Advanced Clinical Research Center

Division of Hematopoietic Disease Control
造血病態制御学分野

Professor	Yasuhito Nannya, M.D., Ph.D.	教　授	博士(医学)	南　谷　泰　仁
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学)	小　沼　貴　晶
Assisstant Professor	Koji Jimbo, M.D., Ph.D.	助　教	博士(医学)	神　保　光　児

The main goal of our research is to elucidate the pathogenesis of hematopoietic diseases and to study the development of therapeutic strategies for these diseases. We will continue to develop the therapeutic targets that have already been identified and advance them to the next stage for clinical application. In particular, we are collaborating with various research groups in Japan and abroad regarding the elucidation of pathogenesis through genome analysis, and have discovered a novel genetic mutation involved in the progression of myeloproliferative tumors. Further advanced genome analysis has yielded interesting insights into clonal evolution in PNH.
We are also leading a whole genome sequencing project collected from major hematopoietic disease centers in Japan, which has identified numerous novel genomic aberrations in acute lymphocytic leukemia and malignant lymphoma. We are also elucidating the role of specialized neutrophils in MSC therapy by proteomics analysis and the significance of genomic abnormalities specific to a particular type of hairy cell leukemia by generating model mice.

1. Whole-genome sequencing of myeloproliferative neoplasms revealed dynamic clonal changes in the fibrotic or leukemic transformation and novel FOXP1 mutations in the fibrotic transformation

Hiroyuki Takamori[1*], Ying-Jung Huang[2*], Hidehito Fukushima[1], Kazuaki Yokoyama[1], Ting-Yu Huang[2], Ming-Chung Kuo[2,3], Seishi Ogawa[4], Yasuhito Nannya[1†], Lee-Yung Shih[2,3†]
1: Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2: Division of Hematology-Oncology, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan.
3: School of Medicine, Chang Gung University, Taoyuan, Taiwan.
4: Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Myeloproliferative neoplasms (MPNs) are characterized by clonal proliferation of hematopoietic stem cells, which can lead to secondary myelofibrosis or acute myeloid leukemia. We explored the changes in genomic alterations during MPN transformation using whole-genome sequencing of samples from both the chronic and fibrotic or leukemic phases of 20 patients. We identified *FOXP1* mutations in 3 of 14 (21.4%) patients with secondary myelofibrosis. This novel mutation was identified in another 5 of the 35 patients (14.3%) in an independent cohort. All these 8 patients with *FOXP1* mutations did not experience leukemic transformation after a median follow-up of 5.1 years. The acquisition of non-canonical MPL^{Y591} mutations was detected in the fibrotic or leukemic phase. Clonal expansion, involving both known and unknown driver genes (in 18 and 2 patients, respec-

tively), was observed in all patients. We determined the patterns of clonal evolution based on myeloid driver mutations in 18 patients: linear clonal evolution in 11 patients and branched clonal evolution in 7 patients. Our results suggested that MPN patients carrying *FOXP1* mutations are unlikely to have leukemia transformation and emphasized that the acquisition of specific genetic mutations and dynamic changes in clonal architecture underlie the pathogenesis in patients undergoing MPN transformation.

2. Inferred Trajectories of Clonal Expansion in Paroxysmal Nocturnal Hemoglobinuria.

Hiroyuki Takamori[1,2], Yasutaka Ueda[1], Yoshikazu Matsuoka[3], Tatsuya Fujioka[3], Hideki Makishima[4], Yoshiko Murakami[5], Taroh Kinoshita[5,6,7], Satoru Miyano[8], Yuzuru Kanakura[1,9], Jun-Ichi Nishimura[1], Naoki Hosen[1], Seishi Ogawa[4], Yasuhito Nannya[2,4]

1. Department of Hematology and Oncology, Osaka University Graduate School of Medicine

2. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo

3. Department of iPS Stem Cell Regenerative Medicine, Kansai Medical University

4. Department of Pathology and Tumor Biology, Kyoto University

5. Laboratory of Immunoglycobiology, Research Institute for Microbial Diseases, Osaka University

6. Immunoglycobiology, WPI Immunology Frontier Research Center, Osaka University

7. Center for Infectious Disease Education and Research, Osaka University

8. M&D Data Science Center, Tokyo Medical and Dental University

9. Department of Hematology Sumitomo Hospital

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematological disorder resulting from clonal expansion of PIGA mutated clones. *PIGA* gene is involved in the first step of glycosylphosphatidylinositol (GPI) anchored protein synthesis. CD55 and CD59 are GPI-anchored proteins that function as a central component of complement regulatory factors in red blood cells. Somatic mutations of *PIGA* gene result in the absence of CD55 and CD59, leading to complement-mediated intravascular hemolysis. It is well known that *PIGA* mutations alone are not sufficient for the clonal expansion of *PIGA* mutated clones. T cell mediated immune pressure or additional mutations other than *PIGA* mutations could confer the clonal expansion. Currently, next-generation sequencing and high-sensitivity flow cytometry enable us to observe the clonal dynamics and clonal architecture before the diagnosis of PNH. Here I present our current study in which we attempt to estimate the clonal expansion in PNH before the diagnosis using the whole genome sequence of single cell derived colonies. This study has the potential to clarify the trajectories of PIGA mutated clones before the significant clonal expansion or the diagnosis, thereby gaining insight into the pathogenesis of the clonal expansion in PNH.

3. Investigation of MSC Therapy and Neutrophil Function in Acute GVHD Using Plasma Proteomics

Tomokazu Seki[1], Xinyu Huang[2], Kazuaki Yokoyama[3], Seiya Imoto[4], Tokiko Nagamura-Inoue[2], Yasuhito Nannya[1,3]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

2. Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

3. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

4. Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Japan.

We analyzed plasma protein profiles in patients with acute graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation using targeted proteomics with Olink®. The results revealed that biomarkers and pathways previously reported to be associated with acute GVHD were identified in the GVHD cohort compared to healthy controls. Notably, in the acute GVHD group—particularly those with skin involvement—neutrophil-secreted proteins and pathways related to secretory vesicles and degranulation were downregulated compared to patients without GVHD. These findings suggest potential neutrophil dysfunction in acute GVHD. We further evaluated the plasma protein profiles of patients with steroid-refractory acute GVHD who responded to umbilical cord-derived mesenchymal stromal cell (UC-MSC) therapy. Compared to pre-treatment profiles, post-MSC therapy profiles demonstrated a time-dependent upregulation of neutrophil-secreted proteins and pathways associated with secretory vesicles and degranulation. These results suggest that MSC therapy can enhance neutrophil function, leading to suppress GVHD. We plan to perform T-cell functional analyses using CFSE-MLR assays via flow cytometry to investigate whether MSC-mediated promotion of neutrophils contributes to GVHD suppression.

4. Multiomics Analysis of Drug Sensitivity in AML

Tomokazu Seki[1], Kimihito C Kawabata[2], Chao Li[1,3], Kazuaki Yokoyama[4], Seiya Imoto[5], Yasuhito Nannya[1,4], Satoshi Takahashi[2]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2. Division of Clinical Precision Research Platform, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
3. Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan
4. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
5. Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Japan.

We conducted drug sensitivity assays using primary tumor specimens (PTS) from AML patients. Based on the assay results, we performed bulk RNA sequencing (RNA-seq) to identify differentially expressed genes (DEGs) and pathways potentially contributing to the therapeutic responses of specific drugs. We plan to increase the number of bulk RNA-seq samples to enable more robust analyses. Additionally, we aim to integrate PTS data with clinical information, whole-exome sequencing (WES), and whole-genome sequencing (WGS) results from AML patients. This approach will facilitate a comprehensive, multi-faceted evaluation of drug sensitivity.

5. Molecular pathogenesis of hairy leukemia Japanese variant based on whole genome-multi-omics information

Iku Kamitani[1], Kazuaki Yokoyama[2], Keisuke Kidoguchi[3], Shinya Kimura[3], Seiya Imoto[4], Yasuhito Nannya[1]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
2. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
3. Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga Unversity, Saga, Japan
4. Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

We have performed whole genome analysis of hairy cell leukemia variants in collaboration with Saga University. Hairy cell leukemia is a rare disease, and hairy cell leukemia without BRAF mutations is extremely rare and has little validation regarding pathogenesis. About 30% of cases showed structural abnormalities such as partial chromosomal deletions and inversions in the TCL1 to IGH region on chromosome 14, which is often observed in other B-cell lymphomas. Therefore, we generated a mouse model in which chromosomal structural abnormalities occur in the IGH region from TCL1A on chromosome 14 using the loxP-Cre system, bred Mb1Cre mice to operate the Cre-loxP system specifically for B cells, and observed the process of disease development.

6. Whole genome sequencing (WGS)-based structural variant detection in Acute lymphoblastic leukemia and other hematological malignancies

Hidehito Fukushima[1], Kaito Mimura[2], Koji Okazaki[3], Hiroyuki Takamori[1], Ryunosuke Saiki[3], Yotaro Ochi[3], Kazuaki Yokoyama[1], Kenichi Yoshida[2], Motohiro Kato[4], Kotoe Katayama[5], Seiya Imoto[5], Seishi Ogawa[3], and Yasuhito Nannya[1]
1) Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan
2) Division of Cancer Evolution, National Cancer Center Research Institute, Tokyo, Japan
3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
4) Department of Pediatrics, University of Tokyo, Tokyo, Japan
5) Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, Tokyo, Japan

Our study aimed to i) overcome major obstacles encountered in SV analysis using short-read sequencing data , ii) propose an optimal SV detection pipeline, and iii) to identify novel structural variants. We enrolled 1,453 cases diagnosed with major hematological disorders. For each case, diagnostic samples along with an oral swab were subjected to a range of genomic analyses, including whole-genome sequencing (n=1,453), RNA-seq (n=888), Optical Genome Mapping (n=12), and long-read sequencing (PacBio, n=46). Additionally, we constructed a panel of normals (PoNs) utilizing multiple SV reference panels (ToMMo, gnomAD SVs) and included 53 remission samples from pediatric leukemia patients that underwent whole-genome sequencing. To validate our findings, we compared the outputs from various SV callers (GRIDSS, Manta, Delly, SVABA, GenomonSV) with those obtained through other sequencing modalities. The intersection of the SV callers revealed that only 6% of all ensembled calls overlapped, potentially attributed to tumor cell contamination in normal samples and the presence of caller-specific calls. Furthermore, we assessed the sensitivity of GRIDSS, Manta, and SVABA in detecting fusion events using RNA-seq as a reference standard. Our results indicated that GRIDSS exhibited improved sensitivity (0.75) with our custom filter compared to Manta and SVABA (0.5~0.7). Moreover, we validated the effectiveness

of PoNs, demonstrating its capacity to eliminate approximately 75% of all calls on average, thus maintaining a higher positive predictive value. Finally, our study has identified a substantial number of novel recurrent structural variants, such as ADD3 deletions observed in acute lymphoblastic leukemia samples (15%). Our next steps involve conducting comprehensive functional analyses on these identified variants.

7. Optical Genome Mapping for Hematological Malignancies

Hidehito Fukushima[1], Kaito Mimura[2], Koji Okazaki[3], Hiroyuki Takamori[1], Ryunosuke Saiki[3], Yotaro Ochi[3], Kazuaki Yokoyama[1], Kenichi Yoshida[2], Motohiro Kato[4], Kotoe Katayama[5], Seiya Imoto[5], Seishi Ogawa[3], and Yasuhito Nannya[1]

1) Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

2) Division of Cancer Evolution, National Cancer Center Research Institute, Tokyo, Japan

3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

4) Department of Pediatrics, University of Tokyo, Tokyo, Japan

5) Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, Tokyo, Japan

Optical Genome Mapping (OGM), developed by Bionano Genomics, represents a novel cytogenetic technology capable of detecting structural variants (SVs) and copy number variations (CNVs) by fluorescently labeling specific sequences within long DNA fragments (approximately 1 Mb) and mapping them to a reference genome. While promising as an alternative to traditional techniques such as SNP arrays, G-banding, and FISH, its limitations include the inability to detect structural variants smaller than 500 bp or copy number variations below 50,000 bp.

This study analyzed tumor samples from 12 patients diagnosed with acute myeloid leukemia using OGM and evaluated its performance by comparison with G-banding and whole-genome sequencing (WGS). The structural variant analysis with WGS employed GRIDSS, while copy number analysis utilized Battenberg. The sensitivity of OGM for detecting chromosomal abnormalities identified by G-banding was 83%, with five out of six chromosomal abnormalities being successfully detected. Events with clone fractions greater than 80% were detectable by OGM, while a +21 abnormality with a 30% clone fraction was not detected.

When comparing OGM with WGS, GRIDSS identified 93 structural variants, with OGM demonstrating a 100% sensitivity for chromosomal translocations and a 94% sensitivity for events larger than 5,000 bp. However, its sensitivity dropped significantly to 14% for structural variants smaller than 5,000 bp. The average number of structural variant calls per sample by OGM was 1,169, though most lacked supporting evidence. Current efforts to establish optimal thresholds for QUAL and VAF values for filtering have been inconclusive. Some structural variants, such as those near the SPG11 gene, remain undetectable due to mapping challenges posed by pseudogenes and paralogs concentrated in the region. Additionally, positional discrepancies averaging 3,564 bp were observed between structural variants detected by OGM and WGS. OGM's tendency to classify inversions as intrachromosomal translocations underscores the need for expert interpretation.

The CNV detection capabilities of OGM revealed a sensitivity of 0% for events smaller than 50,000 bp and 28% for events exceeding this size threshold. The accuracy of CNV detection is influenced by both clone size and the length of the structural variants. OGM showed particular utility in identifying translocations and large-scale deletions, inversions, and duplications with high sensitivity, making it a viable alternative to G-banding for samples with high clone purity. However, the integration of long-read sequencing technologies is necessary to improve the detection of complex structural variants and to address current limitations.

In conclusion, OGM represents a significant advancement in genomic analysis for hematological malignancies, offering high sensitivity for specific structural variants. Nonetheless, its utility is constrained by limitations in detecting small-scale events and by challenges inherent to the technology. Further development and integration with complementary sequencing modalities are essential to maximize its diagnostic and research potential.

8. Whole genome sequencing (WGS) analysis in Malignant Lymphoma

Hidehito Fukushima[1], Kaito Mimura[2], Koji Okazaki[3], Hiroyuki Takamori[1], Ryunosuke Saiki[3], Yotaro Ochi[3], Kazuaki Yokoyama[1], Kenichi Yoshida[2], Motohiro Kato[4], Kotoe Katayama[5], Seiya Imoto[5], Seishi Ogawa[3], and Yasuhito Nannya[1]

1) Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

2) Division of Cancer Evolution, National Cancer Center Research Institute, Tokyo, Japan

3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

4) Department of Pediatrics, University of Tokyo, Tokyo, Japan

5) Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, Tokyo, Japan

In this study, we conducted a comprehensive genomic analysis of 251 cases of malignant lymphoma utilizing whole-genome sequencing (WGS) and RNA sequencing. WGS was applied to identify single nucleotide variants (SNVs), copy number variations (CNVs), and structural variants (SVs), while RNA sequencing enabled detailed profiling of gene expression patterns. This integrative approach allowed us to systematically uncover not only coding-region mutations in genes previously implicated in malignant lymphoma but also non-coding region variants with potential regulatory significance. Importantly, these non-coding mutations exhibited distinct patterns of distribution across subtypes as defined by the LymphGen classification system. Moving forward, we aim to redefine the classification of malignant lymphoma by incorporating these non-coding variants alongside coding-region mutations, with the ultimate goal of refining our understanding of the genomic underpinnings of this disease.

Publications

1. Yamagishi, M., Y. Kuze, S. Kobayashi, et al., *Mechanisms of action and resistance in histone methylation-targeted therapy.* Nature, 2024. **627**(8002): p. 221-228.
2. Wang, Q.S., T. Hasegawa, H. Namkoong, et al., *Statistically and functionally fine-mapped blood eQTLs and pQTLs from 1,405 humans reveal distinct regulation patterns and disease relevance.* Nat Genet, 2024. **56**(10): p. 2054-2067.
3. Tomofuji, Y., R. Edahiro, K. Sonehara, et al., *Quantification of escape from X chromosome inactivation with single-cell omics data reveals heterogeneity across cell types and tissues.* Cell Genom, 2024. **4**(8): p. 100625.
4. Sugiura, H., T. Ishikawa, T. Kuroi, et al., *Comparison of disease and risk classifications of AML before and after incorporation of NGS analysis of bone marrow samples.* Int J Hematol, 2024. **120**(5): p. 594-600.
5. Okuda, R., Y. Ochi, R. Saiki, et al., *Genetic analysis of myeloid neoplasms with der(1;7)(q10;p10).* Leukemia, 2024.
6. Nakamura, N., N. Yamamoto, T. Kondo, et al., *Sustained remission after cord blood transplantation for breast cancer with lung metastases and myelodysplastic syndrome.* Int J Hematol, 2024. **119**(6): p. 762-767.
7. Nakamura, M., K. Chonabayashi, M. Narita, et al., *Modelling and drug targeting of a myeloid neoplasm with atypical 3q26/MECOM rearrangement using patient-specific iPSCs.* Br J Haematol, 2024. **205**(4): p. 1430-1443.
8. Mizuike, J., K. Suzuki, S. Tosaka, et al., *Rewired chromatin structure and epigenetic gene dysregulation during HTLV-1 infection to leukemogenesis.* Cancer Sci, 2024.
9. Kubota, Y., M. Sakurai, Y. Nannya, et al., *Post-transplant transient abnormal myelopoiesis evolving from a GATA1 mutant clone in umbilical cord blood.* Ann Hematol, 2024.
10. Kubota, H., H. Ueno, K. Tasaka, et al., *RNA-seq-based miRNA signature as an independent predictor of relapse in pediatric B-cell acute lymphoblastic leukemia.* Blood Adv, 2024. **8**(5): p. 1258-1271.
11. Konuma, T., M. Hamatani-Asakura, M. Monna-Oiwa, et al., *Donor NKG2D rs1049174 polymorphism predicts hematopoietic recovery and event-free survival after single-unit cord blood transplantation in adults.* Bone Marrow Transplant, 2024. **59**(4): p. 566-568.
12. Konuma, T., M. Hamatani-Asakura, M. Monna-Oiwa, et al., *Recipient IL-17A polymorphism rs2275913 is associated with acute graft-versus-host disease after single-unit cord blood transplantation.* Transpl Immunol, 2024. **86**: p. 102096.
13. Kaito, S., K. Aoyama, M. Oshima, et al., *Inhibition of TOPORS ubiquitin ligase augments the efficacy of DNA hypomethylating agents through DNMT1 stabilization.* Nat Commun, 2024. **15**(1): p. 7359.
14. Iyoda, S., K. Yoshida, K. Shoji, et al., *KRAS G12 mutations as adverse prognostic factors in KMT2A-rearranged acute myeloid leukemia.* Leukemia, 2024. **38**(7): p. 1609-1612.
15. Boddu, P.C., A.K. Gupta, R. Roy, et al., *Transcription elongation defects link oncogenic SF3B1 mutations to targetable alterations in chromatin landscape.* Mol Cell, 2024. **84**(8): p. 1475-1495 e18.
16. Bernard, E., R.P. Hasserjian, P.L. Greenberg, et al., *Molecular taxonomy of myelodysplastic syndromes and its clinical implications.* Blood, 2024. **144**(15): p. 1617-1632.