【Grant-in-Aid for Scientific Research (S)】

Broad Section I

Title of Project : **Comprehensive elucidation of molecular pathogenesis based on genetic abnormalities in malignant lymphoma**

KATAOKA Keisuke
(Keio University, School of Medicine, Professor)

Research Project Number : 21H05051 Researcher Number : 90631383
Term of Project : FY2021-2025 Budget Allocation : 143,600 Thousand Yen
Keyword : Malignant lymphoma, CRISPR screening, single-cell analysis

【Purpose and Background of the Research】

Malignant lymphoma is a heterogeneous disease that includes diffuse large B-cell lymphoma (DLBCL), adult T-cell leukemia/lymphoma (ATL), and extranodal NK/T-cell lymphoma (ENKTL). In recent years, the entire picture of genetic abnormalities in lymphoma has been investigated, and various novel alterations have been identified. We have performed comprehensive genetic analysis of ATL, a T-cell neoplasm caused by HTLV-1 infection, which is common in Japan, and identified dozens of abnormalities such as PD-L1 3′-untranslated region (UTR) truncation. In addition, we recently performed a high-depth whole-genome sequencing of 150 pairs of ATL cases, which provided a different overall picture of genetic abnormalities than previous analyses and identified novel alterations such as gene X (unpublished). However, the biological significance of many of the genetic aberrations, their roles in lymphoma development in vivo, and their effects on the immune microenvironment, remain unclear.

【Research Methods】

In this study, we aim to elucidate the detailed molecular mechanisms in vivo and microenvironmental changes caused by the genetic abnormalities observed in lymphoma by applying newly developed CRISPR screening and single-cell multi-omics analysis technologies, focusing on the abnormalities identified by our studies.

A. Elucidation of the molecular function of abnormalities identified in our genetic analyses using animal models of diseases: we will develop and analyze animal models of *PD-L1* 3′-UTR deletion and *GENE X*.
B. Highly efficient investigation of genetic abnormalities contributing to lymphoma development by in vivo CRISPR screening: We have previously demonstrated that introduction of sgRNA custom libraries targeting genes with loss-of-function abnormalities in ATL and ENKTL induces malignant lymphomas. In this study, we will not only expand our studies in ATL and ENKTL, but also perform the same in vivo CRISPR screening for abnormalities found in other lymphomas such as DLBCL.
C. Elucidation of the regulatory mechanism of B-cell-specific PD-L2 expression by CRISPR tiling screening: We will perform a comprehensive CRISPR tiling screening analysis, and search for transcription factors involved in the regulation of PD-L2 expression by loss-of-function CRISPR screening.
D. Application of single-cell multi-omics analysis to mouse lymphoma models for analyzing the microenvironment: We have developed single-cell multi-omics analysis, which enables the simultaneous assessment of transcriptome, many surface markers, and TCR/BCR repertoires at the same single-cell level. In this study, we will apply the technology to various mouse models.
E. Exploring the possibility of clinical application using comprehensive genetic analysis data derived from humans: Abnormalities newly found in relation to items B and C will be investigated using genetic data derived from human specimens.

【Expected Research Achievements and Scientific Significance】

These results are expected to lead to the development of new therapeutic targets and biomarkers for lymphoma, and at the same time, to their practical application through implementation in next-generation sequencing panels, thereby contributing to cancer precision medicine.

【Publications Relevant to the Project】

- Landscape and function of multiple mutations within individual oncogenes. Saito Y, Koya J, Araki M, Kogure Y, Shingaki S, Tabata M, McClure MB, Yoshifuji K, Matsumoto S, Isaka Y, Tanaka H, Kanai K, Miyano S, Shiraishi Y, Okuno Y, Kataoka K. *Nature*. 2020;582(7810):95-99.

- Aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. Kataoka K, Shiraishi Y, Takeda Y, Sakata S, Matsumoto M, Nagano S, Maeda T, Nagata Y, Kitanaka A, Mizuno S, Tanaka H, Chiba K, Ito S, Watatani Y, Kakiuchi N, Suzuki H, Yoshizato T, Yoshida K, Sanada M, Itonaga H, Imaizumi Y, Totoki Y, Munakata W, Nakamura H, Hama N, Shide K, Kubuki Y, Hidaka T, Kameda T, Masuda K, Minato N, Kashiwase K, Izutsu K, Takaori-Kondo A, Miyazaki Y, Takahashi S, Shibata T, Kawamoto H, Akatsuka Y, Shimoda K, Takeuchi K, Seya T, Miyano S, Ogawa S. *Nature.* 2016; 534(7607):402-6.

【Homepage Address and Other Contact Information】

https://www.ncc.go.jp/jp/ri/division/molecular_oncology/index.html
https://www.keio-hematology.jp/