Advanced Clinical Research Center

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

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The main goal of our research is to elucidate the pathogenesis of hematopoietic diseases and to study the development of therapeutic methods for these diseases. For those therapeutic targets that have already been identified, we will continue to develop them and move them to the next stage of clinical application. In particular, in terms of elucidating the pathophysiology through genome analysis, we are collaborating with various research groups in Japan and overseas, and have made interesting findings on myeloproliferative tumors and the clonal evolution of PIGA. We are also leading a whole-genome sequencing project of more than 1,400 leukemia samples collected from major hematopoietic disease centers in Japan, and will begin analyzing genomic data from these samples this fiscal year. The project aims to comprehensively search for abnormalities that occur in non-genetic coding regions and other regions of the genome, and to elucidate previously undiscovered pathomechanisms. We also aim to identify the genetic predisposition to leukemia.

1. Clonal progression mechanisms of Myeloproliferative neoplasms using whole genome sequencing across disease stages

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Myeloproliferative tumors have a chronic course in true polycythemia vera and essential thrombocythemia, some of which may progress to myelofibrosis and acute myelogenous leukemia. The genomic aberrant changes that occur in such cases remain to be elucidated. We performed whole-genome sequencing of bone marrow fluid samples from 23 patients with myeloproliferative tumors that had converted from chronic to acute myelogenous leukemia before and after conversion. Almost all of the patients had received JAK inhibitors. The results showed that in most cases, driver mutations found in myeloid tumors appeared as subclones of mutations characteristic of MPNs. The mutation signature showed the same pattern between the chronic phase and acute transformation, suggesting that the mutagenicity was identical for both phases. Interestingly, there were two cases of JAK2 mutation-positive MPNs that progressed to JAK2-negative secondary AML, both with a common parental clone, a result contrary to the conventional hypothesis of secondary AML emerging from completely different clones.

2. Insights from Trajectories of PIGA-mutated clones in Paroxysmal Nocturnal Hemoglobinuria

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The pathophysiology of clonal expansion with paroxysmal nocturnal hemoglobinuria (PNH) remains unclear. To elucidate the trajectories of PNH clones, we constructed phylogenetic trees using whole genome sequencing data of single cell-derived colonies of bone marrow samples from two PNH patients (52 colonies for case 1 and 77 for case 2). In case 1, we identified three PIGA-mutated and one BCOR-mutated clone with no shared common somatic variants, showing independent origin. In case 2, one PIGA-mutated clone was detected, in which HMGA2 mutations (Inoue et al. Blood. 2006) were accompanied in all the colonies examined. Bayesian estimation revealed that these PIGA mutations are assumed to be acquired around 14 years before the clinical PNH onset in both cases. PIGA-mutated clones exhibited a higher mutation acquisition rate than unmutated ones (16 vs. 11 mutations/year for case 1 and 14 vs. 12 for case 2). We estimated the effective population size of PIGA-mutated clones using coalesce principles. PIGA-mutated clones grew more slowly than unmutated normal clones of early embryonic development. In case 1, the onset of the significant clonal expansion of two PIGA-mutated clones seems to coincide with diagnosis of the aplastic anemia (AA), which supports the theory that immune attack associated with AA facilitated the expansion of PNH clones. This technique helps to infer the disease development mechanism by depicting the diseased clone's evolutionary history, which cannot be directly observed.

3. A forward-looking analytical study of minimal residual disease after allogeneic hematopoietic stem cell transplantation using circulating tumor DNA in acute myeloid leukemia: KSGCT1702

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Post-allogeneic relapse of AML/MDS is an important clinical issue, and there is a need to develop a non-invasive method to detect relapse at an early stage. To evaluate the usefulness of the minimal residual disease assay, which measures driver mutations identified by comprehensive genetic analysis in post-transplant AML/MDS patients by VAF of circulating tumor DNA in post-transplant patients' serum, in transplant patients in the Kanto Hematopoietic Stem Cell Group.

Patients diagnosed as AML/MDS cases according to WHO Classification 2008, aged 20 to 65 years, who may undergo allogeneic hematopoietic stem cell transplantation with myeloablative pretreatment at KSGCT participating centers, and whose written consent has been obtained from the patient. Target cases will be enrolled and next-generation sequencing will be performed on tumor and control (oral mucosa) specimens. Driver gene mutations will be identified and a Droplet Digital PCR (ddPCR) assay will be designed. Cell free DNA will be extracted from serum samples before and after bone marrow transplantation, and driver gene mutations will be quantitatively measured using ddPCR. 70 cases were enrolled from 12 centers, of which 12 cases dropped out before transplantation. Transplantation was performed in 58 cases, recurrence within 1 year after transplantation in 15 cases, and death within 1 year after transplantation in 10 cases (preimplantation death in 1 case, recurrence death in 4 cases). Of the transplant recipients, 57 underwent NGS to identify driver mutations. 52 were identified as MRD-eligible driver mutations. 48 completed MRD measurement by ddPCR.

4. A forward-looking analytical study of minimal residual disease after allogeneic hematopoietic stem cell transplantation using circulating tumor DNA in acute lymphoid leukemia: KSGCT1901

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Post-allogeneic relapse of ALL is an important clinical issue, and there is a need to develop a non-invasive method to detect relapse at an early stage. To evaluate the usefulness of the minimal residual disease assay, which measures driver mutations identified by comprehensive genetic analysis in post-transplant ALL patients by VAF of circulating tumor DNA in post-transplant patients' serum, in transplant patients in the Kanto Hematopoietic Stem Cell Group.

Patients 16 years of age or older, acute lymphoblastic leukemia according to WHO classification 2016, any history of chemotherapy at the time of transplantation, specimens with tumor volume of at least 20% available, potential for allogeneic transplantation, and written consent obtained from the patient. Target cases will be enrolled and next-generation sequencing will be performed on tumor and control (oral mucosa) specimens. Driver gene mutations will be identified and a Droplet Digital PCR (ddPCR) assay will be designed. Cell free DNA will be extracted from serum samples before and after bone marrow transplantation, and driver gene mutations will be quantitatively measured using ddPCR. There were 54 cases enrolled, 5 cases of pre-transplant dropout, and 43 cases of transplantation performed. NGS of tumor samples was performed in 40 cases and driver gene mutations were identified in 24 cases. A Droplet Digital PCR assay is being designed for each case.

5. Novel recurrent structural variants in hematopoietic malignancies and construction of Japanese-SV-specific panel of normal (PoN)

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Structural Variation analysis was performed with GRIDSS/MANTA/GenomonSV on a total of 1453 hematological paired whole genome sequencing(WGS) data. We found that the intersection of calls from GRIDSS, MANTA, GenomonSV, Delly and SVABA was less than 1%, and the vast majority of them are presumed to be false positive calls. This is mainly due to the inability of every caller to remove alignment errors at repetitive or low-complexity sequences. We, therefore, constructed Japanese specific Structural Variant Panel of Normal (PoN) with recurrent calls from samples of healthy japanese donors, aiming to provide a set of false-positive calls that should be subtracted from the call results of Japanese tumour samples. We confirmed a significant (>80%) reduction in the number of calls using this panel, and currently figuring out novel recurrent pathogenic structural variants.

Publications

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