—Review—

Role of Patient-Derived Tumor Organoids in Advanced Cancer Research

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Cancers originating from the same tissue vary significantly in genetic mutations and patient drug response. Furthermore, tumor tissue is composed of diverse cancer cell clones. This phenomenon, known as "cancer cell heterogeneity," occurs among tumors (between patients) and within individual tumors and is an important mechanism driving resistance to cancer therapy. Therefore, an understanding of cancer cell heterogeneity is essential for the development and delivery of more effective personalized treatments. The cancer cell lines typically used in cancer research cannot accurately replicate this heterogeneity. However, patient-derived tumor organoids (PDTOs), three-dimensional cultures of tumor cells, can precisely replicate the histological, molecular, and cellular heterogeneity of the original tumor. PDTOs generated from human cancers are now widely used as innovative tools in cancer research, including in studies of the mechanisms of cancer development and progression and in screening of anticancer drug. This review summarizes recent advances in human tumor research that uses PDTOs.
(J Nippon Med Sch 2025; 92: 234–241)

Key words: patient-derived tumor organoids (PDTOs), PDTO biobank, genome editing, drug screening

Introduction

Cancer remains a major health risk, with an estimated 20 million new cases and 10 million deaths reported worldwide in 2022[1]. In recent years, significant progress has been made in cancer treatment, particularly in the development of immune checkpoint therapies and molecular-targeted drugs. Despite these advances, many patients continue to die from cancer recurrence. The primary cause of treatment failure is clonal heterogeneity of cancer cells, both among patients and within individual tumors. Even when cancer develops from the same tissue, therapeutic responses vary substantially between patients and cancer cell clones within the same tumor. Understanding and addressing the heterogeneity of cancer cell clones are thus essential for advancing cancer research and improving therapeutic outcomes.

Two-dimensional (2D) cancer cell lines have long been used to study human cancers and for high-throughput drug screening to identify compounds that inhibit tumor growth. However, these cancer cell lines are derived from a limited subset of tumors from individual patients and fail to reflect the inter-patient and intra-tumor heterogeneity of cancer cells. Consequently, the drugs identified in cancer cell lines often lack therapeutic efficacy[2].

Patient-derived tumor organoids (PDTOs) have been established as models to address these issues and replicate the diversity of cancer cells in patient tumors. In 2009, organoid cultures were used to study mouse intestinal stem cell (ISC) function. Single purified Lgr5-positive stem cells produced structures resembling intestinal crypts and villi that differentiated into various mature epithelial cells[3]. Subsequently, this method demonstrated that three-dimensional (3D) "miniature organs" and "miniature tissues" could form *in vitro* from various types of stem cells, including adult stem cells from different tissues, embryonic stem cells, induced pluripotent stem cells, and tumor cells derived from mice and humans. A PDTO model and biobank have been developed for a range of tumors, including colorectal, gastric, pancreatic, breast, lung, head and neck, ovarian, prostate, bladder, and liver cancers, as well as for rare cancer types, such as neuroendocrine tumors, for which preclinical models are lacking[4–6]. As a result, characteristics of human tumors that could not be identified using conven-

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https://doi.org/10.1272/jnms.JNMS.2025_92-310
Journal Website (https://www.nms.ac.jp/sh/jnms/)

Table 1 Usefulness of patient-derived tumor organoids and cancer cell lines

Feature	Patient-Derived Tumor Organoids (PDTOs)	Cancer Cell Lines
Genomic fidelity	Retain patient-specific genetic mutations and heterogeneity	Often acquire genetic drift and lack intratumoral heterogeneity
3D structure	Mimic tumor architecture and microenvironment	2D monolayer culture lacks tissue organization
Drug response predictability	More accurately reflect patient drug responses	Often fail to predict clinical drug efficacy due to adaptation to in vitro conditions
Tumor microenvironment interaction	Can be co-cultured with stromal and immune cells to study interactions	Limited ability to incorporate tumor-stroma interactions
Scalability and high-throughput screening	Amenable to drug screening but more complex and costly to establish	Easy to expand and widely used in large-scale drug screening
Clinical relevance	Better model for precision medicine and personalized therapies	Less representative of patient-specific tumor biology
Establishment efficiency & biobanking potential	Higher establishment efficiency across various cancer types, allowing for creation of large biobanks representing diverse patient populations	Lower success rate; difficult to establish from primary patient samples, limiting biobanking potential

Table 2 Potential applications of patient-derived tumor organoids

Application	Description
Precision oncology	PDTOs allow for patient-specific drug testing to guide personalized treatment strategies.
Drug screening and development	Used in high-throughput screening to assess the efficacy and toxicity of novel anticancer compounds.
Mechanistic studies of tumor biology	Enables investigation of cancer progression, metastasis, and tumor heterogeneity.
Immunotherapy research	Can be co-cultured with immune cells to evaluate immunotherapy responses, including checkpoint inhibitors and CAR-T therapies.
Tumor microenvironment modeling	Facilitates study of tumor-stroma and tumor-immune interactions in a more physiologically relevant context.
Biomarker discovery	Aids in identifying predictive and prognostic biomarkers for cancer therapies.
Gene editing and functional genomics	CRISPR and other gene editing tools can be applied to PDTOs to study the role of specific genes (mutations) in tumor initiation, progression, and drug resistance.
Preclinical models for translational research	Serve as patient-specific ex vivo models to bridge the gap between basic research and clinical applications.

tional cancer cell lines were revealed. **Table 1, 2** summarize information on the utility and application of PDTOs.

Clinical Relevance of PDTOs

PDTOs have been used in tumor biology studies of tumor evolution[7,8], cancer cell clone diversity[9], and metastasis mechanisms[10] and to explore new anti-cancer drugs[11,12]. Additionally, several clinical trials have evaluated the responsiveness of PDTOs to standard treatments, and the results have been used to determine optimal treatments for patients[13,14].

The rapid expansion of PDTO research can be attributed to two key factors. First, PDTOs accurately replicate the features of a patient's tumor tissue (see below). Second, establishment efficiency is much higher for PDTOs than for general cancer cell lines, thus enabling modeling of various cancer clones within the same cancer type. This has led to the creation of organoid biobanks worldwide that collect organoid lines from many cancer patients[4–6]. Findings from PDTO biobank analysis can reveal unknown cancer characteristics and advance oncological research and treatment.

The extent to which PDTOs recapitulate the original tumor tissue can be assessed by analyzing genetic mutations and histological similarities. This section highlights representative studies using PDTOs for colorectal cancer (CRC), which has the largest number of reported PDTO banks. Sato and his colleagues were among the first to establish a CRC organoid PDTO biobank, generating 55 PDTO lines from 43 patients, with a 100% success rate[15]. They compared the *in vitro* tissue morphology of PDTOs and tumor tissue formed by xenotransplanting PDTOs under the renal capsule of immunodeficient (NOD/Shi-scid IL-2Rγ-null, NOG) mice with the original tumor tissue morphology. The histological differentiation of the original tumor was highly reproducible in both PDTOs

and xenograft tumors (PDTOX). In rare CRC subtypes, such as neuroendocrine tumors (NEC) and mucinous adenocarcinomas, characteristics such as increased neuroendocrine or mucus-producing cells were faithfully reproduced by PDTOs and PDTOX[15].

Clevers and his colleagues are also pioneers in this field, having established their own CRC organoid biobank, similar to the Sato group[15]. They reported that somatic mutations in the coding region of PDTOs were highly consistent with those in the corresponding parent tumor tissues (median concordance frequency, 0.88; range, 0.62-1.00)[16].

Several studies have evaluated the correlation between the effectiveness of standard treatments (chemotherapeutic agents and radiation therapy) and the responsiveness of PDTOs derived from the same patient tumor. Yao et al.[17] established organoids from tumor tissues of patients with untreated rectal cancer and investigated the relationship between organoid response to chemotherapeutic agents *in vitro* and the efficacy of neoadjuvant chemoradiotherapy (NACR). The efficacy of NACR was estimated with an accuracy of 84.4% by observing the responses of PDTOs to chemotherapy and radiation[17]. Similarly, Geevimaan et al.[18] established 115 colorectal cancer organoids and compared the response of PDTOs to oxaliplatin with the clinical treatment effects of FOLFOX (5-fluorouracil [5-FU], leucovorin, and oxaliplatin) in patients whose tumors were used to generate the PDTOs. The two results correlated strongly, enabling prediction of the treatment effect with 70.6% accuracy in patients with CRC, when using PDTO response as an indicator[18].

Mo et al.[19] established 50 PDTOs derived from primary and liver metastatic tumors of patients with colorectal cancer liver metastasis and found that the response to FOLFOX and FOLFIRI (5-FU, leucovorin, and irinotecan) correlated with the clinical treatment effect in the original patients[19]. Similarly, to evaluate the efficacy of chemotherapy, Ooft et al.[20] established PDTO lines from 35 biopsies of patients with metastatic CRC. They reported that PDTOs can serve as predictive tools for patients with metastatic CRC that does not respond well to irinotecan-based chemotherapy[20].

A more important aspect of using PDTOs as a preclinical model is the extent to which they can stably maintain the characteristics of a patient's tumor in culture. Early-passage PDTOs retain key genomic and transcriptomic features of the source tumor, making them valuable preclinical models[16,21–26]. Although some mutations are lost or acquired during long-term PDTO culture, the overall effect of this phenomenon seems to be limited[7,27,28]. Furthermore, the drug responses of early- and late-passage PDTOs were very similar. Therefore, it appears that the nature of the drug response, as well as gene mutations, is robustly maintained even after repeated passages[27,29,30].

In summary, the PDTO model accurately replicates patient tumor characteristics, thus enabling prediction of treatment effects before drug administration. Because chemotherapy and radiotherapy can impair patient quality of life, these treatments can be avoided in patients with low predicted response rates.

Genetic Engineering of PDTOs

Cancer development and progression are thought to occur when normal cells acquire mutations in driver genes. These processes can be simulated by applying genome editing technology to organoid models. Genome editing alters the DNA sequences of living organisms at specific sites, making it possible to add, delete, or modify genes with high precision. Clustered regularly interspaced short palindromic repeats-Cas9 (CRISPR-Cas9) is a genome editing technology that modifies specific genes by cutting DNA at target sites. This system consists of a guide RNA comprising CRISPR RNA (crRNA), trans-activating CRISPR RNA (tracrRNA), and the Cas9 protein, which cuts DNA. When DNA is cut by CRISPR-Cas9, the cell repairs it using either the non-homologous end-joining (NHEJ) or homology-directed repair (HDR) pathways. In NHEJ, DNA is repaired with random base insertions and deletions (indels), often resulting in frameshift mutations and loss of function (knockouts) in the target gene. In contrast, HDR uses a homologous donor DNA as a template for precise DNA repair, allowing for insertion or modification of specific gene sequences (knock-in). Using CRISPR-Cas9 gene-editing technology, researchers have introduced driver mutations into organoids derived from normal epithelial tissues to observe changes in cell proliferation, invasion, and metastasis. This section provides representative examples.

Fifty percent of gastric cancers (GC) are classified as the chromosomal instability (CIN) type, and approximately 70% of CIN GC harbor *TP53* loss of heterozygosity mutations, which are strongly associated with aggressive phenotypes and treatment resistance[31,32]. Karlsson et al.[8] used human normal gastric organoids (HGOs) to analyze GC development and evolution *in vitro*. They used CRISPR technology to introduce CIN through TP53 gene loss in HGOs and then continued the culture for approximately 2 years. At 100 days after inducing TP53

inactivation, they used lentiviral vectors to genetically label individual cells with barcodes and tracked the expansion and regression of each clone with chromosomal alterations and gene expression. They observed that when GC occurred through TP53 deletion, clones that had acquired a growth advantage from mutations in well-known tumor suppressor genes, including deletion of the *CDKN2A* gene locus or deletion of the *FHIT/FRA3B* gene locus, were initially selected. Furthermore, although the process of evolution is extremely complex, even when HGO from different donors was used, subclones with increased expression of claudin genes (*CLDN3/4/7*) and carcinoembryonic antigen genes (*CEACAM5/6*), as well as activated NF-κB-mediated TNF signaling pathways and hypoxic pathways, eventually become dominant[8].

Matano et al.[33] generated artificial CRC organoid lines by sequentially introducing driver mutations in tumor suppressor genes *(APC*, *SMAD4*, and *TP53)* and oncogenes (*KRAS* and *PIK3CA*) into normal human colorectal epithelial organoids. Intriguingly, acquisition of these mutations gradually reduced organoid dependency on niche factors[33]. These findings suggest that cancer development and progression are closely associated with deviations from niche factor dependency. Similarly, using CRISPR-Cas9 technology, Drost et al.[34] reported that simultaneous mutations in four commonly altered CRC genes (*APC*, *TP53*, *KRAS*, and *SMAD4*) in normal human ISC organoids resulted in highly proliferative and invasive cancers when transplanted into the cecum of immunodeficient mice.

The CRISPR-Cas9 system is a valuable tool for studying the relationship between mutations in cancer driver genes and drug sensitivity. Ovarian clear cell carcinoma (OCCC), the second most common ovarian cancer worldwide, has a poor prognosis owing to its limited response to platinum-based chemotherapy, making treatment of residual and recurrent disease challenging[35]. OCCC is often associated with a high frequency of mutations in *ARID1 A*, which encodes a subunit of the SWI/SNF chromatin remodeling complex[36]. Notably, OCCCs with *ARID1A* missense mutations are sensitive to the tyrosine kinase inhibitor dasatinib and ATR inhibitor VE-821[37,38]. Hirt et al.[39] established PDTO lines from pancreatic ductal adenocarcinoma (PDAC) tumor tissues, including lines with wild-type *ARID1A* and others with missense mutations. They confirmed that PDAC-derived PDTO lines with an *ARID1A* missense mutation exhibited increased sensitivity to dasatinib and VE-82127[39]. To verify this gene-drug correlation, they introduced a frameshift mutation via CRISPR to delete the *ARID1A* gene in PDTOs with *ARID1A* missense mutations. This deletion significantly increased resistance to dasatinib and VE-821, demonstrating that the dysfunctional *ARID1A* protein caused by the missense mutation is essential for increased drug sensitivity[39].

Furthermore, although still limited, the CRISPR system is now being applied for genome-wide functional screening of PDTOs, similar to its use in cancer cell lines. The TGFβ signaling pathway suppresses cancer cell growth, and inactivation of proteins in this pathway is common in colorectal cancer[40,41]. However, even CRC organoid lines derived from patients without mutations in TGFβ-related genes often exhibit resistance to TGFβ-mediated growth suppression[15]. To investigate this, Ringel et al.[42] conducted dropout screening using the CRISPR-Cas9 system to identify molecular mechanisms underlying TGFβ resistance. Although such screening is common in cell lines[43,44], it is rare in organoid models because of the large number of cells required. By optimizing conditions for introducing pool-type single-stranded guide RNA (sgRNAs), the study revealed that multiple subunits of the SWI/SNF chromatin remodeling complex contribute to TGFβ resistance[44]. These findings indicate that CRISPR-Cas9 dropout screening of PDTOs can reveal the mechanisms underlying cancer progression and drug resistance.

Nevertheless, the CRISPR-Cas9 system presents several challenges, including low target specificity, low editing efficiency, and potential risks to genomic stability. In particular, a major challenge is that sgRNAs can bind to non-target sequences, causing unexpected DNA double-strand breaks (DSBs) and resulting in genetic mutations (off-target effects). In this context, Gaudelli et al.[45] developed an innovative technique, called "base editing," that induces single-base substitutions without causing DNA DSBs, thus avoiding off-target effects[45].

Furthermore, Geurts et al.[46] applied a base-editing technique to simultaneously induce four colorectal cancer driver gene mutations—APC^{Q1406*}, $PIK3CA^{E545K}$, $SMAD4^{R361H}$, and $TP53^{W53*}$—in human colorectal epithelial organoids. In contrast, the conventional CRISPR-Cas9 system requires a step-by-step introduction of edits to each gene. Thus, their method significantly reduced the time and effort required to create artificial cancer models[46].

Prime editing (PE) is a genome editing tool that is similar to base editing and avoids DNA double-strand breaks (DSBs)[47]. It combines nickase-CRISPR-Cas9 with reverse transcriptase as a functional domain, enabling insertion of a modified sequence into the genome through

a reverse transcription reaction guided by a gRNA containing the modified sequence (pegRNA). Using this technique, Geurts et al.[48] were able to introduce Tp53 cancer gene mutations into human colon epithelial and liver organoids with efficiencies of 25% and 97%, respectively[48]. Although PE is a highly precise genome editing technology, its efficiency varies across different genetic loci and cell types, and factors such as chromatin accessibility and PAM availability affect editing success. In addition, the system can introduce unintended edits, including off-target mutations and bystander effects, owing to the misalignment of the pegRNA and the activity of the reverse transcriptase enzyme. Overexpression of reverse transcriptases can also cause cytotoxicity by disrupting normal RNA metabolism and activating DNA damage responses. Although PE is effective for small DNA modifications, its efficiency decreases with larger modifications owing to limitations in the processivity of reverse transcriptase. Despite these issues, ongoing improvements, such as optimized pegRNA designs[49], high-fidelity Cas9 variants[50,51] and alternative strategies such as twin prime editing[52] are helping to increase the efficiency of the technology.

PDTOs as a Future Platform for New Drug Screening

To identify novel and highly effective anti-cancer drugs, preclinical human cancer models that closely resemble patient tumor tissues are essential. Traditionally, 2D cell lines and patient-derived xenograft (PDX) models have been used for drug screening. However, 2D cancer cell lines fail to replicate the heterogeneity of patient tumor tissues, and most drugs screened using these models show limited therapeutic efficacy in clinical practice. While PDX models can replicate the heterogeneity of the original tumor tissue, they are time-consuming, expensive, and have low throughput, making them unsuitable for large-scale drug screening. PDTOs are a promising alternative for screening new drugs. PDTOs exhibit high reproducibility in histology and genetics, with drug responses closely aligned with the effects observed in the patients from whom they were derived[17–20].

Use of PDTOs derived from diverse patients in high-throughput drug screening enables comprehensive analysis of the relationship between drug responsiveness and the genetic characteristics (e.g., gene mutations and expression) of each PDTO. Such analyses are not feasible with conventional cancer cell lines that lack diverse clones. Using 56 PDTOs derived from human head and neck cancers, Gu et al.[11] evaluated the effects of 2,248 small-molecule compounds, including 1,800 FDA-approved drugs, 319 compounds in clinical trials, and 129 preclinical compounds. By integrating the gene mutation and expression data of these PDTO lines with the results of a drug screening study, the authors suggested that the JAK2 inhibitor fedratinib may suppress growth of head and neck squamous cell carcinoma (HNSCC) subtypes with low *KRT18* expression. Additionally, the topoisomerase inhibitor mitoxantrone was effective against the IL6R-activated HNSCC subtype. These results strongly suggest that high-throughput drug screening using diverse PDTOs facilitates cancer stratification and discovery of tailored treatment options for specific subtypes[11]. PDTOs may also play a crucial role in identifying biomarkers that can predict the clinical efficacy of standard treatments. For example, docetaxel is a standard treatment for HNSCC but only 10-30% of patients respond to it, highlighting the need for predictive markers. A study assessed the sensitivity of PDTO lines to docetaxel and correlated the responses with the gene expression profiles. Lines with high *ITGB1* expression were resistant to docetaxel. Further analysis of the clinical trial data from patients with oral squamous cell carcinoma confirmed these results. Tumors with relatively high *ITGB1* protein expression are associated with significantly lower survival rates in patients receiving postoperative chemoradiotherapy, including docetaxel. Thus, low *ITGB1* expression may serve as a valuable biomarker for identifying patients likely to benefit from docetaxel therapy.

Toshimitsu et al.[12] developed a drug screening method using patient-derived CRC organoids. To achieve high-throughput drug screening, they developed a unique method to amplify a large number of CRC organoids under suspension culture conditions. Using this method, they evaluated the effects of nine clinical anti-cancer drugs and 47 small-molecule compounds targeting cancer-related signaling pathways in 58 normal epithelial organoids and 179 CRC organoids. JQ1, an-inhibitor of the BET bromodomain protein involved in the transcriptional activation and elongation of target genes, specifically suppressed the growth of cancer cells[12].

Conclusions

In summary, PDTOs have considerable potential as tools for basic cancer research, as well as for developing new drugs and implementing personalized medicine. However, several challenges remain in cancer organoid research, including differences in organoid establishment

and drug screening methods, the low efficiency of organoid generation for certain cancer types, and high culture costs. Although organoids can replicate the histological and genetic diversity of the original tumor tissue, they are often established from only a small portion of the patient's tumor. Genetic mutations in established organoids vary in relation to the tumor collection site[9]. Therefore, caution is warranted when comparing PDTO responsiveness to the clinical therapeutic effects of the same drugs. This limitation can be addressed by collecting PDTOs from more patients and analyzing a broader range of cancer cell clones. In a previous study, the present author confirmed that tongue cancer organoid models from different patients exhibit distinct genetic mutation patterns and varied responses to chemotherapy agents[53].

Another limitation is that PDTOs do not fully recapitulate the tumor microenvironment (TME), which is composed of not only tumor cells but also various other cell types such as stromal, immune, and endothelial cells. Interactions between these cells may affect tumor progression, metastasis, and treatment response[54–56]. Most PDTO models typically culture tumor cells alone. However, to replicate the TME more accurately, some studies have used models that incorporate cancer-associated fibroblasts (CAFs)[57–60], immune cells such as tumor-infiltrating lymphocytes (TILs)[61], and endothelial cells[62]. These models more accurately represent a patient's tumor response to anti-cancer drugs and radiotherapy.

Although the PDTO model is still under development, future technological advances focused on reducing costs, improving success rates, and integrating new analytical techniques could reveal previously unknown cancer characteristics that analyses of conventional cancer cell line could not. Such information will enable precision medicine to accurately classify cancers and provide the most effective treatments. To achieve this, it is essential to create large-scale PDTO biobanks and comprehensive catalogs of human cancer cell clones.

Acknowledgements: The author thanks the members of the Sato Lab for their help with preparing the manuscript.

Funding: This work was supported by a JSPS Grant-in-Aid for Challenging Research (Pioneering) under Grant Number JP21K18259 (T.S.), a Takeda Science Foundation Research Grant, and the Princess Takamatsu Cancer Research Fund under Grant Number 20-25220 (T.S.).

Conflict of Interest: The author declares no competing interests.

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(Received, January 17, 2025)
(Accepted, March 26, 2025)