새로운 약물 검색 플랫폼: 종양 오가노이드

서울대학교 의과대학 ¹암연구소 세포생물학연구실, ²의과학과, ³암연구소 한국세포주은행, ⁴허혈저산소질환연구소

맹주은^{1,2,3} · 서하영^{1,3} · 김순찬^{1,3,4} · 구자록^{1,2,3,4}

Novel Drug Screening Platform: Tumor Organoid

Ju Eun Maeng^{1,2,3}, Ha-Young Seo^{1,3}, Soon-Chan Kim^{1,3,4}, Ja-Lok Ku^{1,2,3,4}

¹Laboratory of Cell Biology, Cancer Research Institute; ²Department of Biomedical Sciences; ³Korean Cell Line Bank, Cancer Research Institute; ⁴Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Pancreatic ductal adenocarcinoma (PDAC) is known to be one of the most lethal cancers among all cancer types, with a relative 5-year survival rate of less than 8%. Currently, surgery is the only probable curative treatment for PDAC which is available for only 10-15% of the patients diagnosed with the cancer. Organoids resemble the original tissue in morphology and function with self-organizing capacity. Organoids can be cultured with high effectiveness from individual patient derived tumor tissue which makes them an extremely fitting model for translational uses and the improvement of personalized cancer medicine. Before personalized medicine based on organoids can be applied in the clinic, the improvement of drug screening platforms in terms of sensitivity and robustness is necessary.

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Corresponding author: Ja-Lok Ku

Laboratory of Cell Biology, Cancer Research Institute, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Korea

Tel. +82-2-3668-7919 Fax. +82-2-742-0021

E-mail: kujalok@snu.ac.kr

ORCID: <https://orcid.org/0000-0002-7090-537X>

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and fatal cancers among all cancer types, with a relative 5-year survival rate of less than 8%.¹ Surgery, the only probable curative option for PDAC, is available for only 10-15% of the patients diagnosed with the cancer.² The low survival rate is due to diagnosis at late stages which is why new methods to treat PDAC are critical.³

Organoids resemble the original tissue in morphology and function with self-organizing capacity. They are microscopic 3D

structures grown from tissues, pluripotent stem cells, or embryonic stem cells.

Patient derived tumor and corresponding healthy organoids are cultured and biobanked in big groups. These biobanks can be applied to discover if organoids hold predictive value for drug responses for individual patients. In order to strengthen the statistical ability to that required to correspond genetic markers with differences in drug reactivity, increasing the quantity of biobanked organoids will be essential.⁴ Before personalized medicine based on organoids can be applied in the clinic, the improvement of drug screening platforms in terms of sensitivity

and robustness is necessary.⁵ Using organoids to target molecular pathways that contribute to cancer pathogenesis will eventually help for personalized drug screening and the improvement of cancer treatment.

MAIN BODY

1. Establishment of organoids

Organoids are small self-renewing 3D structures that are produced *in vitro*. They show many similarities functionally and structurally in comparison to their complement organs. Accurate prediction of drug responses in a personalized treatment setting can be achieved by organoids.⁶

Original tissue is digested enzymatically or mechanically into small fragments and then embedded in a matrix to produce organoids. To generate 3D organoids, the most commonly used matrices are Collagen and Matrigel (Corning, Corning, NY, USA). In order to provide mesenchymal-based signals, differentiation modulators and various growth factors are essential. For example, epidermal growth factor, fibroblast growth factor 10 (mitogens), Rspo1 (enhances Wnt signaling), Noggin (inhibits bone morphogenetic protein [BMP] signaling), Wnt3a, nicotinamide, N-acetylcysteine, gastrin and A83-01 (Alk inhibitor) are needed. Moreover, for normal human 3D organoids, prostaglandin E2 is required.⁷ Tumors resected from surgery along with biopsies like fine needle aspirates that have limited material can produce

organoid models.⁸⁻¹⁰

In contrast to classical 2D cell lines, organoid application is more efficient in establishing patient derived cultures.⁵ As a result, tumor-derived organoid biobanks have been made with various organs. These organoids can be used to solve translational research questions as well as replicate tumor attributes.¹¹

Organoids are quintessential in examining each stage in tumorigenesis because they can be passaged endlessly and cryopreserved. Also, they are compliant to transcriptomic, genetic, proteomic, and biochemical analyses.³

Patient derived organoids (PDO) maintain morphological features of the primary tissue. Organoid cultures can be controlled in culture by using specific growth factors to sort out cells with tumor specific genetic alterations or by pharmaceutical inhibition. A prominent problem when growing tumor-derived organoids is the overgrowth of contaminating cells in tumor samples. Controlling organoid culture can be a probable solution to this problem.¹¹

2. Applications of pancreatic cancer organoid

1) Pancreatic cancer organoids in basic research

Initially, organoid technology was used to study untransformed healthy tissue. Eventually, the culture system has been employed to examine tumors, including PDAC models.¹²

The success rate of selecting and generating PDAC organoid models was quite alike (>70%) between fine-needle biopsies and

Table 1. Pancreatic cancer organoids in literature

Organ of origin	Species	Number of lines	Histological subtypes	Year	Reference
Pancreas	Human	8	Ductal adenocarcinomas	2015	8
Pancreas	Mouse	19	Ductal adenocarcinomas	2015	8
Pancreas	Human	17	Ductal adenocarcinomas	2015	17
Pancreas	Human	39	Ductal adenocarcinomas	2018	36
Pancreas	Human	114	Ductal adenocarcinomas	2018	9
Pancreas and distal bile duct	Human	30	Ductal adenocarcinomas	2019	11
Pancreas	Human	5	Ductal adenocarcinomas	2019	39
Pancreas	Human	5	Intraductal papillary mucinous neoplasms	2020	37
Pancreas	Human	31	Ductal adenocarcinomas	2020	40
Islets	Human	1	Ductal adenocarcinomas	2020	40
Pancreas	Human	15	Intraductal papillary mucinous neoplasms	2021	38

resected tumor tissues in a large group analysis. Hence, organoid models could be produced from all stages of PDAC.^{13,14}

With the range to be administered to many significant features of pancreatic tissue pathology, organoids denote a potent device for research. Pancreatic 3D organoids can be used for drug screening and assessment of promising diagnostic biomarkers due to the generation of organoids made in a short period of time from small amount of tissue. Being that 3D organoids can be cultured from both surgical samples and biopsy samples or endoscopic

fine-needle aspiration (FNA), various stages of cancer and clinical conditions can be closely resembled with this technology.⁸ Table 1 lists the number of organoids established in paper over the years. Pancreatic cancer organoids display different morphologies. Fig. 1 shows brightfield microscopy images of SNU-5790-TO and SNU-5813-TO that show cystic structures with clear lumen.

Anne Grapin-Botton and Hans Clevers have both established procedures to culture organoids in Matrigel from normal murine pancreata.^{15,16} So as to achieve pancreatic duct development,

Fig. 1. Brightfield microscopy images of pancreatic cancer organoids. Bar indicates 500 μ m.

Fig. 2. Confocal microscopy images of pancreatic cancer organoid. SNU-5577-TO. Red: phalloidin, Blue: DAPI, Green: CD-133. Bar indicates 50 μ m.

Gräpin-Botton and colleagues grew murine embryonic pancreas cells inside Matrigel.¹⁵ These embryonic pancreatic organoids grew rapidly in culture and went through differentiation. The Clevers group used a different approach from Gräpin-Botton. They used adult murine pancreatic duct cells to establish organoids,¹⁶ building on their previous research.³ Pancreatic duct cells formed rapidly growing cystic spheres when inserted into serum-free Matrigel with a combination of growth factors.

These methods were used by The Muthuswamy groups in order to grow organoids with high effectiveness from surgically resected human PDAC tumors.¹⁷ Human PDAC organoids grew as filled spheres and displayed dysplastic morphologies. Organoids transplanted into mice developed adenocarcinomas that mirrored the original tumor. Moreover, organoids could endure cryopreservation and be passaged continually. Similar to the numerous histologies found in primary tumors, a culture of human PDAC organoids exhibited various histologies which suggests that the organoid culture system may represent the intratumoral heterogeneity in primary tumors.³

The Kuo laboratory¹⁸ developed a culture system using an air-liquid interface with essential growth factors such as stromal support cells. This was different from the usual culture system which used medium containing tissue specific growth factors. Induced pluripotent stem cells can also be used to culture organoids.¹⁹

Patient derived organoids from cancer tissue mimic the pancreatic microenvironment very well and they are more developed in research than pluripotent stem cell (PSC) derived organoids. Nonetheless, considering the multipotent characteristic of PSCs and the microenvironment that produces required factors, organoids cultured from PSCs need fewer growth factor supplements.

2) Coculture methods and cancer associated fibroblasts (CAF) subtypes

A coculture model of pancreatic stellate cells and pancreatic cancer 3D organoids was established by Ohlund et al. With this method, they noticed that fibroblasts and organoids proliferated rapidly. Furthermore, they came across different levels of interleukin-6 and smooth muscle actin which showed

heterogeneity between cancer-associated fibroblasts. These characteristics displayed similar functions to the organoids. Consequently, these results demonstrate the intricacy of the stroma and its importance in epithelial tumorigenesis.²⁰

The coculture of pancreatic cancer organoids with pancreatic stellate cells brought about the discovery of pancreatic CAF subtypes. As well as the ones that aided organoid proliferation by secreting interleukin-6.²⁰ Methods to modify CAF formation in tumors were disclosed by biochemical pathways of distinctive CAF subtypes identified by further research with PDO-CAF cocultures.²¹ Researchers and clinicians will be able to evaluate numerous immunotherapy strategies before clinical applications, with a strong patient-matched co-culture system.²²

As a consequence of the poorly immunogenic quality of PDAC, current evidence strongly indicates crucial limiting problems ahead for development of immunotherapy in PDAC cure. Throughout the virulent alteration and development of PDAC, the inborn aggressive quality of the cancer may be associated with its lacking immunogenicity and deficient immune activation. Nevertheless, the immune system is sufficient with diverse immune cells with various effector pathways including natural killer cells, cytotoxic T cells and T helper cells that provides a promising future for overcoming poor immunity in PDAC.²²

3) Precision medicine with organoids

Pancreatic cancer organoids are representations of human PDAC that can be quickly produced from surgically resected tumors.⁸ Organoids imitate the tumor of patients, as well as the stromal components thought to be accountable for chemotherapy resistance.^{8,23,24} Human PDAC organoids can be fabricated rapidly from less material as opposed to patient derived xenografts which need rather large amounts of tumor tissue and may take many months to form in the host organism.²⁵ Hence, by examining possible therapeutic targets, organoids could be employed for personalized cancer treatment.

The study of advanced and metastatic patients is possible by using pancreatic ductal organoids which are ex-vivo models of PDAC that can be set up from small biopsies. Pancreatic cancer research of organoid models proposes an encouraging stage for precision medicine applications.¹²

As genetic characteristics of disease such as driver mutations and chromosomal copy number are maintained, organoids provide a standard model for patient specific assays.²⁶ The discovery of pancreatic cancer frequently requires compilation of a biopsy, like an endoscopic ultrasound-guided FNA. Organoids can be generated from simply one needle pass with high chance of success, distinct from other culture technologies.⁸ PDAC organoids cultured from fine needle biopsies (FNB) allows for procurement of tissue from the tumor before chemotherapy, providing a more general understanding of the pancreatic tumor itself. Organoid models give potentiality of ex-vivo therapeutic testing and genomic characterization in PDAC patients which have been overlooked in research. PDAC organoids could be passaged, genotyped, and tested with authorized standard of care therapies in less than a few weeks once set up. This likely permits for organoid-guided therapeutic options offered to the patient.³

Fig. 2 displays images of pancreatic cancer organoid taken with confocal microscopy. Red indicates phalloidin which stains actin filaments. 4 ,6-Diamidino-2-phenylindole (DAPI) binds to DNA

and the fluorescent dye shows the color blue. CD133 is a cell surface marker expressed by immature hematopoietic stem cells.

3. Drug screening of organoids

Prior to or during the application of clinical therapy for a PDAC patient, organoids can give a platform for drug testing of independent tumors in a small amount of time. After receiving the biopsy of a patient, large-scale drug screens were able to be carried out within 3-4 weeks while some groups have announced a 1-week time period from biopsy to drug selection.²⁷

2D cancer cell lines lacking reflection of the original tumor tissue may have provided the lack of success of newly discovered drugs in clinical trials,^{28,29} despite the fact that drug screen on their compilations have produced major insights into genetic conjectures of drug response.³⁰ Patient derived tumor organoids may better represent diagnosis and test of new anticancer drugs because they recapitulate native tumors well. High-throughput drug screening technologies in patient derived organoids is starting

Fig. 3. Drug screening of pancreatic cancer organoid.

to expand. Organoid biobanks that have carried out small-scale drug screen until now have produced favorable outcomes.⁵

To test for drug sensitivity of pancreatic organoids in our lab, TryPLE solution is used to mechanically dissolve the basement membrane extracts (BME) dome. 6-9 mL of TryPLE is added to the organoid embedded in BME and transferred to a 15 mL conical tube. The tube is incubated in 37°C water bath for 15 minutes. The conical tube is centrifuged and the supernatant is completely aspirated. Human pancreatic culture medium (HPCM) and BME gel is mixed with 1:1 ratio. 60 µL of the solution is added to each well of the 96-well plate using a 12 channel multipipette. After 30 minutes, 20 µL HPCM is added to each well. After 96 hours, drugs are applied to the embedded organoids.

Fig. 3 presents the morphological changes of pancreatic cancer organoid to the drug concentration. As the concentration of Gemcitabine increased, the degeneration of the organoids was noticeable. Similar results can be seen in different passages for the sensitivity test of gemcitabine of SNU-3947-TO.

Pharmacotyping which shows organoid responses to therapeutic testing, matches patient responsiveness to chemotherapy. Either the establishment of new automation methods planned to work with 3D cultures or the adjustment of 3D culture techniques to existing automation systems is necessary for high-throughput drug screening of organoids. Yet, it has the ability to detect transformative treatment approaches.³¹

High throughput drug screening in patient derived organoids discloses sensitivities to a variety of therapeutic agents. A comparable reaction was discovered for factors targeting the identical biological procedure or molecular pathway. Drugs could be distinguished for which the individual PDOs was more responsive than all other PDOs examined for many of the PDOs tested. Once more, the majority of efficient drugs often found were the numerous drugs aiming the same molecular pathway. All things considered, these discoveries support the theory that precise targeted therapies will be successful in only a small percentage of patients. Thence, to choose the proper drug for each individual patient, a personalized application will be needed.¹¹

Organoid biobanks were gathered by Marc van de Wetering and colleagues.³² They came up with significant contrast and discovery

of tumor-specific DNA and RNA differences by completing deep genomic and transcriptomic analyses using both tumor and adjacent-normal organoids. To recognize the compounds the organoids were responsive to, tumor organoids were tested in a high-throughput manner utilizing a custom collection of therapeutic composites. This method brought about the recognition of practical patient-specific therapy. Mutation-based drug sensitivities, which were formerly well-known, were proved by the association between therapeutic feedbacks and mutational position. In essence, sequencing analysis alone was not capable of projecting some therapeutic reactions, emphasizing the importance of such a proposition.³

Next-generation sequencing (NGS) is the key model of precision medicine in pancreatic cancer today. NGS permits for the analysis of many of the established parts of the genome in order to discover different point mutations from a very small amount of tumor tissue gained from the primary pancreatic tumor.³³ Additionally, this allows "panel testing" for particular groups of mutations developed in genes that are linked with pancreatic malignancy.^{34,35} The data can be applied in the choosing of different chemotherapy procedures known to be more successful in the presence of particular types of pancreatic tumors. NGS is in the early phase considering its possible treatments of the disease on the whole and stands for one type of precision medicine. Organoids are a powerful modern establishment for translational research and precision medicine in pancreatic cancer. They can be produced from surgically resected tumors and are basically small tumor models of individual human PDAC.⁸ When reconstituted with fibroblasts and stroma in particular, organoids may imitate the complete range of a patient's tumor.²⁰

CONCLUSIONS

Effective organoid establishment is vital for personalized medicine for patients with pancreatic cancer that can not be removed surgically. Organoids can be used to represent and research cancer initiation and development in many organs. Also, they are genetically and phenotypically stable, can be cryopreserved and passaged long term. Organoid technique can be used to examine signaling pathways and cancer related processes. A

significant benefit of using organoid technology for drug development is that both healthy and tumor tissue can produce organoids. This permits for screening for drugs that particularly select tumor cells while leaving healthy cells undamaged resulting in diminished toxicities in patients. Organoids can be cultured with high effectiveness from individual patient derived tumor tissue which makes them an extremely fitting model for translational uses and the improvement of personalized cancer medicine. To enhance the application of organoid models to basic and translational research, a number of problems need to be addressed. For instance, reducing the costs for organoid culture, cutting the time for organoid growth, improving the productivity and mimicking the tumor micro-environment of the original tumor will further develop research for organoids. Although current problems need to be approached, organoid technology is quickly developing and the chances that this method will have a beneficial effect for basic cancer research and clinical advance is evident.

요약

췌장암은 발생률 비례 생존율이 가장 낮은 암으로, 5년 생존율이 8% 미만이며 최초 진단 환자의 10-15%만 수술이 가능한 것으로 알려져 있다. 오가노이드는 형태와 기능면에서 본래 조직의 형태와 기능을 잘 반영하며, 배양법을 최적화 함에 따라 개별 환자 유래 종양 조직에서 높은 효율로 배양할 수 있다. 오가노이드는 개별 환자 유래 종양 조직에서 높은 수렴율을 보이므로 개인 맞춤형 치료 모델에 적합하다. 하지만 오가노이드를 기반으로 한 개인 맞춤형 의료가 임상에서 적용되기 위해서는 실험의 정확도 측면에서 약물 스크리닝 플랫폼의 개선이 필요하다.

국문 색인: 췌장암; 오가노이드; 약물 스크리닝; 바이오뱅크; 차세대 염기서열 분석

Conflicts of Interest

The authors have no conflicts to disclose.

ORCID

Ju Eun Maeng	https://orcid.org/0000-0002-0900-9816
Ha-Young Seo	https://orcid.org/0000-0001-6063-9680
Soon-Chan Kim	https://orcid.org/0000-0002-7969-4804
Ja-Lok Ku	https://orcid.org/0000-0002-7090-537X

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