Social Cooperation Research Program

Division of Clinical Precision Research Platform

臨床精密研究基盤社会連携研究部門

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Since the opening of our new laboratory under a joint research agreement with Daiichi Sankyo RD Novare Co., Ltd, we have been struggling to establish our experimental flow for drug-sensitivity screening using primary tumor specimens derived from patients with hematological malignancies. In order to finally establish a novel platform for precision medicine projects combining DSS and comprehensive multi-omics analyses, we aimed to optimize our tissue culture methods for PTS from acute myeloid leukemia (AML) patients. Using clinical specimens kindly provided by the Department of Hematology and Oncology, IMSUT Hospital, we were able to evaluate optimal tissue culture conditions for 3 to 9 days of ex vivo incubation with drugs. From these experiments, we were able to establish our in-house tissue culture medium that can maintain hematopoietic stem/ progenitor cell-like components of AML cells for the period of ex vivo drug treatments. While we handle these cell processing procedures, we could also make progress in high-throughput assay system with automation technologies. Our efforts are highly expected to provide us with tools to understand the pathogenesis of hematological malignancies and develop further therapeutics.

1. Clinical precision research for hematological malignancies

Kimihito Cojin Kawabata[1], Hironobu Komori[1,2], Hayato Tsuji[1,2], Yoshiharu Takama[1,2], Seiko Kato[1], Maiko Morita[1], Sanae Suzuki[1], Tetsushi Oka[2], Yoshimasa Ono[2], Kenji Wakabayashi[2], Gen Kudo[2], Yasuhito Nannya[1], Satoshi Takahashi[1].

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The aim of this study is to perform a comprehensive multi-omics analysis covering genetic and epigenetic alterations as well as gene expressions, which can be compared side-by-side with functional analyses such as ex vivo drug responses. All these experiments use primary tumor specimens (PTS) derived from patients with hematopoietic malignancies attending either the IMSUT or collaborating hospitals, together with the Department of Hematology and Oncology. Currently, our special focus is on acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).

Currently, in the aforementioned network, we have performed whole genome or whole exome analyses on the PTS of patients with hematopoietic malignancies, along with RNA expression, transcriptome analyses. We further aim to organize these omics approaches with higher resolution at the single cell level. These omics profiles can be immediately compared with the clinical course of the actual patients. The NGS data compared to the clinical profile are dis-

cussed in detail in regular Tumor Board meetings (biweekly).

Drug sensitivity screening (DSS) using PTS is another axis of the group's efforts. In this topic, several other groups have already published their data on similar aspects (Nature. 2018;562(7728):526-31, Cancer Discovery. 2022;12(2):388-401, etc.). However, the more samples and compounds we use at multiple doses, and the more intensive work and time we need in these high-throughput procedures. In this sense, this field is still in its infancy. Therefore, we are trying to install more updated and modernized technologies in these wet experiments and subsequent analyses. One of them is a fully automated ex vivo drug screening system, which uses a robotic arm to integrate several machines that can automatically handle the entire process from cell seeding, drug injection, cell incubation, liquid handling to flow cytometry. After data acquisition, drug sensitivity profile data is also collected through an automated data processing pipeline. After installation, we have completed the optimization of the entire system so that each PTS we have stored in the laboratory is analyzed in a 384-well plate format. We are currently evaluating the significance of these results.

To date, there is a great deal of variability in the methods used for ex vivo culture of PTS in the field of DSS. These methods need to be compared side-by-side and ultimately further optimized to obtain drug sensitivity profiles using cells in a better state. To reiterate, the limited number of cells collected from patients and the use of all available frozen vials in initial screening experiments are two major problems in experiments using PTS. In order to expand the possibilities of this AML/MDS PTS for more advanced applications, we have conducted experiments to compare different ex vivo culture methods side by side. At the Clinical Precision Research Platform, we have already collected 159 different PTS aliquoted in 1495 cryovials. They are continuously used either in the automated DSS experiments, OMICS analyses or in these ex vivo culture assays. We have found that 1. Short term culture protocols (up to 7 days) optimized for the current DSS assays make some differences regardless of changing concentrations of serum or other supplements for culture media. 2. The use of stromal feeder cells is superior to other conventional stroma-free culture methods currently used for normal hematopoietic stem cells. Moreover, 3. Recently, we have installed the most updated culture methods to challenge for stroma-free, serum-free ex vivo culture system optimized for PTS. The idea for the updated culture media is derived from ongoing collaborative research projects on normal hematopoietic stem cell culture. Initial data on this topic continue to fuel our research motivation on a daily basis. Details of these findings will be updated as they become available.

2. Generation of antigen-specific T cells derived from cord blood

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The aim of this project is to establish a non-gene transfer method for the generation and expansion of viral antigen-specific T cells from naive human cord blood (CB)-derived T cells.

Previous studies have shown that cGAMP, a type of STING ligand, induces an IFN-type response and promotes cross-priming of antigen-specific CD8 + T cells by mature DCs. It has also been reported that cGAMP induces the transcription factor T-bet, which is required for the development of effector CD8 + T cells. When CB-derived naive T cells were cultured with cGAMP and viral antigen peptides twice for 14 days, significant production of inflammatory cytokines such as IFN-γ and TNF-α was observed. As a result of optimization of culture conditions, supplementation with ascorbic acid, ITS-G supplement, and an amino acid was shown to effectively produce inflammatory cytokines from CB-derived CTLs. In some samples, strong inflammatory cytokine production was observed even after 5th stimulation with viral antigen peptides, and its amount correlated with the number of stimulations. However, repeated experiments showed that cell proliferation and cytokine production were largely dependent on individual differences. As a next step, we plan to perform multi-omics analysis or single-cell RNA-seq to investigate the difference between PB-derived CTLs and CB-derived CTLs and to identify the factors for efficient induction of CTLs from naive T cells. Future clinical studies are planned to advance this research and improve the safety of cord blood transplantation by promoting immune reconstitution against viral infection after transplantation.

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