Press release

Keio University

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Keio University Faculty of Pharmacy

Establishing Tumor Organoids for Personalized Treatment of Hepatobiliary Cancer:
An International Multicenter Study Offers Future Perspectives

In an international collaborative study, a research team has analyzed multiple institutions that have successfully created hepatobiliary cancer organoids in order to study the success rate and environmental factors necessary for the successful establishment of such organoids. They also presented an outlook on information related to current issues, potential solutions, and future improvements in establishing hepatobiliary cancer organoids. The team was composed of Professor Yoshimasa Saito from the Faculty of Pharmacy at Keio University and researchers from Johns Hopkins University in the United States, the University of Cambridge in the United Kingdom, and the Erasmus University Medical Center in the Netherlands. The results of this research were published online in the international journal *Cancer Cell* on February 2, 2022 (EST).

1. Main Points of Research

- In this international multicenter study, researchers analyzed the success rate and environmental factors related to establishing hepatobiliary cancer organoids.
- The success rate was 37.2% (32 successes out of 86 attempts) for cholangiocarcinoma (CCA), and 19.3% (17 successes out of 88 attempts) for hepatocellular carcinoma (HCC). There were no significant differences in the success rates among the centers. Researchers also found that the success rate of Hepatobiliary cancer (HBC) organoid establishment was comparatively lower than for colorectal and other types of cancer.
- The research results presented a future outlook on current issues, potential solutions, and future improvements in establishing hepatobiliary cancer organoids.

2. Research Background

Hepatobiliary cancer (HBC) is a general term for hepatocellular carcinoma (HCC), which develops in the liver, cholangiocarcinoma (CCA), which develops where bile passes in the biliary tract. Gallbladder carcinoma is also included in this category. Many of these cancers are difficult-to-treat, malignant tumors that are unlikely to be completely cured with surgery or cancer drugs, making the development of new treatment options highly advantageous. In particular, as the efficacy of cancer drugs on malignant tumors differs between individual patients, researchers are hoping to establish personalized medicine[1] that selects the best drug to treat each patient.

Recently, methods for creating organoid cultures[2] have been developed that allow researchers to replicate in vivo tissues and tumors in a culture dish. This process involves culturing tissue stem cells and cancer stem cells in three dimensions (3D). Organoid established using patient-

derived cells are sometimes referred to as *in vitro* patient "avatars" (stand-ins), as they faithfully reflect the morphology and characteristics of the original organ or tumor. By using these patient-derived organoids, researchers can predict the efficacy of treatments based on results in a culture dish. As a result, personalized medicine using organoids have drawn much attention from the scientific community.

In a previous study, Professor Saito and his research group established organoids using cancer tissue collected from patients with CCA, a variety of cancer that doesn't respond well to treatment. In a world-first, they succeeded in securely culturing and maintaining cultured organoids for more than one year (Saito Y et.al., 2019). Still, the relatively low success rate of establishing HBC organoids compared to other cancers, such as colorectal cancer, remains an ongoing issue. In this study, researchers examined several institutions that have successfully established HBC organoids to compare their success rates and the environmental factors related to successfully establishing HBC organoids.

3. Content of Research and Results

In an international collaborative study at Johns Hopkins University in the United States, the University of Cambridge in the United Kingdom, Erasmus Medical Center in the Netherlands, and Keio University, a research team analyzed the success rates and environmental factors of organoid establishment among multiple institutions that have successfully established HBC organoids.

The overall success rate was 37.2% (32/86) for CCA, and 19.3% (17/88) for HCC. There were no significant differences in the success rates among the institutions. They also found that the success rate of HBC organoid establishment was comparatively lower than colorectal and other kinds of cancers.

Then, in order to investigating the environmental factors involved in the successful establishment of HBC organoids, researchers examined the patient's biological sex, the way in which specimens were collected, tumor differentiation, incidence of lymph node metastasis and cirrhosis, and information related to the used medical equipment and facilities. For HCC, the success rate for establishing organoids using cells taken from female patients was significantly higher than the rate among male patients. For CCA, however, there were no apparent factors related to the establishment success rate. (Fig. 1)

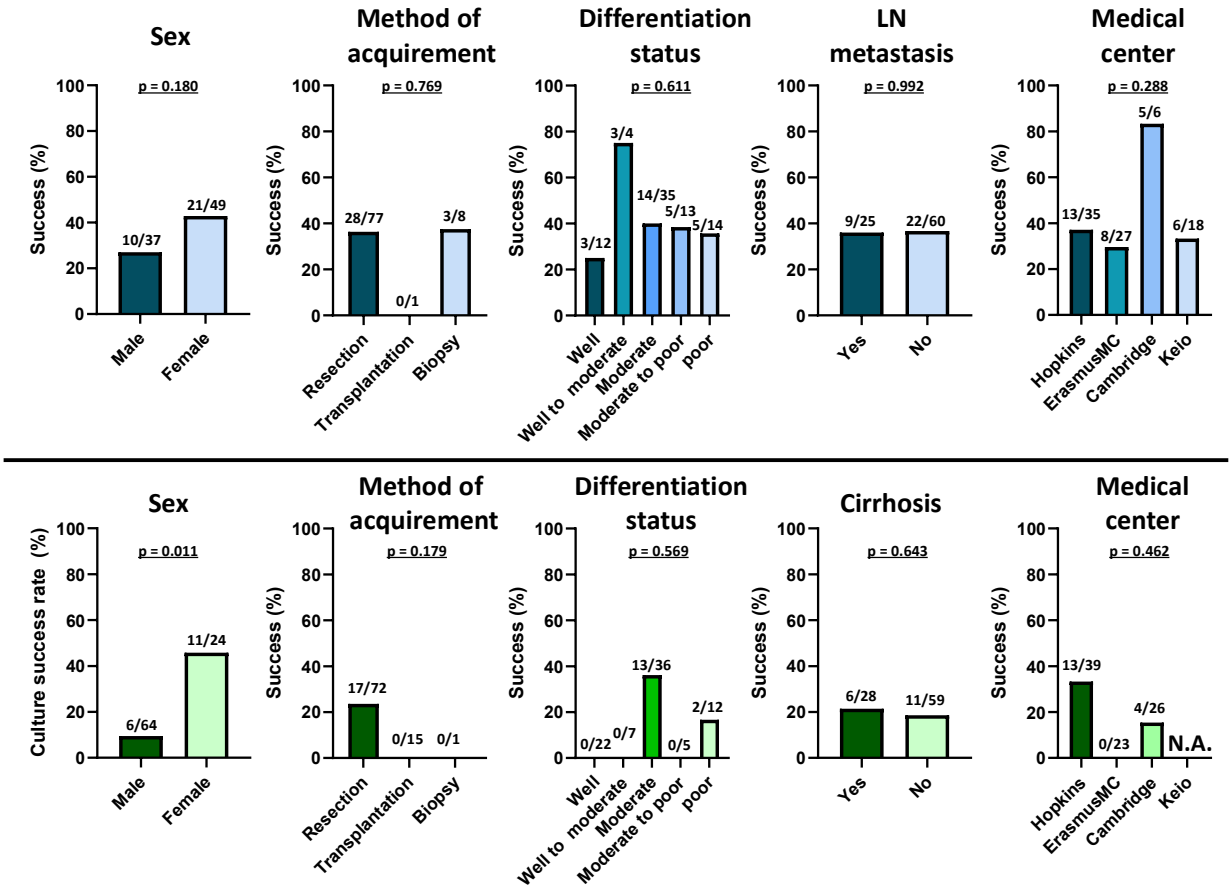

Figure 1. Examination of success rates for organoid establishment with cholangiocarcinoma (CCA, top row), hepatocellular carcinoma (HCC, bottom row), and associated factors. Researchers looked at the patient's biological sex, the methods of acquirement of samples, tumor differentiation status, incidence of lymph node (L/N) metastasis and cirrhosis, and medical center. For HCC, the success rate for establishing organoids using cells taken from female patients was significantly higher than the rate among male patients. For CCA, however, there were no apparent factors related to the establishment success rate.

An overview of the current challenges and potential solutions for establishing HBC organoids is given in Figure 2. As handling and transportation times can impact the viability of cells in specimens provided by patients, it may be necessary to standardize transportation routes and procedures among facilities. The composition of the medium used for organoid cultures is another important factor for the stable cultivation and maintenance of organoids, necessitating exploration into the optimal medium for organoids through confirming signaling pathways for cell proliferation via single-cell analysis, etc.

Contamination by non-cancerous cells proliferating in greater volume than cancerous cells was a key factor that researchers identified as leading to low success rates in establishing HBC organoids. To avoid this issue, researchers must select cancer organoids by handpicking

according to their morphology, by using cytotoxic agents that target non-cancerous cells and ignore cancerous cells with driver mutations, or by utilizing other methods to isolate cancer organoids. Finally, to verify that organoids with expanded cell cultures are indeed derived from HBC, facilities must confirm tumorigenicity through methods such as transplantation into mice, checking for genetic mutations, genomic analyses, and long-term cultures.

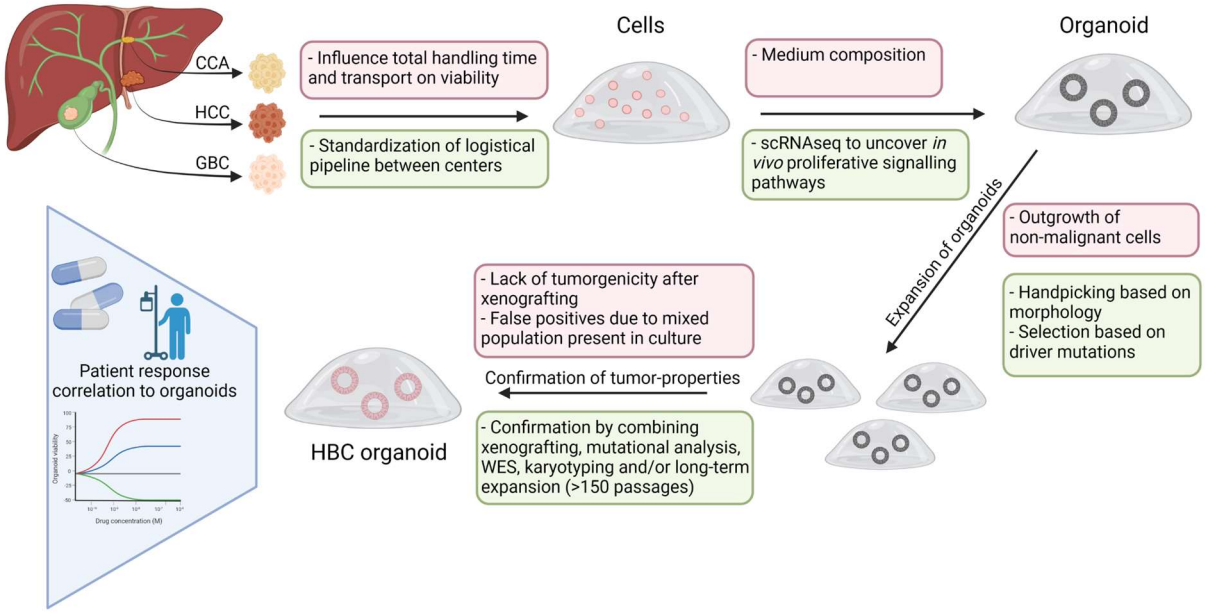

Figure 2. Overview of the current challenges and potential solutions for establishing HBC organoids. The red text boxes indicate current issues, while the green text boxes indicate important considerations and potential solutions.

4. Future Developments

Successfully established HBC organoids have the potential to serve as powerful tools in personalized medicine for patients with these cancers because of their ability to be used to predict the efficacy of drugs and search for biomarkers. By implementing solutions to the issues raised in this study, the researchers of this study hope that the success rate of establishing HBC organoids will improve, leading to better treatment outcomes and prognosis for patients with refractory HBC.

Details of Journal Article

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Article authors: Gilles S. van Tienderen*, Ling Li*, Laura Broutier*, Yoshimasa Saito*, Patricia Inacio, Meritxell Huch, Florin M. Selaru, Luc J.W. van der Laan, Monique M.A. Verstegen. (*Co-first authors)

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Glossary

[1] **Personalized medicine:** medicine that selects the most effective drugs and therapeutic agents for each patient while taking into account the severity of the disease and the patient's genetic information to provide them with optimal treatment. Until now, medicine has treated patients with the same disease all in the same fashion. Personalized medicine is expected to provide more effective treatment with fewer side effects for individual patients. Also known as "tailor-made medicine" or "precision medicine."

[2] **Organoid cultures:** a method of reproducing tissues and tumors in a culture dish by extracting stem cells from tissues and cancer stem cells from cancer tissues and culturing them in 3D. While traditional cancer research has primarily relied on cell-lines involving two-dimensional cell cultures, cancer organoids exhibit morphology and properties similar to those of tumors in the patient's body, making them suitable for pathological analysis, biomarker discovery, and drug screening.

*Please direct any requests or inquiries on press coverage to the contact information provided below in advance.

Inquiries about research:
Division of Pharmacotherapeutics, Keio University Faculty of Pharmacy
Professor Yoshimasa Saito
TEL: +81 3-5400-2647
FAX: +81 3-5400-2647
Email: saito-ys@pha.keio.ac.jp

Inquiries about press release:
Office of Communications and Public Relations
Keio University
Akiko Wakahara (Ms.)
TEL: +81 3-5427-1541
FAX: +81 3-5441-7640
Email: m-pr@adst.keio.ac.jp https://www.keio.ac.jp/en/