatic tumors, other pathways and processes other than RAS signaling, including CDK, TGF-, SHH, JNK, and integrin signaling, are complicatedly changed, with some of the modifications frequently overlapping (42). Several different genes, including ARID1A, ELF3, ERBB2, IDH1, IDH2, KRAS, PIK3CA, and TP53, are mutated in biliary cancers. Biliary malignancies don't frequently exhibit distinctive somatic mutations; as a result, each tumor has a unique mutation pattern. Cancers with somatic mutations frequently present a target for molecularly targeted treatment. However, the variety of somatic mutations present in each patient makes the development of individualized therapy approaches necessary (42).

Organoid-derived pancreatic cancer

Compared to other malignancies, patient-derived PBC organoids have received fewer reports. Despite recent reports of a procedure for cultivating PBC organoids, the success rate of these cultures is still lower than that of other malignancies. There might be a number of variables that make it difficult to culture PBC organoids. First, there is a significant quantity of stroma and just a small number of live tumor cells in PBC resected tissues. This was particularly evident in samples of pancreatic cancer collected following neoadjuvant treatment (43). Second, as discussed in the previous section, PBC has different mutational patterns in comparison to other malignancies. These unique mutational profiles in PBC indicate that specific culture conditions are necessary to produce organoids by modifying growth factors to meet the mutational profile (43). We discovered that the cancer organoids that were grown from surgical specimens' tumor portions were mixed with normal epithelial organoids, and we demonstrated experimentally that it was required to remove the normal organoids in order to culture the cancer organoids. Organoids made from healthy epithelial cells had balloon-like structures and were devoid of primary tumor mutations, but organoids made from cancer cells had solid shapes and did, in fact, contain primary tumor mutations (44). A technique for the selective cultivation of pancreatic cancer organoids was described by Seino et al. by modifying the growth factors given to the culture media. Due of the high frequency of KRAS mutations in pancreatic ductal adenocarcinoma (PDAC), their technique calls for the organoids to first be put in an environment where EGF is deficient in order to enrich cancer organoids with an autonomously active EGFR-RAS pathway. To select probable TP53 or SMAD4 mutant organoids, respectively, organoids that are amenable to EGF removal are alternatively treated with Nutlin3 (an MDM2 inhibitor) or Noggin removal/ BMP4 (44).

As with biliary malignancies, our research and others' findings show that the mutations are not only unique from other cancers but also vary across

different tumors. This means that depending on the tumor, different culture conditions should be used. The presence of heterogeneity in a tumor, such as intraductal papillary neoplasm of the bile duct, is important and frequently poses significant challenges to culture (43, 45).

Recent research on PBC organoids has two main goals. The first is the use of cancer organoids in the screening of medicines. The second is to study cancer biology, including how tumor cells and their surrounding environment interact. Using a panel of 76 distinct therapeutic agents, Driehuis et al. developed a platform with 27 patient-derived pancreatic cancer organoids for high-throughput drug screening (46). Through the use of organoids and PDXs in treatment investigations, Hu et al. discovered SIRT5 to be a significant tumor suppressor in PDAC (47). Cholangiocarcinoma (CCA) organoids were examined for their unique 5- ALA-based photodynamic activity, and Fujiwara et al. proposed that this activity may be used as a diagnostic marker to distinguish CCA from non-tumor tissues (48). Research and therapy methods that target not just cancer cells but also the tumor microenvironment, such as fibroblasts or immune cells, may be necessary for PBC with an abundance of stroma. A co-culturing method for pancreatic organoids, fibroblasts, and T cells was developed by Tsai et al. They observed T cells invading the Matrigel, moving in the direction of the organoids, and spreading out at the boundary of Matrigel domes containing organoids (49). According to Koikawa et al., pancreatic stellate cells (PSCs) and organoids were co-cultured in direct contact and indirect contact systems. Their findings suggested that matrix metalloproteinase (MMP) 2, which binds to membrane type-1 MMP (MT1MMP) on PSCs, causes basement membrane breakdown and stromal invasion of organoids in direct contact with PSCs (50). Ohlund et al. were successful in co-culturing fibroblasts and pancreatic cancer organoids, and they were able to see how well they cooperated together in terms of growth morphology (51).

Personalized medicine

A treatment strategy based on a patient's DNA profile, environmental factors, and lifestyle is known as personalized medicine. The genetic profile of the patient's tumor should ideally serve as the basis for customized cancer treatment. Organoids created from surgically removed tumors of patients might potentially be highly helpful in developing tailored medication since they mimic the in vivo characteristics of the underlying tumor and enable in vitro biochemical testing. Personalized medicine systems that integrate thorough genotyping with organoid culture have, however, only been the subject of a few reports of effective implementation (52, 53).

Ideally, the tumor's genetic profile should serve as the basis for personalized cancer treatment. Finding genotype-oriented targets and choosing candidate-targeted treatments for cancers would be reasonable and effective (54). As an alternative, multidrug screening on cell lines originating from tumors may be useful to verify this impact. However, the generation of 2D cancer cell lines from primary tumor materials is relatively ineffective, and cancer cell lines do not always phenotypically mimic real malignancies (54). The organoid, often known as "a miniature of organ," inherits the gene alterations and characteristics of the parent tumor. Previous publications claim that even after prolonged cultivation of organoids, there is minimal genetic alteration. As a result, it is anticipated that organoids created and cultivated from recent tumor samples would be helpful in developing individualized treatment. Translational research on cancer should benefit greatly from using PDCOs as models (54).

The PDCO has been claimed to have some significant applications as a model for personalized medicine, which is predicted to benefit patients. The first is multidrug screening, like high-throughput screening. Organoids are ideal for multi-drug screening because, with the proper adjustments to the culture conditions, numerous clones may be produced quickly (46, 55, 56). High-throughput screening is acceptable for quickly identifying certain successful medications or finding novel compounds. Saito et al. performed a multi-drug screening of 339 therapeutically available drugs on biliary tract cancer organoids and experimentally showed that 22 substances, including antifungal agents, HMG-CoA reductase inhibitors, and dopamine D2 receptor agonists, inhibited organoid development (54). So, the benefit of multi-drug screening, such as high-throughput screening, is the discovery of novel medications and unexpected outcomes. In order to create an organoid from peritoneal diffused colorectal cancer, Narasimhan et al. conducted multi-drug screens. After conventional surgery and five cycles of chemoradiotherapy, they were able to discover a novel agent from the findings of drug screening that was clinically successful in patients with progressing illness (57). Four patients' main pancreatic cancer tumors and the organoids that were created from them were studied by Driehuis et al. for correlations in gemcitabine sensitivity. In all four cases, the sensitivity to gemcitabine was essentially same between original tumors and organoids, indicating that organoids can be used as a personalized medication in vitro testing paradigm (46).

Additionally, using organoids for the verification of genome-driven targeted treatments is particularly helpful for choosing individualized therapy for specific patients (46, 58, 59). According to certain biological pathways, Broutier et al. confirmed genomic alterations in original tumors and patient-derived cancer organoids. These pathways were the focus of the selection of potential targeted medications, and the effectiveness of those medications was examined in organoids (60).

Organoids have also been used in certain trials on immunotherapy. Nael et al. created a coherent group of endogenous syngeneic tumor-infiltrating lymphocytes (TILs) and primary tumor epithelial cells in co-culture. Immune checkpoint inhibition using anti-PD-1 and/or anti-PD-L1 increasing and activating

tumor antigen-specific TILs and inducing tumor cytotoxicity was successfully reproduced in human and mouse organoids (61). In order to investigate the cytotoxicity of lymphocytes made with a chimeric antigen receptor (CAR) in patient-derived colon organoids, Schnalzger et al. created a platform. They showed effective targeting in a variety of organoids utilizing CAR-engineered NK-92 cells that were targeted toward a common epithelial antigen, and CAR-NK-92 cells that were directed toward organoids expressing EGFRvIII were used to study tumor antigen-specific cytotoxicity. Finally, they presented a sensitive in vitro platform for a tailored assessment of CAR effectiveness and tumor specificity (62). Organoids have also been used in various customized medical models, albeit the number of reports is still fairly modest. Forsythe et al. tested the most effective treatment temperature and medication concentration for hyperthermic intraperitoneal chemotherapy in 23 instances using organoids obtained from appendiceal cancer or colorectal cancer (HIPEC). They came to the conclusion that the best perfusion protocol differed by patient and that organoid technology may provide a platform for customizing HIPEC settings to the level of each individual patient (63).

For the practical use of customized therapy with patient-derived cancer organoids, there are various obstacles to be addressed. First, it is essential to reduce the length of culture period and raise the success rate of organoids. Depending on the tumor, organoids grow at varying speeds and have varying degrees of success in culture (64). The second point is creativity in cultivating from the tiny sample of the following: If it is possible to grow cancer organoids from needle biopsy or liquid cytology samples, they can be used to test medications for neoadjuvant chemotherapy or even for resectable tumors that are not amenable to surgery. There have been a few reports on this strategy thus far, but they are relatively sparse (65). The third point deals with the impact of tumor heterogeneity, which is unavoidably a component of the tumor but does not entirely capture its characteristics. Even from a single-cell clone, a tumor is often a mass of heterogenic clones; the organoids must have only received some of the tumor's traits. Fourth, the candidate-targeted therapy approach using genomic profiling may not produce any therapeutic drugs that are clinically available (66). However, high-throughput multi-drug screening with non-anticancer agents may reveal unexpected drugs that are effective for some cancer cells with the above-mentioned distinct phenotypes, which could result in the discovery of new antitumor drugs. Moreover, investigations on radiation treatment using patient-derived organoids may offer substitutes, even if the number of patients is currently small.

CONCLUSIONS

We examined the technical advancement of cancer organoids and their present uses in cancer research and personalized treatment. In the study of cancer biology and translational medicine, PDCOs offer a lot of potential. It works well for evaluating potential targeted medications found through multi-omics analysis. Organoid-based research is now used in radiation treatment and immunotherapy as well as targeted medication screening. Furthermore, systems have been established that allow for the in vitro study of the tumor microenvironment employing cancer organoids in combination with immune cells and stromal cells, such as lymphocytes and fibroblasts, respectively. There are still certain hurdles to clear for therapeutic applications, such as speeding up, stabilizing, and simplifying methods for cultivating and studying cancer cells. Organoids are expected to grow in versatility in a variety of malignancies and provide a greater contribution to the study of cancer.

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