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**Invited Mini Review**

# Development of bioinformatics and multi-omics analyses in organoids

*Doyeon Ha[1], JungHo Kong[1], Donghyo Kim[1], Kwanghwan Lee[1], Juhun Lee[1], Minhyuk Park[1], Hyunsoo Ahn[2], Youngchul Oh[1] & Sanguk Kim[1,2,*]*

[1]Department of Life Sciences, Pohang University of Science and Technology, Pohang 37673, [2]Graduate School of Artificial Intelligence, Pohang University of Science and Technology, Pohang 37673, Korea

**Pre-clinical models are critical in gaining mechanistic and biological insights into disease progression. Recently, patient-derived organoid models have been developed to facilitate our understanding of disease development and to improve the discovery of therapeutic options by faithfully recapitulating *in vivo* tissues or organs. As technological developments of organoid models are rapidly growing, computational methods are gaining attention in organoid researchers to improve the ability to systematically analyze experimental results. In this review, we summarize the recent advances in organoid models to recapitulate human diseases and computational advancements to analyze experimental results from organoids. [BMB Reports 2023; 56(1): 43-48]**

## INTRODUCTION

The use of cell lines and animal models has greatly improved our mechanistic understanding of human developmental processes and disease initiation. Identifying disease mechanisms using cell lines and animal models has guided the development of potential drugs, however, there exist inherent differences between classical pre-clinical models and humans, which limits the clinical translation of research findings. The development of 3D culture organoid models, which are self-organizing cells that mimic *in vivo* human tissue, can help reduce these limitations.

In this review, we describe examples of organoid models and computational methods and their application in detecting human disease signatures using genetic mutations, expression patterns, multi-omics analyses, and image data. It includes examples of sequencing techniques used in organoid research, as well as computational methods developed to improve the detection of true signals from the sequencing results.

*Corresponding author. Tel: +82-54-279-2348; Fax: +82-54-279-2199; E-mail: sukim@postech.ac.kr

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## GENOMIC ANALYSES OF ORGANOIDS

Organoids are powerful tools for identifying and validating disease mechanisms since (i) they provide an accurate representation of human tissue and (ii) they can be genetically modified. For instance, Li and colleagues observed that genetic modification of tumor suppressors or oncogenes can recapitulate colon tumorigenesis in organoid models (1). In another example, Artegiani and colleagues discovered that loss of BRCA 1 Associated Protein 1 (BAP1), a deubiquitinating enzyme, in liver cancer organoid models resulted in a loss of multiple epithelial characteristics and acquisition of tumorigenic features, supporting that BAP1 can act as a tumor suppressor in the liver (2).

Genetic manipulation in organoid models revealed key oncogenic variants in cancer. Specifically, studies have found context-specific oncogenic signaling pathways and tumor suppressor genes (TSGs) through genome-wide Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 (CRISPR-Cas9) screening of organoids. For example, several TSGs were identified as negative regulators of the Transforming Growth Factor (TGF)-beta pathway in the context of mutant Adenomatous Polyposis Coli (APC), including subunits of the Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex (3). Transforming Growth Factor Beta Receptor 2 (TGFBR2) was known to be the most prevalent TSG in the context of mutant APC and Kirsten rat sarcoma virus (KRAS) for colorectal cancer (4). For genetically defined benign tumor-derived organoids carrying APC and KRAS mutations, tumorigenic ability of the mutation in Activin A Receptor Type 1B (ACVR1B), Activin A Receptor Type 2A (TACVR2A), and AT-Rich Interaction Domain 2 (ARID2) was evaluated by disrupting TSG function and the role of Transformation-related Protein 53 (TRP53) was uncovered in tumor metastasis (5).

Genetic manipulation in organoid models has also been conducted to observe developmental processes or to identify various human disease variants. Ungricht and colleagues found that the combination of inducible genome-editing and longitudinal sampling of human kidney organoids not only provided

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the knowledge of early differentiation into the kidney lineage, but also helped to identify childhood/chronic kidney disease-related genes, such as ciliopathies (6). Beumer and colleagues discovered that CRISPR-Cas9 engineered organoid biobank can be used to identify essential host factors for coronaviruses (7). Furthermore, CRISPR-Cas9 gene editing can be applied to human intestinal organoids to correct disease-causing mutations. Correction of Cystic Fibrosis Transmembrane Conductance Regulator (CFTF) locus by homologous recombination using CRISPR-Cas9 gene editing system in cultured intestinal stem cells of cystic fibrosis patients restored the level of swelling comparable to that of wild-type organoids (8).

Testing all genetic variants for their functional role will require a vast amount of resources. Therefore, evaluating the impact of the genetic variants prior to functional validation in organoid models can effectively reduce experimental time and cost. Many computational methods have been developed to predict the impact of genetic variants using evolutionary selections on noncoding regions (9) or coding regions (10, 11), protein 3D structures, (12) and splicing patterns (13). These computational methods first identify disease variants, which can then be validated in organoids. For instance, to identify and validate cancer driver genes, Arnold and colleagues used a computational tool, PolyPhen-2, to identify potential pathogenic mutations in pancreatic ductal adenocarcinoma patients (14). They identified D48Y and R609H missense mutations in RAD50 Interactor 1 (RINT1), where the variants were suggested to hamper RINT1 function by impairing the interaction with zw10 kinetochore protein (ZW10) and RAD50 Double Strand Break Repair Protein (RAD50). They proved the disease-association of the mutations based on the genetic alteration of RINT1 on patient-derived organoids. These results suggest that computational methods may facilitate the discovery of novel disease variants when combined with functional validation in organoid models.

## GENE EXPRESSION PROFILING OF ORGANOIDS

A cell's functional role is determined by the expressed genes and therefore, gene expression is considered as an intermediate phenotype (15, 16) in understanding various human diseases. Accordingly, gene expression profiling has been conducted on organoid models to measure molecular similarity between organoids and human, especially by comparing the similarities in developmental trajectories or cellular compositions. For example, Cowan and colleagues developed human retinal organoids with functional synapses, and sequenced the organoids at seven developmental time points that span 46 weeks (17). The results showed that the organoids developed into matured state at 30 to 38 weeks, with transcriptomes matching that of a developing human retina *in vivo*.

In addition to retinal organoids, human cerebral organoids have been developed to understand the developmental processes or disease-related signatures of the human brain. For instance, Kanton and colleagues developed human, chimpanzee, and macaque cerebral organoids, and conducted single-cell RNA sequencing (scRNA-seq) to detect human-specific expression patterns, which revealed that human neuronal development was much slower than two other primates (18). Another group analyzed cerebral organoids generated from eight bipolar disorder patients and eight healthy control individuals using a reprogramming human induced pluripotent stem cells, and found downregulation of gene expression in the biological processes including cell adhesion and neurodevelopment (19).

Gene expression profiling also revealed key similarities between cancer organoids and human primary tumors. Liu and colleagues evaluated transcriptomic similarity of organoids and cancer cell lines and showed that organoids more closely resembled metastatic breast cancer samples compared to cancer cell lines (20). Another recent study found that a pancreatic ductal adenocarcinoma cancer (PDAC) organoid has multiple subtypes, suggesting that organoid models are useful in recapitulating intra-tumor heterogeneity (21). In detail, scRNA-seq revealed that the two molecular subtypes (classical and basal) of PDAC coexist in the same organoid sample. Given the transcriptome similarity between organoids and *in vivo* tissue samples, gene expression profiling in organoid models are useful in identifying mechanisms of action of therapeutic drugs and drug biomarkers. For instance, Norkin and colleagues developed a method for systematically conducting drug screening and gene expression profiling in colorectal cancer organoids (22). They applied the method in wild-type and cancer organoids to screen cell differentiation inducing anti-cancer drugs.

Despite the usefulness of gene expression analysis, results derived from expression profiles often do not generate reproducible results (23). One of the approaches that can improve the reproducibility of gene expression analyses is the use of a protein-protein interaction network. Recent studies suggest that genes associated with diseases would likely form clusters in protein interactome (24, 25). Based on these observations, a method has been developed to identify anti-cancer drug biomarkers from drug screening data in organoids (26). Kong and colleagues showed that drug response biomarkers can be derived using a protein-protein interaction network with drug screening and transcriptomics data from patient-derived organoid models, which are predictive of drug response in 5-Fluorouracil-treated colorectal cancer and cisplatin-treated bladder cancer patients.

## MULTI-OMICS ANALYSES OF ORGANOIDS

Multi-omics analysis has played an essential role in identifying physiological similarities between *in vitro* organoids and *in vivo* organs. For the brain, Amiri and colleagues provided evidence that human-induced, pluripotent stem cell (hiPSC)-derived organoids recapitulate human cortical development from the embryonic to the prenatal stage (27). They compared chromatin immunoprecipitation sequencing (ChIP-seq) data

and RNA-seq data obtained from hiPSC-derived organoids with those from isogenic human postmortem fetal samples. They identified enhancer-gene links that are closely related to early cortical development. In addition, they showed that genes related to cortical development during early fetal stages are also associated with neurodevelopmental disorders such as autism spectrum disorder (ASD) (27). Additionally, brain organoids have been developed to model the postnatal stage of brain development. Gordon and colleagues demonstrated that the developmental process observed in 3D organoids derived from human stem cells displayed high transcriptomic and epigenetic similarities with neurodevelopmental processes observed *in-vivo* (28). These findings were further confirmed by an observation that organoids underwent chromatin remodeling, which resembled developmental processes observed in the *in-vivo* human forebrain (29).

In addition to brain organoids, multi-omics analyses demonstrated that 3D organoids derived from primary tumors recapitulated the features of *in-vivo* human tumors. For example, Broutier and colleagues showed that organoid cultures from eight liver cancer patients successfully recapitulated genomic, transcriptomic, and histological features of the primary tumor even after the organoids had been cultured for long-term periods (30). Gao and colleagues leveraged genomic and transcriptomic data to confirm the resemblance of organoids derived from prostate cancer patients (31), and Boj and colleagues utilized transcriptomic and proteomic analyses to comprehensively understand pancreatic organoids (32). Integrated analyses of genomic, transcriptomic, and phosphor-proteomic data derived from colorectal cancer patient-derived organoids also showed that patient-derived organoids could recapitulate patients' tumors at the molecular level (33).

Since various omics data showed that patients-derived organoid models accurately mimicked original tissue development and disease pathology, many precision medicine methods were developed through drug screening in organoid models. Specifically, cancer organoid models have been developed and applied to test the efficacy of various anticancer agents. For example, using a high-throughput drug screening platform for patient-derived colorectal organoids, Toshimitsu and colleagues discovered that bromodomain and extra-terminal (BET) bromodomain protein inhibitor as a cancer-selective growth suppressor that targets genes were aberrantly activated in colorectal cancer (34). Using genome and transcriptome data together, they discovered an association between checkpoint with fork-head and ring finger domains (CHFR) silencing and paclitaxel sensitivity. Similarly, Broutier and colleagues identified novel drugs for liver cancer treatment (30). They developed an organoid model for the three most common primary liver cancer (PLC) subtypes: hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and combined HCC/CC (CHC) tumors. Multi-omics analysis using genomics and transcriptomics data of PLC-derived organoids discovered the ERK inhibitor SCH772984 as a potential therapeutic agent for primary liver cancer. Using tumor organoids from mouse endometrial cancer, Chen and colleagues performed drug screening with a library of small molecules that targets epigenetic factors. Their RNA-seq and CHIP-seq results showed that the hypoxia-inducible factor (HIF) pathway was the most significantly downregulated pathway by MI-136 treatment (35).

Moreover, the accumulation of organoid models has led to the establishment of organoid biobanks and enabled a large-scale drug screening which successfully revealed several unexpected drugs to be tested and approved in clinical trials. Yan *et al*. established a primary gastric cancer organoid (GCO) biobank that comprised normal, dysplastic, cancer, and lymph node metastases (n = 63) from 34 patients with detailed whole-exome and transcriptome analysis (36). They identified gastric cancer therapeutic agents in the clinical phase of development, including Napabucasin, Abemaciclib, and the ATR inhibitor VE-822. The OncoTrack consortium built a large biobank of 106 colorectal cancer patients (stages I-IV) and developed a pre-clinical platform composed of 35 organoids and 59 xenografts, with extensive omics data comprised of a compendium of drug sensitivity data totaling $>$4,000 assays testing 16 clinical drugs (37). Linking molecular profiles with drug sensitivity patterns identifies novel biomarkers, including a signature that outperformed RAS/RAF mutations in predicting sensitivity to the EGFR inhibitor cetuximab.

The proteomics data from organoids is also applied in drug screening. Schumacher and colleagues analyzed a cohort of well-characterized colorectal cancer organoids and found heterogeneous activation of mitogen-activated protein kinase (MAPK) signaling pathway in the targeted proteomics data. They showed that the heterogeneity resulted in variable responses to epidermal growth factor receptor (EGFR) inhibition (38). Drug screening was also conducted in diverse disease models including cancer models. Mills and colleagues performed functional screening of 105 small molecules with pro-regenerative potential in cardiac organoids (39).

The key objective in the multi-omics analysis is to capture a robust biological signature. Common approaches for identifying robust signatures include (i) leveraging regulatory or protein-protein interaction networks (40, 41) and (ii) using machine-learning (ML) techniques, such as deep neural networks (42). As important signatures may be undiscovered when using single-omics data, the application of multi-omics methods in organoid models will become increasingly important as more sequencing data from organoid models become available.

## IMAGE-BASED ANALYSES OF ORGANOIDS

Unlike omics-based sequencing methods, image-based data provide information on cell composition, morphology, and their positional location during cell development, which are closely related to cellular function and phenotypic outcome of an organism (36). Conventional image-based data includes the use of brightfield microscopy, H&E imaging, and immunostain-

ing techniques (36). Interestingly, recent advances in high-resolution microscopy and 3D confocal staining have made it possible to generate 3D organoid images, which in turn have led to the development of high-content screening (HCS) (43, 44). Lukonin and colleagues used HCS to characterize intestinal organoids and to accurately identify their developmental trajectory (45). In brain research, where research using organoids are currently being actively conducted, high-throughput screening results were verified by checking the shape, size, total gene expression, cell composition, and structure through image data (46). Recently, there have been efforts to develop a drug screening platform based on high-resolution 3D imaging technology (34). All of these efforts resulted in an increased number of image data for various organoids.

As high-throughput imaging techniques become more available, it is likely that ML techniques will be actively applied to help automate analyses. Specifically, organoid images form optically opaque aggregates due to dense, diverse, and tightly interacting cells, posing difficulties for human experts to conduct cell segmentation and phenotypic annotation of organoid image data (47). To solve such limitations, recent studies have focused on applying ML techniques to accurately interpret image data from organoids. For instance, Gritti and colleagues utilized a relatively simple ML model (*i.e.* Logistic Regression and Multi-layer perceptron) for efficient segmentation, quantification, and visualization of large-scale image data of organoids (48). Also, there were efforts to handle organoid images with more sophisticated ML techniques including convolutional neural networks (CNNs). Kassis and colleagues utilized a CNN for automated recognition and quantification of human intestinal organoids with brightfield images (49). Kok and colleagues tracked the development of organoids by recognizing histone H2B-mCherry stained organoid images in single-cell resolution relying on a CNN (50). An interesting research opportunity in organoid image-based tasks would be to apply high-performance image recognition ML models that are being developed in the field of computer vision.

## CONCLUSION

Organoids show close similarities in molecular profiles, morphological characteristics, and drug responses with human tissues with respect to it. However, because organoid technology is still in its infancy, the ability to accurately mimic mature organs observed in human requires further investigation. One of the approaches for improving organoid models is to incorporate a tissue microenvironment. Kim and colleagues created a bladder organoid that includes tissue stem cells with stromal components termed assembloids (51). It was able to recapitulate regenerative responses to bladder injury that matches *in vivo* regeneration patterns. Moreover, the bladder assembloid revealed a master pioneer factor, Forkhead Box A1 (FOXA1), that determines tumor phenotype in bladder cancer. Neal and colleagues used an air-liquid interface (ALI) to incorporate tumor organoids with immune cells (52). The organoid model using ALI method was treated with immunotherapies (anti-programmed cell death protein 1 or anti-programmed cell death ligand 1) and successfully observed tumor cell death. Altogether, further investigations to improve organoid models will be the key to the development of experimental techniques that will adequately incorporate stromal and immune cell components that match *in vivo* tissue environments.

A better understanding of the similarities between human and organoid models for improved organoid technology is likely to be an active research subject. In this respect, computational methods developed to quantify similarities between pre-clinical models and humans can be applied in organoid research. Han and colleagues have previously developed computational methods to understand similarities between human and mouse models (53, 54), which can be applied in organoid research as the number of sequencing data and phenotypic profiling in organoid models increases.

## CONFLICTS OF INTEREST

The authors have no conflicting interests.

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