EUS-guided fine-needle technique facilitates the establishment of organoid biobanks

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A biobank, or a biospecimen bank, refers to the standardized collection, processing, and storage of biological samples, such as macromolecules, cells, tissues, and organs; as well as the associated clinical, pathological, treatment, follow-up, informed consent information; along with quality control, information management, and application systems.[1] The rapid growth of various life science fields and the introduction of translational medicine have increased the need for biospecimen resources. Biobanks play an irreplaceable role in the research on the prediction, diagnosis, and treatment of human diseases. As an essential resource for basic research and translational medicine, biobanks have become highly valued by countries over the years.[2-4]

DIFFERENT BIOBANK SAMPLE COLLECTION STRATEGIES

Biobanks are repositories that collect, process, store, and distribute biological samples and associated data for basic, translational, and clinical research. They collect highquality human biological samples (such as tissues, blood, and other body fluids) and associated clinical data, which create an essential scientific foundation for personalized medicine. Identifying biomarkers associated with specific diseases such as tumors, cardiovascular diseases, and neurological diseases is beneficial for the early detection, prevention, and treatment of diseases.[5] The identification of tumor biomarkers for tumor diagnosis and prognosis and to increase the effectiveness of tumor treatment is critical for personalized medicine, which is rapidly changing the treatment approach in many diseases. A vital requirement for personalized medicine is the collection of a large number of patient samples that are annotated with the complete clinical and pathological information.[6] In this respect, biobanks are critical in the development of personalized medicine.

The identification of disease-associated biomarkers from the blood or tissue samples of patients is beneficial for the early detection, prevention, and treatment of these diseases, and this helps predict prognosis and design personalized medicine.[7] Samples collected from patients can be screened for specific gene mutations to formulate personalized medicine. For instance, tests can be performed on tumor samples of patients with non-small cell lung cancer to determine the presence of epidermal growth factor receptor (EGFR) mutations. If the results are positive,

Access this article online
Quick Response Code:
Website: www.eusjournal.com
DOI: 10.4103/eus.eus_79_20

How to cite this article: Yang F, Wang S, Guo J, Liu X, Ge N, Wang G, *et al*. EUS-guided fine-needle technique facilitates the establishment of organoid biobanks. Endosc Ultrasound 2020;9:355-60.

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Received: 2020-09-01; **Accepted:** 2020-10-26; **Published online:** 2020-12-14

the patients may receive personalized medicine with anti-EGFR tyrosine kinase inhibitors, such as gefitinib and erlotinib.[8] Personalized medicine for a patient is the most suitable treatment, with the least toxicity for that particular patient.

The development of personalized medicine depends on readily available, high-quality human biological samples with complete medical history. However, most existing biobanks consist of large-scale paraffin-embedded tissue blocks or DNA banks, which can only provide limited personalized information at the DNA, RNA, and protein levels.[6] As drug action in the body is complex, and the effectiveness of the same drug is different for each patient, the information provided by conventional biobanks cannot estimate the actual *in vivo* drug effectiveness. Thus, an *in vitro* model biobank that allows personalized validation of treatment effectiveness is urgently needed, particularly in the current era where immunotherapy in the field of immuno-oncology is undergoing rapid development.[3] However, given the complexity of the tumor microenvironment, the results obtained using immunotherapeutic agents *in vitro* cannot be translated directly into clinical efficacy. Thus, biobanks that simply provide data at the DNA, RNA, and protein levels cannot meet the current needs of personalized treatment.

Organoid technology provides the technical means to meet this demand. Organoids are cell cultures obtained through three-dimensional (3D) cell cultures or other techniques that exhibit certain key features of the corresponding organs. They may be derived from somatic cells, adult stem cells (including progenitor cells), or pluripotent stem cells. Such *in vitro* culture systems include a self-renewing stem cell population that can differentiate into multiple organ-specific cell types.[9,10] Compared with 2D models, the composition and behavior of organoids more closely resemble that of actual organs. Their genomes are stable, and they are more suitable for use as research models for biological transfections and high-throughput screening. Furthermore, organoid models are more straightforward, yield faster results, and allow more direct observations compared with the commonly used animal models. Since the successful establishment of epithelial organoids in 2009,[11] multiple organoid models have been generated, including colon, liver, pancreas, prostate, stomach, fallopian tube, ovary, taste bud, salivary gland, esophagus, lung, endometrium, mammary gland, and blood vessels.[12]

Research and development on antitumor drugs in the past, relied heavily on tumor cell cultures *in vitro* and rodent (mainly mouse) tumor models. Irrespective of the animal models used, considerable differences exist between these models and humans with respect to the changes during the course of the disease and drug responsiveness. Animal models often represent only one stage of the disease and fail to recapitulate the entire process of tumor development in all other aspects, such as ethology, timeframe, and progression. Thus, the clinical effectiveness of antitumor therapies developed based on these models cannot be predicted. More importantly, the genetic background, growth environment, pathogenic factors, and treatment of laboratory animals are too simple and cannot be applied to the diverse tumor types and different patients in complicated clinical settings. The limitations of animal models have prompted researchers to study patient specimens directly. Human-derived tumor cell cultures provide researchers with an opportunity to study the features of patient-specific tumor cells and their drug sensitivity. However, not all tumors can be successfully expanded *in vitro*. Isolating and culturing tumor cells *in vitro* eliminate their interactions with other components in the tumor microenvironment, which are critical for tumor development and drug responsiveness. Similar problems exist in patient-derived xenografts in immunodeficient mice. First, the success rate of transplantation is low. In addition, the tumor microenvironment in immunodeficient mice differs significantly from the internal environment of the human body and may lead to mouse-specific tumor evolution.[13,14]

Compared with that of the conventional 2D culture and tumor xenografts, the success rate of tumor organoids is significantly higher. Furthermore, tumor organoids can be cultured rapidly at a low cost for a prolonged period. Shorter timeframes and lower costs facilitate genetic modifications and large-scale drug screening. The features of tumor tissues are retained in 3D cultures; thus, the impact of the tumor microenvironment is not lost during the research, thereby providing a more realistic environment for the development of antitumor drugs. Tumor organoids can better recapitulate the diversity and complexity of the tumors, from which they are derived with respect to their genetic makeup, transcription profiles, metabolic status, cytology, and histology. More importantly, *in vitro* cultures do not lead to an apparent homogeneous cell population in tumor organoids. Tumor organoids

recapitulate many features of *in vivo* tumors, and they currently serve as a bridge between basic tumor research and clinical applications.[15-17]

Organoids can be propagated (expanded in cultures), cryopreserved (stored), and resuscitated (immortalized), which increases the feasibility of establishing organoid banks. The establishment of organoid biobanks is also necessary because organoids allow the modeling of human carcinogenesis in a dish, and can be routinely derived from normal human tissue. This allows the *in vitro* mutational modeling of all stages of malignancy. Moreover, unlike the existing large-scale paraffin-embedded tissue blocks or DNA banks, living tumor organoids represent higher pathological and biological value. They are important in many fields of research, including tumor pathogenesis, drug combination/novel drug evaluation, and novel biomarker studies. Currently, an organoid biobank has been established by the nonprofit company Hubrecht Organoid Technology (HUB) in the Netherlands (www.hub4organoids.eu). Similar to the American type culture collection, the HUB organoid biobank contains more than 800 different types of organoids with known features, such as genetic data, for use in scientific research institutions and businesses. Meanwhile, different tumor culture models are also constantly being established and developed as shared resources by The Human Cancer Models Initiative. Numerous studies have also reported attempts to establish organoid biobanks.

ESTABLISHMENT OF AN OVARIAN CANCER ORGANOID BIOBANK

In 2019, the research groups of Hans Clevers and Wigard P. Kloosterman in the Netherlands published the results of their scientific research in *Nature Medicine*. They established *in vitro* ovarian cancer (OC) organoid models capable of long-term expansion, and their models covered most of the OC subtypes.[18] In their study, tissue specimens from patients with OC were used to induce organoids, which were established as *in vitro* OC models for long-term cultures. They established 56°C organoid lines from 32 patients, covering most OC subtypes. Cell morphology and immunohistochemistry confirmed that the features of the established organoids were highly consistent with tissue sections form patients.

In addition, their study demonstrated the applications of OC organoids for drug screening. The response of different tumor subtypes to platinum-based chemotherapy was also investigated, including the development of chemoresistance in recurrent disease. OC organoids could be xenografted, thereby exhibiting potential application in *in vivo* drugsensitivity tests. They provided a vital platform for future research on drug screening and responses of different OC subtypes to chemotherapy.

ESTABLISHMENT OF A BREAST CANCER ORGANOID BIOBANK

Sachs *et al.* also employed organoid technology and established a breast cancer organoid biobank to capture the heterogeneity of breast cancer.[19] They collected tissue samples from more than 150 patients with breast cancer and utilized a protocol for the long-term culture of organoids while maintaining their features. More than 100 breast cancer organoids were successfully established, with a success rate of more than 80%. They also performed histopathology, whole-genome sequencing, transcriptome sequencing, and drug sensitivity tests on the breast cancer organoids. Collectively, their results showed that breast cancer organoids retained the histological and genetic features of the original tumors. Furthermore, these features were retained in the organoids after extended passaging. Ultimately, their research team established a biobank of breast cancer organoids that could facilitate scientific research and drug development.

ESTABLISHMENT OF A BLADDER CANCER ORGANOID BIOBANK

In the US, Lee *et al.* reported the establishment of a patient-derived organoid biobank; these organoids recapitulated the histopathology and molecular diversity of human bladder cancer.[20] Samples from patients of different ethnic groups and sexes, containing two subtypes of bladder cancer at different stages, were used to establish 16 organoid systems. The organoid systems could recapitulate the mutational spectrum of human bladder cancer. The organoids could also be used for clinical drug screening, and their mutational spectrum could be used to predict drug responses.

ESTABLISHMENT OF GASTRIC CANCER ORGANOID BIOBANK

Seidlitz *et al.* established the largest gastric cancer organoid biobank known to date. In-depth analyses

of the gastric cancer organoids regarding genomic variations, molecular profiling, chemotherapy sensitivity, and sensitivity to targeted therapy were performed.[21] A total of 20 gastric cancer organoids were generated, four of which were selected for further investigations. All four selected organoids were stably passaged for more than 1 year, indicating the stability of the organoid models. Transplantation of these organoids into immunodeficient mice resulted in the establishment of xenograft tumors. Intriguingly, the growth rate of the xenograft tumors was consistent with that of the organoids, indicating that the gastric cancer organoids properly recapitulated the biological behavior of gastric cancer. The report by Seidlitz *et al.* is undoubtedly a core study for gastric cancer organoids. Not only a large biobank of gastric cancer organoids was generated but molecular profiling based on the cancer genome atlas datasets was also performed. Furthermore, they also proved the feasibility of targeted therapy testing using gastric cancer organoids and specific targets.

Despite significant progress being made, the field of organoid research still faces many challenges. The most important aspect is the tissue source for organoid cultures. Currently, most tumor organoids are generated from surgically resected tissue specimens. However, most patients are diagnosed with cancer at an advanced stage that is not conducive to surgery. This severely limits the opportunities for generating tumor organoids using surgical specimens. A more challenging issue is that patients with cancer who relapse following radiotherapy and chemotherapy, not only lose the opportunity for surgery, but they also become unfit to undergo surgery even if they meet the criteria. Thus, the conventional approach of generating organoids using surgical specimens followed by drug screening for personalized medicine is simply impossible. This limitation also dramatically hampers the number of organoid biobanks.

The endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) technique effectively overcomes the shortcomings and limitations of generating organoids via surgery.[22] The use of EUS-FNA to obtain organoid cultures exhibits many advantages. First, biopsy through EUS-FNA enables the collection of tissue aspirates through inserting a fine-needle into the target under continuous real-time ultrasound guidance. The cells or tissues obtained from FNA are smeared onto a glass slide to perform real-time analysis of abnormalities and cancer diagnosis. Afterward, the specimens can be used immediately to generate organoid cultures in the laboratory.[23,24] This method eliminates the lengthy pathological diagnosis period of the conventional approach, where surgically resected tissue is used to culture organoids, which sometimes leads to the growth of nontumor organoids. Second, EUS-FNA can be performed at any phase of the disease, including before surgery, before or after radiotherapy and chemotherapy, or even after recurrence. In addition, endoscopic examination procedures are often safer than surgical operations.[25] Finally, EUS-FNA enables the extraction of tissues from almost all structures of the chest, abdomen, and pelvis through the gastrointestinal tract.[26-28] This greatly facilitates the use of EUS-FNA to generate a variety of tumor organoids.[29,30] Researchers have successfully established organoid cultures *in vitro* through needle biopsies of liver cancer,[31] pancreatic cancer,[30] and colorectal cancer with liver metastases.[32] These studies further affirm the feasibility of utilizing EUS-FNA to prepare organoids and the possibility of establishing organoid biobanks.

The quality and quantity of biobanks are critical for determining whether substantial progress can be made in translational medicine and personalized medicine. EUS-FNA enables real-time diagnosis and organoid generation. It is unrestricted by the stage of the disease and can reflect the status in real-time. It can also be used to generate organoids of almost all tissue types. These advantages demonstrate that EUS-FNA can significantly facilitate the establishment of organoid biobanks. It is almost impossible to establish a comprehensive and diverse organoid biobank that covers all diseases without utilizing EUS-FNA.

Other challenges in the field of organoid research also need to be overcome to match scientific ambitions. First, organoids do not contain all components of the microenvironment in the human body. Moreover, co-culturing with immune cells or fibroblasts presents problems, such as the incorporation of blood vessels and nerve cells into the culture systems to establish multidimensional cultures that are yet to be addressed. Second, culturing techniques for tumor organoids are complex, with different tissues and even different subtypes requiring different culturing conditions. Thus, further investigations are needed to identify a more rational approach;[33,34] however, overall, organoids can adequately recapitulate the genetic, physiological, and morphological features of the corresponding *in vivo* tissues. They are also suitable for use in

high-throughput drug screening, which suggests that organoids can serve as research models for personalized therapies.[35]

Thus, with the development and discovery of new technologies, organoids have the potential to become more widely adopted and available in the future. Organoid biobanks, as a new type of a living biological database, will also become valuable resources for primary disease and clinical research, drug development, and novel clinical diagnostic and treatment research. EUS-FNA will also play an irreplaceable role as an important means of obtaining tissues for establishing organoid cultures. Finally, it must be emphasized that the formulation of systematic and elaborated access rules for organoid biobanks is necessary to enhance their ethical management and control the increasing risks of privacy violation.

Financial support and sponsorship

This study was supported by grants from National Key Research and Development Project of China to Siyu Sun (Grant No. 2017YFC0109803), from the National Natural Science Foundation of China to Siyu Sun (Grant No. 81900601), from the Special Project from the central government of Liaoning Province to Siyu Sun (Grant No. 2018107003), from the Liaoning Province Medical Science and Technology Achievements Transformation Foundation to Siyu Sun (Grant No. 2018225120), from the China Postdoctoral Science Foundation to Fan Yang (Grant No. 2020M670101ZX), from the Doctoral Scientific Research Foundation of Liaoning Province to Fan Yang (Grant No. 2019-BS-276), from the Science and Technology Program of Shenyang to Fan Yang (Grant No. 19-112-4-103), from the Youth Support Foundation of China Medical University to Fan Yang (Grant No. QGZ2018058), from the Scientific Fund of Shengjing Hospital to Fan Yang (Grant No. 201801), and from the 345 Talent Project of Shengjing Hospital to Fan Yang (Grant No. 52-30C).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hewitt R, Watson P. Defining biobank. *Biopreserv Biobank* 2013;11:309-15.
2. Hofer-Picout P, Pichler H, Eder J, *et al*. Conception and implementation of an austrian biobank directory integration framework. *Biopreserv Biobank* 2017;15:332-40.
3. Hartman V, Gali B, Dee S, *et al*. Canadian tissue repository network biobank certification program: Update and review of the program from 2011 to 2018. *Biopreserv Biobank* 2019;17:530-8.
4. Paskal W, Paskal AM, Dębski T, *et al*. Aspects of modern biobank activity Comprehensive review. *Pathol Oncol Res* 2018;24:771-85.
5. Gee S, Oliver R, Corfield J, *et al*. Biobank finances: A socio-economic analysis and review. *Biopreserv Biobank* 2015;13:435-51.
6. Caixeiro NJ, Byun HL, Descallar J, *et al*. Health professionals' opinions on supporting a cancer biobank: Identification of barriers to combat biobanking pitfalls. *Eur J Hum Genet* 2016;24:626-32.
7. Lindner M, Morresi-Hauf A, Stowasser A, *et al*. Quality assessment of tissue samples stored in a specialized human lung biobank. *PLoS One* 2019;14:e0203977.
8. Mok TS, Cheng Y, Zhou X, *et al*. Improvement in overall survival in a randomized study that compared dacomitinib with gefitinib in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *J Clin Oncol* 2018;36:2244-50.
9. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: Mechanism and applications. *Science* 2013;340:1190-4.
10. Lancaster MA, Knoblich JA. Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science (New York, NY)* 2014;345:1247125.
11. Sato T, Vries RG, Snippert HJ, *et al*. Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 2009;459:262-5.
12. Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. *Nat Rev Genet* 2018;19:671-87.
13. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer* 2018;18:407-18.
14. Boj SF, Hwang CI, Baker LA, *et al*. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 2015;160:324-38.
15. Crespo M, Vilar E, Tsai SY, *et al*. Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. *Nat Med* 2017;23:878-84.
16. Neal JT, Li X, Zhu J, *et al*. Organoid modeling of the tumor immune microenvironment. *Cell* 2018;175:1972-88.e16.
17. Miura S, Suzuki A. Generation of mouse and human organoid-forming intestinal progenitor cells by direct lineage reprogramming. *Cell Stem Cell* 2017;21:456-71.e5.
18. Kopper O, de Witte CJ, Lõhmussaar K, *et al*. An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nat Med* 2019;25:838-49.
19. Sachs N, de Ligt J, Kopper O, *et al*. A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* 2018;172:373-86.e10.
20. Lee SH, Hu W, Matulay JT, *et al*. Tumor evolution and drug response in patient-derived organoid models of bladder cancer. *Cell* 2018;173:515-28.e17.
21. Seidlitz T, Merker SR, Rothe A, *et al*. Human gastric cancer modelling using organoids. *Gut* 2019;68:207-17.
22. Ang TL, Li JW, Kwek AB, *et al*. The difference in histological yield between 19G EUS-FNA and EUS-fine-needle biopsy needles. *Endosc Ultrasound* 2019;8:255-60.
23. Chong CC, Teoh AY, Tang RS, *et al*. EUS-FNA using 22G nitinol or ProCore needles without on-site cytopathology. *Endosc Ultrasound* 2018;7:56-60.
24. Mizuno S, Nakai Y, Isayama H, *et al*. EUS-FNA of gastric cancer metastatic to the head of pancreas using a forward oblique viewing echoendoscope in a case with Roux-en-Y anatomy. *Endosc Ultrasound* 2018;7:420-1.
25. Cazacu IM, Luzuriaga Chavez AA, *et al*. A quarter century of EUS-FNA: Progress, milestones, and future directions. *Endosc Ultrasound* 2018;7:141-60.
26. Patel S, Jinjuvadia R, Devara A, *et al*. Performance characteristics of EUS-FNA biopsy for adrenal lesions: A meta-analysis. *Endosc Ultrasound* 2019;8:180-7.
27. Mohan BP, Shakhatreh M, Garg R, *et al*. Comparison of Franseen and fork-tip needles for EUS-guided fine-needle biopsy of solid mass lesions: A systematic review and meta-analysis. *Endosc Ultrasound* 2019;8:382-91.
28. Ikarashi S, Tsuchiya A, Hayashi K, *et al*. Delayed pancreatic ductal

leakage after EUS-FNA for autoimmune pancreatitis. *Endosc Ultrasound* 2019;8:277-8.
29. Yang F, Wang H, Liu X, *et al*. EUS-guided fine-needle technique-derived cancer organoids: A tailored "Shennong deity" for every patient with cancer. *Endosc Ultrasound* 2019;8:73-5.
30. Tiriac H, Bucobo JC, Tzimas D, *et al*. Successful creation of pancreatic cancer organoids by means of EUS-guided fine-needle biopsy sampling for personalized cancer treatment. *Gastrointest Endosc* 2018;87:1474-80.
31. Nuciforo S, Fofana I, Matter MS, *et al*. Organoid models of human liver cancers derived from tumor needle biopsies. *Cell Rep* 2018;24:1363-76.
32. Weeber F, van de Wetering M, Hoogstraat M, *et al*. Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc Natl Acad Sci U S A* 2015;112:13308-11.
33. Pauli C, Hopkins BD, Prandi D, *et al*. Personalized *in vitro* and *in vivo* cancer models to guide precision medicine. *Cancer Discov* 2017;7:462-77.
34. Schizas D, Kapsampelis P, Mylonas KM. Adenosquamous Carcinoma of the Esophagus: A Literature Review. J Transl Int Med. 2018;6:70-3.
35. Sak K. A Hypothetical Approach on Gender Differences in Cancer Diagnosis. J Transl Int Med. 2019;7:90-2.